Increased plasma β-hydroxybutyrate levels during the fasting test in patients with endogenous hyperinsulinaemic hypoglycaemia

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Abstract

Objective: The objective of the present study was to determine whether a plasma β-hydroxybutyrate (BOHB) level > 2700 μmol/l during the 72-h fasting test is sufficient to rule out the diagnosis of endogenous hyperinsulinaemic hypoglycaemia (EHH).

Research design and methods: We retrospectively studied BOHB levels in 39 patients with EHH who had undergone a 72-h fasting test to make the diagnosis of EHH, and we compared EHH patients with BOHB levels > 2700 μmol/l (group 1), EHH patients with BOHB levels < 2700 μmol/l (group 2) and 59 controls (median glycaemia: 3.2 mmol/l and median BOHB: 6095 μmol/l).

Results: During a 72-h fasting test, nine patients (group 1) had BOHB levels > 2700 μmol/l (median 6140 and range 2957–7824) and 30 patients (group 2) had BOHB levels < 2700 μmol/l (median 542 and range 0–2607). In group 1, four patients had undergone partial pancreatectomy previously and were evaluated for the recurrence of hypoglycaemia, whereas none of the group 2 patients had been operated. The duration of the fasting test was longer in group 1 than in group 2 (P<0.0001), and at the end of the fasting test, plasma glucose concentrations were not significantly different (P=0.0617), but insulin (P=0.004), C-peptide (P=0.0015) and proinsulin (P=0.0038) levels were significantly lower in group 1 patients than in group 2 patients, suggesting lower insulin secretion and/or impaired glycaemic counter-regulation.

Conclusion: During a fasting test, a BOHB level > 2700 μmol/l is observed in some EHH patients, suggesting that BOHB levels cannot rule out the recurrence of EHH, in particular, after partial pancreatectomy.

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Introduction

The diagnosis of endogenous hyperinsulinaemic hypoglycaemia (EHH), due to insulinomas or nasidioblastosis, in patients with Whipple’s triad is based on hypoglycaemia (plasma glucose concentration < 3 mmol/l), either spontaneously or during a 72-h fasting test, associated with inappropriate secretion of insulin or other β-cell polypeptides (C-peptide and proinsulin) (1). In patients without EHH, inhibition of insulin secretion during fasting is responsible for lipolysis and ketogenesis. The increase of ketone bodies during the 72-h fasting test is thus considered to be one of the best criteria for ruling out the diagnosis of EHH. Indeed, a plasma β-hydroxybutyrate (BOHB) level > 2700 μmol/l combined with a plasma glucose concentration < 3.3 mmol/l has been reported to have 100% sensitivity and specificity for ruling out EHH (2). However, a few cases of EHH patients with plasma BOHB levels > 2700 μmol/l during the fasting test have recently been reported (3, 4, 5).

In addition, there is no specific study regarding the validity of the usual diagnostic criteria of EHH for the diagnosis of recurrence of EHH after partial pancreatectomy.

This prompted us to retrospectively study the BOHB levels observed during the 72-h fasting test in our EHH patients and to try to characterise those patients with BOHB levels > 2700 μmol/l in order to determine i) whether a plasma BOHB level > 2700 μmol/l during the 72-h fasting test is sufficient to rule out the diagnosis of EHH and ii) whether this criterion loses its validity in a specific subgroup of EHH patients.

Subjects and methods

Patients with EHH

From 1988 to 2009, 39 patients with EHH (20 women and 19 men), aged 55 (median) years (range 23–82),
underwent a 72-h fasting test with BOHB measurements. The fasting test was performed to make the initial diagnosis when the patients first consulted (n=35) or the diagnosis of recurrent EHH after transient post-surgical remission (n=4) in two patients with malignant insulinomas and two patients with nesidioblastosis. The median BMI of our 39 patients was 25.0 kg/m² (range 19.0–43.0) and three patients had a BMI > 30 kg/m².

Thirty-one patients had histopathologically proven insulinomas (median insulina size=14 mm and range 6–45). Among them, seven had malignant insulinomas (defined by the occurrence of metastasis) and two had genetically proven multiple endocrine neoplasia type 1 (MEN-1) (one had hyperprolactinæmia and hyperparathyroidism, while the other had been treated previously for hyperparathyroidism and had undergone bilateral adrenalectomy for Cushing’s disease). Three other patients had histopathological findings consistent with nesidioblastosis. The remaining five patients had symptomatic hypoglycaemia with laboratory findings being consistent with EHH, though they had no radiological evidence of a pancreatic tumour. Among them, one patient had hypoglycaemia (1.7 mmol/l) with an insulin level of 17 mIU/l, a C-peptide level of 2.9 ng/ml and a proinsulin level of 73 pmol/l; the second patient had a plasma glucose concentration of 1.6 mmol/l concomitant with insulin, C-peptide and proinsulin levels of 34 mIU/l, 1.2 ng/ml and 50 pmol/l respectively; the third patient had a plasma glucose concentration of 1.9 mmol/l with a concomitant C-peptide level of 3.3 ng/ml; and the fourth had a plasma glucose concentration of 2.6 mmol/l with insulin, C-peptide and proinsulin levels of 20 mIU/l, 8.9 ng/ml and 110 pmol/l respectively, while the last one had a plasma glucose concentration of 2.8 mmol/l with insulin, C-peptide and proinsulin levels of 11 mIU/l, 3.2 ng/ml and 184 pmol/l respectively. All these five patients required treatment with either diazoxide or octreotide in order to control clinical hypoglycaemia. In all EHH patients, no serum sulphonylureas were detected. All patients had normal liver and renal functions.

**Controls**

Fifty-nine controls (39 women and 20 men) aged 40 (median) years (range 17–85 years) were evaluated for suspected hypoglycaemia. EHH was ruled out if the fasting test showed a nadir plasma glucose concentration > 3.0 mmol/l or a nadir plasma glucose concentration between 2.2 and 3.0 mmol/l associated with insulin, C-peptide or proinsulin levels below the diagnostic thresholds defined by the Endocrine Society guidelines (1). Median BMI was 21.9 kg/m² (range 17.0–35.0 kg/m²) and four controls had a BMI > 30 kg/m². Clinical follow-up of the control subjects after the fasting test did not reveal the occurrence of symptomatic hypoglycaemia with neuroglycopenic symptoms. All controls had normal liver and renal functions.

**Methods**

**The 72-h fasting test** The patients and controls underwent a 72-h fasting test under close medical supervision. Briefly, blood glucose, insulin, C-peptide and proinsulin levels were measured every 4 h during the fasting test. Additional samples were collected when the plasma concentration of glucose was below 2.5 mmol/l or if clinical symptoms of hypoglycaemia occurred. The fasting test was discontinued before 72 h if the patients presented with either severe neuroglycopenic symptoms or milder symptoms of hypoglycaemia and confirmed plasma glucose concentration below 2.5 mmol/l.

**Assays** Plasma concentrations of glucose were measured using venous samples drawn in sodium fluoride tubes with a standard method (hexokinase). Serum insulin levels were measured using an IRMA (Bi-Insulin Biorad kit, Pasteur Diagnostics, Marnes La Coquette, France). The inter- and intra-assay coefficients of variation were 8.0 and 3.8% respectively. The detection limit was 0.2 mIU/l (1.4 pmol/l). The normal range for fasting insulin levels is 2–17 mIU/l (14–122 pmol/l).

Serum C-peptide levels were measured using an IRMA (RIA C-peptide CTK, Sorin Biomedica, Saluggia, Italy). The inter- and intra-assay coefficients of variation were 12.6 and 4.4% respectively. The detection limit of the assay was 0.08 ng/ml (0.025 nmol/l). The normal range for fasting C-peptide levels is 0.5–3.0 ng/ml (0.17–1.00 nmol/l). Serum proinsulin levels were measured using an IRMA (Human Proinsulin Kit, Linco Research, St Charles, MO, USA). The inter- and intra-assay coefficients of variation were 7.7 and 6.9% respectively. The detection limit of the assay was 2 pmol/l. The normal range for fasting proinsulin levels is 7.9 ± 1.5 pmol/l. Samples for the detection of serum insulin, C-peptide and proinsulin levels were drawn in dry tubes. Samples for the detection of BOHB levels were drawn in sodium fluoride tubes in ice, and BOHB levels were measured using an automated kinetic method (6).

**Statistical analysis**

Since the data did not show a Gaussian distribution, values are given as median value and range.

Non-parametric tests such as the Mann–Whitney U test and the Spearman correlation test were used for statistical analysis (Statview 5 program, SAS Institute, Inc., Cary, NC, USA). The results were considered to be significant if P < 0.05.
Results

Patients with EHH and BOHB levels >2700 μmol/l (group 1)

Among the 39 EHH patients who underwent a 72-h fasting test, serum BOHB levels in nine patients (four men and five women) with a median age of 55 years (range 32–67) reached values above 2700 μmol/l (median BOHB: 6140 μmol/l and range 2957–7824), and these patients constituted group 1. The clinical and biochemical characteristics of these nine patients are reported in Table 1. All four patients examined for the recurrence of hypoglycaemia after partial pancreatic resection (patients 1 and 2 after caudal pancreatectomy and patients 3 and 4 after pancreaticoduodenectomy for malignant insulinomas) were included in group 1 (Table 2). Moreover, patient 7 had MEN-1 with multiple pancreatic tumours and had previously undergone bilateral adrenalectomy for Cushing’s disease and had adequate mineralocorticoid and glucocorticoid replacement therapy.

During the fast, six of nine patients had neuroglycopenic symptoms, whereas the remaining three patients had milder clinical symptoms of hypoglycaemia. In all patients, nadir plasma glucose concentrations reached values below 3.3 mmol/l (median nadir plasma glucose concentration: 2.5 mmol/l and range 1.6–3.2). During the fasting test, plasma glucose concentrations reached values below 3.0 mmol/l in eight of nine patients and below 2.5 mmol/l in six of nine patients. In patient 8, hypoglycaemia occurred at the beginning of the fasting test, immediately followed by a spontaneous remission, and plasma glucose concentrations remained above 3.3 mmol/l thereafter.

In five of the six patients whose plasma glucose concentrations reached values of 2.5 mmol/l or less (patients 1, 2, 5, 7, 8 and 9), concomitant serum proinsulin levels were inappropriate high, i.e. above 5 pmol/l (median: 9.2 pmol/l and range 7.3–92.0) (proinsulin levels were not measured in patient 9). In three of these five patients (patients 1, 2 and 7), the other traditional criteria for the diagnosis of EHH (insulin and C-peptide) would not have been helpful in making the diagnosis of EHH. In patients 3, 4 and 6, plasma glucose concentrations remained > 2.5 mmol/l throughout the fast (lowest glucose concentrations of 2.6, 3.2 and 2.9 mmol/l respectively). The diagnosis was based on plasma proinsulin levels in patient 6 (> 2.2 pmol/l throughout the fast). Interestingly, during his fasting test, this patient had a transient (at 56 h) decrease in plasma glucose levels (2.9 mmol/l) with a concomitant rise in the levels of insulin and other peptides that are co-secreted by β-cells. For both patients with recurrent malignant insulinomas after pancreaticoduodenectomy (patients 3 and 4), the diagnosis was based on the recurrence of clinical symptoms of hypoglycaemia combined with radiological evidence (CT scan) of the tumour process, i.e. liver metastases in patient 3 and local growth in patient 4. In patient 3, the insulin level was inadequate at the time of hypoglycaemia, whereas the remaining three patients had recurrent malignant insulinomas after pancreaticoduodenectomy and these patients constituted group 1. The clinical and biochemical characteristics of these nine patients are reported in Table 1. All four patients examined for the recurrence of hypoglycaemia after partial pancreatic resection (patients 1 and 2 after caudal pancreatectomy and patients 3 and 4 after pancreaticoduodenectomy for malignant insulinomas) were included in group 1 (Table 2). Moreover, patient 7 had MEN-1 with multiple pancreatic tumours and had previously undergone bilateral adrenalectomy for Cushing’s disease and had adequate mineralocorticoid and glucocorticoid replacement therapy.

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Table 2 Main characteristics of EHH patients with BOHB levels >2700 μmol/l (group 1) and EHH patients with BOHB levels <2700 μmol/l (group 2).

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**Patients with EHH and BOHB levels <2700 μmol/l (group 2)**

Among the 30 EHH patients with BOHB levels <2700 μmol/l, 25 had insulinomas (three malignant and 22 benign tumours) and one had nesidioblastosis. Four patients fulfilled the biochemical criteria of EHH with a negative radiological exploration. No patient had a history of previous pancreatic surgery (Table 2). By the end of the fasting test, all patients had a plasma glucose concentration below 3.0 mmol/l and 22 patients had a plasma glucose concentration below 2.5 mmol/l. C-peptide and proinsulin levels were inappropriately high for all group 2 patients.

**Comparison between the nine EHH patients with BOHB levels >2700 μmol/l (group 1) and the 30 EHH patients with BOHB levels <2700 μmol/l (group 2)**

There was no significant difference in age and BMI between the two groups. However, the duration of the fasting test was longer in the nine EHH patients who had BOHB levels >2700 μmol/l (group 1) than in the other patients with EHH with BOHB levels <2700 μmol/l (group 2). Indeed, the median duration of the fasting test in group 1 was 72 vs 9.5 h in group 2 (P<0.0001), and the mean duration of the fasting test in group 1 was 60 vs 15.7 h in group 2. In group 1, plasma glucose concentrations of the patients reached values below 3.0 mmol/l at a later time point during the fasting test (median: 36 h, range 0–58 vs 2 h, range 0–42, P=0.0049). At the end of the fasting test, plasma glucose concentrations were similar in both the groups of patients, but insulin, C-peptide and proinsulin levels were significantly lower (P=0.004, 0.0015 and 0.0038 respectively) in group 1 than in group 2 (Table 3).

**Comparison between group 1 patients and controls**

Median BMI was significantly higher in group 1 patients than in controls (P=0.0429) (Table 3). There was no significant difference during the first 24 h of the fasting test in median plasma glucose, serum insulin, C-peptide, proinsulin or BOHB levels. However, after 48 and 72 h of fasting, median plasma glucose levels were significantly lower in group 1 patients than in controls (48 h: 2.9 mmol/l, range 2.0–3.7, n=8, vs 3.4 mmol/l, range 2.5–5.5, P=0.03, and 72 h: 2.5 mmol/l, range 1.9–3.8, n=5, vs 3.2 mmol/l, range 2.2–5.3, P=0.0118). There was no significant difference between group 1 patients and controls regarding serum insulin, C-peptide, proinsulin and BOHB levels at the different time points of the fasting test. Finally, the median duration of fast necessary to reach a BOHB level >2700 μmol/l did not differ significantly between group 1 patients (median value =48 h, range 0–72) and controls (median =49 h, range 24–72) (P=0.36).

**Correlation between BOHB and insulin levels in EHH patients and controls**

There was a significant negative correlation between BOHB and serum insulin levels in the 30 EHH patients

Table 3 Comparison between nine EHH patients with BOHB levels >2700 μmol/l at the end of the fasting test (group 1), 30 EHH patients with BOHB levels <2700 μmol/l (group 2) and control subjects (n=59). Data shown for plasma glucose, insulin, C-peptide, proinsulin and BOHB levels are those observed at the end of the fasting test.

<table>
<thead>
<tr>
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<th>Group 1 n=9</th>
<th>Group 2 n=30</th>
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<td>2957–7806</td>
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aMedian duration of fast is 72 h (with a range of 27–72 h) since most (5/9) patients in group 1 completed the 72-h fasting test.
with BOHB levels <2700 μmol/l (P = 0.0068), whereas no significant correlation was found between these two parameters in controls (P = 0.09) or in the nine EHH patients with BOHB levels >2700 μmol/l (P = 0.22).

Discussion

BOHB level observed during the 72-h fasting test has a place of choice in the evaluation of patients with suspected hypoglycaemia. According to the Mayo Clinic study on the diagnosis of 237 patients with surgically proved insulinomas, a plasma BOHB concentration >2700 μmol/l at the time of hypoglycaemia below 3.3 or 2.75 mmol/l rules out the diagnosis of EHH with 100% sensitivity and specificity (2). It is not specified whether this criterion can be applied for the diagnosis of recurrence of EHH after partial pancreatectomy or only for the initial diagnosis. Recently, plasma BOHB levels greater than this threshold have been reported in a few patients with EHH during the fasting test. Wiesli et al. reported of an 80-year-old woman who presented symptoms of hypoglycaemia with a plasma glucose concentration of 2.7 mmol/l and inappropriate insulin (67 pmol/l = 9.3 mU/l) and C-peptide (310 pmol/l = 0.93 ng/ml) levels during a fasting test. Plasma BOHB level at the time of hypoglycaemia was 3005 μmol/l (4, 5). Other authors reported of a 49-year-old man who had a plasma glucose concentration of 1.76 mmol/l with an insulin level of 1.1 mU/l, a C-peptide level of 1.2 ng/ml and a BOHB level of 3200 μmol/l during a fasting test (5). Interestingly, hyperinsulinaemic hypoglycaemia was triggered by a glucagon stimulation test in both of these cases. The authors suggested that the insulinoma of their patients had a secretory profile similar to that of normal b-cells. In these two patients, hypoglycaemia was in fact triggered by two classic secretagogues of normal b-cells (glucagon and glucose during the oral glucose tolerance test). Moreover, histological examination of the insulinoma of the first patient revealed perineural immunostaining with anti-proinsulin antibodies similar to that observed in normal b-cells, whereas it is usually diffuse in insulinoma cells (7).

Among our patients with EHH, nine patients had BOHB levels >2700 μmol/l and a lower median value of insulin levels during the 72-h fasting test than the other patients with EHH. In all of them, plasma glucose concentrations had reached values below 3.3 mmol/l, i.e. glucose concentrations for which concomitant BOHB levels had been reported to have 100% diagnostic specificity and sensitivity to distinguish EHH patients from controls (2). In such patients, higher BOHB and lower insulin levels could be related to different mechanisms.

First, four patients (patients 1, 2, 3 and 7) had a positive fasting test with symptomatic hypoglycaemia (below 2.5 mmol/l in three of them) and low (<3.0 mU/l) though detectable insulin levels. Patients 1, 2 and 3 had undergone caudal pancreatectomy or pancreaticoduodenectomy and patient 7 had been previously treated by bilateral adrenalectomy for Cushing’s disease. In these patients, high BOHB levels could be explained by the lower insulin secretion, and impaired counter-regulation would explain the occurrence of fasting hypoglycaemia despite the lower insulin secretion, which nevertheless remained inappropriate for plasma glucose concentrations. Partial pancreatectomy acts on the secretion of both insulin and glucagon by reduction of b- and α-cell mass. There is a decrease in the secretion of both basal insulin and glucose-stimulated insulin, while the secretion of basal glucagon is higher, but the secretion of glucagon in response to hypoglycaemia is reduced (8, 9).

Second, two patients (patients 5 and 9) had a positive fasting test with symptomatic hypoglycaemia below 2.5 mmol/l and inappropriate insulin or C-peptide levels, attesting that insulin secretion was abnormal and that mechanisms responsible for ketogenesis could overcome the effect of insulin as reported previously (3, 4, 5). Two recent studies have given evidence for such a phenomenon. An escape of ketogenesis from inhibition by insulin during hypoglycaemia has been shown in pancreatectomised dogs undergoing a hyperinsulinaemic clamp with euglycaemia or hypoglycaemia (10). Moreover, similar results were observed during hyperinsulinaemic hypoglycaemia in humans (11). Lipolysis can escape the inhibitory effect of insulin during the rise in counter-regulatory hormone levels. Therefore, in some patients in whom plasma glucose concentrations reach lower values (below 2.75 mmol/l as reported in dogs), ketogenesis could partly escape the inhibitory effect of insulin (10).

Finally, three patients (patients 4, 6 and 8) had a negative fasting test, i.e. their plasma glucose concentrations remained above 3.0 mmol/l at the end of the fast, suggesting that there was little or no secretion of insulin by the tumour during the 72-h fasting test. Similar cases of low-secreting EHH have been reported previously, mainly in patients with nesidioblastosis (12, 13). In the Mayo Clinic study, three insulinoma patients with postprandial hypoglycaemia had negative fasting tests (2). However, none of our three patients had postprandial symptoms or nesidioblastosis, and this may be explained by an erratic and unpredictable insulin release by insulinoma cells (14). The fasting test of patient 6 illustrates this secretory pattern: during the fast, he transiently presented a plasma glucose concentration of 2.9 mmol/l with a concomitant rise in insulin levels. Such isolated and short bursts of insulin secretion by the insulinoma cells could be insufficient to prevent the rise in BOHB levels during the prolonged fast.

Placzkowski et al. (2) reported 100% sensitivity and specificity for BOHB levels with the threshold of
with EHH, two (5%) had BOHB levels reached values above 2700 μmol/l during the fasting test. On the other hand, the group of patients whose BOHB levels reached values above 2700 μmol/l during the fasting test (group 1) comprised all four patients who had recurrence of EHH after partial pancreatectomy and a MEN-1 patient after bilateral adrenalectomy, suggesting that BOHB levels lose their diagnostic accuracy in patients with impaired glycaemic counter-regulation and should not be used for ruling out the diagnosis of recurrence of EHH after partial pancreatectomy.

It can also be observed that four of our group 1 patients had malignant insulinomas (two at the initial diagnosis of EHH and two at the recurrence of EHH after undergoing partial pancreatectomy previously). To the best of our knowledge, there is no previous case report in the literature describing similar findings. Therefore, the prevalence of such patients in our study calls attention to the need for care in interpreting the results of the 72-h fasting test in patients with suspected recurrent malignant insulinomas after partial pancreatectomy.

Finally, the threshold of 2700 μmol/l for BOHB levels had been validated as a diagnostic criterion for plasma glucose concentrations between 3.3 and 2.8 mmol/l during the fasting test; however, for plasma glucose concentrations below 2.8 mmol/l, BOHB level was seen as a complementary diagnostic criterion to C-peptide levels (15). Plasma glucose concentrations below 2.5 mmol/l are rather rare in normal subjects during the fasting test (16, 17), and no control subject was found to have plasma glucose concentration below 2.2 mmol/l (16) or 2.0 mmol/l (18). Based on our results, we suggest that in patients with symptomatic hypoglycaemia, when plasma glucose concentrations reach values below 2.5 mmol/l, the diagnosis of EHH should not be ruled out on the basis of a BOHB level above 2700 μmol/l alone and should be based on inappropriate C-peptide and/or proinsulin levels, which are known to have a high diagnostic specificity for such plasma glucose concentrations (17).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
A Buffet collected and analysed the data and wrote this paper. D Vezzosi, J C Maiza and S Grunenwald provided the data of EHH patients. A Bennet co-directed this work. P Caron directed this work.

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