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Evaluation of Endocrine Tests. D: the prolonged fasting test for insulinoma

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ABSTRACT

Objective: To establish the diagnostic performance of the prolonged fasting test in patients suspected of insulinoma.
Methods: We included all patients who were referred to our department between August 1995 and August 2006 with a clinical suspicion of insulinoma. Insulinoma was diagnosed by a positive Whipple's triad during the prolonged fast in combination with an insulin/C-peptide ratio below 1. The presence of insulinoma was confirmed by histopathological data, which was considered the golden standard. If the prolonged fast was negative, long-term follow-up was obtained.
Results: Ten patients had a positive Whipple's triad during the prolonged fast: eight had a histologically proven insulinoma, and two had factitious hypoglycaemia (insulin/C-peptide ratio >1.0) One additional patient likely had an insulinoma, but the Whipple's triad remained absent at up to 56 hours of fasting. Follow-up (median 53 months (3 to 142) in 76% of patients with a negative fasting test revealed no missed cases of insulinoma. During the prolonged fast the glucose, insulin and C-peptide concentrations overlapped in patients with and without insulinoma.
Conclusion: In our centre, the prolonged fasting test defined as a positive Whipple's triad in combination with an insulin/C-peptide ratio <1 had a sensitivity of 88.9% and a specificity of 100% for the diagnosis of insulinoma.

KEYWORDS

Insulinoma, prolonged fast, Whipple's triad

INTRODUCTION

In patients with a clinical suspicion of endogenous hyperinsulinism a prolonged fast is the recommended first test in the diagnostic work-up. The golden standard for a positive fasting test is the presence of a Whipple's triad and an insulin/C-peptide ratio below 1.[1] The Whipple's triad is positive if biochemical hypoglycaemia is accompanied by neuroglycopenic symptoms, with disappearance of these symptoms after correction of the hypoglycaemia. To differentiate between factitious hypoglycaemia and insulinoma the insulin/C-peptide ratio should be determined at the time point when Whipple's triad is positive. In the case of insulinoma, the ratio has been reported to be lower than 1.0.[1] To further substantiate endogenous hyperinsulinism the presence of sulphonylurea derivatives in serum should be excluded. Our aim was to report our experience with the prolonged fasting test in a group of patients with clinical suspicion of insulinoma referred to our hospital.

MATERIALS AND METHODS

Patients

We included all patients referred to our department between August 1995 and August 2006 because of clinical suspicion of insulinoma. They all were subjected to a supervised prolonged fasting test. When neuroglycopenic signs and symptoms occurred during the fast in the presence of hypoglycaemia (venous glucose below 2.8 mmol/l),[2] a blood sample was taken for determination of the insulin/C-peptide ratio and for the analysis of the presence of sulphonylurea derivatives. In patients meeting the Whipple's triad, factitious hypoglycaemia was diagnosed by an insulin/C-peptide ratio exceeding 1.0 or by the presence of sulphonylurea derivatives. In patients meeting the criteria of Whipple in whom factitious hypoglycaemia had been excluded, abdominal imaging was performed and a definitive diagnosis of insulinoma was sought by pancreas surgery. In patients not meeting the Whipple's triad during

the fast, insulinoma was excluded by extended follow-up. If our patients were no longer visiting our outpatient clinic, we contacted their general practitioners by telephone to look for evidence that insulinoma had been diagnosed during the follow-up. If no information could be obtained, the follow-up period was arbitrarily set at three months.

Prolonged fasting test

Patients were admitted to the Department of Internal Medicine of the Academic Medical Centre, Amsterdam. The first blood sample was withdrawn at midnight, marking the beginning of the prolonged fasting test. Patients were allowed to drink only calorie-free drinks. Blood samples were routinely taken at 8.00 am and 8.00 pm for assay of glucose, insulin and C-peptide. The fast was stopped after 80 hours. If neuroglycopenic symptoms occurred, a venous blood sample was drawn. If the glucose was below 2.8 mmol/l, 50 ml glucose 50% was administered intravenously and the response of the patient was recorded after 15 minutes.

Analytical methods

Insulin was assayed until March 2004 with a RIA (Pharmacia Diagnostics, Uppsala, Sweden) and thereafter on an Immulite system (Siemens Healthcare Diagnostics B.V., Breda, the Netherlands). Both assays had an inter-assay coefficient of variation of 7% at 60 pmol/l and 4.5% at 120 pmol/l and a detection limit of 15 pmol/l. There was no systematic difference in insulin measurements between both assays. Cross-reactivity of pro-insulin in the Pharmacia assay was 25% and on the Immulite system 8%, and there was no cross-reactivity with the C-peptide in either assay.

C-peptide was measured with a RIA (RIA-coat C-peptid, Byk Sangtec Diagnostica, Dietzenbach, Germany). The inter-assay coefficient of variation was 9% at 100 pmol/l and 7% at 500 pmol/l. From April 2004, C-peptide was determined with a RIA of Linco (St. Charles, USA). The inter-assay coefficient of variation was 6% at 100 pmol/l and 4% at 500 pmol/l. All results were transformed to the Linco assay (Linco = 0.8 x Byk-Sangtec). The detection limit was 50 pmol/l. Cross-reactivity of pro-insulin was <4% in the Linco assay and 78.5% in the Byk assay.

Glucose was measured using the hexokinase method on a Hitachi Modular P800 system (Roche Diagnostics, Almere, the Netherlands).

Statistical analysis

Values below the detection limit of the assays were included in the analyses as having a value of 50% of the detection limit. Normality was tested by the Kolmogorov-Smirnov test. Differences in demographic characteristics, glucose, insulin and C-peptide were evaluated using the Student t test and nonparametric tests where appropriate. P values of less than 0.05 were considered statistically significant.

All statistical analyses were performed using the Statistical Package of Social Sciences and Problem Solutions (SPSS version 16.0).

RESULTS

A total of 82 prolonged fasting tests were performed. We excluded one patient who underwent the prolonged fasting test after previous resection of an insulinoma. Among the remaining 81 patients, ten had a positive Whipple's triad: two patients had a factitious hypoglycaemia (insulin/C-peptide ratio >1.0; sulphonylurea derivatives in serum were absent), and eight had an insulinoma (insulin/C-peptide ratio <1.0) (*table 1*). Among the insulinoma patients, the Whipple's triad had occurred within 48 hours in all except one. In that patient neuroglycopenic symptoms developed after 67 hours of fasting; after 56 hours the glucose level was below 2.5 mmol/l, but the insulin (18 pmol/l) and C-peptide (220 pmol/l) were still not suppressed.

Table 1. *Time in hours of prolonged fast at which a positive Whipple's triad was observed*

Patient number	Time hours	Glucose mmol/l	Insulin pmol/l	C-peptide pmol/l	Insulin/C-peptide ratio	Localisation insulinoma
1	5	2.2	71	1150	0.06	Tail, 1.2 cm
2	17	2.2	304	1500	0.2	Tail, 1.9 cm
3	19	2.1	64	500	0.13	Tail, 1.2 cm
4	21.5	1.3	255	713	0.36	Head
5	34	1.9	105	712	0.15	Head, 1.2 cm
6	39	1.8	220	1048	0.21	Corpus/tail
7	44	2.2	80	432	0.19	Tail, 1.5 cm
8	67	1.9	17	190	0.09	Tail, 1.4 and 0.6 cm
10	32	2.8	420	40	10.5	-
11	64	2.7	280	40	7	-

Due to insulinoma in patients 1 to 8 and to factitious hyperinsulinaemia patients in 10 and 11; patient 9 is not mentioned because of a negative Whipple's triad.

Definitive proof of insulinoma in these eight patients was obtained from histopathology of the surgical specimen (*table 1*). One additional case of insulinoma was found in a patient (no. 9) in whom no neuroglycopenic symptoms occurred during the prolonged fast, which for unknown reasons was discontinued after 56 hours. During the fast the lowest measured glucose concentration was 1.9 mmol/l with an insulin level of 32 pmol/l and a C-peptide level of 460 pmol/l (insulin/C-peptide ratio 0.07). Because of repeated findings of low glucose and elevated insulin concentrations on follow-up, imaging studies were performed because of the persistent clinical suspicion of endogenous hyperinsulinaemia. Computed tomography and endoscopic ultrasonography did not show any abnormality of the pancreas. However, positron emission tomography (PET) with fluorine-18 L-3,4- dihydroxyphenylalanine (^{18}F-DOPA) revealed elevated uptake in the head of the pancreas. Surgery was declined by the patient, excluding any histopathological confirmation. We consider the result of the prolonged fast in this patient as a false-negative.

Follow-up was completed in 76% of all the patients with a negative Whipple's triad during the prolonged fast. Evidence had not emerged in any of these patients for the existence of an insulinoma during follow-up (median duration 53 months, range 3 to 142). One patient died due to ruptured aneurysm of the abdominal aorta. There was a tendency for insulinoma patients to be older, more often female, and to have a higher BMI than non-insulinoma patients (*table 2*).

Table 2. *Patient characteristics*

	Insulinoma	Non-insulinoma	Significance p value
N	9	72	
Age (years)	66 (28-83)	43(16-86)	0.06
Women (%)	66.7	59.7	0.69
BMI	25.4 (19.7-29.7)	22.6 (17.7-39.6)	0.29

Values are given as median and range

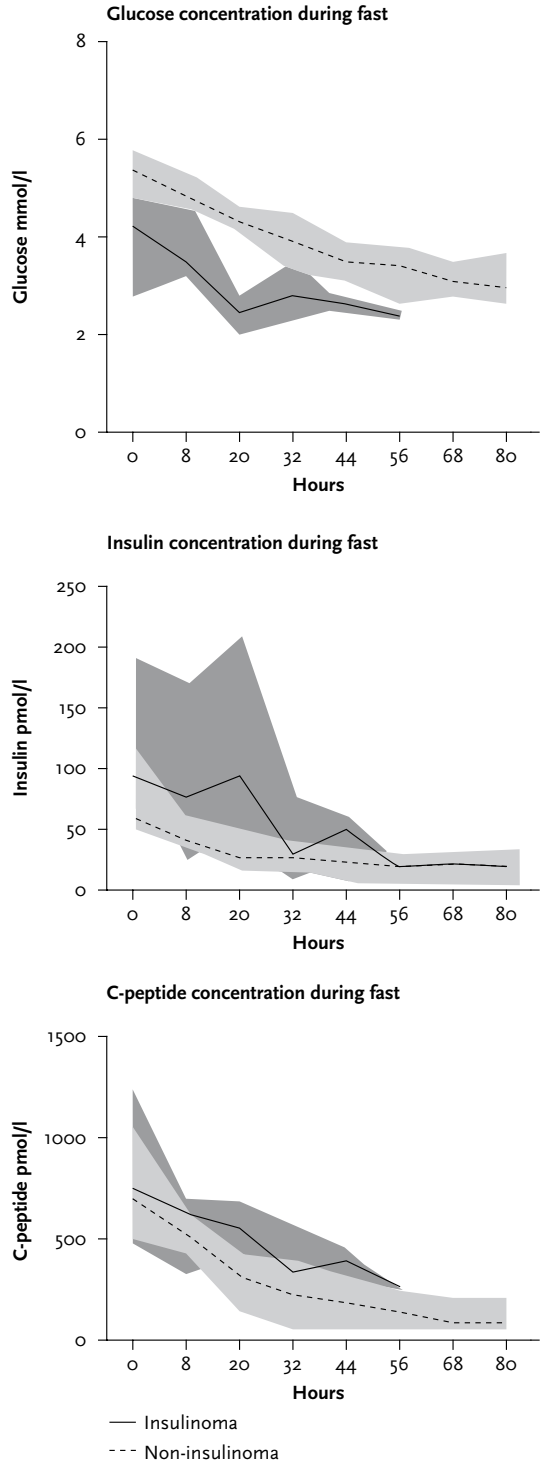
In our further analysis of venous glucose, insulin and C-peptide concentrations during the prolonged fast we excluded five patients in the non-insulinoma group, i.e., the two patients with factitious hypoglycaemia, and three patients because of missing values. Another two patients were analysed only until the time point they had obviously eaten something during the fast, as evident from elevated glucose, insulin and C-peptide levels at t=44 and t=68 hours, respectively. The time course of the biochemical parameters during the prolonged fast in the nine insulinoma and the 67 non-insulinoma patients is given in *table 3* and *figure 1*. There was a considerable overlap between insulinoma and non-insulinoma patients, although mean glucose levels were lower and insulin and C-peptide levels were higher in the insulinoma patients. Consequently, the prolonged fasting test defined as a positive Whipple's triad in combination

Table 3. *Glucose, insulin and C-peptide concentrations during a prolonged fast in insulinoma (I) (n=9) versus non-insulinoma (NI) patients (n=67)*

Hours of fasting	Glucose mmol/l		Insulin pmol/l		C-peptide pmol/l	
	I	NI	I	NI	I	NI
T=0	3.7	5.3	100	57	880	660
	2.9-5.7	4.9-5.7	76-144	41-105	519-1235	496-1048
p value	0.037		0.078		0.665	
T=8	3.5	5.0	66	40	560	488
	3.2-4.6	4.7-5.2	32-153	34.5-60	356-674	381-625
p value	0.001		0.133		0.572	
T=20	2.4	4.4	60	25	488	296
	2.1-2.7	4.1-4.7	35-184	16-35	408-672	152-400
p value	0.000		0.005		0.015	
T=32	2.7	4.2	32	21	344	230
	2.0-3.2	3.7-4.6	11-68	7.5-36	276-500	140-368
p value	0.001		0.394		0.131	
T=44	2.4	3.7	38	20	336	180
	2.1-2.6	3.2-4.1	27-60	7.5-30.8	280-448	40-264
p value	0.023		0.052		0.036	
T=56	2.2	3.4	25	20	350	138
	2.0-2.4	3.0-3.9	18-32	7.5-28	220-480	40-252
p value	0.022		0.416		0.103	
T=68		3.3		16		112
		2.9-3.6		7.5-28		40-200
T=80		3.2		20		128
		2.7-3.7		7.5-30		40-192

Values are given as median and interquartile range

Figure 1. *Glucose, insulin and C-peptide levels during the prolonged fast in nine insulinoma and in 67 non-insulinoma patients*



Values are given as median plus minimum and maximum range.

with an insulin/C-peptide ratio <1 had a sensitivity of 88.9% and a specificity of 100% for the diagnosis insulinoma.

We made two additional observations in the non-insulinoma group. First, 36 patients recognised their symptoms during the prolonged fast, but the Whipple's triad was absent and their glucose, insulin and C-peptide levels were higher than in the insulinoma patients (*table 4*). Second, glucose levels ≤2.5 mmol/l during the fast were observed at n=29 time points in patients without endogenous hyperinsulinaemia and without symptoms (*table 5*).

Table 4. *Plasma concentrations of glucose, insulin and C-peptide at time of symptoms in the non-insulinoma group compared with patients with insulinoma (n=8)*

	Insulinoma	Non-insulinoma	p value
N	**8**	**36**	
Glucose (mmol/l)	1.9 (1.3-2.2)	4.0 (2.5-5.9)	<0.001
Insulin (pmol/l)	93 (17.0-304.0)	29.0 (7.5-124.0)	0.003
C-peptide (pmol/l)	712.5 (190-1500.0)	210 (40.0-680.0)	0.001
Time (hours)	28 (5.0-67.0)	32.0 (4.5-76.0)	0.73

Time is defined as number of hours until Whipple's triad in the insulinoma group or symptoms in the non-insulinoma group. Values are given as median and range (minimum and maximum)

Table 5. *Patients in the non-insulinoma group with glucose concentrations ≤2.5 mmol/l*

Number of patients	Glucose levels mmol/l	Insulin levels pmol/l	C-peptide levels pmol/l
11	2.5	7.5-40	40-240
4	2.4	7.5-28	40-190
3	2.3	7.5-17	40-260
3	2.2	7.5-38	40-328
4	2.1	7.5-27	40-336
1	2.0	32	480
2	1.9	7.5-32	80-368
1	1.8	7.5	150

DISCUSSION

We diagnosed an insulinoma in nine of 81 patients who underwent a prolonged fasting test because of clinical suspicion of endogenous hyperinsulinaemia. The diagnosis of insulinoma was confirmed by surgical pathology in eight patients and is highly likely in the ninth patient. In view of our extended follow-up it seems unlikely that we have missed any further cases of insulinoma. The nine insulinoma patients were slightly older, more often female and tended to have a higher BMI than non-insulinoma patients, which is in accordance with the

existing literature.[3-5] The incidence of insulinoma in our referral hospital is about one case per year.

Our data demonstrate that even in a population with a high pre-test likelihood of endogenous hyperinsulinaemia, the prolonged fast confirmed the clinical suspicion in only eight out of 81 patients (10%) as indicated by a positive Whipple's triad and insulin/C-peptide ratio below 1. Two positive triads of Whipple but a ratio above 1 were caused by factitious hypoglycaemia. Neither of these patients admitted this diagnosis, but during their long-term follow-up no insulinoma was found. Of the nine insulinoma patients, the prolonged fast gave a positive result in eight and a negative result in one patient. The diagnostic accuracy of the prolonged fast for insulinoma in our series is high (sensitivity 89%, specificity 100%). It is well conceivable that the patient with a negative prolonged fast who appeared to have a very high suspicion of insulinoma would have been positive if the prolonged fast had not been stopped prematurely after 56 hours. In this context it is noteworthy that a positive Whipple's triad had occurred in seven out of nine insulinoma patients (78%) within 48 hours of fasting. In large series reported earlier a positive Whipple's triad of fasting had occurred after 48 hours in 95%[5] and 93%,[6] and after 72 hours in 99%.[6] These data are in keeping with our experience, and do not imply that we should change the duration of our test.

Could the accuracy of the prolonged fast be improved? Hirschberg *et al.*[5] observed that serum proinsulin concentrations are poorly suppressed during fasting in insulinoma patients in contrast with non-insulinoma patients; the sensitivity of proinsulin in his series was 90% at the end of 72 hours fast. Vezzozi *et al.*[4] reported that the best criterion for the presence of an insulinoma was a proinsulin concentration above 5 pmol/l if the serum glucose was below 2.5 mmol/l, reporting sensitivity and specificity figures reaching 100%. Others included the assessment of beta-hydroxybutyrate (BHOB) in order to shorten the 72-hour fast.[3] BHOB is suppressed in insulinoma patients but there is an almost linear rise in BHOB after 18 hours fasting in insulinoma patients. If the cut-off level of BHOB is set at 2.7 mmol/l, 74% of persons in the non-insulinoma group will have reached this cut-off before 72 hours of fasting. Recently, the Endocrine Society published a clinical practice guideline about evaluation and management of an adult hypoglycaemia. Their recommendation, in case of a positive Whipple's triad and other potential causes of an hypoglycaemia are excluded, is measurement of glucose, insulin, proinsulin, BHOB, insulin antibodies and derivatives of sulphonylurea.[6]

Glucose, insulin and C-peptide serum concentrations during fasting do not really discriminate between insulinoma and non-insulinoma patients (*figure 1*). However, the analysis of these parameters is an excellent tool to distinguish between insulinoma and factitious hypoglycaemia in patients with a positive Whipple's triad. In keeping with the present study, Service *et al.*[8] reported overlap in glucose concentrations between insulinoma and non-insulinoma patients. Wiesli *et al.*[9] did not find plasma glucose below 2.5 to be indicative for insulinoma. Our study supports these findings: in the non-insulinoma group 11 subjects had a plasma glucose concentration of 2.5 mmol/l while 18 subjects reached a plasma glucose level below 2.5 mmol/l during fasting (*table 5*). Service *et al.*[10] found C-peptide levels higher than 200 pmol/l in all insulinoma patients. In our study C-peptide concentrations were above 190 pmol/l during the Whipple's triad, and C-peptide levels were above 200 pmol/l in the non-insulinoma group if glucose was below 2.5 mmol/l in the absence of neuroglycopenic symptoms (*table 5*).

It is intriguing why some patients have a marked fall in their glucose levels well into the hypoglycaemic range in the absence of hyperinsulinaemia (*table 5*). Ten of these patients participated in an extended metabolic study using stable isotope techniques. During these measurements no hypoglycaemic events occurred and no abnormalities in fatty acid oxidation or in amino acid/organic acid metabolism were observed.[11]

In conclusion a positive prolonged fast defined as a positive Whipple's triad and an insulin/C-peptide ratio below 1 has an excellent discriminative value for the presence of endogenous hyperinsulinaemia. To detect the few patients that become hypoglycaemic after 48 hours, the fast has to be continued for three days.

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