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Extended metabolic evaluation of suspected symptomatic hypoglycemia: the prolonged fast and beyond

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Abstract

The diagnostic evaluation of spontaneous hypoglycemia in adults is mainly directed at detecting an insulinoma. Its interpretation is troublesome in those patients who develop low venous plasma glucose levels with appropriate hypoinsulinemia during a prolonged supervised fast. In this study, we investigated in this group of patients whether abnormalities in intermediary metabolism (fatty acid oxidation and amino/organic acids) could be detected that might explain the hypoinsulinemic hypoglycemia. Ten patients with otherwise unexplained low venous plasma glucose levels (<3 mmol/L) during prolonged fasting were included in the study. The patients participated in an extended metabolic protocol based on stable isotope techniques after an overnight fast to explore abnormalities in endogenous glucose production and intermediary metabolism. Endogenous glucose production, glucoregulatory hormones, plasma acylcarnitines, gluconeogenic amino acids, and rates of fatty acid and carbohydrate oxidation after 16 and 22 hours of fasting were measured. Although during the prolonged fast all patients had low venous plasma glucose level, there were no hypoglycemic events during the extended metabolic protocol. No abnormalities in endogenous glucose production (compared with reference values obtained in young healthy volunteers), fatty acid oxidation, or amino acid/organic acids were found in this patient group. In a group of patients exhibiting low venous plasma glucose levels during prolonged fasting in whom insulinoma was excluded, we found no signs of metabolic disorders. Therefore, the observation of low plasma glucose values in this subgroup of patients probably does not warrant extensive metabolic evaluation.

1. Introduction

The diagnostic evaluation of spontaneous hypoglycemia can be troublesome, and the evaluation and management rely largely on clinical experience as discussed by the recent Endocrine Society Clinical Practice Guidelines on Evaluation and Management of Adult Hypoglycemic Disorders [1]. A prolonged supervised fast is a validated clinical test to confirm and explore suspected hypoglycemia [2]. However, a substantial part of the evaluated patients has plasma glucose levels that fall to less than 3.0 mmol/L in the absence of hyperinsulinemia and signs of neurohypoglycemia (unpublished observation).

Plasma glucose levels decrease during fasting, notwithstanding the integrated metabolic response that prevents energy depletion and protects the central nervous system from hypoglycemia [3-5]. This response consists among others of increased fatty acid oxidation (FAO) to spare glucose consumption. During fasting, the combination of low plasma insulin levels and increased levels of cortisol, growth hormone, glucagon, and catecholamines increases lipolysis of adipose tissue thereby increasing the plasma free fatty acid (FFA) concentration and subsequent FAO in oxidative tissues (eg, skeletal muscle) [6,7]. In addition, the

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increased FFA availability in the liver stimulates ketone body (KB) formation [5,8].

The decrease in plasma glucose levels during fasting is, in adults, mainly due to a decrease in endogenous glucose production (EGP) [4] that is the main denominator of the fasting plasma glucose level [9]. Despite the inevitable lowering of plasma glucose concentration during fasting, levels normally do not fall to less than 3.0 mmol/L [4,5]. However, healthy women may have plasma glucose levels less than 3.0 mmol/L during fasting [4,5,10]. In addition, in a number of inborn errors of metabolism, including glycogen storage diseases, disorders of gluconeogenesis, and disorders of mitochondrial FAO, marked fasting hypoglycemia is one of the most important biochemical signs [11].

Medium-chain acyl–coenzyme A (CoA) dehydrogenase deficiency (MCADD, OMIM 201450) is probably the most common FAO disorder in which fasting induced FAO results in the accumulation of toxic FAO intermediates and their CoA esters [11,12]. The latter are thought to contribute to the clinical presentation of FAO disorders: MCADD patients present with hypoketotic hypoglycemia despite hypoinsulinemia during episodes of fasting [11,12]. A number of other inborn defects of other, chain length–specific, acyl-CoA dehydrogenases, catalyzing the first step in intramitochondrial FAO, have now been characterized. All of these may present with hypoinsulinemic hypoketotic hypoglycemia [13]. Because the accumulating acyl-CoA esters are converted to their matching acylcarnitine esters and released in plasma; analysis of these plasma acylcarnitine profiles by tandem mass spectrometry is the current standard for the diagnosis of FAO disorders at the metabolite level [13].

In this descriptive report, we present 10 patients with hypoinsulinemic low venous plasma glucose levels in whom insulinoma had been excluded. The patients now participated in an extended metabolic diagnostic protocol designed to explore whether abnormalities in EGP (measured with the stable isotope technique), plasma amino/organic acids (defective gluconeogenesis and glycogenolysis), or FAO (whole-body FAO and plasma acylcarnitine profiles) could explain the clinical findings.

2. Subjects and methods

2.1. Subjects

Between 2000 and 2007, 48 patients underwent a prolonged supervised fast (72 hours) because of hypoglycemic complaints (eg, weakness, fatigue, and tremor responding to the intake of food). Of these patients, 33% (n = 16) had plasma glucose levels during the prolonged supervised fast of less than 3.0 mmol/L with concomitant hypoinsulinemia (insulin <42 pmol/L; detection limit, 15 pmol/L) but no clinical signs of neurohypoglycemia. Of these patients, we included 10 patients. The other patients declined to participate, or their physician did not refer them to the Metabolic Unit.

The limit of 3.0 mmol/L was set because β-cell polypeptide secretion is suppressed at values less than that glucose level [2]. Patients had completed the prolonged test in our center or in other hospitals because of unexplained hypoglycemia. The supervised fast was only terminated in the presence of neurohypoglycemia. Other criteria for inclusion in our study were (1) no medication; (2) absence of diabetes or insulin administration; and (3) absence of adrenocortical failure, which was excluded by a corticotropin stimulation test [14]. Patients were informed on the purposes and nature of the diagnostic protocol.

2.2. Experimental protocol

For 3 days before the diagnostic protocol, patients consumed a weight-maintaining diet containing at least 250 g of carbohydrates per day. Patients were studied after 16 hours of fasting: patients were fasting from 6:00 PM the evening before the study day until the end of the study day. They were allowed to drink water only. After admission to the Metabolic Unit of the Academic Medical Center of the University of Amsterdam at 7:30 AM, a catheter was inserted into an antecubital vein for infusion of the stable isotope tracer. Another catheter was inserted retrogradely into a contralateral hand vein and kept in a thermoregulated (60°C) Plexiglas box for sampling of arterialized venous blood. In all studies, saline was infused as NaCl 0.9% at a rate of 50 mL/h to keep the catheters patent; [6.6-$^2H_2$]glucose was used as tracer (>99% enriched; Cambridge Isotopes, Andover, MA) to study glucose kinetics.

At 8:00 AM, blood samples were drawn for determination of background enrichments; and a primed continuous infusion of [6.6-$^2H_2$]glucose was started—prime, 8.8 μmol/kg; continuous, 0.11 μmol/(kg min)—and continued until the end of the study. After an equilibration period of 2 hours (16 hours of fasting), 3 blood samples were drawn for glucose enrichments and 1 for glucoregulatory hormones, FFA, KBs, alanine, and acylcarnitines. Plasma glucose levels were then measured every 30 minutes at the bedside to guard possible developing hypoglycemia. After 22 hours of fasting, blood samples (enrichment and glucoregulatory hormones) were repeated, after which the test ended. The diagnostic protocol was discontinued when plasma glucose levels fell to less than 2.5 mmol/L and/or neurohypoglycemia developed. In such case, an intravenous glucose solution (25 g) was administered; and the patient was fed oral carbohydrates.

2.3. Indirect calorimetry (glucose and FAO)

Glucose and FAO were measured after 16 and 22 hours of fasting during 20 minutes by indirect calorimetry using a ventilated hood system (Sensormedics model 2900; Sensormedics, Anaheim, CA).

2.4. Plasma glucose, FFA, KBs, and lactate

Plasma glucose concentrations were measured with the glucose oxidase method using a Beckman Glucose Analyzer

2 (Beckman, Palo Alto, CA; intraassay variation, 2%-3%). [6.6-$^2H_2$]glucose enrichment was measured as described earlier [15]. For [6.6-$^2H_2$]glucose enrichment (tracer to tracee ratio [TTR]), intraassay variation was 0.5% to 1%, interassay variation was 1%, and detection limit was 0.04%.

Plasma FFA concentrations were determined with an enzymatic colorimetric method (NEFA-C test kit; Wako Chemicals, Neuss, Germany): intraassay variation was 1%, interassay variation was 4% to 15%, and detection limit was 0.02 mmol/L. The KB samples were drawn in chilled sodium-fluoride tubes and directly deproteinized with ice-cold perchloric acid 6% (1:1). The KB's acetacetate (AcAc) and D-3-hydroxybutyrate (D-3HB) were measured with an enzymatic/spectrophotometric method using D-3HB dehydrogenase (COBAS-FARA centrifugal analyzer; Roche Diagnostics, Almere, The Netherlands. Detection limit was 0.05 mmol/L for AcAc and 0.1 mmol/L for D-3HB.

2.5. Glucoregulatory hormones

Insulin and cortisol were determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA). Insulin was measured with a chemiluminescent immunometric assay with intraassay variation of 3% to 6%, interassay variation of 4% to 6%, and detection limit of 15 pmol/L. Cortisol was measured with a chemiluminescent immunoassay with intraassay variation of 7% to 8%, interassay variation of 7% to 8%, and detection limit of 50 nmol/L. Glucagon was determined with the Linco ^{125}I radioimmunoassay (St Charles, MO) with intraassay variation of 9% to 10%, interassay variation of 5% to 7%, and detection limit of 15 ng/L. Norepinephrine and epinephrine were determined with an in-house high-performance liquid chromatography method with intraassay variation of 2% for norepinephrine and 9% for epinephrine, interassay variation of 10% for norepinephrine and 14% to 18% for epinephrine, and detection limit of 0.05 nmol/L. Presented reference values are the reference values of the Laboratory of Endocrinology of the Academic Medical Center.

2.6. Plasma acylcarnitines

Free carnitine and short- (C2 and C4), medium- (C8), and long-chain (C14:1, C16:1, and C18:1) acylcarnitines were measured by a quantitative analysis using electrospray tandem mass spectrometry as described earlier [16]; intraassay variation was 6% to 15% for free carnitine as well as for the different acylcarnitines.

2.7. Organic and amino acid analysis

Organic acids (eg, methylmalonic acid and glutaric acid) in plasma were analyzed by conventional gas chromatography/mass spectrometry after extraction with ethyl acetate/diethyl ether. The acids were separated as their methoxime/trimethylsilyl esters. Amino acids in plasma were analyzed (eg, alanine and glutamine) using reversed-phase high-performance liquid chromatography combined with electrospray tandem mass spectrometry of the underivatized amino acid. Reference values of the separate organic and amino acids were in-house reference values of the Laboratory Genetic Metabolic Diseases of the Academic Medical Center.

2.8. Calculations and statistics

Endogenous glucose production was calculated as the rate of isotope infusion (micromoles per kilogram per minute) divided by plateau TTRs; hence, EGP was expressed as micromoles per kilogram per minute as described earlier [17]. To compare the EGP of patients to a reference population, data on EGP were used from previous studies in healthy lean male volunteers (n = 63) after 14 to 16 hours of fasting [15,18-26]. A central range encompassing 95% of the data values was set, using the 2.5th and 97.5th percentiles as limits.

Glucose metabolic clearance rates ($MCR_{glucose}$) were calculated as EGP/[glucose]. Glucose and FAO rates were calculated from O_2 consumption and CO_2 production as reported previously [27].

Data are presented as individual results and as median (minimum-maximum) when expressed for the total group. Comparisons were performed with the Wilcoxon signed rank test. The SPSS statistical software program version 14.0.2 (SPSS, Chicago, IL) was used for statistical analysis.

3. Results

3.1. Anthropometric characteristics

Ten patients agreed to participate in the diagnostic protocol. The subject characteristics are presented in Table 1. Women outnumbered men (9 vs 1, respectively). The median age of the patients was 40 (21–57)] years, and they had a median body mass index (BMI) of 24 (17.7-29.4) kg/m^2.

3.2. Glucose kinetics

All patients had low plasma glucose levels at the end (72 hours) of the prolonged supervised fast with low plasma insulin levels (Table 1).

In the diagnostic protocol described here, plasma glucose levels decreased in all patients between 16 and 22 hours of fasting, with a median decrease of 12% (2.9%-25.8%) (P = .005, Table 2). All patients had an EGP within our reference values (reference values for EGP after 14-16 hours of fasting: 7.98-14.46 μmol/[kg min]). Endogenous glucose production decreased in all patients between 16 and 22 hours of fasting, with a median decrease of 18.3% (13.0%-29.1%) (P = .005). The $MCR_{glucose}$ was lower after 22 hours of fasting compared with 16 hours of fasting in 9 patients, but did not change in patient 8 (median decrease, 14% [0%-18%]; P = .007 for the whole group).

Table 1
Patient characteristics before inclusion

	Sex	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Glucose (mmol/L)	Insulin (pmol/L)
Patient 1	F	43	158	67	26.8	2.6	<15[a]
Patient 2	F	29	170	85	29.4	2.8	20
Patient 3	F	21	174	69	22.8	2.5	16
Patient 4	F	46	147	51	23.4	2.5	<15[a]
Patient 5	F	40	178	56	17.7	2.4	22
Patient 6	F	35	157	59	23.9	2.5	20
Patient 7	F	57	163	63	23.7	2.1	<15[a]
Patient 8	M	38	172	70	23.7	2.9	<15[a]
Patient 9	F	43	182	70	21.1	2.9	<15[a]
Patient 10	F	40	178	73	23.0	2.1	<15[a]

Data are presented as individual values. Plasma glucose and insulin levels presented in this table were obtained at the end of the prolonged fast. F indicates female; M, male.

[a] Plasma insulin levels were less than the detection limit (15 pmol/L).

3.3. Plasma FFA, KBs, and lactate

Plasma FFA levels were detectable in all patients after 16 hours of fasting (Table 3). No change in plasma FFA levels was observed ($P = .2$). During the diagnostic protocol, AcAc increased (median increase, 72% [0%-400%]) in all patients but 1 (patient 8); D-3HB increased (median increase, 133% [0%-900%]) in all but 2 patients (patients 2 and 8, $P = .008$ and .011 for AcAc and D-3HB, respectively). One of the patients did not display ketosis at all (patient 8). Plasma lactate levels were unremarkable and did not change between 16 and 22 hours of fasting ($P = .7$).

Plasma alanine decreased in most patients between 16 and 22 hours of fasting, with a trend toward a decrease for the total group: median decrease, 9% (-22% to 25%) ($P = .093$). Plasma alanine levels were less than the reference values in 4 patients both after 16 and 22 hours of fasting. Analysis of other plasma amino acids was uneventful in all patients (data not shown).

3.4. Glucoregulatory hormones

Plasma insulin levels were detectable in all patients after 16 hours of fasting and decreased thereafter (median decrease, 47% [10%-78%]) ($P = .005$, Table 4). Plasma glucagon levels increased (median increase, 18% [−12% to 28%]) between 16 and 22 hours of fasting in all but one patient ($P = .038$). Plasma cortisol levels were within the reference range for fasting cortisol [14] and decreased (median decrease, 20% [−68% to 58%]) during the day ($P = .037$). Plasma noradrenaline levels were within the reference range in all patients and decreased (median decrease, 27% [−8% to 46%]) ($P = .017$). Plasma adrenalin levels were within the reference range in all patients without significant change between 16 and 22 hours of fasting ($P = .6$).

3.5. Glucose and FAO

Glucose oxidation rates were lower after 22 hours compared with 16 hours of fasting (median decrease, 55% [−66% to 87%]) in all subjects but one (patient 7, Table 5) ($P = .011$). Likewise, FAO rates were increased (median increase, 72% [−12% to 150%]) after 22 hours compared with 16 hours of fasting in all subjects but one (patient 7) ($P = .015$).

3.6. Plasma acylcarnitines

Plasma levels of free carnitine as well as short-, medium-, and long-chain acylcarnitines did not exceed upper reference limits after 16 hours of fasting except for C2-carnitine in patient 5. Plasma levels of free carnitine as well as short-, medium-, and long-chain acylcarnitines did not exceed upper reference limits after 22 hours of fasting except for C2-carnitine in patient 6. Plasma free carnitine decreased (median decrease, 11% [−12% to 26%]) ($P = .028$), whereas the acylcarnitines did not change between 16 and 22 hours of fasting (Table 6).

4. Discussion

The prolonged fast is a well-validated clinical test to diagnose and explore spontaneous hypoglycemia [2]. In our experience, some patients display unexplained hypoinsulinemic (insulin <42 pmol/L) hypoglycemia (glucose <3.0)

Table 2
Glucose kinetics after 16 and 22 hours of fasting during the extended metabolic protocol

	Glucose (mmol/L)		Δ (%)	EGP[a] (μmol/[kg min])		Δ (%)	$MCR_{glucose}$ (mL/[kg min])	
	16 h	22 h		16 h	22 h		16 h	22 h
Patient 1	4.9	4.1	−16.5	9.2	6.6	−29.1	1.9	1.6
Patient 2	5.2	4.7	−8.3	8.4	7.0	−16.5	1.6	1.5
Patient 3	5.1	4.5	−12.1	10.9	8.9	−18.1	2.1	2.0
Patient 4	4.9	4.1	−15.3	12.1	9.9	−18.5	2.5	2.4
Patient 5	4.5	4.4	−2.9	11.2	9.3	−16.6	2.5	2.1
Patient 6	4.8	4.5	−5.6	10.2	8.1	−20.0	2.1	1.8
Patient 7	4.5	4.4	−2.9	9.8	7.9	−19.8	2.2	1.8
Patient 8	5.3	4.6	−12.5	11.4	9.9	−13.0	2.2	2.2
Patient 9	4.9	4.7	−4.1	11.6	9.6	−17.4	2.4	2.0
Patient 10	4.2	3.7	−11.6	11.2	9.1	−18.9	2.7	2.5

Data are presented as individual values.

[a] Reference values of EGP after 16 hours of fasting: 7.98 to 14.46 μmol/(kg·min).

Table 3
Plasma FFA, KBs, lactate, and alanine after 16 and 22 hours of fasting during the extended metabolic protocol

Reference values	FFA (mmol/L) –		AcAc (mmol/L) –		D-3HB (mmol/L) –		Lactate (mmol/L) 0.5-2.0		Alanine (μmol/L) 182-552	
	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h
Patient 1	0.56	0.8	0.09	0.13	0.1	0.4	0.6	0.6	199	162
Patient 2	0.66	0.84	<0.05[a]	0.07	0.1	0.1	0.6	0.4	280	232
Patient 3	1.12	0.82	0.09	0.12	0.2	0.3	0.8	0.8	236	287
Patient 4	0.7	0.99	0.06	0.24	0.1	0.5	0.5	0.6	159	137
Patient 5	0.84	0.7	0.05	0.25	0.1	0.6	1.1	0.7	161	121
Patient 6	0.49	0.79	0.05	0.21	0.1	0.6	<0.5[a]	0.6	213	202
Patient 7	0.66	0.97	0.18	0.31	0.3	0.7	0.6	0.6	148	138
Patient 8	0.33	0.44	<0.05[a]	<0.05[a]	<0.1[a]	<0.1[a]	0.7	0.6	211	191
Patient 9	0.66	0.57	0.08	0.13	0.3	0.4	0.5	0.6	134	135
Patient 10	0.93	0.82	<0.05[a]	0.18	<0.1[a]	0.5	0.7	0.6	165	133

Data are presented as individual values. Reference values are hospital based.
[a] Less than the detection limit.

(unpublished observation). Although it may be reassuring that a diagnosis of insulinoma is excluded in these patients, a subtle metabolic disorder cannot be ruled out with certainty.

In this report, we describe 10 patients who underwent extended metabolic testing to rule out an inborn error of metabolism, a relatively unexplored area in internal medicine to date.

We investigated a small group of patients because of the low numbers of patients with hypoinsulinemic hypoglycemia, but it included patients referred with this problem over a period of 7 years. From a practical point of view, we chose to test patients after 16 hours of fasting because our intention was to explore the contribution of metabolic defects resulting in hypoglycemia during the prolonged fasting test and these are biochemically detectable even in the absence of an overt hypoglycemia [28].

All the patients we studied showed plasma FFA levels within the expected range for 16 hours of fasting [29]. Plasma FFA levels did not increase in all patients between 16 and 22 hours of fasting, which is explained by the fact that plasma FFA has been shown not to increase markedly until 18 to 24 hours of fasting [6].

Plasma FFA induces KB formation in the liver [8,20], a metabolic adaptation that is hampered in FAO disorders [11]. Indeed, it has been advocated to measure KB levels during the prolonged fast to determine whether a fast is positive: increased plasma KBs prove suppressed pancreatic insulin production [1,30]. All the patients but one (patient 8) showed KB formation after 22 hours of fasting. The lack of KB in patient 8, the only male, may be explained by the plasma FFA levels that were rather low in comparison with female patients and may not have stimulated KB formation sufficiently [8]. Men have lower plasma FFA levels, lipolysis rates, and KBs compared with women during fasting [17]. Otherwise, the lower KBs in patient 8 together with the suppressed plasma insulin levels could be due to circulating insulin-like growth factors (IGFs) such as pro-IGF-II, free IGF-II, and IGF-I for these may cause hypoinsulinemic euglycemic hypoglycemia [1]. Such IGFs are however mostly due

Table 4
Glucoregulatory hormones after 16 and 22 hours of fasting during the extended metabolic protocol

Reference values[a]	Insulin (pmol/L) 34-172		Glucagon (ng/L) 10-140		Cortisol (nmol/L) 150-802		Noradrenaline (nmol/L) 0-3.25		Adrenaline (nmol/L) 0-0.55	
	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h
Patient 1	35	20	41	48	147	159	0.96	0.93	0.18	0.14
Patient 2	71	58	81	71	249	170	1.91	1.03	<0.05[b]	<0.05[b]
Patient 3	92	49	53	64	480	382	1.23	1.33	0.23	0.22
Patient 4	22	<15[b]	46	59	232	236	1.16	1.07	0.06	0.09
Patient 5	61	33	54	67	571	334	0.62	0.55	0.09	0.12
Patient 6	62	56	64	76	107	180	0.74	0.51	0.15	0.23
Patient 7	19	<15[b]	89	90	265	241	1.45	1.00	0.31	0.41
Patient 8	34	<15[b]	44	46	524	309	1.26	0.84	0.26	0.14
Patient 9	33	<15[b]	66	–[c]	306	130	1.69	1.23	0.21	0.16
Patient 10	39	<15[b]	58	67	464	323	1.18	0.74	0.15	0.19

Data are presented as individual values. Reference values are hospital based.
[a] Reference values for overnight fast.
[b] Less than the detection limit.
[c] n = 9.

Table 5
Fatty acid and glucose oxidation rates after 16 and 22 hours of fasting during the extended metabolic protocol

	Fat oxidation (mmol/min)		Δ (%)	Glucose oxidation (mmol/min)		Δ (%)
	16 h	22 h		16 h	22 h	
Patient 1	0.062	0.087	40	0.458	0.197	−57
Patient 2	0.039	0.1	158	1.144	0.425	−63
Patient 3	0.094	0.107	13	0.378	0.051	−87
Patient 4	0.069	0.073	6	0.292	0.126	−57
Patient 5	0.073	0.085	16	0.467	0.264	−43
Patient 6	0.053	0.076	42	0.527	0.307	−42
Patient 7	0.08	0.074	−7	0.212	0.351	65
Patient 8	0.074	0.086	16	0.725	0.45	−38
Patient 10	0.105	0.132	24	0.348	0.000	−100

Data are presented as individual values. n = 9.

to nonislet cell tumors, that is, clinical apparent mesenchymal tumors [1].

Plasma free carnitine levels decrease during fasting because carnitine is esterified intracellularly to form acylcarnitine [31]: this effect was already observed in our patients after 22 hours of fasting. In general, medium- and long-chain acylcarnitines are not increased after 20 hours of fasting in adults [31,32] in contrast to 62 hours of fasting [33].

Medium-chain acyl-CoA dehydrogenase deficiency is probably the most frequently occurring FAO disorder in children with incidence numbers up to 1 in 10 000, but adult presentations have been described [34-36]. Furthermore, the clinical presentation of FAO disorders may vary from asymptomatic to severe disease, explaining the fact that some cases escaped detection until the current neonatal screening was used [34,37,38]. Plasma C8-acylcarnitine is a main parameter to diagnose MCADD [11,28,39] and should be higher than 0.3 μmol/L for its unequivocal diagnosis [39]. In adult MCADD patients, plasma C8-carnitine levels can be as high as 5 μmol/L [28]. As plasma C8-carnitine did not exceed 0.14 μmol/L despite an increase in FAO, MCADD as a possible explanation for hypoinsulinemic hypoglycemia was ruled out in our patients. The lack of increased levels of short- and long-chain acylcarnitines in our patients negates other defects of FAO (ie, short- and long-chain acyl-CoA dehydrogenase deficiency) to be present.

Fasting increases FAO and decreases glucose oxidation. These changes are present within 24 hours of fasting [6]. A normal increase of whole-body FAO was observed in 9 patients between 16 and 22 hours of fasting. Patient 7 showed no increase of FAO, which is not readily explained because no other abnormalities were found.

Defective gluconeogenesis and glycogenolysis may be accompanied by elevated gluconeogenic amino acids and an increase of various metabolites such as lactate and pyruvate [40]. Alanine is a critical gluconeogenic amino acid that is converted into pyruvate, notably during catabolic stress [11,41]. Plasma alanine levels decrease during the first 30 hours of fasting [42]. The lower plasma alanine concentrations in our patients argue against defective EGP. Moreover, total EGP stayed within the reference range. In addition, no abnormalities in other amino acids or organic were detected, excluding amino/organic acid disorders as a cause for hypoinsulinemic hypoglycemia in these patients.

Reference data on EGP were data from young, lean healthy men. It is unlikely that the absence of matched reference data has clouded our results because higher age does not decrease EGP [43]. Women have equal EGP

Table 6
Acylcarnitine levels (micromoles per liter) after 16 and 22 hours of fasting during the extended metabolic protocol

Reference values	Free carnitine 22.3-54.8		C2-carnitine 3.4-13.0		C4-carnitine 0.14-0.94		C8-carnitine 0.04-0.22		C14:1-carnitine 0.02-0.18		C16:1-carnitine 0.02-0.08		C18:1-carnitine 0.06-0.28	
	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h	16 h	22 h
Patient 1	36.63	31.88	7.25	9.49	0.15	0.2	0.07	0.06	0.09	0.04	0.04	0.04	0.11	0.10
Patient 2	37.29	30.25	2.87	8.58	0.07	0.17	0.07	0.14	0.06	0.14	0.02	0.06	0.13	0.19
Patient 3	34.15	31.16	8.03	9.09	0.28	0.14	0.05	0.04	0.16	0.09	0.07	0.05	0.21	0.13
Patient 4	31.07	34.66	14.56	9.23	0.27	0.12	0.08	0.07	0.08	0.09	0.07	0.04	0.23	0.19
Patient 5	23.97	23.37	12.62	13.32	0.16	0.17	0.04	0.03	0.08	0.06	0.04	0.04	0.13	0.12
Patient 6	33.54	24.86	5.58	8.48	0.19	0.22	0.09	0.08	0.04	0.14	0.03	0.04	0.07	0.1
Patient 7	22.78	19.42	9.94	8.9	0.09	0.06	0.04	0.04	0.06	0.07	0.03	0.03	0.11	0.12
Patient 8	46.49	41.5	6.35	7.23	0.16	0.22	0.09	0.04	0.03	0.07	0.04	0.04	0.12	0.11
Patient 9	25.15	25.25	9.73	9.4	0.27	0.29	0.13	0.09	0.07	0.08	0.03	0.04	0.12	0.1
Patient 10	38.4	30.55	6.52	6.27	0.1	0.09	0.08	0.03	0.19	0.11	0.07	0.05	0.14	0.12

Data are presented as individual values. Reference values are hospital based.

compared with men after an overnight fast [4]. In addition, increased weight may result in lower EGP per kilogram of total body mass. Still, the highest BMIs (patients 1 and 2) had normal EGP. If anything, the absence of matched reference EGP data would have led to lower EGP values; but this was not the case. Plasma insulin levels were low in all patients as expected [6,7]. The other glucoregulatory hormones showed no abnormalities, although these hormones have a circadian or pulsatile rhythm; so minor individual changes may not have been discovered [7].

An important question is why these patients have these low venous glucose levels during fasting. Plasma glucose levels are determined by EGP [9] that tends to be lower in women during fasting [4,17]. This may be due to lower gluconeogenic enzymes in women. In addition, ovariectomized mice have higher gluconeogenesis compared with controls [44]. The low glucose levels during fasting may thus be due to the female sex. Indeed, women have higher FFA and KB during fasting, suggesting successful adaptation. Whether sex differences exist in perception of symptoms attributed to low blood glucose in the absence of neurohypoglycemia remains to be elucidated.

It should be emphasized that patients often present with symptoms such as fatigue, malaise, and nausea that then are attributed to lower plasma glucose levels. In contrast, true hypoglycemia should be diagnosed only when the Whipple triad ([1] neurohypoglycemia; eg, confusion, epileptic insults, coma; [2] hypoglycemia; and [3] resolution of symptoms after correction of the hypoglycemia) is present [1,2,45]. Especially, KB production during fasting ensures a sufficient amount of energy for the brain to prevent such neurohypoglycemia [8,46]. It remains to be elucidated whether differences exist in perception of symptoms that are attributed to hypoglycemia, despite the absence of neurohypoglycemia.

In this report, we describe 10 patients with unexplained hypoinsulinemic hypoglycemia who underwent extended metabolic testing to rule out defects in EGP or FAO like MCADD and amino/organic acid disorders. We found normal plasma glucose concentrations and glucose production rates without abnormalities in circulating gluconeogenic amino/organic acids, whereas normal plasma acylcarnitine profiling excluded FAO disorders. The decrease in plasma glucose concentrations and EGP between 16 and 22 hours of fasting seems perfectly in agreement with the earlier reported decrease of glucose concentrations and EGP between 16 and 22 hours of fasting in lean healthy men and women [29,47]. The same holds true for glucose clearance. These data thus show that hypoinsulinemic hypoglycemia in these adults is not caused by an inborn error of metabolism and may be analogous to idiopathic ketotic hypoglycemia in pediatric patients [48]. Our study suggests that plasma glucose values may decrease to less than the “classic” threshold value for hypoglycemia during fasting and therefore may represent the lower tail of the Gaussian curve for plasma glucose concentration and do not warrant extensive metabolic evaluation.

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