The metabolic response to fasting in humans: physiological studies

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8 Hypoinsulinemic hypoglycemia during fasting in adults: a Gaussian Tale

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Submitted

Abstract

Background: The diagnostic evaluation of spontaneous hypoglycemia is mainly directed at detecting an insulinoma, via a prolonged supervised fast. Its interpretation is troublesome in those patients who develop hypoglycemia 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 which might explain the hypoinsulinemic hypoglycemia.

Methods: 10 patients with otherwise unexplained hypoglycemia during prolonged fasting participated in an extended metabolic diagnostic protocol based on stable isotope techniques after an overnight fast in order to explore abnormalities in endogenous glucose production (EGP) and intermediary metabolism.

Results: Although during the prolonged fast, they became hypoglycaemic, during the diagnostic protocol there were no hypoglycemic events. No abnormalities in fatty acid oxidation (FAO) or in amino acid/organic acids were found in this patient group.

Conclusion: In patients exhibiting hypoglycemia during prolonged fasting in whom insulinoma was excluded, we found no signs of metabolic derangements. Therefore, the previously observed low plasma glucose values in this subgroup of patients probably represent the lower tail of the Gaussian Curve of plasma glucose concentrations during fasting.

Introduction

The diagnostic evaluation of spontaneous hypoglycemia can be troublesome (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glucose levels that fall well below 3.0 mmol/liter in the absence of hyperinsulinemia and signs of neurohypoglycemia (*unpublished observation).*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 (3-6). This response consists amongst 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 (GH), glucagon and catecholamines increase lipolysis of adipose tissue thereby increasing the plasma free fatty acid (FFA) concentration and subsequent FAO in oxidative tissues (e.g. skeletal muscle) (7;8). Additionally, the increased FFA availability in the liver stimulates ketone body (KB) formation (6;9).

The decrease in plasma glucose levels during fasting is, in adults, mainly due to a decrease in endogenous glucose production (EGP) (5) which is the main denominator of the fasting plasma glucose level (10). Despite the inevitable lowering of plasma glucose concentration during fasting, levels normally do not fall below 3.0 mmol/liter (5;6). However, healthy women may have plasma glucose levels well below 3.0 mmol/liter during fasting (5;6;11). 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 (12).

Medium-chain acyl-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 (12;13). The latter are thought to contribute to the clinical presentation of FAO disorders: MCADD patients present with hypoketotic hypoglycemia (decreased EGP) despite hypo-insulinemia during episodes of fasting (12;13). 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 hypoketotic hypoglycemia (14). Since 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 (14).

In this descriptive report, we present 10 patients with hypoinsulinemic hypoglycemia, 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.

Subjects and methods

Subjects

Between 2000 and 2007 48 patients underwent a prolonged supervised fast because of hypoglycemic complaints. Of these patients, 33% (n = 16) had plasma glucose levels during the prolonged supervised fast below 3.0 mmol/liter with concomitant hypoinsulinemia (insulin < 42 pmol/L) and no Whipple's triad. 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/liter was set because β-cell polypeptide secretion is suppressed below that glucose level (2). Patients had undergone the prolonged test in our center or were referred by other hospitals because of unexplained hypoglycemia. Other criteria for inclusion in our study were: 1) no medication; 2) absence of diabetes or insulin administration; 3) absence of adrenocortical failure, which was excluded by an ACTH-stimulation test (15). Patients were informed on the purposes and nature of the diagnostic protocol.

Experimental protocol

For three 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 1800 h the evening before the study day until the end of the study day. They were allowed to drink water only. After admission at the Metabolic Unit of the Academic Medical Center of the University of Amsterdam at 0730 h, 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 thermo-regulated (60ºC) 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, USA) to study glucose kinetics.

At 0800 h, 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 two hours (16 h of fasting), 3 blood samples were drawn for glucose enrichments and 1 for glucoregulatory hormones, FFA, KBs, alanine and acylcarnitines. Then blood glucose levels were measured every 30 minutes at the bedside to guard possible developing hypoglycemia. After 6 h (22 h of fasting) again 3 blood samples were drawn for glucose enrichments and 1 for glucoregulatory hormones, FFA, KBs, alanine and acylcarnitines after which the test ended. The diagnostic protocol was discontinued when plasma glucose levels fell below 2.5 mmol/liter and/or neurohypoglycemia developed. In such case, an intravenous glucose solution (25 grams) was administered and the patient was fed oral carbohydrates.

Indirect calorimetry (glucose and fat oxidation)

Glucose and fat oxidation were measured after 16 and 22 h of fasting during 20 min by indirect calorimetry using a ventilated hood system (Sensormedics model 2900; Sensormedics, Anaheim, USA).

Plasma glucose, FFA, ketone bodies, lactate

Plasma glucose concentrations were measured with the glucose oxidase method using a Beckman Glucose Analyzer 2 (Beckman, Palo Alto, USA, intra-assay variation 2-3%). [6.6-$^2H_2$]glucose enrichment was measured as described earlier (16). [6.6-$^2H_2$]glucose enrichment (tracer/tracee ratio) intra-assay variation: 0.5-1%; inter-assay variation 1%; detection limit: 0.04%.

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

Glucoregulatory hormones

Insulin and cortisol were determined on an Immulite 2000 system (Diagnostic Products Corporation, Los Angeles, USA). Insulin was measured with a chemiluminescent immunometric assay, intra-assay variation: 3-6%, inter-assay variation: 4-6%, detection limit: 15pmol/L. Cortisol was measured with a chemiluminescent immunoassay, intra-assay variation: 7-8%, inter-assay variation: 7-8%, detection limit: 50nmol/L. Glucagon was determined with the Linco ^{125}I radioimmunoassay (St. Charles, USA), intra-assay variation: 9-10%, inter-assay variation: 5-7% and detection limit: 15ng/L. Norepinephrine and epinephrine were determined with an in-house HPLC method, Intra-assay variation norepinephrine: 2%; epinephrine 9%; inter-assay variation norepinephrine: 10%; epinephrine: 14-18%; detection limit: 0.05 nmol/L. Presented reference values are the reference values of the Laboratory of Endocrinology of the Academical Medical Center.

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 (17); intra-assay variation 6-15% for free carnitine as well as the different acylcarnitines.

Organic and amino acid analysis

Organic acids (e.g. methylmalon acid and glutaric acid) in plasma were analyzed by conventional gas chromatography/mass spectrometry following extraction with ethyl acetate/diethyl ether. The acids were separated as their methoxime/trimethylsilyl esters. Amino acids in plasma were analyzed (e.g. alanine and glutamine) using reversed-phase HPLC 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 AMC.

Calculations and statistics

EGP was calculated as the rate of isotope infusion (µmol/kg·min) divided by plateau tracer tracee ratios (TTR), hence EGP was expressed as µmol/kg·min. To compare the EGP of patients to a reference population, data on EGP were used from previously studies in healthy lean volunteers (n = 63, partly unpublished) after 14-16 h of fasting (4;16;18-23). 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 fat oxidation rates were calculated from O_2 consumption and CO_2 production as reported previously (24).

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 Inc, Chicago, IL) was used for statistical analysis.

Results

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] yr. As a group, patients had a normal BMI: 24 [17.7 – 29.4] kg/m^2.

Table 1 Patient characteristics prior to inclusion

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

Data are presented as individual values. [a]Plasma insulin levels were below the detection limit.

Glucose kinetics

All patients had low plasma glucose levels during the prolonged supervised fast with low plasma insulin levels (Table 1). Plasma glucose levels decreased in all patients between 16 and 22 hours of fasting with a median decrease of 12 [2.9 - 25.8] %, $P = 0.005$ (Table 2). All patients had an EGP within our reference values (reference values for EGP after 14-16 h of fasting: 7.98 - 14.46 µmol/kg·min) (4;18-20;22;25). EGP decreased in all patients

Table 2 Glucose kinetics

	Glucose			EGP[a]			MCRglucose (ml/kg · min)	
	(mmol/liter)		Δ (%)	(μmol/kg · min)		Δ (%)		
	16h	22h		16h	22h		16h	22h
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. EGP endogenous glucose production. [a]Reference values EGP after 16 h of fasting: 7.98 - 14.46 μmol/kg • min.

between 16 and 22 hours of fasting with a median decrease of 18.3 [13.0 - 29.1] %, P = 0.005. $MCR_{glucose}$ was lower after 22 h of fasting compared to 16 h of fasting in 9 patients, but did not change in patient 8 (median decrease 14 [0 - 18] %, P = 0.007 for the whole group).

Table 3 Plasma FFA, ketone bodies, lactate and alanine

	FFA (mmol/liter)		AcAc (mmol/liter)		*D*-3BH (mmol/liter)	
Reference values						
	16h	**22h**	**16h**	**22h**	**16h**	**22h**
Patient 1	0.56	0.8	0.09	0.13	0.1	0.4
Patient 2	0.66	0.84	<0.05[a]	0.07	0.1	0.1
Patient 3	1.12	0.82	0.09	0.12	0.2	0.3
Patient 4	0.7	0.99	0.06	0.24	0.1	0.5
Patient 5	0.84	0.7	0.05	0.25	0.1	0.6
Patient 6	0.49	0.79	0.05	0.21	0.1	0.6
Patient 7	0.66	0.97	0.18	0.31	0.3	0.7
Patient 8	0.33	0.44	<0.05[a]	<0.05[a]	<0.1[a]	<0.1[a]
Patient 9	0.66	0.57	0.08	0.13	0.3	0.4
Patient 10	0.93	0.82	<0.05[a]	0.18	<0.1[a]	0.5

Data are presented as individual values. FFA free fatty acids, AcAc acetoacetate, *D*-3HB D-3hydroxybutyrate. [a]Below detection limit.

Plasma FFA, ketone bodies, and lactate

Plasma FFA levels were detectable in all patients after 16 h of fasting (Table 3). For the group as a whole, no change in plasma FFA levels was observed (P = 0.2). During the diagnostic protocol AcAc increased (median increase 72 [0 - 400] %) in all patients but one; *D*-3HB increased (median increase 133 [0 - 900] %) in all but two patients (P = 0.008 and 0.011 for AcAc and *D*-3HB respectively). One of the patients did not display ketosis at all (patient 8). All plasma lactate levels were within hospital reference values after 16 as well as 22 h of fasting and did not change (P = 0.7).

Glucoregulatory hormones

Plasma insulin levels were detectable in all patients after 16 h of fasting and decreased thereafter (median decrease 47 [10 - 78] %), P = 0.005 (Table 4). Plasma glucagon levels increased (median increase 18 [-12 - 28] %) between 16 and 22 h of fasting in all but one patient, P = 0.038. Plasma cortisol levels were within the reference values normal reference range for fasting cortisol (15) and decreased (median decrease 20 [-68 - 58] %) during the day, P = 0.037. Plasma noradrenaline levels were within the reference range in all patients and decreased (median decrease 27 [-8 - 46] %) for the group as a whole, P = 0.017. Plasma adrenalin levels were within the reference range in all patients without significant change between 16 and 22 h of fasting (P = 0.6).

	Lactate (mmol/liter)		**Alanine (µmol/liter)**	
			182 - 552	
	16h	**22h**	**16h**	**22h**
	0.6	0.6	199	162
	0.6	0.4	280	232
	0.8	0.8	236	287
	0.5	0.6	159	137
	1.1	0.7	161	121
	<0.5[a]	0.6	213	202
	0.6	0.6	148	138
	0.7	0.6	211	191
	0.5	0.6	134	135
	0.7	0.6	165	133

Table 4 Glucoregulatory hormones

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

Data are presented as individual values. [a]Reference values for overnight fast. [b]Below detection limit. [c]N = 9.

Glucose and fat oxidation

Glucose oxidation rates were lower after 22 h compared to 16 h of fasting (median decrease 55 [-66 - 87] %) in all subjects but one (patient 7, Table 5), $P = 0.011$. Likewise, fat oxidation rates were increased (median increase 72 [-12 - 150] %) after 22 h compared to 16 h of fasting in all subjects but one (patient 7), $P = 0.015$.

Table 5 Fat and glucose oxidation

	Fat oxidation (mmol/min)		Δ (%)	Glucose oxidation (mmol/min)		Δ (%)
	16h	**22h**		**16h**	**22h**	
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.

	Adrenaline (nmol/liter)	
	0 - 0.55	
	16h	22h
	0.18	0.14
	<0.05[b]	<0.05[b]
	0.23	0.22
	0.06	0.09
	0.09	0.12
	0.15	0.23
	0.31	0.41
	0.26	0.14
	0.21	0.16
	0.15	19

Plasma acylcarnitines

Plasma levels of free carnitine as well as short-, medium- and long-chain acylcarnitines did not exceed upper normal reference limits after 16 h of fasting except for C2-carnitne in patient 5. Plasma levels of free carnitine as well as short-, medium- and long-chain acylcarnitines did not exceed upper normal reference limits after 22 h of fasting except for C2-carnitne in patient 6. Plasma free carnitine decreased in the group as a whole (median decrease 11 [-12 - 26] %, $P = 0.028$), whereas the acylcarnitines did not change for the group between 16 and 22 h of fasting (data not shown).

Organic and amino acid analysis

Plasma alanine decreased in most patients between 16 and 22 h of fasting, with a trend towards a decrease for the total group: median decrease 9 [-22 - 25] %, $P = 0.093$. Plasma alanine levels were below reference values in 4 patients both after 16 and 22 h of fasting. Analysis of other plasma amino acids was uneventful in all patients (data not shown).

Table 6. Acylcarnitine levels (µmol/liter) after 16 and 22 h of fasting.

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

Data are presented as individual values.

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 below 42 pmol/L) hypoglycemia (glucose < 3.0) (*unpublished observation*). Although it may be reassuring that a diagnosis of insulinoma is excluded in these patients, a subtle metabolic derangement 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 which was due to the low numbers of patients with hypoinsulinemic hypoglycemia but it included patients referred with this problem over a period of 4 years. From a practical point of view we chose to test patients after 16 h of fasting because our intention was to explore 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 (26).

All the patients we studied showed plasma FFA levels within the expected range for 16 h of fasting (27). Plasma FFA levels did not increase in all patients between 16 and 22 h of fasting which is explained by the fact that plasma FFA have been shown not to increase markedly until 18 to 24 h of fasting (7).

C8-carnitine		C14:1-carnitine		C16:1-carnitine		C18:1-carnitine	
0.04 - 0.22		0.02 - 0.18		0.02 - 0.08		0.06 - 0.28	
16h	22h	16h	22h	16h	22h	16h	22h
0.07	0.06	0.09	0.04	0.04	0.04	0.11	0.10
0.07	0.14	0.06	0.14	0.02	0.06	0.13	0.19
0.05	0.04	0.16	0.09	0.07	0.05	0.21	0.13
0.08	0.07	0.08	0.09	0.07	0.04	0.23	0.19
0.04	0.03	0.08	0.06	0.04	0.04	0.13	0.12
0.09	0.08	0.04	0.14	0.03	0.04	0.07	0.1
0.04	0.04	0.06	0.07	0.03	0.03	0.11	0.12
0.09	0.04	0.03	0.07	0.04	0.04	0.12	0.11
0.13	0.09	0.07	0.08	0.03	0.04	0.12	0.1
0.08	0.03	0.19	0.11	0.07	0.05	0.14	0.12

Plasma FFA induce KB formation in the liver (9), a metabolic adaptation that is hampered in FAO disorders (12). All the patients but one (patient 8) showed KB formation after 22 h 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 (9). Men have lower plasma FFA levels and lipolysis rates compared to women during fasting (28).

Plasma free carnitine levels decrease during fasting since carnitine is esterified intracellular to form acylcarnitine (29): this effect was already observed in our patients after 22 h of fasting. In general, medium and long-chain acylcarnitines are not increased after 20 h of fasting in adults (29;30).

MCADD is probably the most frequent occurring FAO disorder in children with incidence numbers up to 1 in 10,000, but adult presentations have been described (31-33). 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 employed (31;34;35). Plasma C8-acylcarntine is a main parameter to diagnose MCADD (12;26;36) and should be higher than 0.3 µmol/liter for its unequivocal diagnosis (36). In adult MCADD patients plasma C8-carntine levels can be as high as 5 µmol/liter (26). As plasma C8-carnitine did not exceed 0.14 µmol/liter 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 (i.e. short- and long-chain acyl-CoA dehydrogenase deficiency) to be present.

Fasting increases fat oxidation and decreases glucose oxidation. These changes are present within 24 h of fasting (7). A normal increase of whole body FAO was observed in 9 patients between 16 and 22 h of fasting. Patient 7 showed no increase of FAO which is not readily explained since no other abnormalities were found.

Defective gluconeogenesis and glycogenolysis may be accompanied by elevated gluconeogenic amino acids and in increase of various metabolites such as lactate and pyruvate (37). Alanine is a critical gluconeogenic amino acid that is converted into pyruvate, notably during catabolic stress (12;38). Plasma alanine levels decrease during the first 30 h of fasting (39). The lower plasma alanine concentrations argue against defective EGP. Additionally, no abnormalities in other amino acids or organic were detected, excluding amino/organic acid disorders as a cause for hypoinsulinemic hypoglycemia in these patients.

Plasma insulin levels were low in all patients as expected (7;8). 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 (8).

In this report we described 10 patients who underwent extended metabolic testing to rule out decreased EGP in possible combination with FAO disorders like MCADD and amino/organic acid disorders. We found normal plasma glucose concentrations and glucose production without disorders in gluconeogenic amino/organic acids whereas plasma acylcarnitine profiling showed no FAO disorders. These data thus show that hypoinsulinemic hypoglycemia in these adults is not caused by an inborn error of metabolism. The decrease in plasma glucose concentrations and EGP between 16 and 22 h of fasting seem perfectly in agreement with the earlier reported decrease of glucose concentrations and EGP between 16 and 22 h of fasting in lean healthy men and women (27;40). The same holds true for glucose clearance. These data suggest that plasma glucose values may decrease below the "classical" threshold value for hypoglycemia during fasting and therefore could represent simply the lower tail of the Gaussian Curve.

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