Normoglycemic Ketonemia as Biochemical Presentation in Ketotic Glycogen Storage Disease

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Abstract Background: According to the textbooks, the ketotic glycogen storage disease (GSD) types 0, III, VI, IX, and XI are associated with fasting ketotic hypoglycemia and considered milder as gluconeogenesis is intact.

Methods: A retrospective cohort study of biochemical profiles from supervised clinical fasting studies is performed in ketotic GSD patients in our metabolic center. For data analysis, hypoglycemia was defined as plasma glucose concentration <2.6 mmol/L. Total KB was defined as the sum of blood acetoacetate and β -hydroxybutyrate concentrations. If the product of glucose and KB concentrations was greater than 10, a ketolysis defect was suspected.

Results: Data could be collected from 13 fasting studies in 12 patients with GSD III ($n = 4$), GSD VI ($n = 3$), and GSD IX ($n = 5$). Six patients remained normoglycemic with median glucose concentration of 3.9 mmol/L (range, 2.8–4.6 mmol/L) and median total KB concentration of 1.9 mmol/L (range, 0.6–5.1 mmol/L). The normoglycemic patients included type VI (3 out of 3) and type IX (3 out of 5) patients. All type III patients developed ketotic hypoglycemia. Interestingly, in five patients (one GSD III, one GSD VI, and three GSD IX), the biochemical profile suggested a

ketolysis defect.

Conclusion: Normoglycemic ketonemia is a common biochemical presentation in patients with GSD types VI and IX, and ketonemia can precede hypoglycemia in all studied GSD types. Therefore, GSD VI and GSD IX should be added to the differential diagnosis of ketotic normoglycemia, and KB concentrations should be routinely measured in ketotic GSD patients.

Abbreviations

FI	Fasting intolerance
GSD	Glycogen storage disease
KBs	Ketone bodies

Introduction

Fasting intolerance (FI) is biochemically associated with hypoglycemia and/or metabolic acidosis, the latter often caused by increased concentrations of lactate and/or ketones. The differential diagnosis of childhood FI includes many endocrine disorders and inborn errors of metabolism, among which several types of glycogen storage disease (GSD).

There are at least 13 types of GSD, which are classified according to the protein defect and organ distribution (Laforêt et al. 2012). The ketotic GSD types 0, III, VI, IX, and XI are associated with fasting ketotic hypoglycemia and considered relatively mild compared to GSD type I because gluconeogenesis is intact. Traditionally ketotic hypoglycemia is considered the common diagnostic biochemical phenotype upon fasting in patients with ketotic GSD types (Laforêt et al. 2012), although cohort studies

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have demonstrated that this is not always the case (Beauchamp et al. 2007a, b). It has recently been reported that ketotic GSD types can be easily misinterpreted as idiopathic ketotic hypoglycemia (Brown et al. 2014).

Regular monitoring of ketone bodies (KBs) is recommended to titrate dietary management in ketotic GSD patients (Dagli et al. 2010; Dagli and Weinstein 2009; Goldstein et al. 2011), but experimental data are lacking. Therefore, we have performed this retrospective study of supervised clinical fasting studies in patients with ketotic GSD.

Patients and Methods

Subjects

The Section of Metabolic Diseases, Beatrix Children's Hospital, University Medical Center Groningen is a tertiary metabolic center and a reference center for hepatic GSD patients. In the period 1993–2012, 539 supervised clinical fasting studies have been performed in 476 patients. From this cohort all patients with ketotic GSD have been identified to perform a retrospective study of the biochemical profiles of their supervised clinical fasting studies. Data were anonymously retrieved from both the paper and electronic medical files.

Fasting Studies

Supervised clinical fasting studies were performed as described elsewhere (Bonnefont et al. 1990; Van Veen et al. 2011), for either diagnostic or therapeutic reasons, i.e., to titrate dietary management. Most diagnostic studies were performed before 2003, when plasma acylcarnitine profiling became available in our laboratory. Fasting studies were only performed in healthy patients in good nutritional condition. Subjects were admitted 1 day before the fasting test when they were <8 years of age. There was no limitation in water intake. After the last meal and an individually tailored period of fasting, an intravenous catheter was inserted for blood sampling at hourly intervals. Carefully supervised fasting was continued until glucose concentrations dropped below 2.6 mmol/L or until development of symptoms or signs of hypoglycemia.

Statistical Analysis

SPSS Statistics version 22.0 (IBM Corp., Armonk, New York, USA) was used to calculate median and range for concentrations of glucose and KB. For data analysis,

hypoglycemia was defined as plasma glucose concentration <2.6 mmol/L (Koh et al. 1988). Total KB was defined as the sum of blood acetoacetate and β -hydroxybutyrate concentrations. If the product of glucose and KB concentrations was greater than 10, a ketolysis defect was suspected (Touati et al. 2012).

Results

Table 1 presents patient characteristics from 12 patients with GSD type III ($n = 4$), type VI ($n = 3$), and type IX ($n = 5$), in whom a total of 13 supervised clinical fasting studies were performed. A fasting study was performed twice in patient 2 due to therapeutic reasons. For patients 2 and 12, no confirmatory molecular studies were available; however, diagnosis was confirmed enzymatically, in leukocytes and erythrocytes, respectively. Patient 2 died at the age of 27 years by a car accident; his sister was homozygous for the c.2039G>A *AGL* founder mutation from the island of Aruba.

Table 2 presents biochemical data during the fasting studies in the individual patients. Six patients showed normoglycemia during fasting, i.e., median blood glucose concentration in these patients was 3.9 mmol/L (range, 2.8–4.6 mmol/L) with median total KB concentration of 1.9 mmol/L (range, 0.6–5.1 mmol/L). The normoglycemic patients included type VI (3 out of 3) and type IX (3 out of 5) patients. All type III patients developed ketotic hypoglycemia, but interestingly, a remarkable increase in KB preceded hypoglycemia in these patients. Patient 4 displayed hypoglycemia at the end of the fasting study, although her glucose concentration was not below 2.6 mmol/L during the last combined KB and glucose measurement. Patient 9 developed hypoglycemia very quickly without clinical manifestations. In patient 11, the fasting study was terminated because of abdominal pain, nausea, and vomiting, although plasma blood glucose concentrations were normal. At the moment of terminating, the blood KB concentration was 3.3 mmol/L. For patient 12, only one combined measurement of blood glucose and KB concentration could be obtained; however, it showed an elevated KB concentration of 0.6 mmol/L already after 3 h of fasting. Interestingly, the product of glucose and KB suggested a ketolysis defect in five patients. One of these five patients underwent a fasting study for diagnostic purposes.

Figure 1 presents concentrations of glucose and KB longitudinally in time for all fasted GSD type VI patients, demonstrating normoglycemia despite remarkable increase of KB concentrations.

Table 1 Patient characteristics

Case	Age ^a (Y ^m)	Sex ^b	GSD type	Molecular defect						
				Gene	Exon nr	Nucleotide change allele 1	Coding effect allele 1	Exon nr	Nucleotide change allele 2	Coding effect allele 2
1	18 ^{0/12}	F	IIIa	AGL	17	c.2039G>A	p.Trp680X	17	c.2039G>A	p.Trp680X
2 (1) [^]	11 ^{0/12}	M	IIIa	—	—	—	—	—	—	—
2 (2) [^]	16 ^{6/12}	M	IIIa	—	—	—	—	—	—	—
3 [^]	22 ^{5/12}	F	IIIa	AGL	17	c.2039G>A	p.Trp680X	17	c.2039G>A	p.Trp680X
4	1 ^{5/12}	M	III	AGL	13	c.1571G>A	p.Arg524His	—	—	—
5	3 ^{3/12}	M	VI	PYGL	3	c.385G>A	p.Asp129Asn	20	c.2446C>T	p.Arg816*
6	4 ^{10/12}	M	VI	PYGL	3	c.418C>G	p.Leu140Val	11	c.1366G>A	p.Val456Met
7	1 ^{10/12}	M	VI	PYGL	1	c.131G>A	p.Arg44His	16	c.1900G>C	p.Asp634His
8	4 ^{4/12}	M	IX	PHKA2	33	c.3614C>T	p.Pro1205Leu			
9	1 ^{7/12}	F	IX	PHKA2	33	c.3614C>T	p.Pro1205Leu			
10	2 ^{6/12}	M	IX	PHKA2	—	DelXp22.13	—			
11	7 ^{10/12}	M	IX	PHKB	14	c.1265dup	—	27	c.2316-2A>C	p.Asn422fs
12	2 ^{3/12}	M	IX	—	—	—	—	—	—	—

^a Age during the fasting study in years and months^b M male, F female

—: mutation unknown, *: patients are siblings

Discussion

This study demonstrates that normoglycemic ketonemia is a common biochemical phenotype in GSD types VI and IX and that ketonemia can precede hypoglycemia in all studied GSD types. This is important from both a diagnostic and management point of view.

In this study, normoglycemic ketonemia was presented by half of the GSD patients. Five out of twelve patients displayed a biochemical phenotype suggestive of a ketolysis defect (Bonnefont et al. 1990). It was recently reported that especially GSD IX is an unappreciated cause of idiopathic ketotic hypoglycemia (Brown et al. 2014). As this study also included diagnostic fasting studies, to our opinion, it emphasizes the potential risk of underdiagnosing ketotic GSD. Ketotic GSD should therefore be included in the differential diagnosis of childhood FI-associated normoglycemic ketonemia.

Previously, supervised clinical fasting studies have played a central diagnostic role as an informative functional in vivo test (Bonnefont et al. 1990), but nowadays these studies are considered obsolete. Moreover, fasting studies are relatively time-consuming, expensive, invasive, and potentially dangerous. These fasting studies have merely been replaced after the introduction of new laboratory techniques, like acylcarnitine profiling (Millington et al. 1990). More recently, next-generation sequencing and

exome sequencing have developed into powerful diagnostic confirmatory tests (Wang et al. 2012). In our experience, there are few indications for the traditional clinical fasting studies, under exceptional circumstances and well-controlled conditions, to characterize the clinical in vivo implications for patients with unknown variations in the metabolome or genome.

Several factors complicate the recognition of patients with ketotic GSD. During ‘quick’ physical examination at an emergency room, both the soft hepatomegaly (like in GSD types VI and IX) and failure to thrive may be easily overlooked. Simple laboratory tests in blood are not routinely requested in stress samples from patients with FI. In untreated GSD patients, (a specific combination of) plasma concentrations of lactate, transaminases, uric acid, triglycerides, and cholesterol is usually abnormal. In contrast, the traditional hormonal and secondary metabolic tests (like analysis of plasma acylcarnitines and urinary organic acids) are usually normal, even when samples are obtained under critical conditions. The abovementioned investigations are important first-line tests in patients with FI to select candidates for confirmatory molecular and/or enzymatic testing for GSD.

It is not known why some ketotic GSD patients display hypoglycemia and some do not. This variation is especially observed in GSD VI; hepatic phosphorylase deficiency, encoded by the *PYGL* gene (OMIM #232700), and GSD

Table 2 Biochemical data of the fasting studies

Case	Purpose test ^a (D/T)	Duration of fasting (hh:mm)	Glucose (mmol/L)	KB ^b (mmol/L)	FFA (mmol/L)	FFA/KB	KB x Glucose ^c
1	T	11:30	3.5	1.4	0.9	0.6	4.9
		18:30	2.4	4.0	1.2	0.3	9.6
2	T	10:30	2.1	4.8	1.8	0.4	10.1
		11:30	2.0	6.9	1.3	0.2	13.8
2	T	11:00	3.3	3.8	1.2	0.3	12.5
		17:00	1.7	4.8	1.6	0.3	8.2
3	T	11:00	3.2	2.6	0.9	0.3	8.3
		16:30	1.7	3.2	0.8	0.3	5.4
4	D	04:00	4.8	2.0	1.2	0.6	9.6
		05:00	4.4	1.5	0.8	0.5	6.6
5	D	12:30	5.1	1.5	1.0	0.7	7.7
		15:15	3.0	3.5	1.1	0.3	10.5
6	D	08:45	4.1	0.7	0.6	0.9	2.9
		14:45	3.5	0.8	0.8	1.0	2.8
7	D	09:00	2.7	1.4	—	—	3.8
		12:00	3.0	1.9	—	—	5.7
8	T	02:00	4.6	1.6	0.9	0.6	7.4
		08:15	3.8	5.1	1.2	0.2	19.4
9	T	03:00	3.7	0.6	—	—	2.2
		07:00	2.3	3.3	1.1	0.3	7.6
10	T	08:30	3.5	1.8	1.4	0.8	6.3
		14:15	2.5	6.1	1.6	0.3	15.3
11	T	07:50	4.1	1.4	0.9	0.6	5.7
		14:50	3.8	3.3	0.9	0.3	12.5
12	D/T	03:10	4.3	0.6	0.6	1.0	2.6
			—	—	—	—	—

^a D diagnostic, T therapeutic

^b KB is the sum of acetoacetate and β -hydroxybutyrate

^c Suspect ketolysis defect is defined as a product of glucose and KB greater than 10 (Touati et al. 2012)

IX; and hepatic phosphorylase b kinase deficiency, encoded by the *PHKA2* gene (OMIM #300798, X-linked GSD IX), the *PHKB* gene (OMIM #172490), and *PHKG2* gene (OMIM #172471), respectively. Beauchamp et al. reported hypoglycemia in 5 out of 13 GSD VI patients on either fasting or glucose loading tests (Beauchamp et al. 2007a), while in GSD IX, Beauchamp et al. reported hypoglycemia as a presenting sign in 5 out of 15 GSD IX patients (Beauchamp et al. 2007b). The hypoglycemia in GSD IX patients included those with mutations in the *PHKG2* gene, which is in line with Bali et al., who reported fasting hypoglycemia in all five patients with *PHKG2* mutations (Bali et al. 2014). This finding may be very well explained by the fact that mutations of the *PHKG2* gene contains the catalytic site of hepatic phosphorylase b kinase.

Uncooked cornstarch and protein are the keystones of dietary management in ketotic GSD, the latter serving as an alternative source for gluconeogenesis to maintain normoglycemia (Derks and Smit 2015). In ketotic GSD types, increased KB concentrations reflect increased mitochondrial fatty acid oxidation, which is associated with activation of gluconeogenesis and secondary endogenous proteolysis from muscle tissue. Instead of maintenance of normoglycemia, prevention of increasing KB concentrations could therefore be regarded as a more relevant aim in optimizing metabolic control.

At a relatively young age, one GSD III patient (patient 4) displayed a decrease in both KB and glucose concentrations with prolonged fasting. Hypoketosis has been reported before in GSD III patients (Seigel et al. 2008; Clemente

Fig. 1 Longitudinal course of fasting in GSD type VI. *Circled dash*, glucose concentration; *diamond double dash*, ketone body concentration; *spaced dash*, cutoff point hypoglycemia

et al. 2010), in whom exogenous carbohydrate requirements are still relatively high (Derks and Van Rijn 2015). We speculate that, as a consequence of dietary management with frequent high-carbohydrate meals, there may have been a relatively high plasma insulin state together with high intracellular malonyl-CoA levels, physiologically inhibiting long-chain mitochondrial fatty acid oxidation at the level of carnitine palmitoyltransferase type I.

This study has several limitations. First, data have retrospectively been retrieved from electronic and paper files, from fasting studies that have mostly been performed at least 10 years ago. Second, fasting studies have been conducted in only a subset of our GSD patients, which could have introduced a selection bias. Third, these fasting studies originate from a period, in which the general opinion on dietary management and outcome parameters for ketotic GSD types was different. Last, the definition of hypoglycemia is debatable in several ways. We have defined hypoglycemia as a *plasma* glucose concentration

<2.6 mmol/L, measured by calibrated meters with a constant factor of 1.11 for conversion between blood glucose and plasma glucose concentrations (D'Orazio et al. 2005). Therefore, the plasma glucose concentrations are on average 11% higher compared to blood concentrations, depending on the hematocrit and the water component in blood. Also, hypoglycemia defined by a single number does not distinguish the different values at which an individual starts to compensate for inadequate glucose supply to the brain (Cornblath et al. 2000).

To date, in contrast with GSD III (Derks and Smit 2015; Kishnani et al. 2010), there are no formal diagnostic and management guidelines for GSD VI and IX. Based on expert opinion, caregivers are advised to titrate dietary management, aiming at normoglycemia and maintenance of blood β -hydroxybutyrate concentrations lower than 0.3 mmol/L, measured by a portable blood ketone meter (Dagli et al. 2010; Dagli and Weinstein 2009; Goldstein et al. 2011). This study provides short-term, indirect

biochemical evidence substantiating these management advices, but there is a lack of data on long-term clinical outcome parameters, like growth, liver size, laboratory studies, hepatic complications, and bone density.

Conclusion

This is the first study that critically analyzed blood glucose and KB concentrations during fasting in ketotic GSD patients. Normoglycemic ketonemia is a common biochemical presentation in patients with GSD types VI and IX, and ketonemia can precede hypoglycemia in all studied GSD types. Therefore, GSD VI and GSD IX should be added to the differential diagnosis of ketotic normoglycemia, and KB concentrations should be routinely measured in ketotic GSD patients.

Compliance with Ethics Guidelines

Conflict of Interest

Francjan J. van Spronsen has received research grants and consultancy and speaker's fees from Merck Serono and Danone Nutricia. He is a member of the scientific advisory board of Merck Serono and chair of the scientific advisory board of Danone Nutricia. In the last 5 years, Terry G. J. Derks has received speaker's fees from Danone Nutricia, Vitaflo, and Recordati and research fees from Sigma Tau and Vitaflo. Irene J. Hoogeveen, Rixt M. van der Ende, Foekje de Boer, and M. Rebecca Heiner-Fokkema declare that they have no conflict of interest to disclose.

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The authors have no financial relationship relevant to this article.

Contributions of Individual Authors

Irene J. Hoogeveen and Rixt M. van der Ende collected and analyzed data from supervised clinical fasting studies, performed the data analysis, drafted the first version of the manuscript, and wrote the final manuscript.

Francjan J. van Spronsen was involved in clinical management and monitoring, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

Foekje de Boer was involved in dietary management, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

M. Rebecca Heiner-Fokkema supervised the data analysis of the fasting studies, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

Terry G. J. Derks initiated this study, was involved in clinical management and monitoring, drafted the first version of the manuscript, critically reviewed and revised the manuscript, and wrote the final manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors confirm the absence of previous similar or simultaneous publications.

Informed Consent

All procedures followed were in accordance with the ethical standards of the institutional responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Since all data were retrieved retrospectively and analyses anonymously, no informed consent was needed.

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