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Changes in pediatric plasma acylcarnitines upon fasting for refined interpretation of metabolic stress

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Abstract

Background: Childhood fasting intolerance is a life-threatening problem associated with various inborn errors of metabolism. Plasma acylcarnitines reflect fatty acid oxidation and help determine fasting intolerance etiology. Pediatric reference values of plasma acylcarnitines upon fasting are not available, complicating interpretation of stress samples.

Methods: Retrospective analysis of supervised clinical fasting studies between 01/2005-09/2012. Exclusion criteria involved patients with (suspected) disorders, repeated tests or incomplete results. Remaining children were grouped according to age: group A (≤24 months), B (25-84 months) and C (≥85 months). Median and 2.5\textsuperscript{th} to 97.5\textsuperscript{th} percentiles of basic metabolic parameters and acylcarnitines were determined at start and end of testing on the ward and analyzed for significant differences (p < 0.05).

Results: Out of 127 fasting studies, 48 were included: group A (n=13), B (n=23) and C (n=12). Hypoglycemia occurred in 21%. Children from group C demonstrated significantly higher end glucose concentrations while end ketone body concentrations were significantly lower compared to younger children. In all groups, free carnitine and C3-carnitine significantly decreased upon fasting, while C2-, C6-, C12:1-, C12-, C14:1-, C14-, C16:1- and C16:1-carnitine significantly increased. End concentrations of C6-, C12:1-, C12-, C14:1-, C14-, C16:1- and C18:1-carnitine were significantly lower in children ≥85 months compared to younger children.

Conclusions: Fasting-induced counter-regulatory mechanisms to maintain energy homeostasis are age-dependent. This influences the changes in basic metabolic parameters and acylcarnitine profiles. Our data enable improved interpretation of the individual fasting response and may support assessment of minimal safe fasting times or treatment responses in patients.

1. Introduction

During childhood, fasting intolerance is a common, possibly life-threatening problem associated with hypoglycemia, metabolic acidosis and/or hyperammonemia. Hypoglycemia occurs when there is an imbalance between glucose production, utilization and reabsorption [1,2]. Adequate fasting adaptation depends on three main pillars which provide counter-regulatory mechanisms to maintain energy homeostasis: a functioning liver and endocrine system, enzymes for production and utilization of metabolic fuels and sufficient energy substrate stores [1,2]. In the initial postprandial fasting state, hepatic glycogenolysis provides a sufficient energy balance. As fasting duration progresses, glycogen stores get exhausted and hepatic gluconeogenesis from alternative substrates as lactate and alanine becomes the main energy source. Lipolysis is activated and fatty acid oxidation in mitochondria and peroxisomes increases. This in turn provides energy required for gluconeogenesis and triggers ketogenesis [1,2]. After prolonged fasting, ketone bodies (KB) finally replace glucose as the brain’s major energy source [3,4]. Defects in these pillars can cause hypoglycemia.

Hypoglycemia can result in irreversible neurological impairment or even death [1,5,6]. In order to prevent possible future metabolic...
derangements, the knowledge on fasting tolerance etiology is of large importance and therefore needs to be assessed. Possible underlying diagnoses as endocrine disorders, liver failure, and various inborn errors of metabolism (IEM) should be excluded [1,2,6]. The diagnostic work up of fasting intolerance consists of a combination of medical history (i.e. clinical symptoms, age at onset, time of last meal, family history), physical examination and analysis of metabolic parameters in blood samples (e.g. glucose, lactate, insulin, cortisol, growth hormone, 3-hydroxybutyrate (3-HB), free fatty acids (FFA), acylcarnitines, amino acids) and urine (e.g. organic acids) [7–11]. Molecular analysis, and if necessary, confirmation by analysis of enzyme activity, effectively complements metabolite screening for diagnosis of several IEMs [6,11–14].

Plasma acylcarnitine profiles represent an estimation of the mitochondrial fatty acid oxidation (FAO) status and can be measured by tandem mass spectrometry analysis (MS/MS). Deviations in plasma acylcarnitine profiles are used to identify IEMs, mainly disorders of mitochondrial FAO and several organic acidurias [8,10,11]. Reference values for plasma acylcarnitine profiles in a normal, fed state are available [9,15,16]. Contrarily, little is known on changes in acylcarnitine concentrations due to metabolic stress as prolonged fasting [7,17,18]. This complicates the interpretation of plasma acylcarnitines in stress samples. Therefore, the aim of this study is to describe fasting-induced changes in plasma acylcarnitine concentrations in children at various ages.

2. Material and methods

This study involved a retrospective analysis of fasting test results, including basic metabolic parameters and acylcarnitine profiles. The Medical Ethical Committee of the University Medical Center Groningen confirmed that the Medical Research Involving Human Subjects Act did not apply, rendering official study approval unnecessary (METc code 2011/173). The study protocol was performed in accordance with the Declaration of Helsinki and approved for waived consent as it concerned retrospective, anonymous data.

2.1. Subject selection

Children who experienced a prior episode of (suspected) hypoglycemia underwent a supervised clinical fasting study in the metabolic unit of the Beatrix Children’s Hospital, University Medical Center Groningen. Since MS/MS analysis of plasma acylcarnitine profiles has been performed routinely in our hospital since 2005, children who underwent a fasting study between January 2005 and September 2012 were included. Children were excluded from data analysis in case of 1) a proven metabolic or endocrine disorder; 2) suspicion for a causative IEM which could not be excluded by further diagnostic studies (e.g. riboflavin transporter deficiencies, glucose transporter type 1 deficiency, multiple acyl-CoA dehydrogenase deficiency, mitochondrial respiratory chain disorders); 3) a known disease such as a genetic disorder, epilepsy, cardiac anomalies or chronic disorder; 4) mental retardation or developmental delay; 5) repeated fasting tests or 6) only one acylcarnitine profile available from either the start or end of testing on the clinical ward.

2.2. Procedure supervised clinical fasting studies

Depending on the child’s age and the clinician’s appraisal, children started fasting on the day or night (mostly between 18.00 and 20.00 o’clock) before the supervised clinical fasting test, directly after the last regular meal. The next morning at 9.00 AM, testing started on the clinical ward. During the fasting period, children were only allowed to drink water or tea without additions. Throughout the entire test, children were observed for symptoms related to hypoglycemia, such as lethargy, paleness, clamminess, tachycardia, irritability and sweating. Blood glucose concentrations were measured at least hourly, but frequently more often, to guarantee safety. The intended duration of testing was based on age, as young children are known to have a shorter fasting capacity than older children [7,19,20]. Premature termination of the test occurred in case of hypoglycemia, defined as plasma glucose < 2.6 mmol/l [21], and/or symptoms suggestive of hypoglycemia.

At multiple intervals during testing on the ward, blood samples were collected for analysis of plasma whole blood glucose, pyruvate, lactate, acetocacetate, 3-HB and FFA. Plasma acylcarnitine profiles were measured at the start and end of testing on the ward using flow-injection MS/MS analysis, as described previously [22]. No blood samples were obtained before fasting to minimize the inconvenience of blood sampling. Moreover, the response of plasma acylcarnitines concentrations upon fasting is most informative after prolonged fasting, i.e. when fatty acids are mobilized.

2.3. Data analysis

Based on previous studies which demonstrated age-dependent fasting adaptation [7,9,18,20,23] and acylcarnitine concentrations [15], three age groups were defined: group A (≤ 24 months); group B (25–84 months); group C (≥ 85 months). Median (range) and 2.5th to 97.5th percentiles of basic metabolic fasting parameters and plasma acylcarnitine profiles were determined using the percentile function in Microsoft Excel. The relative change in concentrations was calculated by dividing the absolute change by the concentration at the start of the fasting test, multiplied by 100. Data was further analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corporation, Armonk, New York, USA), GraphPad Prism, version 5.0 (GraphPad Software, La Jolla, California, USA) and SIMCA Software, version 14.0 (Umetrics, Umeå, Sweden). Due to small sample sizes, only non-parametric tests were performed. Differences in parameters at the start and end of testing on the ward within each group were analyzed using Wilcoxon Signed Ranks Test. Differences in parameters between groups were first detected via Kruskal-Wallis analysis and subsequently objectified using Mann-Whitney U Test. A p-value of < 0.05 was considered statistically significant. Bonferroni correction was performed if required. A principal component analysis on acylcarnitine profiles and basic metabolic parameters in plasma at the start and end of the fasting test was used to test for population distribution. This multivariate analysis technique is often used to visualize complex data and explore components that determine variation in a dataset. It is also useful to identify outliers in complex datasets.

3. Results

Out of 127 children who underwent a supervised clinical fasting study between January 2005 and September 2012, 79 were excluded based on former mentioned criteria. Fasting test results of 48 children remained for further analysis: group A (n = 13; median age: 17 months (range 1–23 months)); group B (n = 23; median age 45 months (25–81 months)); group C (n = 12; median age 126 months (89–194 months)). 52% of included subjects were male (n = 25) and 48% female (n = 23). Median fasting duration at the end of testing in group A, B and C, respectively, was 18 hours (10–22 hours), 20 hours (17–22 hours) and 21.5 hours (17.5–24 hours).

Out of 48 children, ten (21%) reached hypoglycemia of which most were found in group A (n = 4, 31%) and group B (n = 5, 22%) compared to group C (n = 1, 8%). Fasting duration until hypoglycemia increased with age, as demonstrated in Fig. 1. Concentrations of basic metabolic parameters at the start and end of testing on the ward are presented in Table 1A. Glucose concentrations decreased significantly in all age groups upon fasting, with a smaller decline in children above seven years of age. In all groups, KB concentrations and the product of glucose and KB significantly increased upon fasting, while FFA/3-HB and FFA/KB significantly decreased. FFA significantly increased in
from n = 12; \( b \) calculated from n = 19; \( c \) calculated from n = 11, due to missing data; age is presented in Fig. 2.

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rization from 0.69 to 0.91 mmol/L. End KB concentrations were significantly lower in group B and C, while group A only demonstrated a slight FFA mobilization as a function of time. Legend: Kaplan-Meier plot that visualizes the proportion of children that maintained normoglycemia till the end of fasting duration until hypoglycemia, categorized according to age. \( a \) calculated from \( n = 12 \); \( b \) calculated from \( n = 19 \); \( c \) calculated from \( n = 11 \), due to missing data; \( \text{censored subject} \).

ward are presented in Table 1B. Since only C5 was significantly different between male and female subjects at the end of fasting, no subgroups were formed based on gender. The corresponding acylcarnitine ratios used for diagnostic differentiation and follow-up of IEM-patients \([16,24]\) are demonstrated in Table 2. Upon fasting, all groups showed a significant decrease in free carnitine and C3-, while C2-, C6-, C12:1-, C12-, C14:1-, C14-, C16:1- and C16-carnitine significantly increased. In addition, end concentrations of C18:2, C18:1- and C18-carnitine significantly increased in group B while C8-, C10:1- and C10-carnitine increased significantly only in group C. Significantly higher end concentrations of C6-, C12:1-, C12-, C14:1-, C14-, C16:1-, C16- and C18:1-carnitine were demonstrated in younger children compared to group C. Almost all acylcarnitine ratios changed significantly upon fasting, except for C4/C8 in groups A and C, C14:1/C12:1 in group A, (C16 + C18:1)/C2 in groups B and C, C5/C3 and C8/C10 in group C, respectively. Only C14:1/C2, C14:1/C10 and C14:1/C16 were significantly lower in group C compared to younger children. The relative changes in plasma acylcarnitine concentrations and their corresponding ratios upon fasting are presented in Fig. 3.

A principal component analysis model on the basic metabolic parameters and acylcarnitine profiles in plasma at the start and end of the fasting test demonstrated evenly distributed normo- and hypoglycemic subjects as presented in Supplementary Fig. 1.

4. Discussion

Until now, the influence of metabolic stress on plasma acylcarnitine concentrations has only been studied to a limited extent \([7,17,18]\). This study demonstrates that prolonged fasting causes significant changes in various long-, medium-, and short-chain plasma acylcarnitine concentrations of and their corresponding ratios, together with changes in basic metabolic fasting parameters. Interestingly, the course of fasting-induced counter-regulatory mechanisms to maintain energy homeostasis, and thus the extent of acylcarnitine accumulation was age dependent. In children above seven years of age, concentrations of various plasma acylcarnitines were significantly lower compared to younger children. Although acylcarnitine ratios also changed significantly upon fasting, they seemed more robust for age influence, emphasizing their usefulness for diagnostic purposes. There is a potential risk of false-positive results when reference values for acylcarnitine profiles in a normal, fed state are used for the interpretation of stress samples. Our data facilitate improved, age-dependent interpretation of stress blood samples in order to discriminate normal from abnormal fasting responses. Particularly for the diagnosis of IEMs that may (only) result in biochemical abnormalities during periods of metabolic stress this might be of importance. Furthermore, these data can also refine the assessment of the minimal safe fasting time and evaluation of treatment response in patients.

It is known that younger children demonstrate earlier FFA mobilization and subsequent ketogenesis compared to children above seven years of age. This is probably caused by more rapid depletion of glycogen and gluconeogenic substrate storages, and a relatively higher total energy requirement \([7,10,23]\). In our study, end KB concentrations, the product of end glucose and KB, and end FFA levels were significantly lower in children above seven years of age compared to children aged two to seven years. This indicates less or later activity of mitochondrial FAO and ketogenesis in older children, as reflected in Fig. 2, which results in a lower degree of plasma acylcarnitine accumulation. Interestingly, FFA mobilization upon fasting was not significant in children below two years of age. Although this might be due to the small sample size, other causes can perhaps include less available substrate or an increased flux. The relatively high energy requirement in infancy and childhood appears largely determined by brain development \([25,56]\). The estimated endogenous glucose production rate is highest in early childhood \([27]\). While energy required for growth declines from ~40% of the total energy requirement in the first month to < 2% in the second year of life \([25]\), the brain’s glucose demand maximizes during childhood \([28]\). Moreover, alanine concentrations have been reported lowest from approximately two to six years of life, reflecting low substrate availability for gluconeogenesis \([29]\). At these ages, fasting can thus result in increased FFA mobilization and ketogenesis in order to preserve brain metabolism. This corresponds to our data, where most notable fasting-induced changes in metabolic parameters were found in children between two and seven years of age.

Prolonged fasting causes a significant increase in various long-, medium-, and short-chain plasma acylcarnitine concentrations, with the most significant changes in C12:1- to C16:1-carnitines and C4:1/C2, C14:1/C10, C14:1/C16 and (C4*C5*C8*C14)/(C0*C3) ratios. The relative change in the molar ratios was generally smaller and seemed less affected by age. Therefore, they enable enhanced interpretation of acylcarnitine profiles with improved sensitivity and specificity \([8]\). The (C4*C5*C8*C14)/(C0*C3) ratio that was proposed by Sahai et al. for improved diagnosis of MADD \([24]\), was highly influenced by fasting, which may invalidate its application. Its diagnostic value remains to be investigated. Interestingly, there was a significant decrease in total carnitine, free carnitine and C3-carnitine in all age groups. The decrease in total carnitine concentrations is largely determined by the absolute change in free carnitine, even though acylcarnitine concentrations of several chain lengths increase upon fasting. Possible explanations for the decrease in free carnitine upon fasting include interference of the exogenous supply, increased uptake by tissues, or increased utilization for acylcarnitine formation \([30]\). C3- or propionylcarnitine is mainly derived from valine and isoleucine metabolism, and to some extent also from other sources including threonine, methionine, and odd chain fatty acids \([31]\). Its decrease may suggest increased utilization upon fasting, but this remains to be elucidated.

A study limitation concerns the relatively small sample size. Methodologically, it would have also been preferred to use a cohort...
Table 1
Basic metabolic parameters and acylcarnitine profiles in plasma during fasting.

<table>
<thead>
<tr>
<th>Parameters (mmol/L or ratio)</th>
<th>Group A ≤ 24 months, n = 13</th>
<th>Group B 25-84 months, n = 23</th>
<th>Group C ≥ 85 months, n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5.3 (4.5-6.1)</td>
<td>5.3 (4.6-6.5)</td>
<td>5.2 (4.3-6.1)</td>
</tr>
<tr>
<td>Lowest glucose</td>
<td>3.2 (2.5-3.9)</td>
<td>3.2 (2.7-4.0)</td>
<td>3.2 (2.5-3.9)</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.11 (0.07-0.16)</td>
<td>0.11 (0.08-0.16)</td>
<td>0.11 (0.07-0.16)</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.8 (0.7-0.9)</td>
<td>0.8 (0.7-0.9)</td>
<td>0.8 (0.7-0.9)</td>
</tr>
<tr>
<td>3-HB</td>
<td>0.72 (0.6-0.8)</td>
<td>0.72 (0.6-0.8)</td>
<td>0.72 (0.6-0.8)</td>
</tr>
<tr>
<td>3-HB/AcAc</td>
<td>1.25 (1.0-1.5)</td>
<td>1.25 (1.0-1.5)</td>
<td>1.25 (1.0-1.5)</td>
</tr>
<tr>
<td>FFA/KB</td>
<td>0.45 (0.35-0.55)</td>
<td>0.45 (0.35-0.55)</td>
<td>0.45 (0.35-0.55)</td>
</tr>
<tr>
<td>Glucose/KB</td>
<td>0.41 (0.35-0.47)</td>
<td>0.41 (0.35-0.47)</td>
<td>0.41 (0.35-0.47)</td>
</tr>
</tbody>
</table>

Legend: Basic metabolic parameters (A) and acylcarnitine concentrations (B) in plasma during fasting. Data are presented as median (25th–75th percentile). The presented reference values are used by our center for children > 1 month of age. *Median (range) fasting duration calculated from n = 12; †calculated from n = 19; ‡calculated from n = 11 due to missing data. A p-value of p < 0.05 was considered statistically significant, *between start and end of test; †compared to group A; ‡compared to group B (p < 0.01 after Bonferroni correction for analyses between age groups). Abbreviations: 3-HB, 3-hydroxybutyrate; AC, acylcarnitine; AcAc, acetoacetate; FFA, free fatty acids; KB, ketone bodies.

without medical history of children who underwent a supervised clinical fasting study to evaluate the cause of previous (suspected) hypoglycemia. Ethically however, it would not be justifiable to perform clinical fasting studies in children without any suspicion of a disease. As strict exclusion criteria were adhered, we considered the remaining children as children who suffered (suspected) hypoglycemia, but who remained defined as apparently healthy subjects. All fasting test results included in our study were considered normal. Each child with a glucose concentration of < 3.0 mmol/L at the end of the test demonstrated a KB concentration of > 1.8 mmol/L, which differentiates them from FAO disorder patients [7]. Increased concentrations of C14:1- and C14:2-carnitines appear to be a result of fasting-induced lipolysis. The C14:1/C12:1 ratio may help distinguish C14:1-carnitine elevations due to a physiologic fasting response from elevations due to VLCAD deficiency, avoiding a false-positive test result [32]. In our data, the individual end C14:1/C12:1 ratios did not exceed the range described for physiologic fasting responses.

The ten subjects who developed hypoglycemia during the fasting test were not excluded. Based on fasting parameters in plasma, an underlying metabolic disorder was not considered to be likely. Principal component analyses revealed no differences between normo- (n = 38) and hypoglycemic subjects (n = 10), as shown in Supplementary Fig. 1. Moreover, end FFA and KB concentrations did not significantly differ between both groups. Although some statistical differences were
Fig. 2. Basic metabolic parameters in plasma at the end of fasting in relation to age. Legend: Glucose levels (A) from n = 48; KB concentrations (B) from n = 47 due to missing data; FFA concentrations (C) from n = 46 due to missing data. Filled symbols represent children who maintained normoglycemia during the fasting test, open symbols represent children who reached hypoglycemia.
<table>
<thead>
<tr>
<th>AC ratio</th>
<th>Disorder</th>
<th>Group A (≤ 24 months, n = 13)</th>
<th>Group B (25–84 months, n = 23)</th>
<th>Group C (≥ 85 months, n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Start (t = 14 h)</td>
<td>End (t = 18 h)</td>
<td>Start (t = 15 h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 14 h (5–15 h)</td>
<td>t = 18 h (10–22 h)</td>
<td>t = 15 h (13–15.5 h)</td>
</tr>
<tr>
<td><strong>O3/(C16 + C18)</strong></td>
<td>CPT-I, CPT-II, CACT</td>
<td>133.83 (77.39–204.40)</td>
<td>62.96 (33.15–167.73)</td>
<td>178.99 (79.99–255.51)</td>
</tr>
<tr>
<td></td>
<td>PROP, MUT, Cbl A-D, B12 def, MCD</td>
<td>2.57 (1.50–6.28)</td>
<td>1.54 (0.76–3.27)</td>
<td>2.80 (1.15–8.27)</td>
</tr>
<tr>
<td>*<em>C3/C2 <em>100</em></em></td>
<td>PRO, MUT, Cbl A-D, CPT-I, B12 def, MCD</td>
<td>1.79 (0.96–3.86)</td>
<td>1.20 (0.71–3.11)</td>
<td>2.57 (1.06–4.77)</td>
</tr>
<tr>
<td><strong>C4/C3</strong></td>
<td>SCAD, MADD, IBG, EE, FIGLU</td>
<td>0.81 (0.51–1.17)</td>
<td>0.56 (0.25–0.96)</td>
<td>0.82 (0.33–2.21)</td>
</tr>
<tr>
<td></td>
<td>MCD, B12 def, Cbl A-D, PROP, EE, IBG, FIGLU, MADD, SCAD</td>
<td>0.29 (0.19–0.47)</td>
<td>0.35 (0.26–0.61)</td>
<td>0.25 (0.13–0.63)</td>
</tr>
<tr>
<td>*<em>C4/C8 <em>100</em></em></td>
<td>IBG, SCAD, EE, FIGLU</td>
<td>0.80 (0.37–1.21)</td>
<td>0.83 (0.32–1.10)</td>
<td>0.76 (0.35–2.18)</td>
</tr>
<tr>
<td>*<em>C5/C3 <em>100</em></em></td>
<td>IVA, 2MBG, MADD, EE</td>
<td>0.23 (0.10–0.45)</td>
<td>0.36 (0.14–0.61)</td>
<td>0.23 (0.12–0.44)</td>
</tr>
<tr>
<td>*<em>C5/C2 <em>100</em></em></td>
<td>IVA, MADD, 2MBG, EE</td>
<td>0.52 (0.23–1.89)</td>
<td>0.30 (0.17–0.94)</td>
<td>0.66 (0.27–1.47)</td>
</tr>
<tr>
<td><strong>C5/C3</strong></td>
<td>MCD, B12 def, MUT, Cbl A-B, PROP, IVA, MADD, EE, 2MBG</td>
<td>0.17 (0.10–0.45)</td>
<td>0.20 (0.14–0.47)</td>
<td>0.22 (0.12–0.44)</td>
</tr>
<tr>
<td>*<em>C8/C10 <em>100</em></em></td>
<td>MCAD, MADD</td>
<td>1.14 (0.66–1.88)</td>
<td>0.78 (0.50–1.50)</td>
<td>1.01 (0.70–2.25)</td>
</tr>
<tr>
<td>*<em>C14:1/C2 <em>100</em></em></td>
<td>MCAD</td>
<td>0.84 (0.64–0.98)</td>
<td>0.73 (0.55–0.97)</td>
<td>0.84 (0.61–1.22)</td>
</tr>
<tr>
<td>*<em>C14:1/C2 <em>100</em></em></td>
<td>VLCAD, MADD, LCHAD/TFP</td>
<td>2.14 (0.86 – 4.00)</td>
<td>3.37 (1.09–5.63)</td>
<td>1.47 (0.93–3.65)</td>
</tr>
<tr>
<td><strong>C14:1/C10</strong></td>
<td>VLCAD</td>
<td>1.62 (0.72–2.47)</td>
<td>2.96 (1.02–4.02)</td>
<td>1.16 (0.69–2.36)</td>
</tr>
<tr>
<td><strong>C14:1/C12:1</strong></td>
<td>VLCAD</td>
<td>1.24 (1.00–1.80)</td>
<td>1.29 (0.93–2.00)</td>
<td>1.34 (0.89–1.76)</td>
</tr>
<tr>
<td><strong>C14:1/C16</strong></td>
<td>VLCAD, MADD, LCHAD/TFP</td>
<td>1.23 (0.48–2.87)</td>
<td>2.47 (0.64–4.30)</td>
<td>1.25 (0.50–2.61)</td>
</tr>
<tr>
<td>*<em>C16 + C18/C2 <em>100</em></em></td>
<td>CPT-I, CPT-II, CACT</td>
<td>3.36 (2.32–4.96)</td>
<td>3.08 (1.84–5.78)</td>
<td>3.78 (2.56–5.30)</td>
</tr>
<tr>
<td><em><em>GA-II index: (C4</em> C5</em> C8* C14)/(C0* C3) <em>10000</em>*</td>
<td>MADD</td>
<td>0.04 (0.01–0.27)</td>
<td>0.26 (0.03–0.72)</td>
<td>0.02 (0.00–0.31)</td>
</tr>
</tbody>
</table>

Legend: Data are presented as median (2.5th–97.5th percentile). Some ratios are multiplied by 100 or 10000 to clarify the differences between start and end of test. aMedian (range) fasting duration calculated from n = 12; bcalculated from n = 19; ccalculated from n = 11 due to missing data. A p-value of p < 0.05 was considered statistically significant, bbetween start and end of test; compared to group A; céntreferred to group B (p < 0.01 after Bonferroni correction for analyses between age groups). Abbreviations (in alphabetical order): 2MBG, 2-short/branched chain acyl-CoA dehydrogenase deficiency (OMIM number 610006); AC, acylcarnitine; B12 def, vitamin B12 deficiency; CACT, camitine-acylcarnitine translocase deficiency (212138); Cbl, cobalamin (complementation group); CPT-I, carnitine palmitoyltransferase I deficiency (255120); CPT-II, carnitine palmitoyltransferase II deficiency (255110); EE, ethylmalonic encephalopathy (602473); FIGLU, formiminoglutamic acidemia (229100); IVA, isovaleryl-CoA dehydrogenase deficiency (231680); MCAD, medium-chain acyl-CoA dehydrogenase deficiency (607008); MADD, multiple acyl-CoA dehydrogenase deficiency (201470); VLCAD, very long-chain acyl-CoA dehydrogenase deficiency (201475). The underlined values are ratios multiplied by 100 or 10000 to clarify the differences between the start and end of test.
detected in end plasma acylcarnitine concentrations, the age-dependent fasting response might have been a confounding factor since nine out of ten subjects who reached hypoglycemia (90%) were below seven years of age. After children above seven years of age were excluded from statistical analysis, only C10-carnitine was significantly higher in hypoglycemic subjects with a median value of 0.20 versus 0.13 μmol/L (p=0.003). However, these values are within the C10-carnitine ranges as presented in Table 1B. One of the most commonly used explanations for hypoglycemia in children involves idiopathic ketotic hypoglycemia (IKH). These otherwise healthy children present with hyperketotic hypoglycemia, usually combined with a period of metabolic stress as intercurrent illness or prolonged fasting [6,33]. It remains debatable whether IKH is a pathophysiological condition or rather a physiologic state. Contrarily to previous beliefs, it has been suggested that these children represent the “lower tail of the Gaussian distribution of fasting tolerance in children” in whom there is a discrepancy between glucose production and utilization [34]. Relatively larger peripheral energy demands in young children as a result of higher brain to body proportions and impaired gluconeogenesis due to limited supplies of gluconeogenic substrates, particularly alanine, have both been mentioned as possible key players [34,35]. Therefore, IKH was not defined as an exclusion criterion in this study. However, a recent retrospective cohort study identified pathogenic variations in 20 out of 164 children with IKH concerning five genes associated with milder types of glycogen.

Fig. 3. The relative changes in plasma acylcarnitine concentrations upon fasting. Legend: Each boxplot represents the 2.5th–97.5th percentile of the relative changes in acylcarnitine concentration (A) and the corresponding molar ratios (B) upon fasting, categorized according to age. Corresponding significance is presented in Table 1.
storage diseases including type 0, VI and IX [36]. It cannot be ruled out that synergistic heterozygosity [37,38], which theoretically could result in more, a positive carrier status for one of these diseases or syndromes or undiscovered disorders associated with hypoglycemia. Furthermore, a principal component analysis model on individual acylcarnitine profiles and basic metabolic parameters in plasma at the start and end of fasting demonstrated an even population distribution without outliers, as presented in Supplementary Fig. 1.

Supervised clinical fasting studies have been performed historically for diagnostic and treatment purposes in patients with fasting intolerance. Possible complications, although rare, include severe hypoglycemia, metabolic acidosis, cardiac arrhythmias and organ failure [6,11]. Since the late 1990s, alternative diagnostic techniques have become available, with higher sensitivity and specificity. While diagnostic supervised clinical fasting studies have mostly been abandoned and are considered obsolete, fasting studies after diagnosis can still provide useful information regarding minimal safe fasting time or treatment response, presumed that they are carried out under strict observation [6,11,18].

5. Conclusions

The described age-dependent changes in plasma acylcarnitine concentrations in children upon (prolonged) fasting can, in conjunction with basic metabolic parameters, be used for refined interpretation of individual response to fasting. In patients with a confirmed diagnosis, these parameters can also contribute to the determination of a minimal safe fasting time and evaluation of treatment responses.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2019.06.007.

Author contributions

WR, RE, CV, FS, TD, and RH contributed to the design of the study; FS and TD supervised the clinical fasting studies; WR, RE, and CV contributed to the data collection; WR performed the data analysis and interpretation; TD and RH supervised the data analysis and interpretation; WR and RH drafted the initial manuscript, and revised the manuscript; RE, CV, FS, TD, and RH contributed to the design of the study; WR, RE, CV, FS, TD, and RH critically reviewed the manuscript. All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article to disclose.

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