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Assessment of Fasting Metabolism With Microdialysis Indicates Earlier Lipolysis in Children With VLCADD Than MCADD

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ABSTRACT

Aim: To investigate fasting metabolism in children with very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) and medium-chain acyl-CoA dehydrogenase deficiency (MCADD) using microdialysis technique.

Methods: Twelve patients (7 with VLCADD, 5 with MCADD, mean age 4.9 years, 10/12 diagnosed via newborn screening) were recruited for investigation in connection to clinical fasting examinations at the Karolinska University Hospital (between 2015 and 2024). Patients were subjected to a 9-h night fast after a standard late evening meal. Analysis of glucose, glycerol, lactate, and pyruvate was conducted by continuous microdialysis. Fasting hormones and acylcarnitines were analysed in blood samples at 1-h intervals in patients with VLCADD.

Results: Children with VLCADD showed signs of lipolysis after a median fasting time of 4.5 h, whereas patients with MCADD showed no significant increase in lipolysis during the fast. A shorter time to initiation of lipolysis tended to correlate with a lower residual enzyme activity in patients with VLCADD. All patients maintained euglycemia during fasting.

Conclusion: Children with VLCADD had a shorter time to initiation of lipolysis during fasting than children with MCADD. Clinical evaluation of fasting metabolism in beta-oxidation disorders should include assessment of lipolysis as an early and important determinant.

1 | Introduction

Fatty acid oxidation disorders (FAODs) are a group of recessively inherited disorders caused by enzyme deficiencies involved in mitochondrial beta-oxidation. Patients with beta-oxidation defects have a compromised capacity for cellular energy production using fatty acids as a substrate. Accordingly, this leads to energy deficiency and tissue accumulation of fatty acid intermediates [1]. Acylcarnitines are fatty acids of different chain lengths conjugated with L-carnitine and found in the circulation [2]. The deficient fatty acid oxidation in patients with FAODs leads to an accumulation of acylcarnitines with a disease-specific pattern of

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, Adenosine triphosphate; BMI-SDS, body mass index standard deviation score; CK, creatine kinase; CSS, clinical severity score; EFA, essential fatty acid; FAOD, fatty acid oxidation disorder; GH, growth hormone; L/P, lactate to pyruvate ratio; LC-FAOD, long-chain fatty acid oxidation disorder; LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; LCT, long-chain fatty acid triglyceride; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; MCT, medium-chain triglyceride; MTPD, mitochondrial trifunctional protein deficiency; NBS, newborn screening program; OCR, oxygen consumption levels; PMBC, peripheral mononuclear cells; VLCADD, very long-chain acyl-CoA dehydrogenase deficiency.

Summary

- Patients with beta-oxidation disorders have an impaired ability to produce energy from stored fat and are therefore fasting intolerant.
- Using subcutaneous microdialysis we found that children with very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) had an earlier fasting-induced lipolysis compared to children with medium-chain acyl-CoA dehydrogenase deficiency (MCADD) indicating that children with VLCADD are more vulnerable to energy deficiency.
- Assessment of fasting-induced lipolysis can serve as an early and important determinant of fasting tolerance in beta-oxidation disorders.

elevated acylcarnitine species corresponding to the enzyme deficiency. The clinical manifestations of FAODs are dependent upon the specific disease and its severity. However, common features are fasting intolerance and dysfunction in organs more reliant on fatty acid oxidation, such as the liver, heart and skeletal muscle [3]. Patients may develop hypoketotic hypoglycemia during episodes of reduced energy intake and heightened energy demand, particularly in the presence of intercurrent infections. This condition can lead to severe organ damage [4]. Creatine kinase (CK) and transaminases are increased during decompensation. In addition, lactic acidosis is seen in severe metabolic decompensations, suggesting a secondary dysfunction of the mitochondrial respiratory chain [5].

Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD; OMIM 201475) is a FAOD caused by mutations in the *ACADVL* gene. The clinical panorama of VLCADD spans from asymptomatic to a severe neonatal-onset form with a poor outcome [6]. Treatment for symptomatic individuals involves restricted intake of long-chain fatty acid triglycerides (LCTs), dietary supplementation with medium-chain triglycerides (MCTs) and essential fatty acids (EFAs), along with the avoidance of prolonged fasting [7]. Enzymatic defects in the *ACADM* gene cause medium-chain acyl-CoA dehydrogenase deficiency (MCADD; OMIM 201450). MCADD is one of the most common FAODs and is associated with decompensations with hypoketotic hypoglycemia, leading to neurological impairment and developmental delay or death in undiagnosed patients [8]. The introduction of newborn screening programs (NBSs) and early diagnosis has significantly improved outcome [9].

Another effect of the widespread establishment of NBS for FAODs is the identification of previously undetected mild phenotypes and patients with no detected clinical symptoms. This development has raised issues concerning the need to individualise and evaluate selected treatments or, in some cases withhold treatment altogether [10, 11].

A key treatment intervention in FAODs is the restriction of fasting time. Clinical practice recommendations range from normal age-correlated fasting time to continuous night feeds depending on the patient's age, type of disorder, and the disease phenotype. Published guidelines generally allow for a nightly fast of 10–12 h in older children and adults during stable metabolic conditions but the definition of fasting tolerance in FAODs is yet to be determined [7, 12, 13]. Despite being one of the cornerstones of treatment, evidence for recommended fasting restrictions in FAOD is limited. Derks et al. presented data on fasting in children with MCADD where the primary endpoint was hypoglycemia (blood glucose < 2.6 mmol/L). The median safe fasting duration (to avoid hypoglycemia) was 12 h in children younger than 1 year and 18 h for children over 1 year [14]. This study mirrors the clinical experience managing children with FAODs in our and other centers that hypoglycemia generally only occurs after prolonged fasting or in connection to infections or other triggering events. In addition, several papers have documented a buildup of disease-specific acylcarnitine species in patients with long-chain FAODs (LC-FAODs) during extended periods of overnight fasting [15–18]. These studies have raised the question of whether increased levels of potentially toxic fatty acid metabolites generated by prolonged night fasting should also be included in evaluating fasting tolerance in this group of diseases.

Microdialysis is a method used for the sampling of analytes in the extracellular tissue fluid of an organ or tissue. A thin semipermeable catheter is placed in the specific organ or tissue and is perfused by a salt solution from a connected pump. The perfusate is then collected in a microvial and analytes can be continuously analysed bedside [19].

During lipolysis stored triglycerides in the subcutaneous fat are hydrolysed and free fatty acids and glycerol are released. A detection of increased subcutaneous tissue glycerol levels indicates an initiation of lipolysis.

Using the microdialysis technique our group has previously shown that patients with Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) have an increased and early lipolysis with an increase in subcutaneous glycerol levels already after 4 h of fasting. This indicates a more limited fasting tolerance than generally thought [15].

In this study we used microdialysis in the subcutaneous fat tissue to examine fasting metabolism in paediatric patients with VLCADD or MCADD. The study aimed to determine the onset time of initiation of lipolysis in both disorders and in relation to disease severity, in VLCADD, during overnight fasting. In addition, changes in plasma levels of disease-specific acylcarnitines and fasting hormones were assessed in patients with VLCADD.

2 | Material and Methods

2.1 | Study Cohort

Patients diagnosed with VLCADD (six boys and one girl, mean age 5.5 years) and MCADD (four girls and one boy, mean age 4.1 years) were recruited from the paediatric metabolic unit at the Karolinska university hospital in Stockholm. Five of seven participants with VLCADD and all participants with MCADD were diagnosed following positive newborn screening (NBS). The two clinically diagnosed patients with VLCADD had severe

symptoms at diagnosis age 4 weeks (patient nr.6) and 1 year (patient nr.3) (Table 1).

All participants with VLCADD had dietary treatments ranging from strict regimens similar to those for LCHADD patients to more lenient regimens. Participants with MCADD had no daily dietary treatment, but three patients had carnitine supplementation, and all had plasma carnitine levels within the normal range during the study. All participants had written emergency regimen instructions based on a dextrin maltose supplement (Fantomalt).

The clinical evaluation of patients with VLCADD was summarised as a clinical severity score (CSS) [20]. Briefly, the CSS comprises 15 clinical parameters, including neonatal symptoms, biochemical abnormalities such as increased creatine kinase (CK) levels, frequency of ER visits, cognitive assessments and presence of gastrostomy. The CSS, ranging from 0 to 15 points, denotes the severity and burden of the patient's disease (Table 1).

The study was performed in conjunction with a planned inpatient clinical visit investigating fasting tolerance.

2.1.1 | Genetic Analysis

Genetic analysis was performed at the Center for Inherited Metabolic Diseases, Karolinska University Hospital, Stockholm, Sweden as part of routine diagnostic procedures. All exons and exon-intron junctions of the *ACADVL* (study participants with VLCADD) and *ACADM* gene (study participants with MCADD) were sequenced from amplified genomic DNA using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and a DNA analyser (model 3730 or 3500XL, Applied Biosystems).

2.1.2 | Residual VLCAD Enzyme Activity Analysis

Residual enzyme activity analysis using the palmitoyl-CoA oxidation rate assay was performed at the University of Freiburg, Germany, using purified lymphocytes in 6 of 7 VLCADD patients. The palmitoyl-CoA oxidation rate was measured as described previously [21], with minor modifications [20]. The results showed that the median activity was 7% of that of healthy controls (range 1.1%–12.5%) (Table 1).

2.2 | Study Protocol

The study participants were admitted to the paediatric metabolic ward at the Karolinska University Hospital, where they underwent a clinical examination by the attending physician. To exclude current infection and metabolic instability/decompensation at admission the following parameters were analysed in blood: CRP, CK, AST, ALT, myoglobin and glucose. In the VLCADD group an additional analysis of insulin, beta-hydroxybutyrate, acyl carnitines and cortisol were performed. The samples were drawn via peripheral intravenous catheters inserted under mild sedation with nitric oxide. During the same session, the microdialysis catheter was placed in the abdominal subcutaneous fat tissue. Figure 1 presents a scheme of the study protocol.

2.2.1 | Microdialysis

The microdialysis device was prepared according to the manufacturer's instructions, whereby the first 2 h of microdialysis were used for calibration [22]. Thereafter, subcutaneous tissue dialysate samples were collected every 30 min during the microdialysis sessions and analysed bedside with results available within a few minutes (Figure 1). Dialysate concentrations of glucose, glycerol, lactate and pyruvate were assessed using the CMA 600 Microdialyser (CMA Microdialysis AB, Solna, Sweden). Participants followed their individually prescribed dietary plan throughout the day. A standardised late evening meal based on their individualised dietary guidelines was administered 1 h before the start of the fasting period (at 11 pm). The pre-fasting late evening meal contained 20% of their total daily energy intake. In the VLCADD group, carbohydrates represented 50% (49–60), fat 40% (15–41) (LCT 7.5%–15%) and protein 10% (9-25) of total energy in the pre-fasting meal. For the MCADD group, carbohydrates represented 55.5% (49–57), fat 29% (25–38) and protein 14.5% (12-20) of total energy in the pre-fasting meal (Table 1). For one patient with MCADD we could not determine the exact composition of the late evening meal since the patient mostly drank juice and only ate a minor part of the served meal (Table 1).

Fasting was discontinued after 9 h (8 am) when patients were served breakfast based on their dietary requirements. Microdialysis was continued for an additional hour, and the study was concluded at 9 am (Figure 1). The fasting time of the study protocol was set to 9 h based on the reported fasting time the participating children had during everyday life conditions (range 6–12 h).

The initiation of lipolysis was defined as the time point during the night fast after which there was a steady increase in subcutaneous glycerol levels in the following hours of monitoring compared to baseline glycerol levels. The glycerol values from the individual investigations were assessed both visually by looking at the individual graphs as well as the included data points in a table.

2.3 | Statistical Analysis

Statistical analysis was performed using Prism 9 (GraphPad Software LLC, San Diego, CA, USA) and IBM SPSS Statistics version 26 (IBM corporation, Armok, NY, USA). Non-parametric statistics were used. Correlation analysis was conducted using Spearman's rank correlation coefficient test. The Mann–Whitney test was used to compare independent groups. All statistical tests were two-sided and a p-value of ≤ 0.05 was considered significant. Results are presented as median and range or mean as appropriate.

3 | Results

3.1 | Study Cohort

All participants had a body mass index (BMI) standard deviation score (BMI-SDS) within ±2.5 BMI-SDS. Median BMI-SDS (SD) for the VLCADD group was 1.2 (−1.1–2.3) and −0.3 (−0.7–0.4) for the MCADD group. The common c.848T>C mutation in the

TABLE 1 | Characteristics of the study cohort.

Patient	Diagnosis	Age (years)	BMI-SDS	Mutation allele 1	Mutation allele 2	Enzyme activity Palmitoyl-CoA Oxidation rate1[a]	Clinical Severity Score[b]	Clinical description	Pre-fasting meal[c]
1. ♂	VLCADD	2.5	1.5	c.848T>C	c.1816T>C	4.5	3	Strict diet, 2 episodes with increased transaminases.	40% F (LCT 7.5%), 50% CH, 10% P.
2. ♂	VLCADD	4	-0,1	c.848T>C	c.1837C>T	6.6	4	Strict diet, 2 episodes with increased transaminases.	41% F (LCT 7.5%), 49% CH, 9% P.
3. ♂	VLCADD	8	−1.1	c.848T>C	c.963C>A	—	8	Strict diet, decompensation at 1 year of age leading to severe motor and cognitive impairment	40% F (LCT 7.5%), 50% CH, 10% P.
4. ♀	VLCADD	6	0.6	c.848T>C	c.1837C>T	9.4	2	Strict diet, 2 episodes with increased CK and transaminases	40% F (LCT 7.5%), 50% CH, 10% P.
5. ♂	VLCADD	3.5	1.6	c.848T>C	c.848T>C	7.4	5	Strict diet, frequent ER admissions, gastrostomy	40% F (LCT 7.5%), 50% CH, 10% P.
6. ♂	VLCADD	8	2.3	c.343DelG	c.343DelG	1.1	11	Strict diet, severe phenotype with neonatal hypoglycemia and very frequent rhabdomyolysis episodes	40% F (LCT 7.5%), 50% CH, 10% P.
7. ♂	VLCADD	6.5	1.2	c.848T>C	c.1838G>A	12.5	3	Dietary treatment, benign clinic, one episode of increased CK and transaminases	15% F (LCT 15%), 60% CH, 25% P.
8. ♀	MCADD	4	−0.3	c.985A>G	c.107A>T	—	—	Benign clinic, emergency regimen	28% F (LCT 28%), 56% CH, 16% P.
9. ♀	MCADD	3.5	−0.2	c.985A>G	c.629A>G	—	—	Benign clinic, emergency regimen	30% F (LCT 30%), 57% CH, 13% P.
10. ♀	MCADD	1.5	−0.6	c.985A > G	c.985A > G	—	—	Benign clinic, emergency regimen and carnitine supplementation	38% F (LCT 38%), 49% CH, 13% P.
11. ♂	MCADD	6.5	0.4	c.158G > A	c.388-3T > G	—	—	Benign clinic, emergency regimen and carnitine supplementation	—

(Continues)

TABLE 1 | (Continued)

Patient	Diagnosis	Age (years)	BMI-SDS	Mutation allele 1	Mutation allele 2	Enzyme activity Palmitoyl-CoA Oxidation rate1[a]	Clinical Severity Score[b]	Clinical description	Pre-fasting meal[c]
12. ♀	MCADD	5	−0.7	c.985A > G	c.985A > G	—	—	Benign clinic, emergency regimen and carnitine supplementation	25% F (LCT 25%), 55% CH, 20% P.

[a]Percentage of healthy controls.
[b]Clinical Severity Score (CSS): 0–15 points. 1 point each for hypoglycemia, 1 episode of elevated CK, ≥ 3 episodes of CK elevation, 1 episode of elevated transaminases, 1 episode of both elevated CK and transaminases, 1 episode of rhabdomyolysis, ≥ 3 episodes of rhabdomyolysis, 1 admittance to PICU, ≥ 3 ER visits during the 1st year of life, strict diet (LCT < 15%), gastrostomy + night feeds, presence of motor impairment, developmental delay, epilepsy, neonatal symptoms.
[c]Pre-fasting meal contents: Percent of total meal energy content. Fat (F), long-chain triglycerides (LCT), carbohydrates (CH) and protein (P). Patient number 11 did not finish the meal and meal contents could not be calculated.

ACADVL gene was present in 50% of all *ACADVL* alleles. In the MCADD group the common c.985A>G mutation was detected in 6 of 10 alleles (Table 1).

The median CSS for the patients with VLCADD was 4 [2-11] (Table 1).

3.2 | Microdialysis

Subcutaneous tissue levels of glycerol remained stable or showed a slight decrease during the first 4 h of overnight fasting in patients with VLCADD. A steady increase in glycerol was initiated after a median time of 4.5 h (3–5 h) of fasting, indicating increased lipolysis (Figure 2). Four of five patients with MCADD showed stable glycerol levels throughout the fast. One patient showed an increase in glycerol after 7 h of fast. This patient ate a minor part of the prescribed night meal and drank 100 mL of fruit juice before fasting began (Figure 2).

The median tissue glycerol levels during the overnight fasting period were similar in the VLCADD and MCADD groups 142 μM (51–358) and 162 μM (105–268). During the first 4 h of fasting, before observing a steady rise in glycerol, median levels were significantly lower in the VLCADD group compared to the MCADD group 117 μM (51–187) and 163 μM (121–219), $p = 0.008$.

In the VLCADD group the correlation between the time to initiated lipolysis and residual enzyme activity ($R = 0.74$, $p = 0.12$) showed a tendency but not for the CSS ($R = -0.38$, $p = 0.47$). No correlation was seen between time to initiation of lipolysis and participant age ($R = 0.03$, $p = 0.98$).

The tissue glucose levels during the night fast were comparable in the VLCADD and MCADD groups, 4.8 mmol/L (3.6–6.5) and 4.9 mmol/L respectively (3.4–5.7) and remained stable throughout fasting (Figure S1). The tissue glucose levels in the VLCADD group were slightly lower than the plasma glucose levels (Figure 3).

Subcutaneous tissue lactate levels remained stable throughout the overnight fasting period. The median levels were significantly higher in the VLCADD group than in the MCADD group, 1.7 mM (0.5–4.4) and 1.3 mM respectively (0.6–3), $p < 0.001$ (Figure S1). The levels of pyruvate in the subcutaneous tissue gradually declined over the initial 5-h fasting period and then increased somewhat until the end of the night fast. Median pyruvate levels were similar for VLCADD compared to MCADD, 124 μM (74–289) for VLCADD and 139 μM (76–243) for MCADD, $p = 0.25$ (Figure S1). The median lactate to pyruvate ratio (L/P) during fasting was higher in VLCADD 11.8 (8.9–31) than in MCADD 9.5 (7.8–15.8) (Figure S1).

3.3 | Baseline and Fasting Levels of Hormones and Metabolites in the VLCADD Group

Baseline blood glucose was 5.3 mmol/L (4.2–6.5), and blood ketones were 0.1 mmol/L (0–0.2). The serum insulin level was 2.6 mIE/L (1.4–8.9) and serum cortisol 152 nmol/L (70–387). CK, AST and ALT were within the normal range (≤ 3.8 microkat/L, ≤ 0.78 microkat/L and ≤ 0.52 microkat/L, respectively) in all

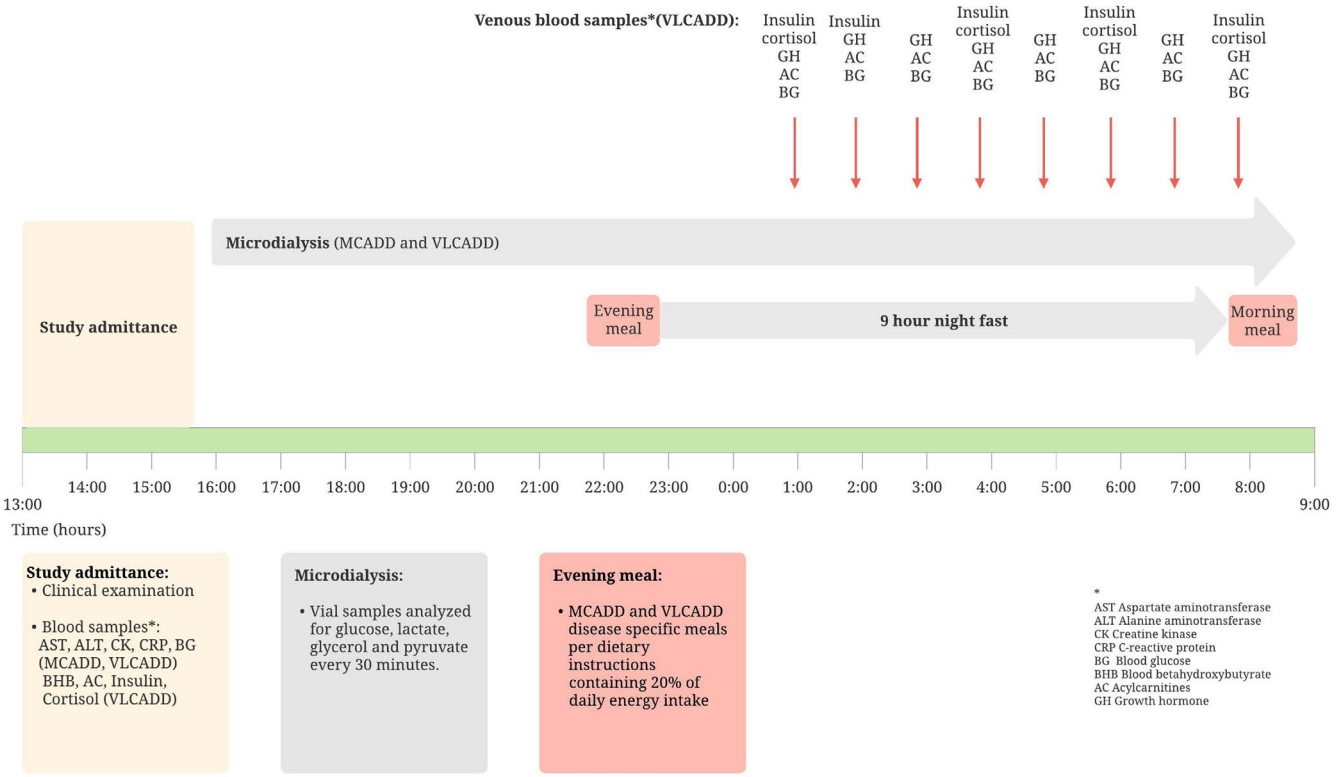

FIGURE 1 | Study protocol.

participants. Plasma acylcarnitine species were not normal at baseline in 5/7 VLCADD participants. In the five abnormal profiles only tetradecaenoylcarnitine (C14:1) exceeded the cut-off (0.79–1.4 μmol/L, normal range <0.3) in four patients. One patient also showed elevated levels of C16:1 (0.38 μmol/L, normal range <0.2) and C18:2 (0.4 μmol/L, normal range <0.3).

During the night fast median blood glucose was within the normal range 5 mmol/L (3.6–6.5) and decreased gradually with fasting time (Figure 3). Peak levels coincided with the maximum growth hormone (GH) secretion levels after 3 h of fasting. In addition, a marginal increase in mean glucose levels was noted for the early morning cortisol peak (Figure 3). A steady decrease in serum insulin levels was observed during fasting, from 13mIE/L (5.9–34) after 2 h of fasting to 6.3 mIE/L (1.9–8.7) before breakfast (Figure 3). Blood ketones were <0.2 mmol/L throughout the overnight fasting for all participants. Serum cortisol peaked at 6 am, after 7 h of fasting (399 nmol/L (377–422)) and decreased towards the end of fasting. Serum GH peaked after 3 h of fasting (2.3 μg/L (0.9–3.9)) and declined throughout the fasting period. Plasma C14:1 remained stable near the upper normal reference limit (0.3 μmol/L) during the first 6 h of fasting, followed by a pronounced increase and peak after 8 h (Figure 3). Throughout the night fast, all other acylcarnitine species remained below the normal reference limit.

4 | Discussion

This study, in a small but well described cohort of children with VLCADD and MCADD, shows that children with VLCADD had an earlier initiation of lipolysis during overnight fasting compared to the children with MCADD. The time to initiation of lipolysis tended to correlate with the residual enzyme activity levels of the VLCAD enzyme in the VLCADD group, suggesting reduced fasting tolerance in patients with more severe disease. Children with MCADD had no increase in lipolysis during the night fast, implying a better tolerance to fasting. Glucose levels remained within normal range in both groups during the entire fasting period.

The median time to initiation of lipolysis in the VLCADD group was 4.5 h, which is slightly longer compared to the time to lipolysis of 3.5 h that we previously determined in a cohort of patients with LCHADD.

During fasting, the body increasingly depends on energy production from stored triglycerides [15, 23]. In patients with beta-oxidation disorders the increased lipolysis will lead to the production of abnormal fatty acid metabolites that can accumulate in the liver and the heart muscle as well as in other organs [24]. The clinical benefits of monitoring the increased acylcarnitine metabolites found in FAODs are being questioned. Previous studies have shown an elevation of plasma C14:1, an acylcarnitine which specifically increase in VLCADD patients, following both physical activity and after prolonged fasting [16]. The considerable diurnal variations of acylcarnitines in patients with LC-FAODs [16] underscore the need for a cautious evaluation of test results. Within the group of FAODs, conditions such as LCHADD and MTPD are known to cause persistent clinical symptoms, including retinopathy and peripheral neuropathy. These symptoms are absent in VLCADD and MCADD [25–27]. A previous report suggests a correlation between high levels of long-chain 3-hydroxylated acylcarnitines and the development of retinopathy [17]. Hence, evaluating substrate dynamics and determining

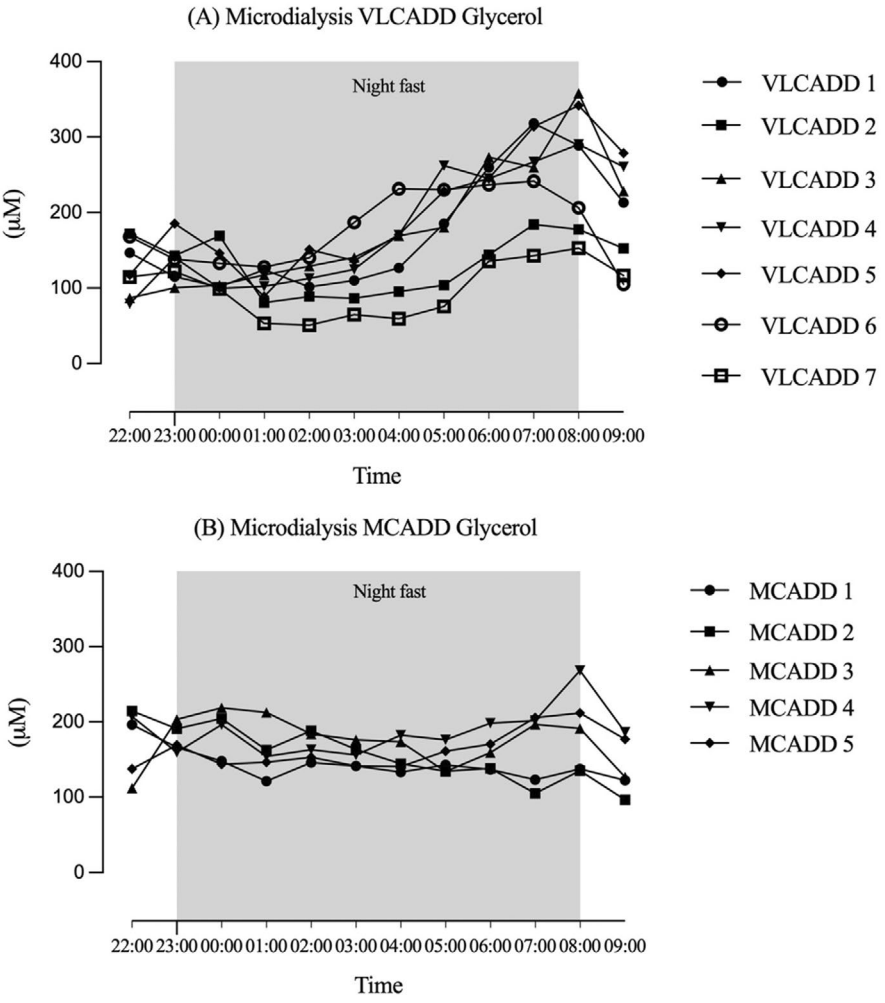

FIGURE 2 | Microdialysis monitoring of glycerol.

when increased lipolysis occurs during fasting in FAODs holds significance, both from the perspective of cellular energy deficiency from impaired fatty acid oxidation and the potential accumulation of toxic fatty acid metabolites.

Mean subcutaneous glycerol levels during the first part of the night fast (from midnight until 4 am) in the VLCADD group were comparable to those described in a microdialysis investigation of healthy prepubertal children aged 7–10 years [28] (Figure 2). In the VLCADD group the median time until the onset of increased lipolysis was 4.5 h. Notably, patients with lower residual enzyme activity tended to display earlier initiation of lipolysis compared to those with higher activity levels. Although age could be a factor influencing time to lipolysis, we did not find a correlation between time to lipolysis and the age of the participants. Even though the patient with the highest CSS (patient nr. 6) had the shortest time to lipolysis, the multifactorial CSS did not correlate significantly with time to lipolysis. Possible reasons for the weak correlation between CSS and time to lipolysis could be that the patients were young and therefore had a low CSS or the small sample size.

Diekman et al. [29] presented another clinical score based on episodes of hypoglycemia, cardiomyopathy/arrhythmia and myopathy to study predictive factors in clinically diagnosed patients with VLCADD. They found a correlation between LC-FAO flux results and the clinical score [29]. The CSS used in our study includes several factors related to hospital admissions and treatment aspects which also reflect the disease burden for the patients. These additional factors are relevant for the overall impact of the disease for the patients but may, in part, be related to other factors than intrinsic severity of the disease.

Four of the five patients with MCADD did not show any clear increase in glycerol during fasting. The patient who drank juice but did not have the full night meal showed a slight increase in glycerol after 7 h, likely reflecting reduced energy intake at the start of the fast. This finding underscores the importance of a standardised food intake before the start of the fast. As no data on residual enzyme activity in the MCADD patients were available and the patients had few clinical symptoms, we were unable to perform any correlation analysis for this group. It is possible that MCADD patients with more severe variants could have a lower fasting tolerance than those in our study. In a previous study from our group on children with LCHADD the onset of increased lipolysis was observed, as increased glycerol levels, after a fasting duration of 3.5 h. These findings provide evidence that microdialysis and determination of time to initiation of lipolysis can be used to assess individual fasting tolerance and possibly disease severity in specific FAODs. Moreover, the differences in time to lipolysis between LCHADD, VLCADD and MCADD patients show that

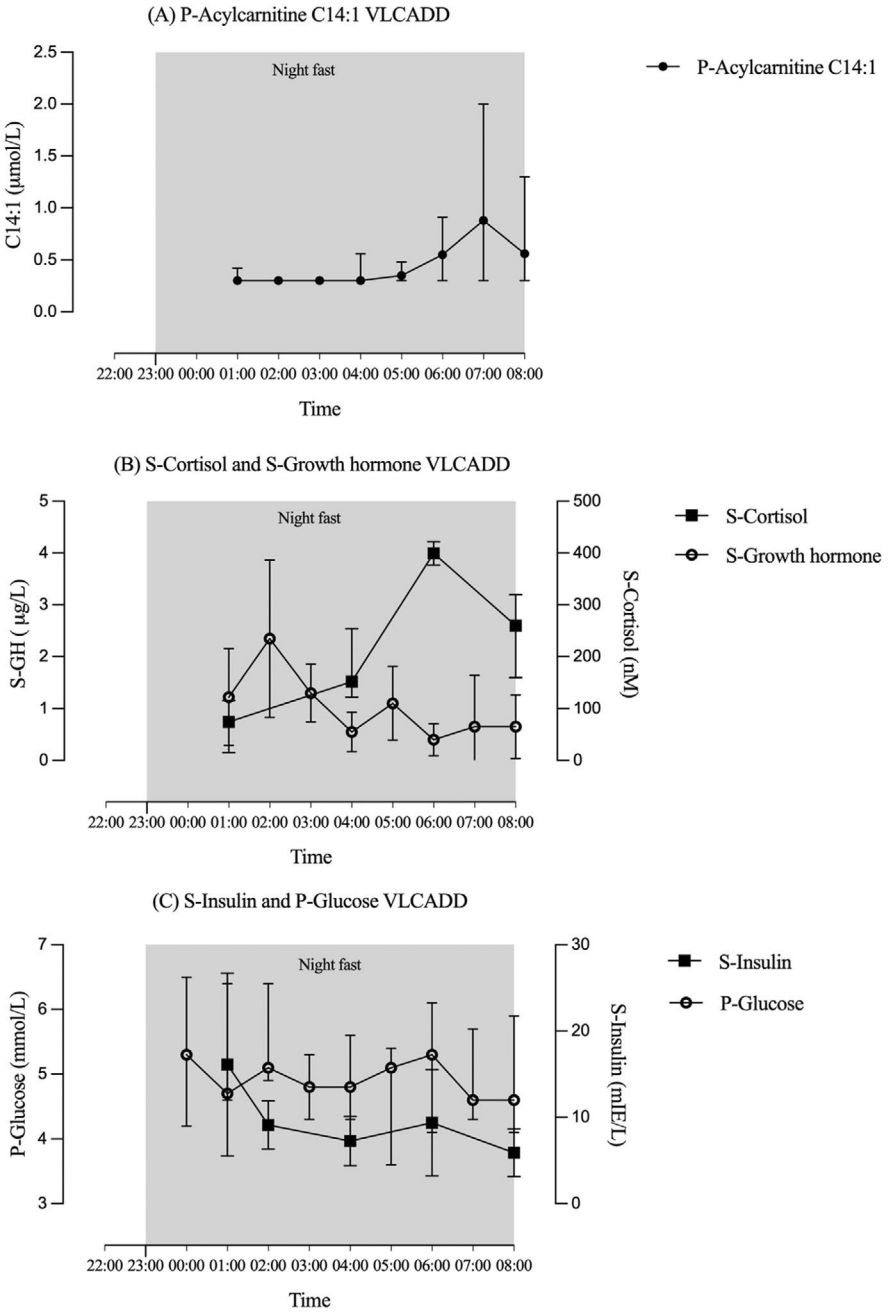

FIGURE 3 | Acylcarnitines, fasting hormones and plasma glucose in patients with VLCADD.

the more severe LC-FAODs tend to have shorter time to lipolysis than MCADD, rendering them more vulnerable to prolonged fasting.

When comparing lipolysis during a fast many factors such as age, body composition, activity level, total energy intake and especially carbohydrate intake prior to fasting can affect outcome. While we did not control for all these factors the variations between the groups in carbohydrate intake were small.

All study patients were normoglycemic during night fasting, and glucose levels in the microdialysate were comparable between the MCADD and VLCADD groups. The diurnal fluctuations of growth hormone and cortisol in healthy participants are associated with a physiological increased insulin resistance during the night [30–32]. Children with VLCADD showed a similar hormonal pattern in the present study compared to what we saw in children with LCHADD in a study previously published by our research team [15]. Although mean plasma glucose was stable through the night fast, small transitory increases coincided with peak levels of growth hormone secretion (occurring after 3 h of fast) and, to a lesser extent, cortisol secretion (at 6 am). These findings point to a physiological rise in insulin resistance.

In clinical practice lactic acidosis has been associated with beta-oxidation disorders during metabolic decompensations, particularly in LC-FAODs [33]. The underlying pathophysiology of this clinical aspect of metabolic decompensations in LC-FAODs is unclear. Previous preclinical studies using patient fibroblasts or animal disease models have documented morphological changes

in mitochondria [34] and reduced ATP production [35]. A previous study by our research team showed significantly lower basal oxygen consumption levels (OCR) in peripheral mononuclear cells (PMBC) in children with VLCADD compared to PMBCs in children with primary carnitine deficiency [36]. Patients in the present study were in stable metabolic condition, with subcutaneous lactate remaining within normal levels throughout the testing phase. We found that the L/P ratios during the nocturnal fast were higher in the VLCADD group compared to the MCADD group. As there are, to our knowledge, no published normal L/P ratios in subcutaneous microdialysis in healthy children this finding is difficult to interpret but could be speculated to be a reflection of mitochondrial dysfunction.

Microdialysis is a useful and informative method for prolonged investigations of changes in tissue levels of several analytes important for body energy metabolism [19]. Contrary to frequent intravenous sampling, microdialysis is suitable for paediatric patients because it does not require any blood samples and can be monitored at the bedside. In addition, microdialysis is clinically useful since it enables evaluation of different dietary interventions in the individual patient. The current dietary strategy evaluated on the first day of analysis can be compared to different dietary interventions on the following day or days. The concentration of an analyte in the dialysate is a sum of the locally produced analyte and the analyte diffused from nearby tissues. Thus, the levels of a specific analyte may vary from the plasma levels. This methodological limitation is less important as the microdialysis in this study was used to investigate analyte dynamics and rate of change. As the microdialysis monitors metabolism in situ, it is more sensitive to changes in metabolism in the local fat tissue compared to plasma monitoring. This was illustrated in a study by Kamel et al. [22] investigating metabolism during an insulin-arginine test where distinct changes in subcutaneous glycerol levels following arginine and insulin infusion was seen while the plasma glycerol levels remained unchanged. In a previous study by our group in children with LCHAD deficiency [15], non-esterified fatty acids and triacylglycerol showed no increase during a 6-h fast while after 3–4 h of fasting, microdialysate glycerol increased steadily. This indicates that microdialysis and measurement of subcutaneous fat tissue glycerol, at the site where the lipolysis occur, is a more reliable method of investigating lipolysis.

The limitations of the present study refer to the small number of participants inherent to the rarity of these disorders. In addition, only patients who underwent a clinical fasting investigation in our clinic were asked to participate. Consequently, there are differences in age, BMI, and gender distribution between the VLCADD and MCADD group as well as potential differences in activity level and total energy intake. The rate of liver glycogen depletion, which could be affected by differences in meal intakes prior to the study, is an important factor to consider when evaluating fasting-induced lipolysis and this was not investigated in the present study. While gender-related factors may influence metabolism it is important to note that all participants were prepubertal.

To conclude, we show that children with VLCADD had an earlier lipolysis during the night fast compared to children with MCADD, who did not show increased lipolysis during the 9-h fast. Patients with VLCADD and a low residual enzyme activity tended to have an earlier initiation of lipolysis than those with higher residual enzyme activity. This pilot study represents the first published data using subcutaneous microdialysis for evaluation of fasting metabolism in children with VLCADD and MCADD, expanding on our previous report on LCHADD [15]. Microdialysis is useful for assessing individual fasting metabolism and fasting tolerance in FAODs and between FAODs of different severity. In addition, microdialysis enables evaluation of different dietary interventions in the individual patient.

Our findings suggest that the assessment of fasting tolerance in children with FAODs should consider the role of increased lipolysis in determining clinical fasting restrictions.

Author Contributions

David Olsson: conceptualization, writing – original draft, investigation, writing – review and editing, methodology, visualization, formal analysis, data curation, validation. **Charlotte Bieneck Haglind:** conceptualization, investigation, writing – review and editing, supervision, methodology, visualization, formal analysis, data curation, validation. **Maria Halldin:** conceptualization, writing – review and editing, supervision, methodology, visualization, formal analysis, validation. **Svetlana Lajic:** conceptualization, writing – review and editing, supervision, methodology, visualization, formal analysis, validation. **Anna Nordenström:** conceptualization, writing – original draft, investigation, writing – review and editing, supervision, methodology, funding acquisition, visualization, formal analysis, resources, data curation, validation.

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Ethics Statement

This study was approved by the Ethical Review Board of Uppsala University (registration number 2006:005) and the Swedish Ethical Review Authority (PM, 2021-02305, 2021-08-12). The study was performed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

Consent

Informed consent documents were signed by all legal guardians of the study participants.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All original data supporting the reported results can be obtained by contacting the corresponding author.

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