Short-chain acyl-CoA dehydrogenase deficiency

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Chapter 4

Fasting and fat-loading tests provide pathophysiological insight into short-chain acyl-CoA dehydrogenase deficiency

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Abstract

Objective To gain insight into the pathophysiological and clinical consequences of short-chain acyl-CoA dehydrogenase deficiency (SCADD).

Study design A retrospective study of 15 fasting and 6 fat-loading tests in 15 Dutch patients with SCADD, divided into 3 genotype groups. Metabolic and endocrinologic measurements and the biochemical characteristics of SCADD, ethylmalonic acid (EMA) and C4-carnitine (C4-C) were studied.

Results Three patients had development of hypoglycemia during fasting; all of these had originally presented with hypoglycemia. Metabolic and endocrinologic measurements remained normal during all tests. The EMA excretion increased in response to fasting and fat-loading, and plasma C4-C remained stable. Test results did not differ between the 3 genotype groups.

Conclusions The metabolic profiles of the 3 patients with development of hypoglycemia resemble idiopathic ketotic hypoglycemia. Because hypoglycemia generally requires a metabolic work-up and because SCADD is relatively prevalent, SCADD may well be diagnosed coincidently, thus being causally unrelated to the hypoglycemia. If SCADD has any other pathological consequences, the accumulation of potentially toxic metabolites such as EMA is most likely involved. However, the results of our study indicate that there is no clear pathophysiological significance, irrespective of genotype, supporting the claim that SCADD is not suited for inclusion in newborn screening programs.
Introduction

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is an autosomal recessive inborn error of mitochondrial fatty acid oxidation (FAO). Most patients with SCADD have been diagnosed as a result of investigations for neurological symptoms and/or hypoglycemia. SCADD is screened for in the United States, although it does not meet newborn screening criteria, and there is no evidence that early detection would be clinically useful.

The short-chain acyl-CoA dehydrogenase (SCAD) protein is the first enzyme involved in the short-chain fatty acid β-oxidation spiral. When SCAD activity is impaired, its substrate, butyryl-CoA (C4-CoA), accumulates and is subsequently converted into different metabolites, including butyrylcarnitine (C4-C), butyrate, and ethylmalonic acid (EMA). C4-C can be measured in blood and EMA can be measured in urine.

DNA analysis is the most reliable method to confirm a diagnosis of SCADD. Most patients with SCADD are homozygous or compound heterozygous for 2 common SCAD-encoding gene (ACADS) variants, or for ACADS variants in combination with an inactivating mutation. Homozygosity for ACADS variants has been found in the general population with a remarkably high prevalence, at approximately 0.3% for the c.511C>T and 5.5% for the c.625G>A variant, and is considered to confer susceptibility to development of clinical disease. However, the factors involved in turning this “susceptibility” into clinical symptoms have not yet been elucidated.

Mitochondrial FAO plays an important role in energy production, especially during periods of prolonged fasting. Pathophysiological mechanisms of inborn errors in FAO include the following: (1) inadequate supply of energy; (2) sequestration or loss of vital components of intermediary metabolism; and (3) accumulation of toxic metabolites. Although these metabolic consequences have been partially elucidated for long- and medium-chain FAO disorders, the pathophysiological mechanisms involved in SCADD are less clear.

We carried out fasting and fat-loading tests on patients with SCADD to assess the potential risks of the disorder. This study was designed to critically review the data of 15 fasting and 6 fat-loading tests in order to gain insight into the pathophysiological and potential clinical consequences of SCADD.

Methods

Patients

Fifteen patients were included in this study (Table). All patients were initially investigated because of clinical symptoms (4 patients because of a hypoglycemic episode). They were subsequently diagnosed with SCADD on the basis of increased C4-C in plasma and/or
increased EMA in urine under non-stressed conditions on at least 2 occasions, along with the presence of a mutation and/or the c.511C>T or c.625G>A variants on each ACADS allele. Patients were classified in 3 different groups on the basis of their ACADS genotype, which include the following: ACADS mutations on both alleles (mutation/mutation [mut/mut] group; n=3); an ACADS mutation on 1 and an ACADS variant on the other allele or both alleles (mutation/variant [mut/var] group; n=8); and ACADS variants on both alleles (variant/variant [var/var] group; n=4) (Table). All patients were part of the Dutch SCADD cohort that was previously described.²

Tests
Fasting tests were performed in all patients except for patient 1, for whom parental consent was not obtained. Fat-loading tests were only performed in the first 6 presenting patients (patients 1, 4-7, and 13) and none thereafter because these tests appeared not to provide additional information beyond the fasting test data. All tests were performed between January 2001 and January 2008. Written informed consent was obtained from the parents or legal representatives of all participating patients. This study was reviewed and approved by the Medical Ethics Committee of the Academic Medical Center.

Fasting protocol
Patients were admitted to our hospital 1 day before the test and only fasted if food intake during three days prior to admission had been normal and only if they were well. During the test, patients were continuously observed by experienced staff. The maximum duration of the fast was determined by the age of the child. Patient 11 (13 years old) fasted for a maximum of 46 hours. All other patients (1 to 8 years old) fasted for a maximum of 22 hours. The fast was terminated earlier if the child showed clinical signs of hypoglycemia or if a blood glucose (BG) level ≤45 mg/dL (2.5 mmol/L) was measured. In the case of the 22-hour fasting test, fasting started after a meal at 18:00 hours. BG was monitored every 2 hours after the first missed meal and every hour after 19 hours of fasting. The measurement frequency was increased if BG concentrations fell rapidly or if there were clinical concerns. Blood samples for the measurement of metabolites and hormone levels were taken after 15 and 19 hours and at the end of the test. Blood samples were drawn using intravenous access while avoiding cuffing. Urine for organic acid analysis was collected from 0-12 hours of fasting, from 12 hours fasting until the end of the test, and from the end of the test until a urine sample of at least 50 mL was obtained. In the case of the 46-hour fasting test, the above blood and urine collection schedules were adjusted.

Fat-loading protocol
Preparations were similar to those used in the fasting test. On the day of admission, patients fasted from 21:00 hours onward. Twelve hours later (t=0) 1.5 mL/kg (maximum of 50 mL) of sunflower oil was administered either orally or through a nasogastric
tube. The sunflower oil was purchased from the regular hospital supplier. Its fatty acid composition was essentially oleic (21%) and linoleic (63%) acids. Blood samples for the determination of metabolite concentrations were obtained at 0, 60, 90, 120, 180, 240, and 360 minutes after fat-loading. Urine was collected in 3 portions: from 9 hours before fat-loading until fat-loading, from fat-loading until 6 hours after fat-loading, and from 6 hours to 10 hours after fat-loading.

Blood and urine analysis

Blood samples were analyzed for BG, nonesterified fatty acids (NEFA) and ammonia levels in both tests. Endocrinologic measurements (insulin, c-peptide, growth hormone, and cortisol) were examined in the fasting tests only. In addition, biochemical analyses of blood samples were performed to explore lactate, pyruvate, ketone bodies (KB), and acylcarnitine profiles in both tests, and amino acids profiles were assessed in the fasting tests. Acylcarnitine profiles were by use of electrospray tandem mass spectrometry. Blood samples for lactate, pyruvate, and KB were immediately deproteinized with perchloric acid and stored on ice, followed by quantitative determination of metabolites with standard spectrophotometric or fluorimetric methods. Blood samples for determination of ammonia levels were immediately stored on ice. All samples were delivered to the laboratory within 10 minutes of collection. Urine samples were analyzed for organic acids by gas chromatography/mass spectrometry, and stored at -20°C until analysis.

Interpretation of test results

For the purposes of the tests described, hypoglycemia was defined as a BG level ≤45 mg/dL (2.5 mmol/L) or 47-54 mg/dL (2.6-3.0 mmol/L) in combination with hypoglycemic symptoms (decreased consciousness and/or vegetative symptoms). KB concentrations during fasting were considered subnormal if the total blood KB concentration was <1.8 mmol/L and abnormal if <0.8 mmol/L, both in the presence of a BG <54 mg/dL (3.0 mmol/L). KB concentrations at the end of the fasting test were also considered abnormal in the case of a NEFA/KB ratio <2.6. The KB response to fat-loading was defined as abnormal if the KB concentration increased by less than twice its initial (t=0) concentration. KB response after fasting and fat-loading was considered abnormal if no statistically significant decrease in the FFA/KB ratio was observed. During hypoglycemia onset, patients were considered to have hyperinsulinism if plasma insulin levels were >15 pmol/L, hypocortisolism if plasma cortisol levels were <400 nmol/L, and growth hormone deficiency if growth hormone levels were <15 mE/L.

Statistical analysis

The Kruskal-Wallis test was used to compare the 3 different genotype (mut/mut, mut/var, and var/var) groups and 3 or more different periods. The Wilcoxon matched pairs signed ranked sum test was used to compare baseline values with values from the end of the tests. The level of significance was set at P <.05. Analyses were performed with
Results

Patients

The genotypes and clinical phenotypes of the participating patients are shown in the Table.

Fasting tests

One fasting test was terminated prematurely because of possible hypoglycemic symptoms that were not confirmed by BG measurement (patient 2, Table). Hypoglycemia developed in 3 patients during fasting (Table). In these patients, insulin levels were appropriately

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<th>Sex</th>
<th>Genotype†</th>
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Pt, patient; *Age at time of test; †Gene variants in regular type, mutations in bold type

Graphpad Prism 3.0 (Graphpad Software, San Diego, California) and SPSS 12.0.1 (SPSS, Chicago, Illinois) software.
decreased, and growth hormone and cortisol levels were appropriately increased by the end of the test. Ammonia, lactate, pyruvate, and amino acid levels remained normal during all tests. To assess the need for continuation of a late evening feeding, patient 12 underwent a second fasting test 2 years after the first one. KB production was subnormal during the first fasting test of patient 12 (Figure 1, A). In all other fasting tests, including the second fasting test in patient 12, KB production was within the normal reference range (Figure 1). No statistically significant differences were observed in KB production between the mut/mut, mut/var, and var/var groups ($P = .24$ for glucose x KB and $P = .34$ for NEFA/KB; Figure 1).

The plasma free carnitine concentration decreased during fasting in all patients, but remained within the normal range (22.3-54.8 μmol/L). The concentrations of C4-C, as well as the C4-C/free carnitine ratios, did not significantly change during fasting in the mut/var ($P = .27$ and $P = .65$, respectively) and var/var ($P = .75$ and $P = .28$, respectively) groups (Figures 2, A and B). They were significantly different between the 3 genotype groups ($P = .027$ for both), with the highest levels in the mut/mut and the lowest in the var/var group.

### Table

<table>
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<tr>
<th>Clinical signs and symptoms</th>
<th>Fast duration, (hours)</th>
<th>Hypoglycemic symptoms during test</th>
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Pt, patient; *Age at time of test; †Gene variants in regular type, mutations in bold type
The EMA levels increased in a statistically significant manner in the mut/var \((P = .025)\) and var/var \((P = .04)\) groups (Figure 2, C).

Fat-loading tests

Two of the 6 fat-loading tests (in patients 1 and 6) were terminated prematurely (after 3 and 4 hours, respectively) because of a loss of intravenous access. BG and ammonia levels were normal during all tests. KB production was sufficient in all patients with a statistically significant, on average approximately 6-fold, increase of KB levels \((P = .03; \text{Figure 3, A})\), and a statistically significant decrease of the ratio of NEFA to KB \((P = .01; \text{Figure 3, B})\) over the course of measurement. C4-C values did not change significantly during the test \((P = .73; \text{Figure 3, C})\). EMA excretion increased during the test; the change in excretion was not statistically significant \((P = .27; \text{Figure 3, D})\).

Discussion

Fasting-induced hypoglycemia has been reported in several patients with SCADD\(^1;8;18;19\) and was the presenting symptom in 19% of the Dutch patients previously described.\(^2\) However, it was not known whether all patients with SCADD are at risk for fasting-induced hypoglycemia. Moreover, a true causal relationship between SCADD and hypoglycemia has not been established. Our study indicates that only those patients with SCADD who had a history of hypoglycemia had development of hypoglycemia during a fasting test,
whereas 11 out of our 14 patients (79%) did not have development of hypoglycemia. Therefore diagnosing SCADD should not routinely be followed by a fasting test or the introduction of a late evening meal, unless the clinical history suggests hypoglycemia.

Apart from the biochemical characteristics of SCADD (increased C4-C in plasma and/or increased EMA in urine, as observed in all patients), metabolic evaluation failed to detect any other metabolic or endocrine abnormalities in patients with development of hypoglycemia. Therefore, hyperinsulinism does not contribute to hypoglycemia in SCADD, as it does in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency, another short-chain FAO disorder. The observed ketotic hypoglycemia in the 3 SCADD patients resembled idiopathic ketotic hypoglycemia (IKH or “toddlers hypoglycemia”), the most common cause of hypoglycemia in children, which is caused by an inability to maintain adequate glucose production levels. IKH is a diagnosis of exclusion, and this diagnosis can be made in hypoglycemic patients only when proper investigations fail to...
detect a cause for the observed hypoglycemia. The high frequency of IKH among the SCADD patients group (3 of 14) could be due to ascertainment bias as a metabolic work-up, which might lead to the diagnosis of SCADD, is normally requested in all patients presenting with hypoglycemia. Because the c.625G>A ACADS variant is common in the general population (5.5% homozygosity in the Netherlands), and the c.1058C>T mutation has been found to be the most common Dutch ACADS mutation, a diagnosis of SCADD due to this ACADS variant and mutation is relatively prevalent and could well be unrelated to the hypoglycemia in these patients. Indeed, we have recently suggested

![Figure 3](image-url)  
**Figure 3.** Ketogenesis expressed as A, KB production and B, the ratio of NEFA to KB and biochemical characteristics of SCADD; C, C4-C and D, EMA in response to fat-loading in 6 patients with SCADD. The dashed lines represent the upper limit of normal levels: 0.58 μmol/L for C4-C and 8 μmol/mmol creatinine for EMA.
that, for at least some patients, SCADD might be a life-long biochemical phenomenon that is not correlated with clinical disease. Our observation that only 3 of the 14 SCADD patients had development of a ketotic hypoglycemia during fasting, and only those who had a prior hypoglycemia history, supports this claim. The observation that patient 12 did not have development of hypoglycemia during a second fasting test 2 years later might be considered an indication for transient disease in SCADD, as has been previously suggested. However, this observation also fits the transient nature of IKH.

The normal ketotic response observed during the fasting tests in all 14 patients with SCADD is further substantiated by the normal ketotic response observed during the 6 fat-loading tests. Long chain (C₁₆-C₂₀) fatty acids are the predominant substrates for FAO during fasting, comprising most of the fatty acids present in sunflower oil, and can undergo 6 to 8 FAO cycles, resulting in 6 to 8 molecules of acetyl-CoA before the decreased activity of SCAD impairs the final β-oxidative cleavage of C₄-CoA. This final step in β-oxidation would normally result in the production of 2 additional acetyl-CoA units. Apparently, the ability to produce at least (depending on residual SCAD activity) 75% of the normal amount of acetyl-CoA units, and thus at least 75% of normal KB production, is sufficient for a normal ketogenic response under fasting conditions and after fat-loading. It also appears to be sufficient for adequate formation of N-acetylglutamate, which activates the first enzyme of the urea cycle, because ammonia levels did not increase (in contrast to medium-chain acyl-CoA dehydrogenase deficiency patients) during hypoglycemia.

Hypoglycemia caused by disorders of mitochondrial FAO is believed to be caused by a reduction in gluconeogenesis in combination with a decrease in the glucose-sparing effect of ketone bodies. However, in 11 out of 14 patients with SCADD, fasting was well tolerated, and normoglycemia was sustained. In addition, the concentrations of lactate and alanine, which are both precursors of gluconeogenesis, did not increase during fasting, (data not shown), suggesting normal gluconeogenesis. Furthermore, ketogenesis was adequate in all patients. Therefore the results of our study do not provide evidence for a role of inadequate energy supply in the pathophysiology of SCADD.

Free carnitine levels decreased in patients with SCADD during fasting, comparable with levels in control subjects, but remained within the normal range. These findings suggest that sequestration or loss of carnitine is also not involved in the pathophysiology of SCADD.

A statistically significant increase in EMA excretion was detected in all 3 genotype groups during fasting. In addition, a clear, but not statistically significant increase was observed after fat-loading. This increase in EMA excretion is likely due to the accumulation of C₄-CoA, the substrate of SCAD, which is converted into ethylmalonyl-CoA by propionyl-CoA carboxylase and subsequently into ethylmalonic acid by one of the mitochondrial acyl-CoA hydrolases. Remarkably, levels of C₄-C, as produced from C₄-CoA by the mitochondrial enzyme carnitine acetyltransferase, did not increase in the plasma of patients with SCADD, which suggests a preference for the EMA pathway during fasting and fat-loading. The observation that the activation of FAO by fasting
and fat-loading results in an increase of EMA suggests that if SCADD is associated with any pathophysiological effect, the accumulation of metabolites like EMA is most likely involved in the pathophysiology of SCADD. EMA inhibits the activity of mitochondrial CK at 1.0 mmol/L in rat brain slices.\(^{25}\) It is, however, questionable whether concentrations this high will occur in the brains of patients with SCADD. Butyrate, another metabolite, which might accumulate as a result of SCADD, has been suggested to be cytotoxic to the human brain;\(^{3,18,26}\) however, no studies have been conducted to demonstrate this effect. Finally, if accumulation of EMA and C4-C contributes to the pathophysiology of SCADD in vivo, it would be expected that patients with the highest levels of these metabolites be the most severely affected. However, we demonstrated here that patients with the highest levels of EMA and C4-C (the mut/mut genotype group) were not more severely affected with respect to ketogenesis when compared to patients with lower EMA and C4-C levels (the mut/var or var/var genotype groups). Furthermore, in our previous study\(^2\) and in the study of Pedersen et al,\(^3\) similar observations were made with respect to clinical symptoms. Therefore the question remains as to whether EMA, C4-C, and/or other metabolites derived from butyryl-CoA, such as butyrate, are related to the clinical signs and symptoms of patients with SCADD.

SCADD is part of newborn screening programs in the majority of states in the United States.\(^{27}\) However, we recently suggested that SCADD is not suited for inclusion in newborn screening programs because it lacks clear clinical significance in many patients and does not meet major newborn screening criteria.\(^2\) Australian screening programs therefore no longer include SCADD in their screening panel.\(^{28}\) The normal metabolic responses observed in this study and the absence of a direct relationship between accumulating metabolites and clinical symptoms in patients with SCADD support the clinical irrelevance of SCADD.

Inadequate supply of energy, as reflected by impaired ketogenesis or gluconeogenesis, or loss of vital components of intermediary metabolism, such as carnitine, does not appear to be relevant in SCADD. Because the level of EMA increased significantly during fasting and fat-loading, the accumulation of potentially toxic metabolites is more likely to be involved in the pathophysiology of SCADD. Because this hypothesis is not sufficiently supported by experimental data, further research is necessary to explore whether these metabolites have pathophysiological effects in SCADD.

Acknowledgments
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References


