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ORIGINAL ARTICLE

Increased lipolysis in LCHAD deficiency

M. U. Halldin · A. Forslund · U. von Döbeln · C. Eklund · J. Gustafsson

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Summary An increasing number of fatty acid oxidation defects are being detected owing to diagnostic improvements and a greater awareness among clinicians. The metabolic block leads to energy disruption, fatty infiltration, and toxic effects on organ functions exerted by β-oxidation metabolites. This investigation was undertaken to assess the influence of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency on lipolysis and energy turnover. We addressed the question whether the lipolysis and glucose production rates would be altered in the fasting state in a child with this disease. Lipolysis, glucose production and resting energy expenditure (REE) were studied in a 17-month-old girl with LCHAD deficiency and her healthy twin sister. Lipolysis and glucose production were determined after a 4–6 h fast by constant-rate infusion of [1,1,2,3,3-$^2H_5$]glycerol and [6,6-$^2H_2$]glucose and analysis by gas chromatography–mass spectrometry. REE was estimated by indirect calorimetry. The affected girl showed 50% higher lipolysis than did her sister, whereas the glucose production rates were similar. Plasma levels of dicarboxylic acids of 6–12 carbon atoms chain length, 3-hydroxy fatty acids of 6–18 carbon atoms chain length, total free fatty acids, and acylcarnitines were increased in the patient, as was REE. Since glucose production rates and plasma glucose levels were similar in the two girls, the increased lipolysis observed in the patient probably represents a compensatory mechanism for energy generation. This is achieved at the price of an augmented risk for fatty acid infiltration and toxic effects of β-oxidation intermediates. This highlights the importance of avoiding fasting in these patients.

Abbreviations

GH	growth hormone
LCHAD	long-chain 3-hydroxyacyl-CoA dehydrogenase
MCT fat	medium-chain triacylglycerols
NEFA	nonesterified fatty acids
REE	resting energy expenditure
SDS	standard deviation score
TAG	triacylglycerols

Introduction

Mitochondrial β-oxidation of fatty acids plays an important role in energy production during exercise, fasting and starvation. Hence, disorders of fatty acid oxidation will affect energy balance. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD, long-chain (*S*)-3-hydoxyacyl-CoA:NAD^+ oxidoreductase, EC 1.1.1.211) deficiency is a rare inborn error of fatty acid oxidation (Hagenfeldt et al 1990; Wanders et al 1990). It has been regarded as clinically more severe and life-threatening than disorders affecting oxidation of fatty acids with shorter chains (Riudor 1998; Ventura et al 1998). The metabolism of fat and glucose is intertwined in a complex mode; thus, energy produced in β-oxidation drives the glucose production in gluconeogenesis (Salway 1999a, b).

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M. U. Halldin (✉) · A. Forslund · C. Eklund · J. Gustafsson
Department of Women's and Children's Health, University Children's Hospital, SE-751 85 Uppsala, Sweden
e-mail: maria.halldin@kbh.uu.se

U. von Döbeln
Centre for Inherited Metabolic Diseases, Karolinska University Hospital, Huddinge, Stockholm, Sweden

Apart from the mechanism underlying energy depletion, knowledge of the pathogenic processes in LCHAD deficiency is limited (Lund et al 2003). Accumulation of toxic fatty acid intermediates has, however, been considered to be one of the mechanisms (Lund et al 2003). Furthermore, increased fatty infiltration in muscles and fibre degeneration have been demonstrated, as well as abnormalities of mitochondrial morphology and of respiratory chain function (Tyni et al 1996).

Fatty acids are released for energy utilization by lipolysis, which is increased during fasting. We considered it important to find out whether the fasting rates of lipolysis and glucose production would be affected in patients with LCHAD deficiency compared to those in healthy individuals. The use of a stable-isotope technique allows evaluation of energy substrate production. This technique has not, to our knowledge, been used before in the studies of patients with this particular inborn error of metabolism. Insight into the energy requirements during fasting is necessary in order to improve guidelines for optimal treatment of these patients.

In this paper we report on lipolysis, glucose production and resting energy expenditure (REE) following a period of starvation in a child with genetically confirmed LCHAD deficiency. The results were compared with corresponding data from her heterozygous twin sister with the aim of identifying possible compensatory mechanisms to meet the energy demand in this disorder.

Subjects and methods

Subjects

The twin girls were delivered prematurely by Caesarean section at 29 weeks of gestation because of pre-eclampsia in the mother. The family history was noncontributory. Both children were given full Apgar scores.

The patient was smaller than her sister at birth, with a birth weight corresponding to −2 SDS and a birth length corresponding to −0.5 SDS, compared to −0 SDS for both variables in the sister (Niklasson and Karlberg 1993).

The neonatal period of both girls was uneventful apart from the need for prolonged feeding by gastric tube in the girl with LCHAD deficiency because of difficulties in normal feeding.

At 8 months of age the patient was referred to the University Children's Hospital because of lethargy. Physical examination revealed low muscle tone and sparse movements of the extremities. She was still somewhat smaller than her sister (Table 1). In retrospect, it was found that she had had drop-attacks of the head and fatigue several times prior to admission. Her motor development was retarded in comparison with that of her sister. Her mental development was adequate for age. Analysis of organic acids in the urine, dicarboxylic acids and 3-hydroxy fatty acids in the plasma, blood chemistry and mutation analysis led to a diagnosis of LCHAD deficiency, with homozygosity for the mutation 1528G>C.

At the time of diagnosis there were no signs of pigmented retinopathy or of cardiomyopathy or arrhythmia. The liver size was at the upper normal limit and there were signs of fatty infiltration of the liver. The healthy twin sister was heterozygous for the 1528G>C mutation.

Prior to this investigation the patient had been treated with a low-fat, high-carbohydrate diet, with a total long-chain fatty acid intake of less than 3 E%. Part of her diet consisted of a [-16pc] special formula (Monogen, Scientific Hospital Supplies [15.5pc] (SHS), Liverpool, England) which is a nutritionally complete formula for infants with LCHAD deficiency. In addition, she was supplemented with essential fatty acids (walnut oil). She was not supplemented with do-

Table 1 Basal characteristics of the patient (P) and the control (C). Anthropometric data are given as SDS[a]

	P	C	Reference value or range
Age (months)	17	17	
Height (cm)	76.3	77.1	
(SDS)	−1.1	−1.0	
Weight (kg)	9.7	10.3	
(SDS)	−1.0	−0.4	
BMI (kg/m^2)	16.4	16.0	
ALT (μkat/L)	0.83	0.48	<0.7
AST (μkat/L)	0.92	0.65	<1.0
TAG (mmol/L)	1.3	1.1	0.3–1.1
Cholesterol (mmol/L)	4.1	3.5	2.7–7.1
Dietary intake			
Caloric intake (kJ (kcal))	4600 (1100)	5000 (1200)	
Fat (total) E%	23	35	
MCT fat E%	20	3	
Carbohydrate E%	69	50	
Protein E%	8	15	

[a]Albertsson-Wikland and Karlberg (1994)

cosahexaenoic acid (DHA), but received L-carnitine (Carnitor), Sigma TAU Pharmaceuticals, Gaithersburg, MD USA, 50 mg/kg per day (Table 1). The girl showed satisfactory psychomotor development after the diagnosis and gained in weight and height (Table 1). As this research group already had IRB approval for studies of energy substrate production in infants, the ethical committee of Uppsala University did not find it necessary to apply for a new ethical approval for this specific study. Informed consent for the investigation was obtained from the parents.

Study design

The girls were admitted to the hospital on the morning of the first day. An indwelling cannula was inserted into each of two antecubital veins, one for blood sampling and the other for infusion of [1,1,2,3,3-$^2H_5$]glycerol and [6,6-$^2H_2$]-glucose. The time schedule was as follows:

Day 1 (day of admission): Blood sampling started at 12:00. Samples were collected for measurements of HbA1c, blood gas, creatine kinase, lactate, calcium, PTH and carnitine. Blood was also drawn before and 90 min after supper for ammonia assay.

Day 2: Both girls fasted for 6 h, from 03:00 to the end of the investigation at 09:00. Blood sampling for measurements of IGFBP1, IGF-I, cortisol, glucagon, dicarboxylic acids, NEFA, triacylglycerols and blood gas was performed at 06:55, before infusion of [1,1,2,3,3-$^2H_5$]glycerol and [6,6–$^2H_2$]glucose (see below), and at 09:00 after the discontinuation of the infusions. Samples for measurement of growth hormone GH were taken every 30th minute, starting at 05:00, and of insulin every 30th minute from 07:00 to 09:00. At 09:00 creatine kinase and lactate were also measured.

Samples for plasma glucose determinations were taken every 4th hour of the sampling period on the day of admission, every 30th minute between 03:00 and 08:00 on the next day, and every 10th minute during the last hour of the tracer infusion.

Tracer infusions: At 06:55 a bolus dose of 1.20 mg/kg (12.3 μmol/kg) of [1,1,2,3,3-$^2H_5$]glycerol and a bolus dose of 5.6 mg/kg (30.8 μmol/kg) of [6,6-$^2H_2$]glucose were given over a 5 min period. Thereafter, between 07:00 and 09:00 constant-rate infusions of [1,1,2,3,3-$^2H_5$]glycerol and [6,6-$^2H_2$]glucose were given in one of the peripheral catheters. The infusion rates corresponded to 0.03 mg/kg per min (0.3 μmol/kg per min) and 0.11 mg/kg per min (0.6 μmol/kg per min), respectively. Saline was used throughout the study period to rinse the catheters used for blood sampling. The tracers were infused with two calibrated volumetric pumps (IMED 965 micro, IMED, Oxford, UK). Blood samples for determination of isotope dilution of glycerol and glucose were obtained before the start of the tracer infusion and every 10th minute during the last hour of the study period (8 samples in total), i.e. during the 6th hour of fasting. The samples were immediately collected into ice-cold EDTA tubes (Fig. 1).

After discontinuation of the tracer infusions, resting energy expenditure (REE) was estimated by indirect calorimetry. While the subjects were at rest, sitting in their parent's lap, a transparent canopy was placed over their head, and the measurements were continued until a steady state was achieved (after approximately 15–20 min).

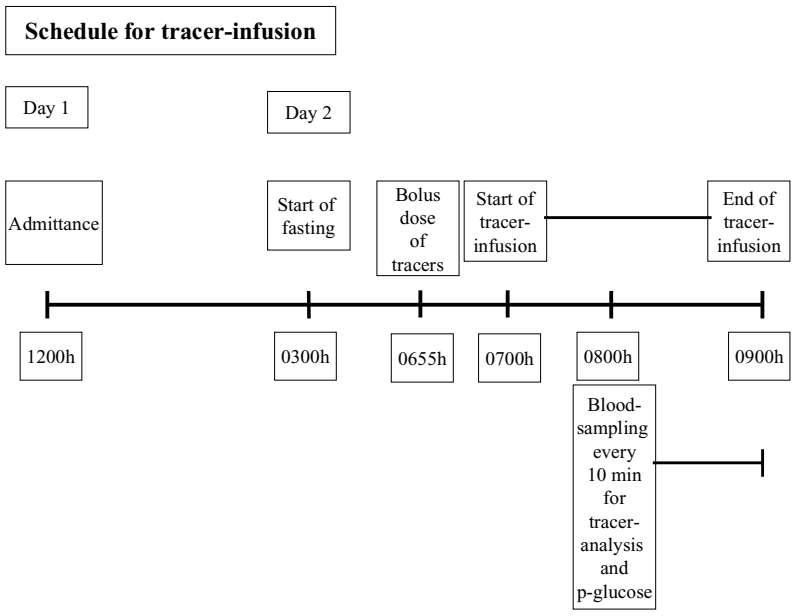

Fig. 1 Timeline for the tracer experiment

Chemical methods

Isotope tracers: The tracers [6,6-$^2H_2$]glucose (isotopic purity 98 atom%) and [1,1,2,3,3-$^2H_5$]glycerol (isotopic purity 98 atom%) were purchased from Cambridge Isotope Laboratories, Woburn, MA, USA. They were dissolved in 0.9% saline solution in concentrations of 4.5 and 1.2 mg/ml, respectively. The solutions were sterile in microbiological cultures and pyrogen-free when tested by the Limulus lysate method (Food and Drug Administration 1987).

Chemical procedures: Plasma was immediately separated by centrifugation and frozen at −70°C pending analysis. Plasma proteins were precipitated with acetone, and the pentaacetate derivative of glucose and the triacetate derivative of glycerol were prepared by addition of equal amounts of pyridine and acetic anhydride. The isotopic enrichments of [6,6–$^2H_2$]glucose and [1,1,2,3,3-$^2H_5$]glycerol were determined by gas chromatography/mass spectrometry (GCMS). A Finnigan SSQ 70 mass spectrometer (Finnigan MAT, San José, CA, USA) equipped with a Varian 3400 gas chromatograph (Varian Associates Inc, Sunnyvale, CA, USA) with a nonpolar (DB 1) capillary column (15 m × 0.25 mm) was used. The temperature of the oven was set to 180°C and 130°C for glucose and glycerol, respectively. Chemical ionization with methane was used with selective monitoring of ions. For glucose the ions monitored were *m*/*z* 331 and 333, reflecting unlabelled and dideuterated glucose (M+2). For glycerol *m*/*z* 159 and 164 corresponded to the unlabelled and pentadeuterated compounds (Halldin et al 2002; Kalhan et al 1980; Sunehag et al 1996).

Calculations: Standard curves were used to calculate isotopic enrichments (given as molar ratios in %) in the individual samples. These were obtained by a gradual increase of the amounts of stable-isotope-labelled compounds. Since the isotopic enrichments of glucose and glycerol in plasma were approximately constant, they were considered to be at steady state. Rates of glucose and glycerol production were calculated as follows (Halldin et al 2002; Sunehag et al 1996): Rate of production $= i \times 100/MR$, where the rate of production is expressed in μmol/min per kg, i is the infusion rate of stable-isotope-labelled compound (μmol/kg per min) and MR is the molar ratio (labelled/unlabelled compound in %).

Resting energy expenditure: REE was estimated by indirect calorimetry using a ventilated hood (canopy) with Oxyconsigma ergospirometer (Erich Jaeger GmbH, Hoechberg, Germany). Carbon dioxide (CO_2) production and oxygen (O_2) consumption were continuously measured until steady state was achieved (CVs for CO_2 production were 2.7% and 2.2%, respectively, and for O_2 consumption were 5.6% and 5.1%, respectively, in the patient and the control). The respiratory quotient (RQ) was obtained from these data ($RQ = CO_2/O_2$). REE was calculated using the Weir equation (Weir 1949) and expressed as kJ/day (kcal/day). The percentage predicted REE was calculated by using World Health Organization (WHO) (FAO/WHO/UNU 1985) and Shofield data.

Plasma dicarboxylic acids and 3-hydroxy fatty acids were assayed as described by Hagenfeldt and colleagues (1990) with the following internal standards: 2,2-dimethylglutarate and 3-hydroxy acids with chain lengths of 9 and 15 carbon atoms. These internal standards and 0.5 ml of saturated sodium chloride were added to 0.5 ml serum and then acidified with one drop of 2 mol/L HCl. After extraction with ethyl acetate, evaporation and silylation, the samples were analysed by GC-MS. The mass spectrometer was focused on *m*/*z* 261, 275, 289, 303,317, 331 and 359 and on *m*/*z* 233 as described by Hagenfeldt et al 1990.

Plasma 3-hydroxybutyrate was measured according to the method described by Persson (1970).

Plasma free fatty acids were assayed by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany) adjusted for analysis on a centrifugal analyser (Cobas Fara, Roche Diagnostic Systems, Inc., Montclair, NJ, USA) at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Göteborg, Sweden.

Acylcarnitine and free carnitine in serum were measured by a method for determination in the picomole range as described by Cederblad and colleagues (Cederblad and Lindstedt 1972).

Glucose was assayed by a bedside glucose oxidase method (Hemo-Cue, Ängelholm, Sweden).

HbA1c was measured by HPLC (ion-exchange chromatographic method, mono-S, high performance liquid chromatographer). The coefficient of variation was 1.2%.

The *insulin* concentration in serum was measured by radioimmunoassay (Pharmacia Insulin RIA, Pharmacia Uppsala, Sweden). The coefficients of variation were within 5–6%.

Growth hormone in serum was determined by an automated immunological assay (Immulite 2000, DPC, Los Angeles, CA, USA).

IGF-I was measured by radioimmunoassay after precipitation of binding proteins with acidified ethanol. ^{125}I-labelled des (1–3) IGF-I radioligand was used to reduce interference by IGFBPs not removed by the extraction procedure (Bang et al 1991). The recovery of unlabelled IGF-I was 95% and the intra-assay and inter-assay coefficients of variation were 5% and 11%, respectively. The lowest detectable amount of IGF-I was 0.01 ng per tube. Cross-reactivity with insulin was less than 0.1%, and with IGF-II less than 2%.

IGFBP1 was measured by radioimmunoassay as described by Povoa and colleagues (1984). The intra-assay and inter-assay coefficients of variation were 3% and 11%, respectively, and the detection limit was 3.0 μg/L. Cross-reactivities with IGFBP2 and IGFBP3 were less than 0.5% and 0.05%, respectively.

Table 2 Rates of glucose production (μmol/min per kg) and glycerol production (μmol/min per kg) in the patient (P) and the control (C)

Subject	Glucose production rate	Glycerol production rate
P	35.9	4.4
C	34.9	2.9

Results

Lipolysis and glucose production rate

The rate of lipolysis was more than 50% higher in the patient than in her healthy twin sister (Table 2). There was no difference in glucose production rate (GPR) between the two girls (Table 2).

Glucose

During the fasting period the blood glucose level showed only minor variations in both girls (mean value 4.7 ± 0.42 and 4.9 ± 0.80 mmol/L in the patient and the control, respectively). Normoglycaemia was maintained throughout the study period. Neither of the girls had any episodes of hypoglycaemia.

Dicarboxylic acids, 3-hydroxy fatty acids and fatty acids

After 4 h fasting the girl with LCHAD deficiency had almost three times higher concentrations of total free fatty acids than her twin sister. However, in contrast to the situation in her sister, the concentrations of free fatty acids did not increase further after two additional hours of fasting (Table 3). The patient had higher concentrations of dicarboxylic and 3-hydroxy fatty acids of almost all chain lengths after both 4 and 6 h of fasting, while those of her sister were normal (Table 3). In fact, in the patient the levels of β-oxidation intermediates were higher after 4 h than after 6 h of fasting, while the β-hydroxybutyrate concentration was higher after 6 h fasting (Table 3).

Resting energy expenditure

Resting energy expenditure was 34% higher in the patient than in her sister. Respiratory quotient (RQ) differed slightly between the girls, the patient having a somewhat lower value (Table 4).

Carnitine

The levels of acylcarnitines were higher in the patient than in her sister both before lunch on the day of admission (14 and 9 μmol/L, respectively) and after 6 h of fasting (19 and 7 μmol/L). The ratios between nonesterified and esterified carnitine were similar on admission day (3.6 in the patient and 3.8 in the control, respectively). However, while the ratio of the patient decreased after the fasting period, that of her sister increased (2.5 and 4.7, respectively), pointing to a relatively higher concentration of acylcarnitines in the patient.

The serum levels of total and free carnitine were normal both in the patient and in her sister (total carnitine 68 and 40 μmol/L, respectively (normal range 24–70 μmol/L); free carnitine 48 and 33 μmol/L, respectively (normal range 18–58 μmol/L)).

Table 3 Plasma levels of 3-hydroxy fatty acids (μmol/L) and total NEFA (mmol/L) in the patient (P) and the control (C) after 4 and 6 h of fasting[a]

	4 h P	4 h C	6 h P	6 C
Dicarboxylic acids				
DC 5:0	0.3	0.8	0.3	1.0
DC 6:0	0.9	0.2	0.5	0.2
DC 8:0	0.2	<0.1	0.2	<0.1
DC 10:0	0.5	<0.1	0.4	<0.1
DC 12:0	<0.2	<0.1	<0.2	<0.1
3-Hydroxy fatty acids				
3OHC4[b]	0.109	0.070	0.223	0.13
3OHC6:0	0.3	0.1	<0.2	0.2
3OHC8:0	1.1	0.4	0.8	0.4
3OHC10:0	0.7	0.1	0.4	0.2
3OHC12:1	0.2	<0.1	<0.2	<0.1
3OHC12:0	0.4	<0.1	0.3	<0.1
3OHC14:1	1.2	<0.1	0.6	<0.1
3OHC14:0	0.6	<0.1	0.4	<0.1
3OHC16:0	2.0	<0.1	1.2	0.1
3OHC18:1	2.2	<0.1	1.3	<0.1
3OHC18:0	2.1	<0.1	1.2	<0.1
Total NEFA	1.00	0.36	0.91	0.62

[a]Hagenfeldt et al (1990)
[b]Measured by a quantitative enzymatic method (mmol/L) (Sunehag et al 1996)

Hormones

The[-10pc] insulin levels were low and similar in the two girls during the investigation (mean level 2.3 ± 0.9 mU/L in

Table 4 Resting energy expenditure of the patient (P) and the control (C)

Parameter	P	C
CO_2 production (ml/min)	126.0	95.7
O_2 consumption (ml/min)	136.3	96.0
RQ	0.93	0.98
Resting energy expenditure (kJ/day (kcal/day))	4080 (974.8)	3053 (729.6)

the patient and 2.1 ± 0.8 in the healthy sister). The glucagon levels in the patient and her sister were comparable (86 and 79 ng/L in the patient and 100 and 82 ng/L in the sister after 4 and 6 h fasting, respectively). Both girls had slightly elevated plasma cortisol levels (497 and 349 nmol/L in the patient and 593 and 428 nmol/L in the sister after 4 and 6 h of fasting, respectively). The plasma levels of growth hormone were of the same magnitude in the patient and her sister (mean value of nine samples for each subject: 6.2 and 10.5 mU/L in the patient and the control, respectively). IGF-I (67 and 54 μg/L in the patient and the sister, respectively) and IGFBP1 (179 and 144 μg/L in the patient and 125 and 149 μg/L in the sister after 4 and 6 h of fasting, respectively) were similar in the two girls. Thyroid hormones were normal in both subjects.

Chromogranin A, reflecting catecholamine secretion, was slightly elevated in both girls during the infusion of stable-isotope-labelled glucose and glycerol (at 07:00 the value in the patient was 6.1 mmol/L and that in her sister was 5.7 mmol/L; at 09:00 the corresponding values were 5.0 mmol/L and 5.9, respectively (normal value <4.0)).

Blood chemistry

The patient had slightly elevated creatine kinase level, 9.8 μkat/L (normal range <5 μkat/L) on the day of admission and a somewhat lower value the day after, 6.4 μkat/L. The sister had normal concentrations of 3.7 and 3.5 μkat/L, respectively. Both girls had normal levels of haemoglobin, glycosylated haemoglobin and blood gases as well as lactate, ammonia and calcium.

Discussion

The present investigation was undertaken to assess lipolysis and glucose production and resting energy expenditure after fasting in LCHAD deficiency. The stable-isotope technique used in this investigation, is, to our knowledge, a new approach in the study of metabolic derangements in patients with inborn errors of metabolism.

Although there is a limitation to the study with regard to the number of subjects, the patient with LCHAD deficiency in fact had a rate of glycerol production >50% higher than her healthy twin sister, indicating a definite increase of lipolysis. The high lipolytic activity may serve the purpose of compensating for the block in β-oxidation by providing increased amounts of fatty acids. The finding of elevated nonesterified fatty acids (NEFA), fatty acid metabolites and acylcarnitines is in line with this observation. In contrast to the situation in the healthy sister, there was no further increase in NEFA levels in the patient during the last two hours of fasting, indicating that stimulation of lipolysis was already maximal after 4 h of fasting. Since ketone bodies exert a negative feedback on lipolysis, the lack of an additional increase in lipolysis between 4 and 6 h of fasting can be explained by the increased levels of β-hydroxybutyrate at this time of fasting (Lund et al 2003).

The two girls had the same rate of hepatic glucose production and this was close to that expected for children with this weight (Bier et al 1977). In addition, both girls were normoglycaemic during the study. Hepatic glucose production is dependent on glycogenolysis and gluconeogenesis. Gluconeogenesis is supported by lipolysis and fatty acid β-oxidation for provision of glycerol, which can be converted to glucose, as well as for supply of energy for the gluconeogenic process. Defective β-oxidation of fatty acids would thus primarily affect gluconeogenesis. However, it is reasonable to assume that after only 4 h of fasting glycogenolysis still predominates (Benyon 1998). Since one consequence of a metabolic block in the β-oxidation of fatty acids would be acute energy depletion, a compensatory increase in glucose production might be expected. However, this was not observed. In contrast, elevated lipolysis was observed, resulting in an increased availability of different fatty acids, which probably can be metabolized by overlapping activities of other enzymes involved in mitochondrial β-oxidation (Wanders et al 1999). The similarity of glucose production rates in the patient and her sister would indicate that the increased availability of fatty acids represents a sufficient compensatory mechanism for energy supply in LCHAD deficiency.

Fatty acids with chain lengths of 14–18 carbon atoms and 0–3 double bonds constitute up to 97% of human adipose tissue triacylglycerols (Raclot 2003). Since the fatty acid composition of adipose tissue is influenced by dietary fat (Raclot 2003), the diet of the patient with LCHAD deficiency, with a high proportion of medium-chain fatty acids (MCT fat), could influence the fatty acid pattern. In fact, the patient had higher plasma levels both of short- and medium-chain fatty acids as compared to her sister (Table 3), and increased levels of β-hydroxybutyrate.

The girl with LCHAD deficiency had a markedly increased resting energy expenditure compared both to her sister and to age- and sex-matched references (FAO/WHO/UNU 1985). The combination of a higher energy turnover and a slightly lower RQ in the patient indicates higher total oxidation of both fat and carbohydrates. This could not be explained by the diet, as the patient had a lower energy intake with larger amounts of carbohydrates than her sister. The increased lipolysis and elevated levels of NEFA are indicative of an increased recycling of fatty acids (Carlson et al 1994; Elia et al 1987). In LCHAD deficiency, the defective conversion of long-chain 3-hydroxyacyl-CoA intermediates to their corresponding 3-ketoacyl-CoA moieties results in ω-oxidation in the endoplasmic reticulum (ER), yielding the corresponding dicarboxylic acids, which are then metabolized by β-oxidation in peroxisomes. These processes are energy-consuming, not least because they include transport

of intermediates from the mitochondria and ER into the peroxisomes (Clarke 2000; Hagenfeldt et al 1990).

The mechanism underlying the elevated lipolysis in the patient with LCHAD deficiency is not clear as there were no differences between the patient and her healthy sister in the insulin/glucagon ratios or in the cortisol concentrations. Growth hormone levels and chromogranin A concentrations were also of the same magnitude in both subjects. Since lipolysis is an insulin-sensitive process (Stumvoll and Jacob 1999), the increased lipolytic activity could be due to decreased insulin sensitivity in the adipose tissue, as the insulin levels were similar in the two subjects. The hepatic insulin sensitivity, on the other hand, was seemingly comparable, as the plasma levels of IGF-I, IGFBP1 and GH were the same in the two girls.

In conclusion, we observed a more than rates of lipolysis after fasting that were 50% higher in the girl with LCHAD deficiency than in her healthy twin sister. As the rates of glucose production were similar and both girls were normoglycaemic during the study, this observation indicates that the increased lipolysis represents a sufficient compensatory mechanism to meet the energy demand after a few hours of fasting. However, this may be achieved at the cost of a risk of fatty acid infiltration and of toxic effects of β-oxidation intermediates on organ functions. The high resting energy expenditure observed in the patient illustrates that energy-consuming processes may be involved in metabolism of intermediates accumulating in LCHAD deficiency. Although we did not evaluate the metabolic situation postprandially, the findings in this study point to the importance of avoiding fasting in individuals with LCHAD deficiency.

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