Altered body composition and energy expenditure but normal glucose tolerance among humans with a long-chain fatty acid oxidation disorder

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1Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, Oregon; 2Graduate Programs in Human Nutrition, Oregon Health & Science University, Portland, Oregon; 3Department of Pediatrics, Oregon Health & Science University, Portland, Oregon; 4Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, Wisconsin; 5Biochemical Genetics Laboratory, Mayo Clinic, Rochester, Minnesota; and 6Department of Medicine, Oregon Health & Science University, Portland, Oregon

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Gillingham MB, Harding CO, Schoeller DA, Matern D, Purnell JQ. Altered body composition and energy expenditure but normal glucose tolerance among humans with a long-chain fatty acid oxidation disorder. Am J Physiol Endocrinol Metab 305: E1299—E1308, 2013. The development of insulin resistance has been associated with impaired mitochondrial fatty acid oxidation (FAO), but the exact relationship between FAO capacity and glucose metabolism continues to be debated. To address this controversy, patients with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency underwent an oral glucose tolerance test (OGTT) and measurement of energy expenditure, body composition, and plasma metabolites. Compared with controls, patients with LCHAD deficiency had a trend toward higher total body fat and extramyocellular lipid deposition but similar levels of intramyocellular and intrahepatic lipids. Resting energy expenditure was similar between the groups, but respiratory quotient was higher and total energy expenditure was lower in LCHAD-deficient patients compared with controls. High-molecular-weight (HMW) adiponectin levels were lower and plasma long-chain acylcarnitines were higher among LCHAD-deficient patients. Fasting and post-OGTT levels of glucose, insulin, and ghrelin, along with estimates of insulin sensitivity, were the same between the groups. Despite decreased capacity for FAO, lower total energy expenditure and plasma HMW adiponectin, and increased plasma acylcarnitines, LCHAD-deficient patients exhibited normal glucose tolerance. These data suggest that inhibition of the FAO pathway in humans is not sufficient to induce insulin resistance.

long-chain 3-hydroxy acyl-coenzyme A dehydrogenase deficiency; acylcarnitines

SEVERAL LINES OF EVIDENCE have linked decreased mitochondrial fatty acid oxidation (FAO) with the development of insulin resistance. Reduced FAO has been associated with the subsequent accumulation of cytosolic lipid by-products such as diacylglycerols (DGs), ceramides, and triglycerides in skeletal muscle and liver, also known as ectopic fat (5, 24, 32, 36). In turn, these cytosolic lipid intermediates have been shown to activate serine stress kinases and signaling pathways that impair normal insulin signaling and are the basis of the lipotoxicity hypothesis for insulin resistance (32, 38). Indeed, decreasing FAO and reducing ectopic fat are thought to be mechanisms that contribute to improved insulin sensitivity by various agents or genetic manipulations (1, 6, 7, 17, 37, 55).

In contrast, others have proposed that increased FAO without a concomitant increase in tricarboxylic acid cycle function results in mitochondrial dysfunction and the subsequent development of insulin resistance. Some studies have shown that blocking fatty acid entry into mitochondria can alleviate high-fat-diet-induced insulin resistance, suggesting that enhanced FAO and subsequent mitochondrial stress is the cause of insulin resistance (22, 23). Impaired mitochondrial function and subsequent production of partially oxidized substrates, such as intermediate metabolites from FAO in the form of fatty acylcarnitines (23, 30, 34), and increased reactive oxygen species (16, 25), have been associated with insulin resistance. These metabolites may serve as biomarkers of mitochondrial function or may be involved as potential mediators of insulin resistance (2). The question, therefore, is whether decreased or increased FAO is sufficient to induce insulin resistance. Following the first description of inherited deficiencies of FAO in humans in the 1980s, ~22 different defects have been identified (40). Patients with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency have a dramatically reduced ability to oxidize fatty acids and typically present with hypoketotic hypoglycemia in infancy, accumulate long-chain acylcarnitine and long-chain hydroxycarboxylcarbinic acid species in their blood, and have exercise intolerance later in life (41, 42, 52, 53). Treatment for these disorders consists of limiting long-chain fats in the diet and providing alternative fuel sources such as carbohydrates and protein (49). In practice, subjects with LCHAD deficiency consume very high carbohydrate diets and eat frequently to avoid fasting (13, 14). Some, but not all, patients become overweight. Although this constellation of features would typically be expected to increase the risk of impaired glucose clearance, to date no individual with LCHAD deficiency and glucose intolerance has been reported in the literature.

Because subjects with these rare disorders could potentially inform us about the relationship between reduced FAO and insulin sensitivity, we measured energy expenditure and body composition and performed oral glucose tolerance tests in patients with LCHAD deficiency compared with age-, sex-, and body mass index (BMI)-matched control subjects. We hypothesized that impaired ability to oxidize fat would not be sufficient to induce insulin resistance among subjects with a long-chain FAO disorder.

METHODS

Patients with a FAO disorder were recruited by announcements posted on family support networks and physician referral. The diag-
nosis of a FAO disorder was confirmed by reviewing medical records and evidence of two of the following four diagnostic criteria: 1) history of rhabdomyolysis with absence of urinary ketones, 2) abnormal acylcarnitine profile suggestive of a FAO disorder, 3) one or two known disease-causing mutations, or 4) decreased enzymatic activity in cultured fibroblasts (4, 39). Based on these criteria, initially 12 patients were identified and studied, consisting of 2 subjects with defects in the carnitine palmitoyltransferase (CPT)-2 enzyme, 1 subject with a mild adolescent-onset LCHAD deficiency, and 9 subjects with infant-onset LCHAD deficiency. Because the patients with CPT2 and mild LCHAD introduced minor enzymatic and phenotypic heterogeneity, it was decided to include only those patients with classical LCHAD defects in this analysis. Control subjects were recruited who were the same gender and had a similar age and BMI for a one-to-one matching design from the Portland area through the Oregon Health & Science University (OHSU) research recruitment webpage and referrals. The groups were matched for age, gender, and BMI. This study was approved by the OHSU institutional review board (IRB no. 817) and is listed on clinical trials.gov (NCT00654004). All subjects and their guardians gave written informed consent after the study had been explained to them.

Patients with LCHAD deficiency and control subjects were admitted to the OHSU Clinical and Translational Research Center for completion of all study procedures except for the doubly labeled water (DLW) sampling. Ingestion of the DLW and subsequent collection of urine samples for analysis took place after returning home to control for the change in ground water isotope enrichment.

Resting energy expenditure. Resting energy expenditure was measured after an overnight fast by indirect calorimetry using a SensorMedics Vmax 29N encore. Participants lay supine in a room with ambient temperature and low lighting for 30 min. The Plexiglas canopy was then placed over their head and chest, and gas exchange was measured for 45 min. Urine was collected for 24 h and analyzed for total urinary urea nitrogen concentration. Substrate oxidation was estimated using resting VO2 (l/min) inspired, VCO2 (l/min) expired, and 24-h urea excretion (g/min) (18).

Total energy expenditure. Total energy expenditure was measured with DLW. Baseline urine samples were collected immediately before and 24-h urea excretion (g/min) (18).

Patients with LCHAD deficiency and control subjects were admitted to the OHSU Clinical and Translational Research Center for completion of all study procedures except for the doubly labeled water (DLW) sampling. Ingestion of the DLW and subsequent collection of urine samples for analysis took place after returning home to control for the change in ground water isotope enrichment.

Resting energy expenditure. Resting energy expenditure was measured after an overnight fast by indirect calorimetry using a SensorMedics Vmax 29N encore. Participants lay supine in a room with ambient temperature and low lighting for 30 min. The Plexiglas canopy was then placed over their head and chest, and gas exchange was measured for 45 min. Urine was collected for 24 h and analyzed for total urinary urea nitrogen concentration. Substrate oxidation was estimated using resting VO2 (l/min) inspired, VCO2 (l/min) expired, and 24-h urea excretion (g/min) (18).

Total energy expenditure. Total energy expenditure was measured with DLW. Baseline urine samples were collected immediately before and three times within a 6-h period following labeled water consumption. Two final urine samples were collected 7 days later about 1 h and three times within a 6-h period following labeled water consumption. Two final urine samples were collected 7 days later about 1 h apart. Samples were analyzed for H2 and 18O enrichment by isotope ratio mass spectrometry as previously described (Laboratory of Schoeller, University of Wisconsin-Madison, Madison, WI). Total energy expenditure was calculated from the difference in elimination curves of H2 and 18O enrichment in urine assuming a respiratory quotient of 0.85 (43).

Body composition. Weight and height were measured in light clothing without shoes after an overnight 10-h fast. Body composition was measured by dual-energy X-ray absorptiometry (Hologic QDR 4500 Densitometer; Hologic, Bedford, MA) to determine total and regional fat and fat-free body mass.

Magnetic resonance imaging and spectroscopy. All magnetic resonance images (MRI) and spectroscopy (MRS) were obtained at the OHSU Advanced Imaging Research Center using a Siemens Magnetom Tim Trio 3 Tesla (Siemens Medical Solutions, Malvern, PA, and Erlangen, Germany) whole body system following an overnight fast. MRI of the abdomen for visceral and subcutaneous fat was obtained as previously described (21). A single slice at the level on the umbilicus was used for analysis and calculation of the visceral fat and subcutaneous fat deposition areas. Liver (intrahepatic lipid, or IHL) and muscle (extramyocellular, or EMCL, and intramyocellular, or IMCL) lipid deposition were measured using image-guided, 3H-localized MRS following high-resolution T-weighted spin-echo image (51). Volumes of interest within muscle were centered over the midsoleus muscles (12–15 cm2) with minimal contribution from gastrocnemius. Similarly, volumes of interest in liver were located away from major vascular structures, typically within the right lobe, with size varying from 18 to 27 cm2. Localized proton spectra within muscle were collected using a PRESS sequence with the following parameters: repetition time = 5 s, time to echo = 40 ms, 1,024 data points over 2,000 kHz spectral width. Liver spectra were collected using the same sequence and parameters except for a method of signal averaging to correct for liver motion due to patient respiration.

Oral glucose tolerance test. Following completion of the indirect calorimetry and continuing in a fasting state, an antecubital intravenous catheter was placed for blood sampling. Two fasting blood samples were drawn 15 min apart to account for pulsatile insulin secretion. Following this, subjects were given a standard flavored glucose to consume orally over 10 min (Glucola: 75 g for ⩾16 yr; 50 g for ⩽16 yr). Postprandial blood samples were obtained via the intravenous catheter at 30, 60, 90, and 120 min following glucose ingestion.

Blood chemistries. Concentrations of glucose were measured by a colorimetric enzyme assay (Raichem Glucose Color Reagent; Cilniaq, San Marcos, CA). Insulin concentrations were measured by radioimmunoassay (EMD Millipore, Billerica, MA). Phenylmethanesulfonyl fluoride and hydrochloric acid were added to plasma at the time of collection to prevent ghrelin degradation. These samples were assayed in duplicate using a ghrelin sandwich assay to measure both des- and acyl-ghrelin as previously described (27). Tetrahydrodipotstatin was added to EDTA plasma at the time of collection to inhibit lipase hydrolysis of free fatty acids ex vivo. Free fatty acid concentrations were measured by a commercially available enzymatic colorimetric kit (Wako Chemicals, Richmond, VA). Plasma was analyzed for acylcarnitines by electrospray tandem mass spectrometry at the Biochemical Genetics Laboratory, Mayo Clinic (46). Plasma was analyzed for amino acids by high-performance liquid chromatography using an amino acid analyzer at the Biochemical Genetics laboratory of the Knight Diagnostic Laboratories (Portland, OR). Protease inhibi-

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Mutation</th>
<th>LCHAD-Deficient Patients</th>
<th>Matched Controls</th>
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<tr>
<td></td>
<td>Age, yr Gender</td>
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<td>140.2</td>
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<td>7 M</td>
<td>135.9</td>
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<td>c.1525G&gt;C/1525G&gt;C</td>
<td>8 M</td>
<td>131.8</td>
</tr>
<tr>
<td>c.1525G&gt;C/1525G&gt;C</td>
<td>14 M</td>
<td>183.1</td>
</tr>
<tr>
<td>c.1525G&gt;C/1525G&gt;C</td>
<td>14 M</td>
<td>162.1</td>
</tr>
<tr>
<td>c.1525G&gt;C/1525G&gt;C</td>
<td>16 M</td>
<td>167.4</td>
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<td>172.6</td>
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<td>16 M</td>
<td>155.4</td>
</tr>
<tr>
<td>c.1525G&gt;C/1525G&gt;C</td>
<td>17 F</td>
<td>155.6</td>
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</table>

LCHAD, long-chain 3-hydroxy acyl-CoA dehydrogenase; BMI, body mass index; M, male; F, female.
itors were added to fasting serum to prevent adiponectin degradation. Adiponectin and high-molecular-weight (HMW) adiponectin were measured by ELISA (EMD Millipore). Fasting leptin concentrations were measured by radioimmunoassay (EMD Millipore) (9).

Data analysis. Insulin sensitivity was estimated by the homeostatic model assessment using the fasting glucose and insulin levels (glucose × insulin/405) (29) and the insulin sensitivity index (ISI) of Matsuda and DeFronzo [10,000/(fasting glucose × fasting insulin) × (mean glucose × mean insulin) during the oral glucose tolerance test] (28). Insulin secretion capacity was estimated using the insulinoergic index, the area under the curve (0–120 min) of insulin/area under the curve for glucose (31).

Differences in body composition, lipid deposition, energy expenditure, substrate oxidation, fasting leptin, and adiponectin between LCHAD-deficient patients and matched control subjects were determined by paired t-test using Prism 4.0 (Graphpad, La Jolla, CA). Because the data were matched one-to-one, it is necessary in the analysis to take into account the matching. The appropriate analysis, the paired t-test, will be more efficient than the unmatched analysis if there is moderate positive correlation between the paired observations. Although this method is more commonly seen when pairs of observations are obtained on the same subject, it is also widely used for more general matched pairs (10, 20). Differences between groups in plasma glucose, insulin, ghrelin, and acylcarnitines over time were compared using a paired repeated-measures ANOVA (Prism 4.0). If there was a significant difference between groups, a Bonferroni post hoc test was used for analysis of difference between individual time points. For all analysis, P < 0.05 was considered statistically significant.

RESULTS

The patients with LCHAD deficiency ranged in age from 7 to 17 yr (Table 1), and their BMIs ranged from 15.3 to 28.8

A

Patients with LCHAD deficiency

Control Subjects

% body composition

Fat Free Mass

Fat Mass

p = 0.08

p = 0.09

B

kg/m²

p = 0.31

p = 0.18

C

% of water peak

p = 0.08

p = 0.12

p = 0.42

Lipid Deposition

IMCL

EMCL

Liver

D

Liver Lipid Spectra

LCHAD deficient patient

Control Subject

E

Muscle Lipid Spectra

LCHAD deficient patient

Control Subject

Fig. 1. Body composition and lipid deposition. Data are presented as means ± SD. A: there is a trend for long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD)-deficient patients (n = 9; closed bars) to have less fat-free mass and more fat mass compared with control subjects (n = 9; open bars) when expressed as %body mass. B: there was no difference in fat-free or fat mass expressed as mass/surface area between groups. C: there was a trend for LCHAD-deficient patients (n = 9; closed bars) to have more extramyocellular lipid (EMCL) but no difference in intramyocellular lipid (IMCL) compared with control subjects (n = 9; open bars). Liver lipid content was not significantly different between groups. D: a representative magnetic resonance imaging (MRI) image of the abdomen and proton spectra of the liver for one LCHAD-deficient patient and the matched control subject are presented. The lipid peak is expressed as a percent of the water peak. E: a representative MRI image of the calf and proton spectra for one LCHAD-deficient patient and the matched control subject are presented. The lipid peak is expressed as a percent of the water peak.
kg/m². No participant met criteria for obesity in an adult. Patients with LCHAD deficiency tended to have a higher percent fat mass and lower percent fat-free mass than the control subjects (Fig. 1A). This difference in body composition appears to be due to both a slight increase in fat mass and decrease in lean mass, as suggested by similar fat mass and lean mass indexed to height between groups (Fig. 1B). The fat content was distributed equally between the trunk and extremities in the subjects (Fig. 2, lower in LCHAD-deficient patients compared with control subjects). Total energy expenditure was similarly lower in LCHAD-deficient patients and control subjects (Fig. 1, C and D). LCHAD-deficient patients exhibited a trend for more EMCL deposition but no difference in IMCL in the soleus muscle compared with control subjects (Fig. 1, C and E).

Resting energy expenditure was similar but respiratory quotient was higher and total energy expenditure was lower among patients with LCHAD deficiency. Resting energy expenditure was not significantly different between LCHAD-deficient patients and control subjects (Fig. 2, A and B). Respiratory quotient was significantly higher in patients with LCHAD deficiency after an overnight fast compared with control subjects (Fig. 2C). There was no significant difference in protein oxidation between the groups, but patients with LCHAD deficiency oxidized more carbohydrate and less fat at rest than controls (Fig. 2D). Only six control subjects completed the measurement of total energy expenditure measured by the DLW method. Total energy expenditure was ~15% lower in LCHAD-deficient patients compared with control subjects (Fig. 2, E and F) regardless whether the groups were compared using a paired (n = 6 pairs) or unpaired t-test (subjects n = 9; controls n = 6; P = 0.04).

Table 2. Regional fat mass and blood levels of lipids, glucose, and insulin and estimates of insulin sensitivity and secretion in patients with LCHAD deficiency and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regional fat mass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat of trunk, %</td>
<td>28 ± 7</td>
<td>26 ± 11</td>
<td>0.6</td>
</tr>
<tr>
<td>Body fat of arms and legs, %</td>
<td>33 ± 7</td>
<td>28 ± 9</td>
<td>0.17</td>
</tr>
<tr>
<td>Visceral adipose tissue, cm²</td>
<td>3 ± 1.4</td>
<td>4 ± 3.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue, cm²</td>
<td>19 ± 9</td>
<td>21 ± 15</td>
<td>0.38</td>
</tr>
<tr>
<td>Lipid levels</td>
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<td></td>
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<tr>
<td>Total cholesterol, mg/dl</td>
<td>139 ± 16</td>
<td>169 ± 33</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>79 ± 20</td>
<td>60 ± 26</td>
<td>0.15</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>86 ± 16</td>
<td>106 ± 30</td>
<td>0.18</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>36 ± 3</td>
<td>51 ± 12</td>
<td>0.011</td>
</tr>
<tr>
<td>Glucose homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>96 ± 13</td>
<td>106 ± 20</td>
<td>0.21</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>17 ± 10</td>
<td>14 ± 5</td>
<td>0.22</td>
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<tr>
<td>HOMA-IR</td>
<td>4.85 ± 2.75</td>
<td>3.61 ± 1.41</td>
<td>0.31</td>
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<tr>
<td>Insulin sensitivity index</td>
<td>2.87 ± 1.92</td>
<td>2.98 ± 1.52</td>
<td>0.89</td>
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<tr>
<td>Insulinogenic index</td>
<td>0.77 ± 0.47</td>
<td>0.53 ± 0.14</td>
<td>0.22</td>
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</table>

Data are means ± SD. Body composition expressed as %body fat or cm² on magnetic resonance imaging image and blood lipid, glucose, and insulin levels expressed as mg/dl were measured after a 10-h overnight fast. LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance, calculated using the following formula: fasting glucose × fasting insulin/405. The insulin sensitivity index of Matsuda and Defronzo (28) was calculated by 10,000√(fasting glucose × fasting insulin) × (mean glucose × mean insulin) during the oral glucose tolerance test. The insulinogenic index was calculated by the area under the curve (0-120 min) of insulin/area under the curve for glucose. P value is from a 2-sided paired t-test.

Responses to oral glucose loads were identical in LCHAD-deficient patients and matched control subjects. Neither fasting nor postoral plasma glucose or insulin levels (expressed as either absolute values or as a percent change from basal levels) were different between the LCHAD-deficient patients and control subjects (Fig. 3). All estimates of insulin sensitivity were likewise similar between groups (Table 2).

Plasma long-chain acylcarnitines were higher among patients with LCHAD deficiency. LCHAD-deficient patients had similar fasting free fatty acid levels compared with controls (Fig. 4A), and, in both groups, free fatty acid levels decreased similarly after an oral glucose load. Plasma acylcarnitine concentrations, an indirect marker of acetyl-CoA in the tissue (45), were not different between the two groups (Fig. 4B). As expected given the known enzymatic defects in the LCHAD-deficient patients, the sum of the long-chain acylcarnitines (including C12:1, C12:0, C14:2, C14:1, C14:0, C16:1, C16:0, C18:2, C18:1, and C18:0) was significantly greater, both before and after an oral glucose load, in the patients with LCHAD deficiency compared with controls (Fig. 4E). All of the long-chain acylcarnitine species were significantly greater among LCHAD-deficient patients compared with control subjects (Fig. 5). On the other hand, levels of summed short-chain acylcarnitines (including C3:0, C4:0, C5:1, C5:0, C4-OH, and C5-OH carnitines) (Fig. 4C) and medium-chain acylcarnitines (including C6:0, C8:0, C10:2, C10:1, and C10:0) (Fig. 4D) were not different between the patients and controls. Because some short-chain acylcarnitine species, such as propionylcarnitine, are derived from branched-chain amino acid (BCAA) oxidation and elevated BCAA have been associated with insulin resistance, we measured fasting plasma amino acid profiles and found there was no difference in any of the fasting plasma amino acids, including the BCAA, between patients with LCHAD deficiency and control subjects (Table 3).

Serum leptin and plasma ghrelin levels were similar but plasma high molecular weight adiponectin was lower among patients with LCHAD deficiency. There was no difference in fasting leptin levels, either total or per kilogram of fat mass, between LCHAD-deficient patients and controls (Fig. 6, A and B). Total adiponectin was not different between groups but HMW adiponectin levels were lower in LCHAD-deficient patients compared with control subjects (Fig. 6, C and D). Plasma total and acyl-ghrelin were similar both before and after an oral glucose load between groups (Fig. 6, E and F).

DISCUSSION

Studying patients with LCHAD deficiency allows a unique opportunity to test relationships between mitochondrial function, accumulation of FAA intermediates, and the effects of these changes on body composition and glucose metabolism. As might be expected when the ability to oxidize fat is impaired, we found a trend for subjects with LCHAD deficiency to have higher body fat content than control subjects. Possibly contributing to this excess adiposity were lower daily energy expenditures in the LCHAD-deficient patients. We found that this deficit in 24-h energy expenditure was not due to a lower resting energy expenditure than controls, which is consistent with our finding of similar acylcarnitine levels in the patients with LCHAD deficiency and controls. Acylcarnitine levels were a surrogate for tissue accumulation of
Fig. 2. Energy expenditure and substrate oxidation. Data are presented as means ± SD. Indirect calorimetry was measured after a 10-h overnight fast. LCHAD-deficient patients (n = 9; closed bars and closed circles) have a similar resting energy expenditure as control subjects (n = 9; white bars and open squares) expressed as mean kcal/day (A) or kcal/kg of fat-free mass (B). Resting respiratory quotient was significantly higher in the LCHAD-deficient patients (n = 9; gray box plot) compared with control subjects (n = 9; white box plot). C: LCHAD-deficient patients (n = 8; closed bars) oxidized more carbohydrate and less fat than controls (n = 8; white bars). Total energy expenditure was lower in LCHAD-deficient patients (n = 9; closed bars and closed circles) compared with control subjects (n = 6; white bars and open squares) expressed as mean kcal/day (E) or as kcal/kg of fat-free mass (F).

Fig. 3. Plasma glucose and insulin with an oral glucose tolerance test. Data are presented as means ± SD. Participants fasted overnight (10 h), and 2 fasting blood samples were drawn 15 min apart (−15, 0 min). After the fasting blood samples were collected, participants consumed a standard glucose drink (Glucola) orally. Additional blood samples were taken at 30, 60, 90, and 120 min after the oral glucose load. LCHAD-deficient patients (n = 9; closed circles) have similar glucose concentrations (A) and percent change in plasma glucose (B) compared with control subjects (n = 9; open squares) before and after an oral glucose load. LCHAD-deficient patients (n = 9; closed circles) have similar insulin concentrations (C) and percent change in plasma insulin (D) compared with control subjects (n = 9; open squares) before and after an oral glucose load.
acetyl-CoA and suggest that energy production at rest was normal despite having partial FAO deficiency. Instead, we suspect that the lower daily energy expenditure in the LCHAD patients is likely explained by reduced activity levels, which have been described among subjects with a FAO disorder who fear metabolic decompensation and rhabdomyolysis triggered by excessive physical activity (48). This remains speculation, though, since we did not formally measure activity in either group in this study.

Despite their FAO defect, patients with LCHAD deficiency did not accumulate excess IHL and tended to store excess lipid as EMCL rather than IMCL in the muscle compared with controls. In some animal models, decreasing FAO by pharmacological inhibition of CPT-1 with etomoxir or by reducing long-chain acyl-CoA dehydrogenase activity has been reported to lead to intracellular lipid accumulation (3, 56). Our study is the first report to demonstrate that, in humans with LCHAD deficiency, an enzyme distal to CPT-1, there may be preferentially lipid deposition in the EMCL space. The mechanism for preferential storage of lipid in the EMCL space is unknown, but a recently published study in mice treated with oxfenicine, a pharmacological inhibitor of CPT-1B, the predominant isoform of CPT-1 found in muscle, reported similar results (19). Obese mice treated with oxfenicine had lower acyl-CoA, DAG, and ceramides compared with untreated mice. The authors speculate that these reduced levels of CPT-1B were related to a compensatory decrease in fatty acid uptake at the plasma membrane because they observed lower C36, a key fatty acid transporter, in oxfenicine-treated mice. However, this mechanism would not be consistent with elevated long-chain acylcarnitines in our patients because fatty acids must enter the cell and the mitochondria before they are metabolized to these partial FAO products.

HMW adiponectin levels were lower in patients with LCHAD deficiency compared with controls. Although the regulation of adiponectin synthesis, release, and the feedback signaling that controls these processes is not fully understood, low HMW adiponectin has been associated with increased adiposity. Adiponectin signaling in muscle and liver increases FAO through activation of AMP kinase (54). Perhaps the absence of the downstream increase in FAO or accumulation of a product upstream from the FAO defect lowered adiponectin synthesis or secretion from adipose in our patients. Leptin concentrations were similar between patients with LCHAD deficiency and control subjects, so

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Fig. 4. Plasma free fatty acids and acylcarnitines with an oral glucose tolerance test. Data are presented as means ± SD. Participants fasted overnight (10 h), and a fasting blood sample (0 min) was analyzed for free fatty acids and plasma acylcarnitines. After the fasting blood sample was collected, participants consumed a standard glucose drink (Glucola) orally. Additional blood samples were taken at 30, 60, 90, and 120 min after the oral glucose load and analyzed similarly. A: LCHAD-deficient patients (n = 9; closed circles) have similar fasting free fatty acids as control subjects (n = 9; open circles) after an overnight fast or following an oral glucose load. B, C, and D: acetylcarnitine, the sum of the short-chain acylcarnitines, and the sum of the medium-chain acylcarnitines were not different between LCHAD-deficient patients (n = 9; closed circles) and control subjects (n = 9; open circles) after an overnight fast or following an oral glucose load. E: the sum of the long-chain acylcarnitines was significantly higher among LCHAD-deficient patients (n = 9; closed circles) compared with control subjects (n = 9; open circles) after an overnight fast and following an oral glucose load.
the decreased adiponectin is not related to an overall effect on adipokine synthesis and release.

A large body of evidence suggests a connection between impaired FAO and development of insulin resistance (23, 34). We show that patients with LCHAD deficiency had lower daily energy expenditures, increased percent body fat, increased long-chain acylcarnitine levels, and lower levels of HMW adiponectin, all characteristics that would be expected to be associated with impaired glucose metabolism. Yet, despite their inherent mitochondrial defect in FAO and these phenotypic characteristics, our data show that glucose and insulin responses following an oral glucose load, as well as estimated insulin sensitivity, were the same in LCHAD-deficient patients compared with normal controls. In addition, patients with LCHAD deficiency had similar suppression of free fatty acid levels after the oral glucose load as control subjects, suggesting no impairment of adipose tissue hormone-sensitive lipase responsiveness to insulin. These findings are consistent with the existing literature in which, to our knowledge, no patient with LCHAD deficiency has been reported to have developed either impaired glucose tolerance or type 2 diabetes mellitus (T2DM). In the context of glucose and lipid metabolism, the low HMW adiponectin concentration deserves additional consideration. LCHAD-deficient patients are the first patient group we are aware of who have low HMW adiponectin levels but normal estimates of insulin sensitivity, IMCL, and plasma triglyceride levels. This appears to be a unique population in which circulating HMW adiponectin levels are incongruent to, or disconnected from, both measures of glucose clearance or IMCL accumulation.

Lipids are thought to play a key role in islet cell secretion. Pancreatic β-cell FAO is associated with decreased insulin secretion, whereas fatty acid/glucose cycling in the cytosol of the β-cell is associated with increased insulin secretion (11, 12, 50). Patients with the FAO disorder short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) deficiency present with infantile hyperinsulinemic hypoglycemia (8). It was recently demonstrated that null mutations in the SCHAD gene result in loss of a protein/protein interaction between SCHAD and glutamate dehydrogenase (GDH), causing an overstimulation of GDH and increased insulin secretion from the islet cells (15, 26). The present study is the first that we are aware of to investigate insulin secretory capacity among humans with impaired long-chain FAO. We observed normal insulin secretory capacity as estimated by ISI in the LCHAD patients, suggesting the defect in long-chain FAO did not impair β-cell function. It is possible the block in long-chain FAO results in increased fatty acid/glucose cycling in the cytosol that potentiates insulin secretion in patients with LCHAD deficiency, but more detailed study of insulin secretion is needed to determine if that is true. In contrast to SCHAD deficiency, subjects with LCHAD deficiency do not exhibit hyperinsulinemia.

We can make several observations regarding accumulation of mitochondrial metabolic intermediates and glucose metabolism. Despite similar substrate availability for oxidation in target tissues, LCHAD-deficient patients had dramatically increased long-chain acylcarnitines. Increased acylcarnitines, by-products of partial FAO, have been associated with insulin resistance in both rodent and human studies (2, 23, 30). We observed extremely elevated long-chain acylcarnitines in our LCHAD-deficient patients in the context of normal carbohydrate metabolism. With levels of long-chain acylcarnitines nearly 100-fold higher than have been reported in patients with diabetes (2, 30), our data suggest that mitochondrial dysfunction at the level of long-chain FAO that results in the accumulation of long-chain acylcarnitines is not sufficient by itself to impair glucose clearance or alter insulin secretion. Instead, our data are consistent with elevations in long-chain acylcarnitines...
being related to increased adiposity in diabetic populations (30) rather than resulting from insulin resistance.

Medium-chain acylcarnitine species have been reported to be elevated among subjects with T2DM and in diet-induced insulin-resistant animal models (2, 22, 23, 30, 35). We observed similar levels of medium-chain acylcarnitines and glucosed tolerance in the LCHAD-deficient patients compared with controls, so we cannot draw conclusions about relationships between this FAO intermediate and glucose metabolism. Similarly, many reports have observed elevated short-chain acylcarnitines among subjects with T2DM (30, 35). Short-chain acylcarnitines can arise from the catabolism of long-chain fatty acids to shorter chain lengths as well as from various amino acids. Because subjects with LCFAO disorders do not generate short-chain acylcarnitines through oxidation of fats, we considered whether oxidation of alternative substrates that contribute to these metabolites is occurring. The primary alternative substrate is the BCAA. However, we found no difference in BCAA concentrations between the patients with LCHAD deficiency and control subjects. Because the synthesis of short-chain acylcarnitines from BCAA occurs distal to the rate-limiting step in BCAA catabolism, branched-chain α-keto acid dehydrogenase complex, it is possible that this process would not alter circulating concentrations of these abundant amino acids.

The current study has several limitations. LCHAD deficiency is very rare, and recruiting and studying subjects is both costly and difficult, resulting in small numbers of study subjects. Knowing this, we chose very sensitive methods to quantify energy expenditure and body composition, including ectopic fat accumulation. To reduce subject burden, though, we relied on parameters derived from an oral glucose tolerance test to estimate insulin sensitivity and secretion. It is possible that, with more direct measurement techniques, such as the hyperinsulinemic-euglycemic clamp, or using muscle tissue derived from biopsy samples, subtle differences in insulin sensitivity could have been detected between the patients and controls. In addition, all subjects with LCHAD deficiency were treated with a fat-restricted diet and sufficient calories from carbohydrates to prevent metabolic decomposition, although the control subjects were not required to adhere to a similar diet. By studying the LCHAD subjects on a low-fat diet, our patients avoided a dietary fat challenge that would be potentially needed to sufficiently “stress” mitochondrial function and result in the accumulation of intermediate products of FAO (e.g., DAGs, BCAAs) and IMCL that would predict insulin resistance. However, a high-fat diet is contraindicated in these patients because of high risk for hypoglycemia and rhabdomyolysis. In addition, our patient group demonstrated significantly impaired fat oxidation, even in the fasting state, as evidenced by verified clinical symptomatic episodes of hypoglycemia and rhabdomyolysis, lower fat substrate oxidation, and markedly elevated long-chain acylcarnitines than controls; yet, no differences in fasting glucose or insulin levels were detected. An-
other confounding factor could be an adaptive response in glucose metabolism among patients dependent upon glucose for energy production, but no data on this possible difference is currently available.

In conclusion, we found that, despite markedly lower FAO, decreased total energy expenditure, and lower HMW adiponectin levels compared with controls, LCHAD-deficient patients have normal glucose tolerance and estimated insulin sensitivity and secretion. Interestingly, patients with LCHAD deficiency did not store more lipid in the IMCL but preferentially stored more lipid in the EMCL compared with controls. Long-chain acylcarnitines were significantly elevated among LCHAD-deficient patients but medium-chain acylcarnitines and BCAAs were not different between groups. Increased levels of long-chain acylcarnitines did not alter glucose tolerance among our subjects, which suggests that the specific acylcarnitine species resulting from incomplete LCFAO are biomarkers of incomplete FAO and potentially of mitochondrial dysfunction but do not independently induce insulin resistance. Inherited defects of long-chain FAO with an obligatory requirement for glucose to produce ATP did not lead to impaired glucose metabolism, suggesting a substantial decrease in the FAO pathway by itself is not sufficient to induce insulin resistance or impaired glucose tolerance in humans.

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