Cholesterol Metabolism Is Enhanced in the Liver and Brain of Children With Citrin Deficiency

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Context: Citrin-deficient infants present neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), which resolves at 12 months. Thereafter, they have normal liver function associated with hypercholesterolemia, and a preference for lipid-rich carbohydrate-restricted diets. However, some develop adult-onset type II citrullinemia, which is associated with metabolic abnormalities.

Objectives: To identify the causes of hypercholesterolemia in citrin-deficient children post-NICCD.

Design and Setting: We determined the concentrations of sterol markers of cholesterol synthesis, absorption, and catabolism by liquid chromatography–electrospray ionization–tandem mass spectrometry and evaluated serum lipoprotein profiles.

Subjects: Twenty citrin-deficient children aged 5 to 13 years and 37 age-matched healthy children.

Intervention: None.

Abbreviations: 4β-OHC, 4β-hydroxycholesterol; 7α-OHC, 7α-hydroxycholesterol; 24S-OHC, 24S-hydroxycholesterol; apo, apolipoprotein; ATP, adenosine triphosphate; CETP, cholesteryl ester transfer protein; C4, 7α-hydroxy-4-cholesten-3-one; CoA, coenzyme A; GH, growth hormone; CYP7A1, cholesterol 7α-hydroxylase; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LC-ESI-MS/MS, liquid chromatography–electrospray ionization–tandem mass spectrometry; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; MA shuttle, malate-aspartate NADH shuttle; MC shuttle, malate-citrate NADH shuttle; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; SD, standard deviation; TC, total cholesterol.

Main Outcome Measures: Relationship between serum lipoproteins and sterol markers of cholesterol metabolism.

Results: The citrin-deficient group had a significantly higher high-density lipoprotein cholesterol (HDL-C) concentration than did the control group (78 ± 11 mg/dL vs 62 ± 14 mg/dL, P < 0.001), whereas the two groups had similar low-density lipoprotein cholesterol and triglyceride concentrations. The concentrations of markers of cholesterol synthesis (lathosterol and 7-dehydrocholesterol) and bile acids synthesis (7a-hydroxycholesterol and 27-hydroxycholesterol) were 1.5- to 2.8-fold and 1.5- to 3.9-fold, respectively, higher in the citrin-deficient group than in the control group. The concentration of 24S-hydroxysterol, a marker of cholesterol catabolism in the brain, was 2.5-fold higher in the citrin-deficient group. In both groups, the HDL-C concentration was significantly positively correlated with that of 27-hydroxycholesterol, the first product of the alternative bile acid synthesis pathway.

Conclusions: HDL-C and sterol marker concentrations are elevated in citrin-deficient children post-NICCD. Moreover, cholesterol synthesis and elimination are markedly enhanced in the liver and brain of citrin-deficient children. (J Clin Endocrinol Metab 103: 2488–2497, 2018)

Citrin is a liver-type aspartate/glutamate carrier isoform-2 that plays an important role in generating adenosine triphosphate (ATP) from carbohydrate. During glycolysis in the liver, NADH2+ is produced in the hepatocyte cytosol. Because NADPH2+ cannot cross the mitochondrial membrane, NADH2+ -derived protons and oxaloacetate are converted to malate for uptake by mitochondria by the malate/2-oxoglutarate antiporter (Fig. 1). In mitochondria, NADPH2+ is regenerated in the reverse reaction and used for ATP synthesis by the

Figure 1. Carbohydrate and lipid metabolism in hepatocytes of citrin-deficient children. Citrin is a liver-type aspartate/glutamate carrier isoform-2 that plays an important role in generating ATP from carbohydrate. Schematic representations of citrin-related carbohydrate and lipid metabolism are shown. (A) Malate-aspartate NADH shuttle (MA shuttle). (B) Malate-citrate NADH shuttle (MC shuttle). CoA, coenzyme A; TCA, tricarboxylic acid.
electron transport system (1, 2). Mitochondrial oxaloacetate is converted to aspartate by aspartate aminotransferase (Fig. 1, hatched circles). Next, mitochondrial aspartate and cytosolic glutamine are exchanged by citrin (Fig. 1, round gray rectangle). This pathway is known as the malate–aspartate NADH shuttle (MA shuttle; Fig. 1A). Citrin is one of the main components of the MA shuttle, and it functions to maintain a low and high NADH/NAD+ ratio in the cytosol and mitochondria, respectively. Because the MA shuttle is nonfunctional in patients with citrin deficiency, the malate–citrate NADH shuttle (MC shuttle; Fig. 1B) is activated in these patients by a compensatory mechanism (3–5). As a result, the cytosolic level of acetyl-coenzyme A (CoA) derived from citrate is increased and is preferentially used for cholesterol and fatty acid synthesis.

Citrin deficiency is a pan-ethnic inborn error of metabolism caused by mutations of the SLC25A13 gene (6, 7). The prevalence of citrin deficiency is 1:17,000 in Japan, but a number of patients remains to be diagnosed. In typical cases, citrin-deficient children present with intrahepatic cholestasis and diverse metabolic abnormalities, including citrullinemia, hyperammonemia, galactosemia, and hypoglycemia in the neonatal period; this condition is referred to as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) (6–8). The clinical features of NICCD generally resolve at 6 to 12 months of age, probably due to compensatory changes. During the post-NICCD period, citrin-deficient children show a strong preference for a lipid-rich carbohydrate-restricted diet, which is associated with hyperlipidemia (9). After this silent remission period, ~20% of patients develop a fatal metabolic disease [adult-onset type II citrullinemia (CTLN2)], which is characterized by profound liver steatosis accompanied by hyperammonemia, mental derangement, and sudden episodes of unconsciousness (1, 4, 6). Because the age of onset of CTLN2 varies widely [usually 20 to 50 years (10)], metabolic compensation to a lipid-rich carbohydrate-restricted diet would prevent or delay the development of CTLN2. However, little is known about cholesterol metabolism in citrin-deficient children.

To investigate the cause of hypercholesterolemia in citrin-deficient children during the post-NICCD period, we determined lipoprotein concentrations, together with the serum concentrations of sterols and oxysterols that reflect cholesterol metabolism, in citrin-deficient and control children. Our data strongly suggest that a lipid-rich carbohydrate-restricted diet induces compensatory changes in cholesterol metabolism in not only the liver but also the brain.

Materials and Methods

Subjects

Twenty children with citrin deficiency (12 boys and 8 girls; 5 to 13 years of age) were enrolled in this study. The diagnosis of citrin deficiency was confirmed by SLC25A13 gene analysis. The method used to genotype the study subjects was reported previously (10, 11). We identified the following seven mutations: (I) 851del4; (II) IVS11 +1G > A; (III) 1638ins23; (IV) S225X; (V) IVS13 +1G > A; (VIII) E601X; and (XIX) IVS16ins3kb. Combinations of these mutations yielded the following 11 genotypes: I/I (n = 4), I/II (n = 2), I/IV (n = 2), I/IX (n = 1), II/II (n = 1), II/IV (n = 2), II/IX (n = 3), III/VIII (n = 1), III/XIX (n = 1), IV/XIX (n = 2), and V/V (n = 1), respectively. The allele frequencies were I (32.5%), II (25.0%), III (2.5%), IV (15.0%), V (12.5%), VIII (2.5%), and XIX (10.0%), respectively. We also recruited 37 healthy children (19 boys and 18 girls; 5 to 13 years of age) as an age-matched control group.

In neonatal mass screening at the age of 5 days, 14 of the 20 citrin-deficient children had the following metabolic abnormalities: hypergalactosemia (n = 7), hyperphenylalaninemia (n = 4), hypermethionemia (n = 2), and coincident hyperphenylalaninemia and hypermethionemia (n = 1). At the age of 1 to 4.5 months, the remaining six children were admitted to local hospitals due to jaundice noticed by their parents. All citrin-deficient children had cholestatic liver disease, together with high serum citrulline, arginine, threonine, tyrosine, and phenylalanine concentrations. By the age of 7 to 18 months, liver dysfunction had ameliorated in all of these children.

Although the citrin-deficient and control children were similar in terms of their total daily energy intake, the former depended more on fat than on carbohydrates. In the citrin-deficient children, fat accounted for 33% to 54% of the total energy intake (25% to 30%, reference value for normal Japanese children), and carbohydrates for 23% to 50% (54% to 58% reference value). The fatty acid and cholesterol intakes were 1.4- to 1.6-fold and 1.1- to 1.2-fold, respectively, greater in the citrin-deficient children than in the control children (9).

Study design

Blood samples were collected at 10:30 to 11:30 AM before lunch following 4 to 5 hours of fasting. After low-speed centrifugation, fresh serum was obtained and used for lipoprotein measurements. The remaining serum was frozen at −80°C for measurement of sterol markers of cholesterol metabolism using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI-MS/MS), as described previously (12). All patients and their parents were informed of the methods and purpose of the study, and their informed consent was obtained prior to enrollment. The study protocol was approved by the institutional Medical Ethics Committee of Takarazuka City Hospital.

Determination of lipoprotein profiles

Total cholesterol (TC) and total triglyceride concentrations were determined using enzymatic methods. High-density lipoprotein (HDL) cholesterol (HDL-C) and low-density lipoprotein (LDL) cholesterol (LDL-C) concentrations were measured by homogenous assays (Cholestest HDL and Cholestest LDL, Sekisui Medical, Tokyo, Japan), the accuracy of which has been demonstrated (13–15). Apolipoprotein (apo)
concentrations were determined by turbidimetric immunoassay (apoA-I, apoB, apoE Auto Daiichi, Sekisui Medical). Cholesterol ester transfer protein (CETP) mass was measured by a two-site sandwich enzyme immunoassay (CETP ELISA Daiichi; Sekisui Medical).

Analysis of serum concentrations of sterols and oxysterols

We evaluated cholesterol metabolism by determining the concentrations of seven sterol and five oxysterol markers. The sterol markers included five cholesterol precursors reflecting hepatic cholesterol biosynthesis (desmosterol, lathosterol, zymosterol, 7-dehydrocholesterol, and 8-dehydrocholesterol) and two phytosterols reflecting cholesterol absorption in the small intestine (campesterol and sitosterol) (16). We calculated the ratios of these markers to TC to correct the amounts of carrier lipoproteins. We also determined the ratio of phytosterols to lathosterol, which reflects the balance between cholesterol absorption and synthesis. Oxysterol concentrations were determined to assess cholesterol catabolism in the brain [24S-hydroxycholesterol (24S-OHC)] and in the peripheral tissues [4β-hydroxycholesterol (4β-OHC), 7α-hydroxycholesterol (7α-OHC), 7α-hydroxy-4-cholesten-3-one (C4), and 27-hydroxycholesterol (27-OHC)] (17–22). Of these oxysterols, 4β-OHC reflects the activity of cytochrome p450 3A4/5, which detoxifies xenobiotics and toxic endogenous substances. The other oxysterols are produced as bile acid precursors in the liver (7α-OHC and C4) and in the systemic peripheral tissues (27-OHC).

Sterol and oxysterol measurement by LC-ESI-MS/MS

After adding internal standards to serum samples, all sterols and oxysterols except C4 were hydrolyzed in 1 N ethanolic KOH in the presence of butylated hydroxytoluene at 37°C for 1 hour. The lipid phase was extracted with organic solvents and derivatized to picolinyl esters. Individual markers were analyzed by a fast liquid chromatography system (Shimadzu, Kyoto, Japan). The instrument consisted of a TSQ Vantage triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an HESI-II probe and a Prominence ultrafast liquid chromatography system (Shimadzu, Kyoto, Japan).

Statistical analyses

Differences between the citrin-deficient and control children were analyzed by unpaired Student two-tailed t tests or nonparametric Mann-Whitney U tests. A Pearson correlation analyses for two variables and multivariate linear regression analysis were performed using Statcel software (OMS, Tokorozawa, Japan) by two-sided analysis. In multivariate linear regression analysis, we used HDL-C concentration as the dependent variable and possible confounding variables (age, 24S-OHC, 27-OHC, and CETP) as independent variables. A P value of <0.05 was considered indicative of statistical significance.

Results

Anthropometric and biochemical parameters

Although the mean age of the two groups was similar, the citrin-deficient group had significantly lower standard deviation (SD) scores for height and body weight compared with the control group (Table 1). In contrast, the liver function parameters, including serum albumin concentration, did not differ between the two groups.

Lipoprotein profiles and CETP concentrations

The citrin-deficient group had dyslipidemia, which is attributable to increased HDL levels. There was no difference in LDL-C or apoB concentrations between the two groups (Table 2). In contrast, HDL-C and apoA-I concentrations were 26% and 32% higher, respectively, in the citrin-deficient group than in the control group. Although a high HDL-C concentration is associated in the citrin-deficient group with a low CETP mass in CETP deficiency and in patients taking CETP inhibitors, the mean CETP mass was higher in the citrin-deficient group than in the control group.

Table 1. Baseline Parameters of the Citrin-Deficient and Control Children

<table>
<thead>
<tr>
<th></th>
<th>Citrin-Deficient Group (n = 20)</th>
<th>Control Group (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>12/8</td>
<td>19/18</td>
</tr>
<tr>
<td>Age, y</td>
<td>9.5 ± 2.4</td>
<td>10.1 ± 2.1</td>
</tr>
<tr>
<td>Age manifesting NICCD, mo</td>
<td></td>
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<tr>
<td>From</td>
<td>2.0 ± 0.5</td>
<td>N/A</td>
</tr>
<tr>
<td>To</td>
<td>9.6 ± 2.4</td>
<td>N/A</td>
</tr>
<tr>
<td>Body height, cm (SD score)</td>
<td>129.0 ± 9.5 (−0.55 ± 0.49)</td>
<td>130.5 ± 9.3 (0.06 ± 0.029)</td>
</tr>
<tr>
<td>Body weight, kg (SD score)</td>
<td>24.1 ± 3.6 (−1.01 ± 0.33)</td>
<td>27.8 ± 3.9 (−0.02 ± 0.33)</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>6.9 ± 0.3</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>3.8 ± 0.3</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>AST, u/L</td>
<td>20 ± 3</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>ALT, u/L</td>
<td>23 ± 3</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>γ-GT, u/L</td>
<td>16 ± 3</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>TBA, μmol/L</td>
<td>7 ± 3</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

Data are means ± SD.

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; N/A, not applicable; TBA, total bile acid; γ-GT, γ-glutamyl transferase.

*P < 0.01 vs control group.

**P < 0.001 vs control group.
In the citrin-deficient group, LDL-C and apoB concentrations showed significant inverse correlations with age (Fig. 2A and 2C, closed circles and solid lines). In contrast, HDL-C and apoA-I concentrations showed inverse correlations with age only in the control group (Fig. 2B and 2D, open circles and dotted lines). Although the LDL-C and apoB concentrations were similar in the two groups, HDL-C and apoA-I concentrations were higher in age-matched citrin-deficient children than in control children (Fig. 2B and 2D).

Because we had 11 genotypes with 7 mutations in 20 citrin-deficient children, we divided the study subjects into two groups according to the presence and absence of the individual mutations. We found no difference in age, anthropometric parameters, and lipoprotein profiles between the allele-positive and allele-negative groups.

We also compared the same variables between the groups picked up, or not, by neonatal mass screening. The mean age was higher in the neonatal mass screening-positive cases than in the mass screening-negative cases (10.3 ± 2.1 vs 7.6 ± 2.1 years, \( P < 0.01 \)), whereas the mean LDL-C concentration was lower in the positive cases than in the negative cases (72 ± 15 mg/dL vs 91 ± 16 mg/dL, \( P < 0.01 \)).

**LC-ESI-MS/MS analysis**

The sterol marker concentrations showed that cholesterol and bile acid synthesis were enhanced in the citrin-deficient group. The concentrations of all cholesterol precursors were 1.5- to 2.8-fold higher in the citrin-deficient group than in the control group (Table 2). The concentrations of markers of cholesterol synthesis were...
higher in the citrin-deficient group than in the control group, irrespective of age. The lathosterol and 7-dehydrocholesterol concentrations are shown in Fig. 2E and 2F as representative examples. Additionally, the cholesterol precursors-to-TC ratios were significantly higher in the citrin-deficient group than in the control group (Table 2). In contrast, among phytosterols, which reflect intestinal cholesterol absorption, only the mean campesterol concentration was significantly higher in the citrin-deficient group than in the control group. After correcting for phytosterol concentrations with TC, however, the ratios of phytosterols (campesterol and sitosterol) to TC did not differ between the two groups (Table 2). Serum phytosterol concentrations did not change with age, and their distributions were similar (Fig. 3G and 3H). The ratios of phytosterols to cholesterol precursor (lathosterol) did not differ significantly between the two groups (Table 2).

We also examined the concentrations of oxysterols, which are produced in the liver (4β-OHC, 7α-OHC, and C4), brain (24S-OHC), and peripheral tissues (27-OHC), by LC-ESI-MS/MS. The mean concentrations of all oxysterols were significantly higher in the citrin-deficient group than in the control group (Table 2). In particular, 7α-OHC, 24S-OHC, and 27-OHC concentrations were 3.9-, 2.5-, and 1.5-fold higher, respectively, in the citrin-deficient group than in the control group. The 24S-OHC concentrations in both groups changed with age, although the regression line of the citrin-deficient group was considerably steeper than that of the control group (Fig. 2K).

In the subgroup analysis, we did not detect any effect of SLC25A13 genotype or neonatal mass screening test results on the markers of cholesterol synthesis, absorption, and elimination, as well as oxysterols.

**Determinants of apoAI and HDL-C**

In the citrin-deficient group, 27-OHC was the only determinant of apoAI and HDL-C concentrations (Fig. 3A and 3B, right panel). In the control group, however, both 24S-OHC and 27-OHC were determinants of HDL-C concentration (Fig. 3A). We carried out stepwise regression analysis using HDL-C as the dependent variable, and age, 24S-OHC, 27-OHC, and CETP mass as the independent variables. In the citrin-deficient group, only 27-OHC was selected as a significant
determinant of HDL-C concentration ($\beta = 0.530$) ($R = 0.530$, $P < 0.05$). In the control group, age and 27-OHC were selected as significant determinants of HDL-C concentration (age, $\beta = 0.518$; 27-OHC, $\beta = 0.392$) ($R = 0.595$, $P < 0.01$).

**Discussion**

Our findings indicate that HDL-C concentrations are elevated in citrin-deficient children during the post-NICCD period, and that cholesterol synthesis and elimination are markedly enhanced in their liver and brain. The mean HDL-C concentration was 26% higher in the citrin-deficient children than in the control children (Table 2). LC-ESI-MS/MS analysis revealed that the concentrations of sterol makers of cholesterol synthesis (Fig. 2E and 2F), bile acid synthesis (Fig. 2I, 2J, and 2L), and cholesterol elimination from the brain (Fig. 2K) were markedly increased in the citrin-deficient children.

In the citrin-deficient children, clinical features including dyslipidemia changed dramatically in an age-dependent manner. In our previous study, citrin-deficient children had significantly higher LDL-C and HDL-C concentrations than did control children (LDL-C, 116 ± 23 mg/dL vs 85 ± 13 mg/dL, $P < 0.001$; HDL-C, 79 ± 7 mg/dL vs 54 ± 11 mg/dL, $P < 0.001$) (24). In the current study, by contrast, only HDL-C concentrations were elevated in the affected children. It is noteworthy that 80% of citrin-deficient children were ≤ 6 years of age in the earlier study, whereas 90% were ≥ 6 years of age in the current study. The concentrations of markers of hepatic cholesterol synthesis were persistently high in our school-age patients (Fig. 2E and 2F). In citrin-deficient children, a lipid-rich and carbohydrate-restricted diet is more likely to increase production of acetyl-CoA, the basic two-carbon unit, for cholesterol biosynthesis than is a normal diet (Fig. 1). Because cholesterol absorption (other than campesterol) did not differ between the citrin-deficient and control children (Fig. 2H; Table 2) and the enhancement of cholesterol synthesis and elimination was likely to be greater than that of cholesterol absorption (Fig. 2E, 2F, 2I, 2J, and 2L; Table 2), compensatory metabolic changes may act to normalize LDL-C concentrations in school-age citrin-deficient children.

Two factors may be responsible for the reduced serum LDL-C concentrations in school-age citrin-deficient children. The first is increased catabolism of cholesterol into bile acids. In the liver, excess cholesterol is converted to 7α-OHC by cholesterol 7α-hydroxylase (CYP7A1), as the first step of the classical bile acid synthesis pathway. CYP7A1 is the rate-limiting enzyme of this pathway (18–20). CYP7A1 activity is absent in the fetus, but increases to a sufficient level after the age of 4 years (18). Other groups have found that CYP7A1 activity is correlated with 7α-OHC and C4 concentrations in humans (7α-OHC, $n = 38$, $r = 0.87$, $P < 0.001$; C4, $n = 16$, $r = 0.84$, $P < 0.001$) (19, 20). In the present study, 7α-OHC and C4 concentrations were significantly higher in the citrin-deficient children than in the control children, which confirms accelerated hepatic bile acid synthesis in the school-age citrin-deficient children. This activation of CYP7A1 is likely to be a result of the increased hepatic cholesterol content caused by enhanced cholesterol biosynthesis.

The second factor that may be responsible for the reduction in serum LDL-C concentrations in school-age citrin-deficient children is enhanced uptake of LDL by LDL receptors. In adults, serum LDL is removed mainly by hepatic LDL receptors. In growing children, however, not only the liver but also peripheral tissues play an
important role in uptake of serum LDL. The cholesterol demand of various tissues is much greater in children than in adults. Instead of de novo cholesterol synthesis, peripheral cells incorporate and use serum LDL as a source of cholesterol. The in vitro and in vivo results suggest that growth hormone (GH) increases the expression of LDL receptor (25–27). In our previous studies, GH treatment reduced the LDL-C concentration significantly in GH-deficient children and small-for-gestational age children without catch-up growth (28, 29). In the current study, LDL-C concentrations were inversely correlated with age in the citrin-deficient children (Fig. 2A), which is consistent with our hypothesis. If this is the case, LDL-C concentrations may increase again in citrin-deficient adults. This remains to be determined in future studies.

The high HDL-C concentrations in citrin-deficient children are likely to be due to enhanced catabolism of cholesterol in peripheral tissues. In this study, 27-OHC concentrations were elevated in citrin-deficient children (Table 2). In the alternative bile acid synthesis pathway, 27-OHC is converted directly from cholesterol by sterol 27-hydroxylase, a ubiquitously expressed enzyme. In vitro experiments have shown that 27-OHC is the ligand of liver X receptor α, and activated liver X receptor α upregulates the messenger RNA levels of apoA-I and ATP-binding cassette transporter A1 (30, 31). ApoA-I is the major constituent of HDL particles, and ATP-binding cassette transporter A1 promotes apoA-I–dependent cholesterol efflux from macrophages. Because 27-OHC had positive correlations with HDL-C and apoA-I concentrations (Fig. 3A and 3B, right panel), the elevated HDL levels may reflect increased cholesterol catabolism in peripheral tissues.

We cannot quite rule out the possibility that a low-carbohydrate and high-fat diet might play a role in increased HDL-C concentrations in citrin-deficient children. In overweight male subjects (body mass index, 25 to 27 kg/m²), a low-carbohydrate and high-fat diet (10% to 15% energy from carbohydrate and 640 mg/d dietary cholesterol) increased HDL-C concentrations (32). In a recent meta-analysis, 5 of 13 studies of obese patients found that a low-carbohydrate diet (<50 g carbohydrate/d) decreased body weight and increased HDL-C concentrations (33). Improved insulin resistance would make a considerable contribution to such HDL-C–raising effects of a low-carbohydrate diet. However, it is difficult to compare the effect of normal and low-carbohydrate diets on HDL-C concentrations in citrin-deficient children because they are not obese and cannot eat a normal-carbohydrate diet.

We speculate that cholesterol synthesis is increased in the brain of citrin-deficient children (especially at younger ages), which stimulates cholesterol elimination from the brain. In the brain of adults, cholesterol turnover is very slow; the half-life is 5 years (34). In the brain of children, in contrast, a considerable amount of cholesterol is physiologically synthesized for myelination. In citrin-deficient children, ketone bodies are produced due to consumption of a lipid-rich diet by means of a compensatory activation for the MC shuttle (Fig. 1). In fact, we reported recently that the mean serum 3-OH butyrate concentration was 3.0-fold higher in citrin-deficient children than in controls (134 ± 164 μmol/L vs 45 ± 52 μmol/L, P < 0.05) (5). Ketone bodies can cross the blood-brain barrier by active transport by monocarboxylic acid transporter 1 (35). Monocarboxylic acid transporter 1 is also expressed in neurons and glial cells, where ketone bodies are reconverted to acetyl-CoA and used for de novo cholesterol synthesis (36). A ketogenic diet has been used to treat not only refractory epilepsy but also neurodegenerative disorders, such as Alzheimer disease and Huntington disease (37). The ketogenic diet not only improves energy metabolism but also inhibits inflammation, apoptosis, and oxidative stress in neuronal cells (37). Furthermore, recent findings suggest that 3-OH butyrate reduces the activity of histone deacetylase and suppresses oxidative stress via the regulation of several transcriptional factors, and its derivative has been found to prevent mitochondrial damage in in vitro and in vivo models (38, 39). Although these reports strongly suggest that ketone bodies are metabolized in the brain, it is still unclear whether acetyl-CoA is increased by a ketogenic diet and used for cholesterol synthesis in the brain.

To maintain cholesterol homeostasis in the brain, excess cholesterol is converted to 24S-OHC by cholesterol 24S-hydroxylase in neurons and glial cells (17). Because 24S-OHC is oxidized at the side chain, this cholesterol metabolite can cross the blood–brain barrier by diffusion (40). It may be relevant that 24S-OHC concentrations were markedly higher in the citrin-deficient children than in the control children, but at around 13 years of age decreased to similar concentrations to those of the control children (Fig. 2K). Similarly, in patients with Alzheimer disease, 24S-OHC levels are high in the preclinical phase but low in a later phase (41). Moreover, we have reported that 24S-OHC concentrations are negatively correlated with phenylalanine concentrations in adults with phenylketonuria (42). A longitudinal study is needed to determine whether the decline in 24S-OHC concentrations in later life is a physiological phenomenon or reflects brain damage in citrin-deficient children.

Our study had the following limitations. First, the sample size was too small to carry out a comprehensive
analysis of the genotype–phenotype correlation. Citrin deficiency is a rare congenital disease caused by various mutations of the SLC25A13 gene. Even siblings with identical mutations do not exhibit the same phenotypes (43). It is possible that unspecified modulating factors—including food intake, alcohol consumption, and oxidative stress—have a greater impact on the phenotypes of citrin-deficient children than do genetic factors (44). A large-scale longitudinal clinical study is warranted. Second, we could not directly examine ketone body metabolism in the brain of the citrin-deficient children; the cause of the elevated 24S-OHC concentrations thus remains to be determined. To reproduce the metabolic environment of the brain of citrin-deficient children, in vitro experiments using induced pluripotent stem cells may be useful.

In this study, conclusions that HDL-C concentrations are elevated in citrin-deficient children during the post-NICCD period, and that cholesterol synthesis and elimination are markedly accentuated in the liver, and probably also in the brain. Serum sterol concentrations can be used to assess compensatory changes in cholesterol metabolism in citrin-deficient children.

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