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Analysis of daily energy, protein, fat, and carbohydrate intake in citrin-deficient patients: Towards prevention of adult-onset type II citrullinemia

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ABSTRACT

Patients with citrin deficiency during the adaptation/compensation period exhibit diverse clinical features and have characteristic diet of high protein, high fat, and low carbohydrate. Japanese cuisine typically contains high carbohydrate but evaluation of diet of citrin-deficient patients in 2008 showed a low energy intake and a protein:fat:carbohydrate (PFC) ratio of 19:44:37, which indicates low carbohydrate consumption rate. These findings prompted the need for diet intervention to prevent the adult onset of type II citrullinemia (CTLN2). Since the publication of the report about 10 years ago, patients are generally advised to eat what they wish under active dietary consultation and intervention. In this study, citrin-deficient patients and control subjects living in the same household provided answers to a questionnaire, filled-up a maximum 6-day food diary, and supplied physical data and information on medications if any. To study the effects of the current diet, the survey collected data from 62 patients and 45 controls comparing daily intakes of energy, protein, fat, and carbohydrate. Food analysis showed that patient's energy intake was 115% compared to the Japanese standard. The confidence interval of the PFC ratio of patients was 20–22:47–51:28–32, indicating higher protein, higher fat and lower carbohydrate relative to previous reports. The mean PFC ratio of female patients (22:53:25) was significantly different from that of male patients (20:46:34), which may explain the lower frequency of CTLN2 in females. Comparison of the present data to those published 10 years ago, energy, protein, and fat intakes were significantly higher but the amount of carbohydrate consumption remained the same. Regardless of age, most patients (except for adolescents) consumed 100–200 g/day of carbohydrates, which met the estimated average requirement of 100 g/day for healthy individuals. Finally, patients were generally not overweight and some CTLN2 patients were underweight although their energy intake was higher compared with the control subjects. We speculate that high-energy of a low carbohydrate diet under dietary intervention may help citrin-deficient patients attain normal growth and prevent the onset of CTLN2.

1. Introduction

Citrin deficiency is caused by mutations of the *SLC25A13* gene on chromosome 7q21.3, which encodes the aspartate glutamate carrier protein in the mitochondrial inner membrane of hepatic, renal, cardiac, and intestinal cells [1–4]. Citrin deficiency is associated with various metabolic abnormalities in the liver, including; *1)* shortage of aspartate supply from the mitochondria to the cytoplasm, causing high citrulline and high ammonia due to impaired urea synthesis cycle, *2)* impairment

Abbreviations: AGC, aspartate-glutamate carrier; AMDR, Acceptable Macronutrient Distribution Range; CTLN2, adult-onset type II citrullinemia; MCT, Medium-chain triglyceride; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; NADH, reduced nicotinamide adenine dinucleotide; NAD, oxidized nicotinamide adenine dinucleotide; PFC ratio, protein, fat, carbohydrate ratio.

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of glycolysis caused by the high cytosolic reduced nicotinamide adenine dinucleotide/oxidized nicotinamide adenine dinucleotide (NADH/NAD^+) ratio, following the incomplete malate-aspartate shuttle activation caused by citrin deficiency, *3)* inhibition of gluconeogenesis as a result of disturbances in the NADH / NAD^+ balance (stoichiometry), and *4)* increase in hepatic levels of fatty acids, glycerol-3-phosphate, and steatogenesis [5,6].

Adult-onset type II citrullinemia (CTLN2) is characterized by hyperammonemia, citrullinemia, liver dysfunction, liver steatosis, and neuropsychiatric impairments such as disorientation, delirium, mental derangement, and episodes of sudden unconsciousness [5,7–9]. Citrin deficiency was initially thought to affect only adults but mutations in the *SLC25A13* gene were identified early this century in neonates with intrahepatic cholestasis and citrullinemia [10–12], a condition to be named later as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). The majority of patients with NICCD present with jaundice, acholic stool, failure to thrive, hypoproteinemia, hypoglycemia, and multiple amino acidemias, such as citrullinemia [13–16]. These conditions almost disappear between 6 and 12 months of age following conservative treatment with medium-chain triglyceride (MCT) milk, lactose-free milk, and lipid-soluble vitamins. However, some cases progress to severe hepatic dysfunction that may require liver transplantation [13,17].

In the past it was thought that the majority of pediatric patients after NICCD period had no notable clinical symptoms nor problems with adaptation and compensation for the metabolic disorders except for a preference to high protein, high fat, and low carbohydrate diet [5]. However, recent studies have shown that various clinical symptoms and signs, such as hypoglycemia, fatigue, failure to thrive, and fatty liver can affect the quality of life of these individuals [9,18,19]. In fact, these symptoms may be observed relatively frequently among patients whose condition is not well-maintained. The poor maintenance of the physical condition leads to pre-CTLN2 symptoms including weight loss, anorexia, psychiatric symptoms, hyperammonemia, and citrullinemia [9,20–22]. Failure to thrive with dyslipidemia caused by citrin deficiency (FTTDCD) is recently reported in poorly controlled patients [22]. It is estimated that less than 10% of patients with citrin deficiency progress to CTLN2 [5,23], which actually indicates that many patients can remain in the silent period (known as the adaptation/compensation period) only with mild symptoms throughout their lives. It is important for patients to maintain a good physical condition during the adaptation/compensation period to prevent the future development of CTLN2 [18,19,24].

When CTLN2 was first identified, several case reports described the strong preference of patients for high-protein/high-fat food, such as peanuts and meat, and aversion of high-carbohydrate food, such as bread and rice [5], but the exact meaning of this unusual diet remains unclear. Recent studies reported that alcohol, high-glucose/high-calorie infusion, glycerol infusion, and high-carbohydrate diet induce CTLN2 [9,25–27]. In contrast, a high-protein/high-fat/low-carbohydrate diet prevents the onset of CTLN2 [9,20,21]. Diet therapy, in addition to possible use of arginine, sodium pyruvate, and medium-chain triglyceride (MCT), has become the main treatment for CTLN2 before liver transplantation [28–32]. This unique regimen may counterbalance the metabolic dysfunction caused by citrin deficiency.

In 2008, Saheki et al. [33] analyzed the nutrition of 18 patients with citrin deficiency aged 1–33 years. Their study reported high protein (19%), high fat (44%), and low carbohydrate (37%) in calorie-based nutrition ratio. Since that study, the importance of high-protein/high-fat/low-carbohydrate diet in maintaining the well-being of patients with citrin deficiency under active dietary intervention has been acknowledged. Similarly, Nakamura et al. [30] also reported a similar diet pattern by nutritional analysis of 5 patients aged ≥39 who developed CTLN2 and later recovered successfully.

The purpose of the present study was to provide an updated evaluation of food and nutrition intake of patients, compared with the control living in the same household, and offer new dietary recommendations to prevent the onset of CTLN2.

2. Materials and Methods

2.1. Study Design and Ethics

This is a cross-sectional study. A survey package including food diary and questionnaire on diagnosis, clinical phenotype at the time of diagnosis, physical data, and current treatment was distributed to patients through six large medical institutions in Japan and the Patient Association of Citrullinemia, which serves as the Citrin Deficiency Patient Association in Japan. The participants were asked to use the food diary for 3–6 days and fill out the survey on one occasion between the time the package was received and time of submission. Surveys were completed between March 2018 and March 2019 were analyzed and compared between patients of citrin deficiency and the control.

The study was approved by the Ethics Committee of Hyogo College of Medicine, and was carried out in accordance with the principles of the Declaration of Helsinki. All subjects and/or guardians gave written informed consent.

2.2. Study Subjects

Sixty-seven patients from 58 families diagnosed with citrin deficiency by genetic analysis and 52 control subjects enrolled in this study. The family members living in the same household served as the control subjects. The selection of the control group from the same household was based on the premise that evaluation of the diet under the same household allows elimination of various potentially biased factors arising from regional or familial characteristics of food culture and averaging out the evaluation bias by dietitians. Due to the incompletion of the survey in some subjects, the actual numbers of participants in this study became 62 patients (36 males, 26 females) aged 2–63 (average: 18 years) and 45 control subjects (22 males, 23 females) aged 2–61 (average: 25 years). Furthermore, 39 of the 43 patients aged 2–16 years had been diagnosed with NICCD during infancy, which allowed early intervention. The remaining 4 patients were diagnosed during the adaptation/compensation period. Out of 10 patients aged 17–39 years, 3 were diagnosed with NICCD during infancy, 5 during the adaptation/compensation period, and 2 were diagnosed with CTLN2 during adulthood. Among the 9 patients aged ≥40 years, 8 were diagnosed with CTLN2 and 1 was in the adaptation/compensation period. None of the CTLN2 patients had undergone liver transplantation at the time of the study. All patients had received nutrition counseling, and some patients were being treated with MCT oil ($n = 12$), sodium pyruvate ($n = 10$), or arginine ($n = 10$).

2.3. Nutritional Evaluation

We conducted nutritional evaluation based on the 3–6 day food diary provided by the patients and control subjects between March 2018 and March 2019, and assessed the energy contents and macronutrients (namely protein, fat, and carbohydrate) using the Standard Tables of Food Composition in Japan 2015 [34]. The average length of the diary was 4.6 ± 1.8 days (mean ± SD). The intake of energy, protein, fat, and carbohydrate was converted into percentage using the National Health and Nutrition Survey in Japan 2016 as reference values (100%) per sex and age [35] (Supplemental Table S1). The nutritional value of MCT oil (mostly at a dose of 8–10 ml/day) was included in the calculation.

2.4. Anthropometry

The height and weight used are based on the survey data collected from subjects. The obesity index was used as the anthropometric parameter for subjects younger than 17 to define overweight and

underweight. The following formula was used: Obesity index (%) = [(measured weight − standard weight)/standard weight] × 100. The standard weight based on height was defined in the Ministry of Health, Labor and Welfare's report on infant physical development published in 2000 (for 0–6 years old) and the Ministry of Education, Culture, Sports, Science and Technology's report on school health in 2000 (for 6–17 years old). The median weight for height was regarded as the standard weight. A cut off value of ±15% of the median weight for height was used to define underweight and overweight for subjects aged less than 7 years and −15% for underweight and + 20% for overweight in subjects aged ≥7.

On the other hand, body mass index (BMI) was used as the anthropometric parameter for subjects over 17 years and was calculated with the survey data, using the following formula: BMI = weight (kg) ÷ height^2 (m). Underweight was defined as BMI ≤18.5 kg/m^2 for subjects aged below 49, and ≤20.0 kg/m^2 for subjects aged over 50. Overweight was defined as ≥BMI 25 kg/m^2.

2.5. Statistical Analysis

Differences between the patient group and the control group were examined for statistical significance using the two-tailed Student's *t*-test on Excel 2016 of Microsoft Office. A *p* value less than 0.05 was considered statistically significant.

3. Results

3.1. Energy and Macronutrients Intake

Fig. 1 shows a scatter plot of energy, protein, fat, and carbohydrate intakes of the patients and controls using the National Health and Nutrition Survey in Japan 2016 as a reference. Table 1 shows the mean intakes of energy, protein, fat, and carbohydrate for all ages and age groups of 2–16, 17–39, and ≥40 of patients and the control.

3.1.1. Energy Intake

The energy intake of the entire group of patients and that of the 2–16 age group were 115% and 122% of the Japanese standard, respectively, and both were significantly higher than those of the control subjects (98% and 108%, respectively; Fig. 1, Table 1). On the other hand, there were no significant differences in these parameters between the patients and controls for ages 17–39 and ≥ 40 groups. However, the energy intake of patients older than 17 years (combined age groups 17–39 and ≥40) was significantly higher than that of the age-matched control ($p < 0.05$). The differences in energy intake between the patients and controls were 14% for the 2–16 group, 14% for the 17–39 group, and 13% for the ≥40 group (Table 1). In other words, the energy consumption was higher in the patients, regardless of age, relative to the control subjects.

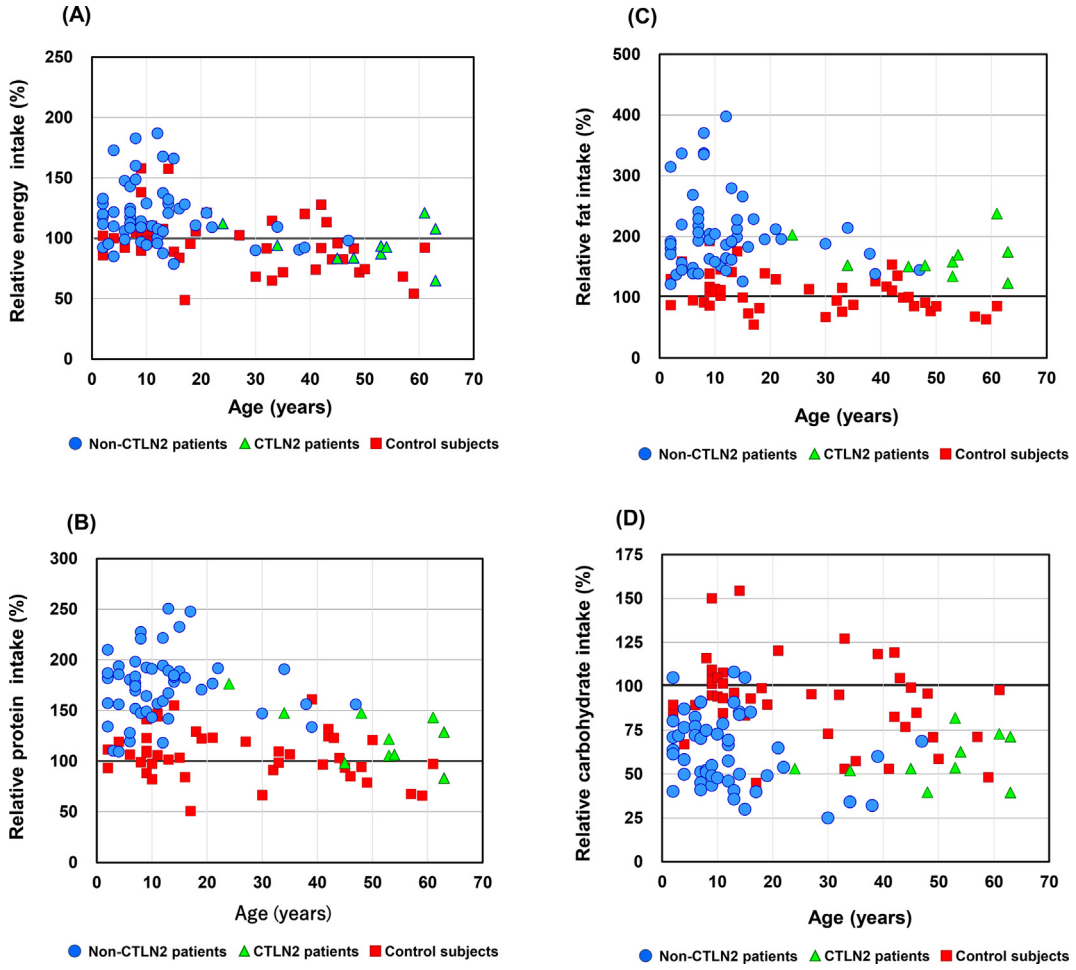

Fig. 1. Scatter plot of intake of energy and the three major food components by age in patients and control subjects. Reference values per age and sex for Japanese standard energy, protein, fat, and carbohydrate are those of the National Health and Nutrition Survey 2016 [35] (Supplemental Table S1). The relative intake of each subject was calculated with the following formula: Relative intake (%) = Absolute intake of each subject/ absolute Japanese standard intake x 100. The distribution of the intake of energy (A), protein (B), fat (C), and carbohydrate (D) is shown in blue circles for citrin deficient patients who did not develop CTLN2, green triangles for CTLN2 patients, and red squares for the control subjects.

Table 1
Intake of energy, protein, fat, and carbohydrate stratified by age in citrin-deficient patients and control subjects using the Japanese standard set as 100%.

Age (years)	Energy (%)	Protein (%)	Fat (%)	Carbohydrate (%)
Patients				
Total (n = 62)	115 ± 26¶	166 ± 36 ¶	199 ± 62¶	62 ± 20¶
2–16 (n = 43)	122 ± 26*	174 ± 32¶	209 ± 68¶	67 ± 20¶
17–39 (n = 10)	106 ± 13	174 ± 32¶	188 ± 29¶	46 ± 13¶
≥40 (n = 9)	99 ± 16	121 ± 25*	161 ± 33¶	60 ± 15*
Control subjects				
Total (n = 45)	98 ± 23	107 ± 24	110 ± 33	92 ± 24
2–16 (n = 21)	108 ± 21	113 ± 22	123 ± 36	101 ± 20
17–39 (n = 11)	92 ± 25	107 ± 30	99 ± 28	88 ± 28
≥40 (n = 13)	86 ± 19	99 ± 21	98 ± 26	82 ± 21

Values are mean ± SD.
*$p < 0.05$, ¶$p < 0.001$, between patients and control subjects of the same age group (by Student's *t*-test).

3.1.2. Protein Intake

The protein intake was significantly higher in the patients' group (166% of the Japanese standard) than that of the control group (107%; Fig. 1, Table 1). The protein intake was significantly higher in patients aged 2–16 and 17–39 (174% and 172%, respectively) and tended to be higher in the ≥40 group (121%), compared with controls. The differences in protein intake between patients and controls were 61% for the 2–16 age group, 67% for the 17–39 group, and 22% for the ≥40 group (Table 1).

3.1.3. Fat Intake

The fat intake was significantly higher in the whole patient group (199% of the Japanese standard) than the control group (110%; Fig. 1, Table 1). In particular, the fat intake was significantly higher in patients aged 2–16 and 17–39 (209%, 188%, respectively), and to a lesser extent in the ≥40 group (161%). The differences in the fat intake between the patients and controls were 86% for the 2–16 group, 89% for the 17–39 group, and 63% for ≥40 group (Table 1).

3.1.4. Carbohydrate Intake

The carbohydrate intake was significantly lower in the patients' group (62% of the Japanese standard) than the control group (92%; Fig. 1, Table 1). The lowest intake (46%) was in patients aged 17–39. The differences in carbohydrate intake between the patients and controls were − 44% for the 2–16 age group, −40.0% for the 17–39 group, and −22% for the ≥40 group.

Fig. 2 shows a scatter plot of daily carbohydrate intake (in grams) in patients and control subjects. In the latter group, carbohydrate intake was above 100 g/day in both sexes irrespective of age, with the highest intake of 380 ± 122 g/day in the 12–16 male group, together with high energy intake (Table 2). In patients, the carbohydrate intake generally ranged between 100 and 200 g/day in both sexes with the exception of 273 ± 95 g/day in the 12–16 male group on the high end (Table 2) and 60.4 g/day (2-year-old boy), 55.2, 76.0, and 71.3 g/day (3 females in their 30s) on the low end. The low levels in the latter three patients contributed to the mean low intake recorded in the 17–39 age group (48.5% of the Japanese standard).

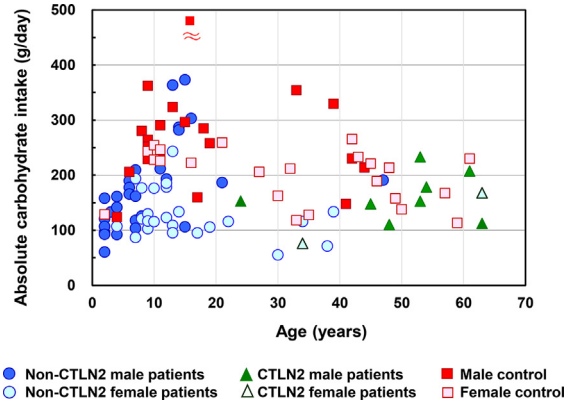

Fig. 2. Scatter plot of absolute daily carbohydrate intake by age in patients and control subjects.

3.2. Energy Intake and Protein, Fat, and Carbohydrate (PFC) Ratio by Age and Sex

We examined the energy intake and the PFC ratio in patients and control subjects stratified by sex (Table 3). The average energy intake in patients was 115% of that of the Japanese general population and no difference was observed between male and female patients. In contrast, a sex difference (male>female) was noted in energy intake in the control group. In this study, as younger subjects tended to consume more energy than older subjects, compared to the Japanese standards, it was assumed to be related to differences in age (average age, females: 31.4, males: 19.6).

The PFC ratio of patients included higher protein, higher fat, and lower carbohydrate than that of the control. Interestingly, the PFC ratio was significantly different between the male and female patients, with the latter showing 1.8% higher protein, 7.0% higher fat, and 8.8% lower carbohydrate, relative to the male patients. There was no notable difference in the PFC ratio between male and female control subjects.

Table 4 shows the PFC ratio stratified by age in patients and control subjects. Subjects aged 2–16 were divided into three groups to illustrate the differences in more detail. The PFC ratio showed a similar trend in the 2–6 and ≥40 age groups, with relatively lower protein, lower fat, and higher carbohydrate ratios than the other ages. No such age differences were noted in the control subjects.

3.3. Effects of Dietary Therapy on Energy Intake and PFC Ratio Over 10-Year Period

There was no active dietary consultation and intervention while the patients' rather distinct food preference and aversion were somewhat recognized for some years since the reporting of the SLC25A13 variants. In 2008, Saheki et al. first reported the unique PFC ratio of patients with citrin deficiency [33] and the report became the starting point for dietary intervention. We compared the data available for 43 patients and 21 controls aged 2–16 from the year 2018 with those of 16 patients aged 2–15 from the year 2008 reported by Saheki et al. [33], using the Japan National Health and Nutrition Survey in 2016 as a reference [35]. Fig. 3 illustrates the dietary differences between the two 10-year apart studies. The energy, protein, and fat intakes were significantly higher in our patients from 2018 than our control group and the 2008 patients. On the other hand, while the carbohydrate intake was lower in our patients than the control, it was almost the same in 2008 and 2018 patients. The protein and fat in the PFC ratio were higher in 2008 patients than in the Japanese general population. The amount of the absolute intakes of protein and fat were similar in 2008 patients and 2018 control, while the absolute intake of carbohydrate was lower in 2008 patients than 2018 controls, which contributed to the lower total energy intake. Of note, there was no significant change in the standard Japanese diet itself between 2008 and 2018 that needs to be taken into consideration for the discussion of the present study.

3.4. Anthropometry

Fig. 4A shows the obesity index of 2-16-year-old patients and control subjects. The mean obesity index was similar in the patients (0.1 ± 9.0%, n = 43) and the control subjects (3.8 ± 12.5%, n = 21). There were 3 underweight subjects (obesity index: −15%, −15%, and

Table 2
The averaged daily absolute intake of energy and three major food components by age in citrin-deficient patients and control subjects.

Age (years)	Patients					Controls				
	n	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	n	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Males										
2–6	3	1484	69.4	75.9	130.5	3	1254	51.1	48.7	152.7
7–11	6	2171	115.0	121.1	155.1	8	2098	78.3	78.8	269.0
12–16	7	3070	161.1	148.4	272.6	3	2893	104.1	106.3	379.8
17–39	2	2481	133.2	141.1	169.7	5	2098	87.3	70.5	277.3
≥40	8	1937	91.8	100.4	166.6	3	1749	80.4	70.9	197.3
Females										
2–6	1	1589	85.4	91.1	107.3	1	1002	37.8	37.3	128.8
7–11	9	2133	105.6	129.8	135.5	5	1843	72.3	66.1	239.7
12–16	7	2450	130.2	146.6	152.6	1	1532	55.5	46.6	222.5
17–39	8	1745	107.3	103.5	96.1	6	1444	61.7	52.6	180.9
≥40	1	1878	85.9	96.0	167.6	10	1487	60.8	52.5	192.9

Table 3
Energy intake and PFC ratio by sex for citrin-deficient patients and control subjects.

	Energy (%)		Protein (%)		Fat (%)		Carbohydrate (%)	
	Mean	CI	Mean	CI	Mean	CI	Mean	CI
Patients								
Total (n = 62)	115	109–122	20.9	20–22	49.1	47–51	30.1	28–32
Males (n = 36)	114	105–122	20.1*	19-21	46.2¶	44–48	33.7¶	31–36
Females (n = 26)	117	106–129	21.9	21–23	53.2	51–56	24.9	22–28
Control subjects								
Total (n = 45)	98	91–104	16.2	15–17	32.6	31–34	51.2	50–53
Males (n = 22)	104*	93–115	15.9	15-17	33.2	31–36	50.8	48–54
Females (n = 23)	91	83–100	16.5	15–18	32	31–33	51.6	50–54

*$p < 0.05$ and ¶$p < 0.001$, between males and females (by Student's *t*-test.).
CI: confidence interval.

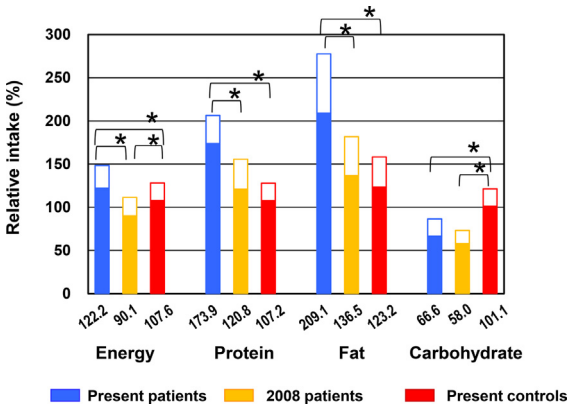

Fig. 3. Comparison of intake of energy and three macronutrients in patients of the present study in 2018, patients of the 2008 study, and control subjects of the present study in 2018. The standard intake in Japan according to age and sex in the 2016 National Health and Nutrition Examination Survey (Supplementary Table S1) [35] was calculated as 100%. The horizontal axis represents the average value of each bar. The open box shows the range of one standard deviation. *$p < 0.05$.

Table 4
PFC ratio stratified by age in citrin-deficient patients and control subjects.

Age (years)	Protein (%)	Fat (%)	Carbohydrate (%)
Patients			
2–6 (n = 14)	19.3 ± 2.8^{a}	45.4 ± 8.1^{ab}	35.3 ± 8.3^{A}
7–11 (n = 15)	20.7 ± 2.8^{a}	52.2 ± 8.0^{B}	27.1 ± 7.7^{ab}
12–16 (n = 14)	21.7 ± 4.3	48.4 ± 6.5	29.9 ± 8.1^{b}
17–39 (n = 10)	23.8 ± 2.3^{A}	52.8 ± 5.3^{A}	23.4 ± 6.6^{ab}
≥40 (n = 9)	19.0 ± 3.0^{a}	46.6 ± 4.7^{a}	34.4 ± 5.6^{B}
Control subjects			
2–6 (n = 4)	15.9 ± 0.9	34.1 ± 8.3	50.0 ± 8.8
7–11 (n = 13)	15.2 ± 2.2	32.9 ± 4.4	51.9 ± 5.2
12–16 (n = 4)	14.5 ± 1.2	31.8 ± 3.9	53.7 ± 3.6
17–39 (n = 11)	17.0 ± 3.0	31.7 ± 3.8	51.1 ± 5.5
≥40 (n = 13)	17.0 ± 2.9	32.8 ± 4.2	50.2 ± 5.7

Data are mean ± SD.
A significant difference ($p < 0.05$) by Student's *t*-test is marked in letters (A, B, a, b) for each nutrient.
Capital letter A denotes significantly higher value than those marked with (a) within the same nutrient. Capital letter B denotes significantly higher value than those marked with (b) within the same nutrient.
For example, protein % is significantly higher in 17–39 years old patient group (marked 'A') than in 2–6, 7–11, and ≥ 40 years old patient groups (marked 'a').

−18%, energy intake ratio: 109%, 97%, and 87%, respectively) and 1 overweight subject (obesity index: 25%, energy intake: 106%) in the patient group while there were no underweight subject and 2 overweight subjects (obesity index: 20% and 40%, energy intake: 84% and 158%, respectively) in the control group. The proportion was not significantly different between the patient and control groups.

Fig. 4B shows the BMI of patients and control subjects aged ≥17 years. The mean BMI of patients (n = 19, 20.7 ± 2.5 kg/m^2) was not significantly different from that of the control (n = 24, 21.6 ± 3.6 kg/m^2). The patient group included 7/19 underweight subjects, a proportion not significantly different from that of the control group (4/24). The patient group did not include any overweight subject while the control group included 4 subjects, and the proportion was not significantly different between the two groups, either. Of the 9 patients aged ≥40, 8 developed CTLN2, and 5 of them were underweight.

In summary, the number of overweight subjects in the patient group was 1/62 while in the control group was 6/45, and the number of underweight subjects in patients was 10/62 while in controls was 4/45. It suggests that there are significantly more overweight subjects in the control group than in the patient group.

4. Discussion

The typical Japanese cuisine contains high carbohydrate in general (PFC ratio 15:30:55) [35]. This often resulted in smaller food consumption in patients to take less carbohydrate, which was naturally associated with less energy intake in the absence of active nutrition consultation and intervention for citrin deficiency. In fact, Saheki et al. [33] reported that the PFC ratio of patients during the adaptation/

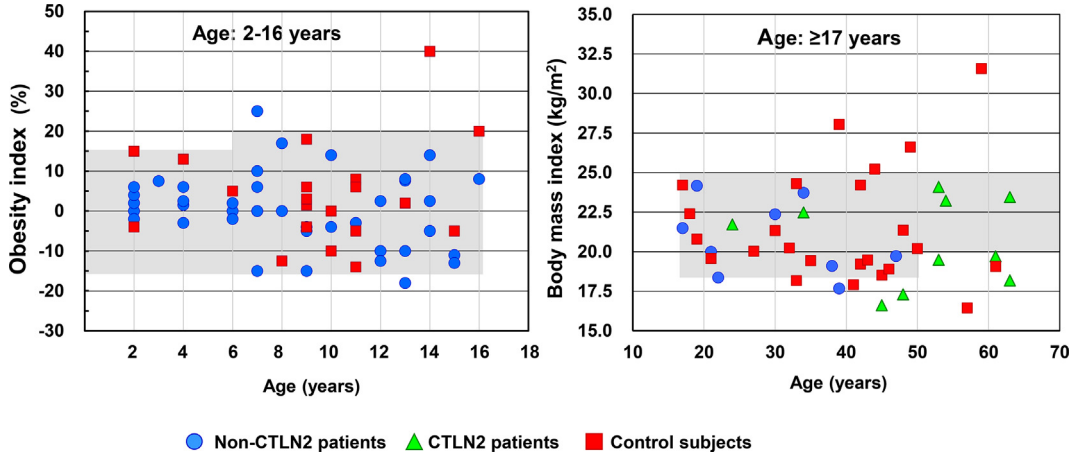

Fig. 4. Scatter plot of physical constitution of patients with citrin deficiency and the control by age. Gray area indicates the Japanese standard range.

compensatory period included high-protein (19%), high-fat (44%), low-carbohydrate (37%), but the energy intake was only 87% of the Japanese standard. Their report published at that time called for dietary intervention. Recently Pinto et al. [36] reported that the PFC ratio of the self-selected diet was 19:42:40% and the energy intake was median 86% of the estimated average requirement in most patients in the UK, and poor growth has been observed among them. It is speculated that the poor growth may be associated with the low energy intake.

The present study showed that the energy intake of patients with citrin deficiency was 115% of the Japanese standard, and it was significantly higher than that of the control group (Table 1). This may be the reflection of the recent nutrition consultation and dietary intervention for the patients implemented since early ages, with no limit to food intake. This can be clearly seen in patients aged 2–16 who had the early intervention. Their energy intake was 122% of the Japanese standard and significantly higher by 15% than the age-matched control. Despite the high energy intake, the average obesity index of this group was merely 0.1%, which was lower than that of the control (3.8%), and none of patients in this group was obese except for one. On a further note, the mean obesity index of 9 patients of this group with the excess energy consumption of over 140% of the Japanese standard was ±0% (minimum: −10%, maximum: +17%), whereas 2 controls with excess energy consumption scored 18% and 40% on the obesity index. It is speculated that patients with citrin deficiency during early childhood and adolescence need 1.2 times more energy than the Japanese standard. In contrast, the energy intake of patients of the 17–39 and ≥40 age groups was similar to that of the Japanese standard (106% and 99%, respectively). Interestingly, these patients consumed 14% and 13% more energy than the age-matched control, respectively. Yet the patients were not overweight (BMI 20.7 kg/m^2, which was similar to the control of 21.6 kg/m^2). This finding suggests that post-adolescent patients also need more energy and perhaps more energy is required for patients to grow, maintain physical status, and compensate for the metabolic failure caused by the condition.

The previous study of Kobayashi et al. [37] reported that their patients with citrin deficiency were underweight; with BMI of ≤20 kg/m^2 in 90% and ≤17.5 kg/m^2 in 40% of their patients. The weight deficiency followed poor control and preceded the CTLN2 onset, but intervention with diet therapy and treatment improves the condition [9,20,21]. Another study of CTLN2 patients without liver transplantation showed that the condition of 5 patients improved by diet therapy and medical treatment and the energy intake was increased to 112% of the Japanese standard, although 2 out of 5 remained underweight [30]. Two other studies reported 8 of 10 patients with CTLN2 were underweight at the time of onset, and 6 of them gained weight after diet therapy and MCT oil supplementation, although the BMI remained low [31,32]. In the present study, 5 out of 8 CTLN2 patients aged ≥40 were still underweight even after diet therapy and hence our results are consistent with the previous studies.

Analysis of the diet of the ≥40 patient group showed a daily absolute carbohydrate intake of 167 g/day, which was roughly similar to that of the age-matched control subjects (194 g/day; Table 2). The PFC ratio of these patients was 19:47:34, which was somewhat lower in protein and fat and higher in carbohydrates, compared to the other age groups (Table 4). In addition to being underweight, the diet of this age group seemed slightly unbalanced relative to the typical citrin-deficiency diet.

It is important to elucidate whether the illustrated diet and physical status of patients aged ≥ 40 merely reflect age-related changes or induced by CTLN2 onset. Diet, body constitution, and the lifestyle of CTLN2 patients aged ≥ 40 should be compared to the age-matched patients during the adaptation/compensation period. The findings will be important in clarifying the therapeutic index of citrin deficiency. Underweight is an important factor in the pathophysiology and severity of citrin deficiency. It is particularly important to stay within the standard weight range with sufficient energy intake and avoid toxicity from excessive carbohydrate consumption in order to prevent the onset of CTLN2.

The patients in this study under the dietary intervention consumed food high in protein (21%), high in fat (49%), and low in carbohydrate (30%; Table 3). The male-female survey found that the PFC ratio for female patients was 22:53:25%, which was significantly different from that of male patients by 1.9% higher protein, 6.8% higher fat, and 8.7% lower carbohydrate. Citrin deficiency is caused by biallelic pathogenic variants and there is no sex difference in the incidence rate of NICCD and citrin deficiency, yet the number of CTLN2 cases in males is twofold that in females [5], and no reason has been identified to explain this phenomenon. One possibility is due to the practice of less carbohydrate diet of female patients based on the findings of the present study. If so, the target PFC ratio for patients with citrin deficiency should be close to that of the female patients.

Fig. 3 shows that the average carbohydrate intake of the patients subjects of the present study was merely 67% of that of the general Japanese, which is roughly a similar result to that of the 2008 report [33]. This finding led us to speculate that patients with citrin deficiency may just satisfy the “minimum required amount” of carbohydrate regardless of the total amount of energy intake. An interesting observation in this study is that the carbohydrate consumption across the patients excluding male adolescents was similar at 100–200 g/day (138 ± 44 g/day, mean ± SD; Fig. 2, Table 2). On the other hand, it was 218 ± 62 g/day in the control subjects excluding male adolescents. Carbohydrate metabolism is different in patients with citrin deficiency. Their liver does not convert lactate to pyruvate well, causing

impairment of liver gluconeogenesis. Fat or protein can be converted to energy, but they may not be utilized enough in gluconeogenesis [5,9,19]. The estimated carbohydrate requirement of healthy individuals of all ages is 100 g/day [38–40] but the ideal amount of carbohydrate for patients has not been defined yet. It is strongly suggested that patients take 100–200 g/day carbohydrate to satisfy the minimum requirement on the smaller side of the scale and avoid carbohydrate toxicity on the bigger side of the scale. Patients with much less than 100 g/day carbohydrate consumption need close attention to avoid adverse conditions such as hypoglycemic episodes.

It is important to determine whether the energy intake and PFC ratio reported by the patients in this nutritional survey are optimal for patients with citrin deficiency. The present study only addressed the diet of the current patients and it will be the future task to define the optimal diet which would bring about the improved QOL for the patients. In addition, there is a concern that the daily intake of such a high-protein and high-fat diet may affect the health of patients. To date, there are no reports of adverse health effects by excessive protein or fat intake in healthy adults and the tolerable upper limits are not defined. One reference for protein is found in the US Acceptable Macronutrient Distribution Range (AMDR), which states the acceptable range of protein in total energy being 10 to 35% for healthy adults and 10 to 30% for 4–18 years old [38,39,41]. In our study, the average protein in the PFC ratio of patients was 21% with a maximum of 26%, which was within the acceptable range as defined by the AMDR. For fat, previous studies reported no adverse effect in healthy individuals with significant increase in the fat ratio from 10% to 50% and from 9% to 79% of the total energy [38]. In the present study, the average fat in the PFC ratio in patients was 49% with a maximum of 67% which seems acceptable according to the previous study. However, it is not uncommon for patients with citrin deficiency to have high blood cholesterol and LDL cholesterol levels as well as fatty liver [9,20,42–45]. High levels of fatty acids and glycerol-3-phosphate, and steatogenesis in the liver in response to the lack of citrin are thought to be the major factors [6,42,46] although a high-fat diet may also contribute to these conditions as well. The periodical liver examination by a hepatic ultrasonography is advised for citrin deficiency patients.

In considering a more suitable diet for patients with citrin deficiency, the mouse model of human citrin deficiency provides suggestions on diet [47–50]. In this mouse model, a low-protein/high-carbohydrate diet results in poor weight gain, while a high-protein/low-carbohydrate diet restores weight gain under a constant fat component ratio. Similar recovery effects were observed with alanine, sodium glutamate, MCT, and sodium pyruvate [47–50]. However, it is not easy to increase the protein amount only while keeping fat low with the natural food components in a low-carbohydrate diet. Protein supplements potentially solve the difficulty. In this regard, Dimmock et al. [51] reported previously that increased protein intake improved citrin deficiency. Furthermore, a favorable clinical course was achieved by maintaining the PFC ratio at 30:40:30 using protein supplementation (personal communication). It is speculated that a good diet control during the adaptation/compensation period with a high-protein/moderately high-fat/low-carbohydrate diet can potentially solve the health problems arising from high-fat food.

5. Conclusions

1) Energy intake in patients with citrin deficiency was approximately 15% higher than the standard. Adequate energy intake may be one of the factors that prevent the onset of CTLN2.
2) The confidence interval of the target PFC energy ratio should be 20%–22%:46%–53%:25%–33%. Patients should limit carbohydrate to the appropriate amount.
3) The lower carbohydrate in females diet may possibly explain the fewer cases of CTLN2 in females.
4) Correction of body weight is important in underweight patients to prevent possible progression to pre-CTLN2 or CTLN2. The BMI should be monitored carefully and longitudinally.

Declaration of Competing Interests

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2021.03.004.

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