Serum carnitine as an independent biomarker of malnutrition in patients with impaired oral intake

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Carnitine was first discovered in muscle tissue in 1905,(1) but its physiological roles remained unknown for many years. In the 1950s, carnitine was recognized as a vitamin-like compound that plays important roles in fatty acid β-oxidation and the control of the mitochondrial coenzyme A/acetyl-CoA ratio. However, carnitine is not added to ordinary enteral nutrition or total parenteral nutrition. In this study, we determined the serum carnitine concentrations in subjects receiving ordinary enteral nutrition (EN) or total parenteral nutrition (TPN) and in patients with inflammatory bowel diseases to compare its levels with those of other nutritional markers. Serum samples obtained from 11 EN and 11 TPN patients and 82 healthy controls were examined. In addition, 10 Crohn’s disease and 10 ulcerative colitis patients with malnutrition who were barely able to ingest an ordinary diet were also evaluated. Carnitine and its derivatives were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The carnitine concentrations in EN and TPN subjects were significantly lower compared with those of the control subjects. Neither the serum albumin nor the total cholesterol level was correlated with the carnitine concentration, although a significant positive correlation was found between the serum albumin and total cholesterol levels. Indeed, patients with CD and UC showed significantly reduced serum albumin and/or total cholesterol levels, but their carnitine concentrations remained normal. In conclusion, only a complete blockade of an ordinary diet, such as EN or TPN, caused a reduction in the serum carnitine concentration. Serum carnitine may be an independent biomarker of malnutrition, and its supplementation is needed in EN and TPN subjects even if their serum albumin and total cholesterol levels are normal.

Key Words: carnitine, total parenteral nutrition (TPN), enteral nutrition (EN), inflammatory bowel diseases

Introduction

Carnitine Homeostasis in the Body

Carnitine is a vitamin-like compound that plays important roles in fatty acid β-oxidation and the control of the mitochondrial coenzyme A (CoA)/acetyl-CoA ratio. However, carnitine is not added to ordinary enteral nutrition or total parenteral nutrition. In this study, we determined the serum carnitine concentrations in subjects receiving ordinary enteral nutrition (EN) or total parenteral nutrition (TPN) and in patients with inflammatory bowel diseases to compare its levels with those of other nutritional markers. Serum samples obtained from 11 EN and 11 TPN patients and 82 healthy controls were examined. In addition, 10 Crohn’s disease and 10 ulcerative colitis patients with malnutrition who were barely able to ingest an ordinary diet were also evaluated. Carnitine and its derivatives were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The carnitine concentrations in EN and TPN subjects were significantly lower compared with those of the control subjects. Neither the serum albumin nor the total cholesterol level was correlated with the carnitine concentration, although a significant positive correlation was found between the serum albumin and total cholesterol levels. Indeed, patients with CD and UC showed significantly reduced serum albumin and/or total cholesterol levels, but their carnitine concentrations remained normal. In conclusion, only a complete blockade of an ordinary diet, such as EN or TPN, caused a reduction in the serum carnitine concentration. Serum carnitine may be an independent biomarker of malnutrition, and its supplementation is needed in EN and TPN subjects even if their serum albumin and total cholesterol levels are normal.

Key Words: carnitine, total parenteral nutrition (TPN), enteral nutrition (EN), inflammatory bowel diseases

Subjects, Materials and Methods

Subjects and sample collection. In this study, we evaluated 22 patients who could not ingest orally due to Parkinson’s disease or sequelae of cerebral infarction, cerebral hemorrhage or meningoencephalitis and who had been receiving TPN (n = 11) or EN (n = 11) for a long period (2–73 months). The baseline characteristics of the patients and sex- and age-matched controls are shown...
in Table 1. The patients received an average of 30 kcal/kg/day and 1.0 g of amino acids/kg/day (range 25–35 kcal/kg/day and 0.8–1.2 g of amino acids/kg/day) from TPN or an average of 30 kcal/kg/day and 1.2 g of protein/kg/day (range 25–40 kcal/kg/day and 0.9–1.5 g of protein/kg/day) from EN. Carnitine was not added to TPN or EN.

Twenty patients with IBDs (10 CD and 10 UC) who were diagnosed by clinical, endoscopic, histopathological and radiological examinations were also evaluated. Among the 10 patients with CD, three were ileum type, and seven were ileum + colon type. One of the patients with CD had a history of ileal resection, whereas the other nine patients had no previous surgeries. Among the 10 patients with UC, one was proctitis type, four were left-side colitis type, and five were pancolitis type. The CD Activity Index score of the CD patients was 171 ± 74, and the Mayo score of the UC patients was 4.9 ± 1.5. All of the CD and UC patients were treated with 5-aminosalicylates, two CD and one UC patient were treated with corticosteroids, five CD and five UC patients were treated with 5-aminosalicylates, two CD and one UC patient were treated with azathioprine, and five CD patients were treated with corticosteroids, five CD and five UC patients were treated with 5-aminosalicylates, two CD and one UC patient were treated with azathioprine, and five CD patients were treated with corticosteroids.

Blood samples were obtained from the TPN patients in the morning and from the EN and IBD patients in the morning before breakfast after an overnight fast, and the sera were stored at −20°C until further analyses. Control sera obtained after fasting from healthy volunteers without obesity, hyperlipidemia, diabetes or liver dysfunction were collected from another study group (courtesy of Professor T. Teramoto, Teikyo University). Informed consent was obtained from all of the subjects, and the experimental protocol was approved by the Ethics Committee of Tokyo Medical University Ibaraki Medical Center.

Materials. Acetyl-L-carnitine HCl and palmitoyl-L-carnitine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), and acetyl-L-[3H]carnitine HCl and palmitoyl-L-[3H]carnitine HCl were obtained from C/D/N Isotopes Inc. (Quebec, Canada). L-[3H]carnitine was synthesized by the alkaline hydrolysis of acetyl-L-[3H]carnitine in 1 N aqueous NaOH at 60°C for 1 h, subsequent neutralization with 1 N HCl, and extraction with 10 volumes of ethanol. L-carnitine and any additional reagents and solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan). MEDIF® SOY BAG was obtained from Ajinomoto Co., Inc. (Tokyo, Japan), ISOCAL® 2K Neo was purchased from Nestle Health Science Company (Tokyo, Japan), A1.5 and MA-R2.0 were obtained from Clinico Co., Ltd. (Tokyo, Japan), ENSURE LIQUID® was obtained from Abbott Japan Co., Ltd. (Tokyo, Japan), RACOL®-NF was purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan), and Meibalance® 1.0 and Inslow® were purchased from Meiji Co., Ltd. (Tokyo, Japan).

Determination of serum carnitine and acetylcarnitine concentrations. The carnitine and acetylcarnitine concentrations in serum and EN were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method was adapted from a report by Ghoshal et al. and was modified as follows. Five microliters of serum or EN was placed in a microcentrifuge tube (1.5 ml), and 25 ng of [1H3]carnitine and 12.5 ng of acetyl-[1H3]carnitine in 50 μl of acetonitrile-water (19:1, v/v) containing 0.1% formic acid were added as an internal standard. The sample tube was vortexed for 1 min and centrifuged at 2,000 × g for 1 min, and the liquid phase was collected and evaporated to dryness at 80°C under a nitrogen stream. The residue was redissolved in 65 μl of 0.1% aqueous formic acid solution, and an aliquot (1 μl) was analyzed using LC-MS/MS. The LC-electron spray ionization (ESI)-MS/MS system consisted of a TSQ Vantage triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an HESI-II probe and a Prominance ultra fast liquid chromatography (UFLC) system (Shimadzu, Kyoto, Japan). Chromatographic separation was performed using a Hypersil GOLD aQ column (150 × 2.1 mm, 3 μm, Thermo Fisher Scientific) at 40°C. The mobile phase consisted of methanol-water (1:9, v/v) containing 0.1% formic acid and was used at a flow rate of 200 μl/min. The MS/MS conditions were as follows: spray voltage, 3,000 V; vaporizer temperature, 450°C; sheath gas (nitrogen) pressure, 50 psi; auxiliary gas (nitrogen) flow, 15 arbitrary units; ion transfer capillary temperature, 220°C; collision gas (argon) pressure, 1.0 mTorr; collision energy, 20 V; ion polarity, positive; and selected reaction monitoring (SRM), m/z 162 → m/z 103 and m/z 204 → m/z 85 for carnitine and acetylcarnitine, respectively, and m/z 165 → m/z 103 and m/z 207 → m/z 85 for the [1H3] variants.

Table 1. Characteristics of patients who received EN or TPN

| Control (n = 20) | EN (n = 11) | TPN (n = 11) |
|-----------------|------------|-------------|
| Age (years)     | 79.5 ± 5.9 [72–92] | 79.8 ± 6.4 [71–89] | 81.8 ± 6.8 [63–93] |
| Gender (male/female) | 9/11       | 4/7         | 5/6         |
| Duration of no oral intake (months) | —         | 22.7 ± 22.8 [2–73] | 10.0 ± 9.1 [2–31] |
| Albumin (g/dl)  | 4.4 ± 0.3 [3.7–4.7] | 3.4 ± 0.3* [2.8–3.9] | 3.0 ± 0.5* [2.0–3.9] |
| Total cholesterol (mg/dl) | 191 ± 25 [112–249] | 165 ± 40 [113–257] | 115 ± 37* [57–187] |

The data are expressed as the means ± SD [range]. EN, enteral nutrition; TPN, total parenteral nutrition. *p<0.001, significantly different from the control. †p<0.05, significantly different from the EN.

Table 2. Characteristics of patients with IBDs

| Control for CD (n = 20) | CD (n = 10) | Control for UC (n = 20) | UC (n = 10) |
|------------------------|------------|-------------------------|-------------|
| Age (years)            | 32.3 ± 8.5 [25–46] | 32.3 ± 10.2 [22–51] | 45.8 ± 14.5 [29–69] | 45.2 ± 17.5 [26–76] |
| Gender (male/female)   | 15/5       | 8/2                     | 11/9        | 6/4         |
| Albumin (g/dl)         | 4.3 ± 0.3 [3.5–4.8] | 3.4 ± 0.3* [2.8–3.9] | 4.4 ± 0.3 [3.6–4.7] | 3.9 ± 0.4* [2.9–4.4] |
| Total cholesterol (mg/dl) | 182 ± 37 [121–272] | 120 ± 37* [105–181] | 180 ± 43 [124–284] | 174 ± 33 [118–221] |
| CRP (mg/dl)             | 0.02 ± 0.02 [0.01–0.42] | 1.77 ± 0.82 [0.01–4.71] | 0.03 ± 0.01 [0.01–0.38] | 1.56 ± 0.59 [0.02–4.58] |
| ESR (mm/h)              | 3.5 ± 1.7 [1.0–4.0] | 23.5 ± 7.5 [3.0–4.10] | 3.4 ± 1.6 [1.0–5.0] | 19.7 ± 7.2 [2.0–38.0] |

The data are expressed as the means ± SD [range]. IBDs, inflammatory bowel diseases; CD, Crohn’s disease; UC, ulcerative colitis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. *p<0.01, significantly different from the control. †p<0.005, significantly different from the control. ‡p<0.001, significantly different from the control.
Determination of serum palmitoylcarnitine concentration. The serum palmitoylcarnitine concentration was also measured using the LC-MS/MS method previously described with the exceptions that 2 ng of palmitoyl-[2H3]carnitine was used as the internal standard and the liquid phase obtained following centrifugation was directly injected into the LC-MS/MS system. Different LC mobile phases and flow rates were used, and the SRM for palmitoylcarnitine and its variant were m/z 400 → m/z 85 and m/z 403 → m/z 85, respectively. The mobile phase initially consisted of methanol-water (1:9, v/v) containing 0.1% formic acid and was used at a flow rate of 300 μl/min for 1.5 min, and the system was programmed in a linear manner to reach 0.1% formic acid in methanol over a period 4.5 min. The final mobile phase was maintained constant for an additional 4 min.

Statistics. The data are expressed as the means ± SD. The statistical significance of the differences between the results in the different groups was evaluated using Student’s two-tailed t test. The correlation was tested by calculating Pearson’s correlation coefficient, r. For all of the analyses, significance was determined at the level of p<0.05.

Results

Carnitine and acetylcarnitine concentrations in EN. The concentrations of carnitine and acetylcarnitine in ordinary ENs are shown in Table 3. The first three brands were administered to our patients. All of the ENs contained very low concentrations of carnitine, which was estimated to be less than 0.3 μmol/kg of body weight/day, whereas the endogenous biosynthesis of this compound is estimated to be 1.2 μmol/kg of body weight/day.18 The acetylcarnitine concentration was markedly lower than the carnitine concentration in all ENs and is not thought to contribute to the amount of carnitine intake even if it is hydrolyzed into carnitine in the intestine.

Circadian rhythm of serum carnitine and its derivatives in a healthy control subject. The circadian rhythm of the serum concentrations of carnitine, acetylcarnitine and palmitoylcarnitine in a healthy male is shown in Fig. 1. Pre-prandial increases and postprandial decreases were observed in the acetylcarnitine and palmitoylcarnitine concentrations, which suggests that the diurnal variation of serum acetylcarnitine and palmitoylcarnitine concentrations is controlled mainly by food intake. In contrast, the serum carnitine concentrations were relatively stable and not affected by food intake.

Serum carnitine and acetylcarnitine concentrations in healthy control subjects. The relationships between age and serum concentration of carnitine or acetylcarnitine in healthy subjects are shown in Fig. 2. In both males and females, no age-related changes in the serum carnitine concentrations were observed, whereas a significant age-related increase in the serum acetylcarnitine concentrations was observed in both genders.
Serum carnitine and acetylcarnitine concentrations in long-term EN or TPN patients. Serum carnitine and acetylcarnitine concentrations were compared among EN and TPN patients and age-matched healthy controls (Fig. 3a). Both the carnitine and acetylcarnitine levels in the EN and TPN patients were significantly lower compared with those of the controls. There were no significant differences in the carnitine or acetylcarnitine levels between the EN and TPN patients.

The relationships between the duration of no oral intake (EN and TPN) and the serum concentrations of carnitine compared with the albumin and total cholesterol levels were studied. In EN and TPN patients, both the carnitine and albumin levels were significantly reduced compared with those of the controls (Table 1). The total cholesterol levels in TPN patients were also reduced, but the levels in EN patients were not significantly different from those of the controls. As shown in Fig. 4a, there was no significant change in the serum carnitine, albumin or total cholesterol levels based on the duration of no oral intake, but the carnitine concentrations tended to decrease over time ($p = 0.148$).

Serum carnitine and other markers of nutrition. The relationships between the serum carnitine levels and other markers of nutrition, i.e., serum albumin and total cholesterol concentrations, are shown in Fig. 4b. Neither the serum albumin nor the total cholesterol level was correlated with the carnitine concentration, although a significant positive correlation was found between the serum albumin and total cholesterol levels.

Serum carnitine and acetylcarnitine concentrations in IBD patients. The concentrations of carnitine and acetylcarnitine in CD and UC patients and age-matched controls are depicted in Fig. 3b. As shown in Table 2, the serum albumin concentrations were significantly lower in both CD and UC patients, and the serum total cholesterol concentration was significantly lower in CD patients. Thus, these IBD patients’ nutritional states were not good because these individuals were barely able to eat a normal diet. Nevertheless, the serum concentrations of carnitine and acetylcarnitine were not significantly different between CD or UC and the corresponding control subjects.

Discussion

In previous investigations, carnitine derivatives were usually measured enzymatically as acylcarnitine. In the present study, we used the LC-MS/MS method and quantified the levels of acetylcarnitine and palmitoylcarnitine, which are the two major acylcarnitines after and before fatty acid $\beta$-oxidation in the mitochondria, respectively. Both the acetyl- and palmitoylcarnitine concentrations changed in parallel, and the levels increased at pre-prandial times, which suggests that the serum acylcarnitine concentration may reflect $\beta$-oxidation activity rather than the nutritional state. In contrast, carnitine (free carnitine) is much more abundant than acylcarnitine, and its levels were not affected by food intake. Thus, carnitine is proposed to be a
better marker than acetylcarnitine for the estimation of carnitine deficiency.

Little is known regarding the effects of aging and gender on the blood and tissue carnitine or acetylcarnitine concentrations. An age-dependent decrease in carnitine and acetylcarnitine concentrations in mice and human muscles was shown by Costell et al.\(^{15}\) These researchers also showed that the blood carnitine concentrations in humans remained unchanged with age in males, whereas an age-dependent increase was observed in females. These results suggest that the blood carnitine levels are maintained despite the slight decrease in the tissue carnitine concentrations. Conversely, the decreased concentration of serum carnitine may represent a marked reduction of carnitine in the tissues. In our study, although the serum carnitine concentrations in females tended to increase with age (Fig. 2b), significant age-related changes were not observed in both genders, which is similar to the results of a previous report. Furthermore, significant age-dependent increases in the serum acetylcarnitine concentrations were observed in both genders. Although the mechanism underlying the latter observations remains unclear, this finding also supports the contention that carnitine is a better nutritional marker than acetylcarnitine.

Although reduced blood carnitine concentrations due to long-term TPN have been observed in children,\(^{9-11}\) the blood
concentrations were followed up for only two months in adult TPN patients. In addition, the blood concentrations in long-term EN patients have not been studied. Our data demonstrated that the serum carnitine levels are significantly decreased to approximately half in patients who could not intake food orally and received TPN or EN for a long term (Fig. 3a). In addition, the carnitine levels tended to decrease in proportion to the duration of no oral intake, although this change was not statistically significant (Fig. 4a). These results suggest that sufficient amounts of carnitine were not supplied to our patients by EN and TPN. This finding was supported by additional data that only trace amounts of carnitine are contained in the ENs that were administered to our patients (Table 3). Recently, injectable carnitine and EN containing sufficient amounts of carnitine became available in Japan. Although carnitine is endogenously biosynthesized to some extent, its supplementation is needed for patients unable to orally intake food.

The serum carnitine concentrations in UC patients have been reported as normal, and our results supported this previous observation. In addition, decreased serum carnitine concentrations in CD patients were demonstrated approximately 40 years ago. However, the treatment of CD is now markedly improved, and our CD patients who were barely able to eat an ordinary diet did not show significantly reduced serum carnitine concentrations. Thus, carnitine supplementation was not needed for a majority of the tested CD patients with the exception of those with severe malabsorption. A patient with short bowel syndrome showed severe malabsorption, and oral carnitine supplementation was not sufficient to restore the low serum carnitine levels. These results suggest that the intravenous administration of carnitine appeared to be necessary to supply a sufficient amount of carnitine to patients with severe malabsorption.

It has been reported that encephalopathy, myopathy and cardiomyopathy are included in the complication of carnitine deficiency,

![Fig. 4.](image-url)
which is known as carnitine deficiency syndrome.\(^{(6)}\) It was difficult to evaluate carnitine deficiency syndrome in our carnitine-reduced cases because most of our patients had disuse syndrome caused by underlying diseases, such as Parkinson’s disease, sequel of cerebral infarction, cerebral hemorrhage and meningoencephalitis. A previous report suggested that the degree of carnitine deficiency in muscle tissue is greater than that in plasma.\(^{(15)}\) Thus, the disuse syndrome in our cases might have been affected by profoundly reduced carnitine levels in the muscle tissue. In addition, the serum carnitine concentrations in various diseases were previously reported, and patients with advanced liver cirrhosis showed a significant reduction presumably due to reduced biosynthesis.\(^{(6)}\) Thus, we should pay specific attention to carnitine deficiency in patients with liver cirrhosis who are receiving EN or TPN.

The relationships of serum carnitine concentrations with other nutritional markers, such as the serum albumin and total cholesterol levels, have not been previously investigated. Our IBD patients showed normal serum carnitine concentrations but had significant malnutrition with low serum albumin and/or low total cholesterol levels. In contrast, the IBD patients showed malnutrition but had normal levels of carnitine because they were able to ingest some ordinary food. Thus, we must be cautious regarding carnitine deficiency in patients with complete artificial feeding even if their other nutritional markers are normal. The serum carnitine concentration could be an independent biomarker of malnutrition for NST.

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**Conflict of Interest**

No potential conflicts of interest were disclosed.

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