Liver-Oriented Acute Metabolic Effects of A Low Dose of $\alpha$-Carnitine under Fat-Mobilizing Conditions: Pilot Human Clinical Trial

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Summary The acute metabolic effect of low dosages of $\alpha$-carnitine under fat-mobilizing conditions was investigated. Healthy subjects (Study 1: $n=5$; Study 2: $n=6$) were asked to fast overnight. Then, 30 min of aerobic exercise on a cycle ergometer was performed after supplementation, followed by a 3.5-h sedentary recovery phase. The following ingestion patterns were used: Study 1 (i) noningestion, (ii) 750 mg of $\alpha$-carnitine (LC), and (iii) 750 mg of LC + 50 g of carbohydrate (CHO); Study 2 (iv) noningestion, (v) 500 mg of LC, (vi) 30 mg of CoQ$_{10}$, and (vii) 500 mg of LC + 30 mg of CoQ$_{10}$. The energy expenditure (EE) and nonprotein respiratory quotient (npRQ) were measured during the pre-exercise, postexercise, and recovery periods. Serum free carnitine, acetylcarnitine, total carnitine (Study 1 and 2), and ketone bodies (Study 2) were measured. The 750 mg LC treatment significantly facilitated fat oxidation during the recovery phases ($p<0.05$) without elevating EE. The higher fat oxidation associated with LC was completely suppressed by CHO. CoQ$_{10}$ affected neither npRQ nor EE. npRQ was significantly correlated with the serum total ketone bodies ($R=-0.68, p<0.001$) and acetylcarnitine ($R=-0.61--0.70, p<0.001$). The highest correlation was found between acetylcarnitine and total ketone bodies immediately after exercise ($R=0.85, p<0.001$). In conclusion, LC enhanced liver fat utilization and ketogenesis in an acute manner without stimulating EE under fat-mobilizing conditions.

Key Words $\alpha$-carnitine, healthy subjects, acute effect, fat mobilization, $\beta$-oxidation, acetylcarnitine, ketone bodies, ketogenesis

The prevention of lifestyle-related diseases and so-called metabolic syndrome is a pressing health issue in many countries with a very large middle-aged population (1). Because metabolic syndrome is brought about by the accumulation of visceral fat, weight management with proper diet and exercise is considered to be crucial as the first-line countermeasure from the prophylactic viewpoint (2).

$\alpha$-Carnitine is an essential substance for transporting long-chain fatty acids into the mitochondrial matrix to obtain catabolic energy (3). To evaluate the effects of oral supplementation with $\alpha$-carnitine, a variety of human clinical trials have been conducted. Experiments using $^{13}$CO$_2$ exhalation gas analysis with $^{13}$C-labeled long-chain fatty acid probes showed that 10 d of supplementation with 3 g/d $\alpha$-carnitine significantly promoted $\beta$-oxidation of exogenously ingested fatty acids (4, 5). Pooyandjoo et al. performed a systematic review with a meta-analysis of nine studies on the effects of oral $\alpha$-carnitine intake on weight management and concluded that the supplementation resulted in significant weight loss (6), which meant that exogenous $\alpha$-carnitine worked for the consumption of stored body fat. In the clinical trials evaluated in this systematic review, the duration of supplementation was 30–360 d with a dosage level of 1.8–4 g/d. $\alpha$-Carnitine is endogenously synthesized in the liver, kidney, brain, etc. (7). In addition, it is obtained from daily meals, typically lean meats and/or dietary supplements (8). Orally ingested $\alpha$-carnitine is absorbed in the small intestine and reaches diverse target organs mainly through the sodium-dependent active transporter organic cation transporter 2 (OCTN2), which is specific to carnitines; e.g. free carnitine, acetylcarnitine (9, 10); then, $\alpha$-carnitine is retained in each body part with unique organ turnover (11). In particular, more than 90% of $\alpha$-carnitine is stored in muscle tissue and used for fat catabolism (12). Therefore, to obtain the effects from $\alpha$-carnitine, long-term supplementation has generally been considered a prerequisite for sufficient muscular accumulation. Wall et al. reported that muscle carnitine was efficiently accumulated by the combined feeding of $\alpha$-carnitine (2.7 g/d) and carbohydrate (160 g/d) for 12 wk (13). Gene functional analysis demonstrated that insulin-mediated muscle carnitine transport was stimulated via the upregulation of three major enriched pathways: insulin signaling, peroxisome proliferator-
activated receptor signaling, and fatty acid metabolism (14).

In Japan, L-carnitine was approved as a food ingredient by the Ministry of Health, Labor and Welfare in 2002. Like other countries, a daily dosage of no more than 1,000 mg was suggested by the authority for food applications to prevent excessive consumption by end consumers due to extravagant expectations of weight loss. On the other hand, most of the L-carnitine-related clinical trials were implemented with more than 2,000 mg of daily dosages as exemplified above (4–6, 13, 14). We conducted a 4-wk double-blinded, randomized, and controlled human clinical test with a low dosage, 500 mg/d, of L-carnitine supplementation and observed a significant reduction in moderately high levels of serum triglycerides and body weight loss; additionally, subjects received motivation training during the trial (15). The study suggested that even the small dose of L-carnitine had positive effects on fat utilization when accompanied by proactive improvements in meals and moderate exercise during daily activities over 4 wk.

In this study, we aimed to investigate the acute effects of L-carnitine supplementation with a dosage of less than 1,000 mg on energy metabolism in healthy untrained subjects. To focus on the utilization of endogenous fat, a protocol inducing fat mobilization was established with overnight fasting followed by moderate aerobic exercise.

**MATERIALS AND METHODS**

**Subjects.** Two independent series of open-labeled pilot trials were organized. Untrained healthy university students aged 22 y were enrolled in the experiments (Study 1: n = 5, 1 male and 4 females; Study 2: n = 6 females). All subjects were checked for health conditions before and after the trials using biochemical examinations (SRL, Inc., Komaki Motomachi, Aichi, Japan). The use of dietary supplements and functional foods was not permitted for the 3 mo preceding the tests and throughout the test periods. Body weight and height were measured to determine the body mass indices. Resting energy expenditure (REE) was measured for 10 min to obtain the baseline in the morning of the first visit after a 30-min rest by an indirect calorimeter, a breath-by-breath device employing the computerized standard open-circuit system (AERO MONITOR AE-300S; Minato Medical Science Co. Ltd., Osaka, Japan). The studies were approved by the Ethical Committee of Aichi Gakusen University (approval number 2015004 and 2018025 for Study 1 and 2, respectively), and they were conducted in accordance with the Declaration of Helsinki.

**Study design.** The testing procedures are shown in Fig. 1.

Exercise and blood and urine sampling: In Study 1 and 2, the subjects came to a laboratory at Aichi Gakusen University (Okazaki, Aichi, Japan) between 7:00 and 10:00 am after 12–15 h of overnight fasting. Subsequent visits were scheduled at the same time as the first visit for each subject. The subjects were asked to main-
tain a normal diet and to avoid alcohol and unusually strenuous physical activity for 24 h prior to each visit. Right after arriving at the laboratory, a set of normalization procedures was introduced as follows. The participants rested for 30 min in a sitting position followed by 30 min of walking on a treadmill (AUTO RUNNER AR-100, Minato Medical Science Co. Ltd.) at 3 km/h; then, the participants rested a second time for 30 min. After the normalization step, a cycle ergometer exercise was performed at 60 W for 30 min, which corresponds to approximately 50–60% of the maximum workload. The subsequent 3.5 h were allocated for the recovery period during which the participants remained sedentary without ingesting anything other than water. Blood (6 mL) and urine samples (10 mL) were collected after the first rest period (1 h prior to supplementation) and 4 h after supplementation in Study 1 and 2. In Study 2, an additional set of blood and urine samples was taken immediately after the exercise. The blood samples were centrifuged at 4°C, and the sera were collected into other vessels and stored at −20°C until use. The urine samples were stored at −20°C until use. The serum samples were subjected to an analysis of the total carnitine and free carnitine concentrations with an enzymatic cycling method using NADH-dependent carnitine dehydrogenase (17) (Kainos Co., Tokyo, Japan). The acylcarnitine level was calculated by subtracting the serum free carnitine concentration from the concentration of total carnitine. Theoretically, the serum acylcarnitine analyzed by this method does not directly measure “acetyl-carnitine”; however, it was understood that the majority of serum acylcarnitine is “acetyl-“ (18). Therefore, the obtained “acyl-“ values were regarded as “acetyl-“ in this study. The urine carnitine was measured to convert the respiratory quotient (RQ) values into nonprotein respiratory quotient (npRQ) values. In Study 2, the concentrations of 3-hydroxybutyric acid and acetocetic acid in sera were measured. The term “total ketone bodies” refers to the sum of those two chemical species in this report.

Supplementation: The L-carnitine capsules were prepared as follows: 250 mg of L-carnitine was encapsulated as a 50 w/w% water solution (approximately 0.5 mL in a total liquid volume) in a hard capsule made from indigestible hydroxypropyl methylcellulose (HPMC) (Licaps®, Lonza/Capsugel Japan, Inc.). In Study 1, the carbohydrates were provided as follows: 147 g of retort cooked steamed rice (Sato Foods Co. Ltd.) was ingested with 180 mL of water within 5 min. The carbohydrate sample included 50 g of available carbohydrate and 3.1 g of protein. The availability of the carbohydrate sample was confirmed by examining the glycemic response with every subject enrolled in Study 1 in a few months prior to the entry of the study. The glycemic response was examined by the following procedure. After 12–15 h of overnight fasting, steamed rice was ingested by the procedure described above. Fingerprick capillary blood samples were taken at 30-min intervals for 120 min, and the samples were analyzed with an electrode blood glucose measuring kit. Glutest Sensor Neoα (Sanwa Kagaku Kenkysuyos Co., Ltd.). In Study 2, CoQ10 was provided as a formulation of soft gelatin capsules (“Kokyuten,” Nissin Pharma, Inc.). The sample, 3.2 kcal/capsule, contains CoQ10 (30 mg), protein (120 mg), fat (290 mg) and carbohydrate (30 mg). In each case, the capsules were swallowed with 100 mL of water. For the control, only a 100-mL glass of water was served.

Procedure: Study 1: Based on the supplementation patterns, three sessions, 1A–1C, were conducted: Session 1A (1st visit): noningestion, Session 1B (2nd visit): 750 mg of LC (L-carnitine), and Session 1C (3rd visit): 750 mg of LC+CHO (carbohydrate). During Session 1B and 1C, the testing samples were ingested immediately prior to the cycle ergometer exercise. The energy expenditure (EE) and npRQ were measured at time points −1 h, −0.5 h, 2 h, 3 h, and 4 h from the commencement of the exercise. Blood and urine samples were collected twice, at −1 h (baseline) and 4 h (endpoint of the recovery period) from the commencement of the exercise. For washout, a 1-wk interval was allocated between every session.

Study 2: Based on the supplementation patterns, four sessions, 2A–2D, were conducted. Session 2A (1st visit): noningestion, Session 2B (2nd visit): 500 mg of LC, Session 2C (3rd visit): 30 mg of CoQ10, and Session 2D (4th visit): 500 mg of LC+30 mg of CoQ10. During Session 2B, 2C and 2D, the testing samples were ingested immediately before the cycle ergometer exercise. The EE and npRQ were measured at time points −1 h, −0.5 h, 0.5 h, 2 h, 3 h, and 4 h from the commencement of the exercise. Blood and urine samples were collected three times, at −1 h (baseline), 0.5 h (immediately after the exercise), and 4 h (endpoint of the recovery period) from the commencement of the exercise. The allocation of the washout period between the sessions was the same as used in Study 1, 1 wk.

Analysis of biochemical parameters. For the safety assessment, part of the blood samples obtained at time 0 h and 4 h of Study 2 (Session 2A and 2B) was used to analyze the following biochemical parameters: serum albumin, total protein, renal function (blood urea nitrogen, uric acid, and creatinine) and liver function (Asp aminotransaminase, Ala aminotransaminase, triglyceride, total cholesterol, high-density lipoprotein, and low-density lipoprotein). All of the analyses were made at SRL, Inc.

Evaluation of baseline comparabilities. The comparabilities of the subject characteristics between Study 1 and Study 2 were statistically evaluated by comparing the values on height, body weight, body mass index, npRQ, REE and plasma levels of carnitine species obtained at the time of entry into the studies immediately after the 1st rest (see Fig. 1) of Session 1A and Session 2A. Washout completion between the sessions and the order-effects, the possible trends due to the order of the treatments among the sessions within each study, were estimated by comparing the values of npRQ, REE and plasma level of the carnitine species immediately after the 1st rest (see Fig. 1) of every session.
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(i) Comparison of subjects characteristics enrolled in Study 1 and Study 2 at the time of entry into the studies

|                  | Visit 1     | Visit 2     | Visit 3     | Difference   |
|------------------|-------------|-------------|-------------|--------------|
| Visit 1          |             |             |             |              |
| Study 1 (n=5)    | Visit 1A    | Visit 1B    | Visit 1C    |              |
| Age (y)          | 22          | 22          | 22          |              |
| Height (cm)      | 160.2±2.9   | 163.4±5.7   | 163.4±5.7   |              |
| BW (kg)          | 53.3±6.7    | 52.4±4.7    | 52.4±4.7    |              |
| BMI (kg/m²)      | 20.8±2.3    | 19.6±1.0    | 19.6±1.0    |              |
| npRQ             | 0.84±0.02   | 0.84±0.02   | 0.81±0.08   | NS           |
| REE (kcal/kgBW/d)| 27.8±2.5    | 29.3±3.6    | 27.8±3.9    | NS           |
| LC (µM)          | 37.9±5.9    | 33.7±7.3    | 35.8±4.7    | NS           |
| ALC (µM)         | 8.44±1.8    | 8.14±2.4    | 9.56±4.1    | NS           |
| TC (µM)          | 46.3±8.0    | 41.9±9.2    | 45.4±8.2    | NS           |

Study 2 (n=6)

Mean±SD.
Baseline comparabilities of the subject characteristics between Study 1 and Study 2 on height, body weight, BMI, REE and RQ were statistically estimated by unpaired t-test. Differences resulting in p-values less than 0.05 were considered statistically significant.

(ii) Baseline value comparisons of npRQ, REE and serum carnitines on every visit

|                  | Visit 1     | Visit 2     | Visit 3     | Difference   |
|------------------|-------------|-------------|-------------|--------------|
| Visit 2          |             |             |             |              |
| Study 2 (n=6)    | Visit 2A    | Visit 2B    | Visit 2C    |               |
| Age (y)          | 22          | 22          | 22          |              |
| Height (cm)      | 160.2±2.9   | 163.4±5.7   | 163.4±5.7   |              |
| BW (kg)          | 53.3±6.7    | 52.4±4.7    | 52.4±4.7    |              |
| BMI (kg/m²)      | 20.8±2.3    | 19.6±1.0    | 19.6±1.0    |              |
| npRQ             | 0.84±0.06   | 0.83±0.04   | 0.79±0.05   | 0.77±0.03    | NS           |
| REE (kcal/kgBW/d)| 28.0±2.8    | 27.2±1.9    | 28.0±1.9    | 27.5±1.9     | NS           |
| LC (µM)          | 37.2±6.8    | 37.7±9.3    | 41.7±2.9    | 37.0±8.1     | NS           |
| ALC (µM)         | 10.8±6.3    | 8.3±1.8     | 10.6±1.7    | 9.4±3.4      | NS           |
| TC (µM)          | 48.0±11.9   | 46.0±9.9    | 52.3±3.8    | 46.4±10.1    | NS           |

Mean±SD.
Statistical analyses were made on each parameter among the values of every paired visit by multiple comparisons with Tukey-Kramer method. Differences resulting in p-values less than 0.05 were considered statistically significant.

Statistical analysis. The data presented in the main text, tables and figures are expressed as the mean±SD.

The statistical analysis on the followings were performed by parametric procedures regarding the parameters investigated were in normal distribution considering that the subjects were of the same age of healthy young Japanese adults: (I) Confirmation of the baseline comparabilities of the subject characteristics between Study 1 and Study 2 on body compositions, REE, RQ and the serum levels of carnitine species (unpaired t-test); (II) Evaluation of the order effects and washout confirmation among the sessions (Tukey-Kramer test); (III) Glycemic response after the carbohydrate ingestion, the blood glucose levels at 0 min against 30 min, 60 min, 90 min and 120 min (Dunnett method (19)).

For the results after interventions (Study 1: Session 1A–1C; Study 2: Session 2A–2D), multiple comparison analyses on npRQ and EE at every time point among each session were performed by a nonparametric procedure (Steel-Dwass test (20)) due to the unpredictability on normality with small number of sample sizes. The correlations between npRQ and blood parameters and between total ketone bodies and carnitines were evaluated by a nonparametric method (the Spearman’s cor-
relation coefficient by rank test (21) because the ketone body distribution was apparently non-normal. All statistical analyses were performed with BelleCurve for Excel software (ver. 3.209) (Social Survey Research Information Co., Ltd.). Differences resulting in p-values less than 0.05 were considered statistically significant.

**RESULTS**

**Subject characteristics and baseline comparisons**

The characteristics of the subjects and baseline com-

| Sessoin      | npRQ                          | Time after ingestion | Mean 2–4 h |
|--------------|-------------------------------|----------------------|------------|
|              | 2 h                           | 3 h                  | 4 h        |            |
| 1A Noningestion | 0.79±0.05 b                 | 0.81±0.03 b         | 0.80±0.01 b | 0.80±0.03 b |
| 1B LC 750 mg     | 0.73±0.02                | 0.74±0.02 b         | 0.74±0.03 b | 0.74±0.02 b |
| 1C LC 750 mg + CHO | 0.82±0.06 b            | 0.84±0.04 b         | 0.82±0.05  | 0.83±0.04 b |

| Sessoin      | REE (kcal/d/kgBW)           | Time after ingestion | Mean 2–4 h |
|--------------|-----------------------------|----------------------|------------|
|              | 2 h                         | 3 h                  | 4 h        |            |
| 1A Noningestion | 30.7±2.6                  | 27.9±3.0             | 29.8±4.4  | 29.5±3.2   |
| 1B LC 750 mg     | 30.3±4.4                   | 28.7±2.9             | 31.0±3.2  | 30.0±3.3   |
| 1C LC 750 mg + CHO | 32.8±3.4               | 31.6±2.7             | 30.5±3.7  | 31.6±2.9   |

| Sessoin      | npRQ                          | Time after ingestion | Mean 2–4 h |
|--------------|-------------------------------|----------------------|------------|
|              | 0.5 h*                        | 2 h                  | 3 h        | 4 h        |            |
| 2A Noningestion | 0.90±0.03 b                 | 0.78±0.03            | 0.77±0.04  | 0.77±0.03  | 0.77±0.03  |
| 2B LC 500 mg     | 0.83±0.03 a*                | 0.77±0.03            | 0.76±0.03  | 0.76±0.03  | 0.76±0.03  |
| 2C CoQ10       | 0.90±0.03 b                 | 0.79±0.02            | 0.78±0.02  | 0.78±0.02  | 0.78±0.02  |
| 2D LC 500 mg + CoQ10 | 0.83±0.04 b           | 0.75±0.02            | 0.75±0.03  | 0.75±0.02  | 0.75±0.02  |

| Sessoin      | REE (kcal/d/kgBW)           | Time after ingestion | Mean 2–4 h |
|--------------|-----------------------------|----------------------|------------|
|              | 0.5 h*                        | 2 h                  | 3 h        | 4 h        |            |
| 2A Noningestion | 37.6±4.0 b*                | 27.5±1.3             | 27.4±2.4   | 28.5±3.0   | 27.8±1.8   |
| 2B LC 500 mg     | 31.3±1.6 a                 | 28.1±1.7             | 27.9±1.8   | 27.7±2.0   | 27.9±1.7   |
| 2C CoQ10       | 33.9±3.2                  | 28.4±1.9             | 28.5±2.1   | 29.1±2.9   | 28.7±2.2   |
| 2D LC 500 mg + CoQ10 | 30.8±1.3 a*             | 27.3±1.7             | 27.8±1.7   | 27.6±1.2   | 27.6±1.2   |

Statistical analyses were performed by Steel-Dwass test (20) among Session 1A, 1B and 1C within each time slot. *p<0.05. Means at the same time slot containing different letters within the superscripts differ significantly.

LC: l-carnitine, CHO: 50 g of available carbohydrate, npRQ: non-protein respiratory quotient, REE: resting energy expenditure, BW: body weight.
parisons in each trial are summarized in Table 1. Having evaluated the baselines between Study 1 and Study 2 on the body composition (height, BW, BMI, npRQ, REE) and serum levels of carnitine species (free carnitine, acetyl-carnitine, total carnitine) at the time of entry into the studies, no significant differences were detected (Table 1 (i)). Visit-by-visit parameter baselines (Session 1A–1C for Study 1, Session 2A–2D for Study 2) are presented in Table 1 (ii). Multiple comparison analyses on every two visits within the same parameters showed no significant differences, thus confirming no order effects or carry-over effects among the sessions in each study.

Effects of L-carnitine supplementation on npRQ and EE

Table 2 shows the effects of single ingestions of L-carnitine on npRQ and EE under the two different dosage patterns, 750 mg and 500 mg. The 750 mg L-carnitine treatment (Session 1B) significantly reduced npRQ vs. noningestion (Session 1A) during the postexercise recovery period (3 h, 4 h and mean 2–4 h after ingestion) ($p < 0.05$). However, the EE levels during that timeframe were not significantly affected by L-carnitine.

With the ingestion of the steamed rice sample which included 50 g of available carbohydrate, the change in glycemic levels was investigated on the subjects enrolled in Study 1 in a few months prior to the entry of the study. The blood glucose concentrations at time 0 (baseline), 30 min, 60 min, 90 min and 120 min were 80.2 ± 8.1 mg/dL, 140.8 ± 4.3 mg/dL, 115.6 ± 17.5 mg/dL, 116.2 ± 14.1 mg/dL, and 107.8 ± 13.5 mg/dL, respectively, which showed significant elevation from the baselines to each timeline tested ($p < 0.05$). When 750 mg L-carnitine was co-ingested with the carbohydrate sample (Session 1C), the npRQ reduction seen in Session 1B was no longer observed.

In Session 2B, the fat utilization immediately after exercise was significantly enhanced by a single shot of 500 mg of L-carnitine ($p < 0.05$) compared with noningestion (Session 2A). Meanwhile, the supplementation significantly decreased the mean EE value by 17% compared with the control ($p < 0.05$).

In summary, L-carnitine supplementation increased fat utilization acutely after aerobic exercise unless carbohydrate was supplied; additionally, the EE levels were largely maintained or even reduced in that timeframe.

In Study 2, L-carnitine (500 mg) was administered in the presence or absence of CoQ10 (Session 2B and 2D). Comparing those two sessions with noningestion (Session 2A) immediately after exercise, although not significant, LC + CoQ10 ingestion (Session 2D) enhanced fat utilization accompanied by significant suppression of EE at similar extent to LC alone (Session 2B). The CoQ10 alone case, on the other hand, showed no significant effects on either npRQ or EE (Session 2C vs. 2A) so far as the time slots tested.

Correlations of npRQ vs. serum acetylcarnitine and ketone bodies

The relationship between npRQ and the serum acetyl-carnitine level was evaluated. As shown in Fig. 2 (a scattergram created with the assembled data sets in Session 1A and 1B) and Fig. 3a (a scattergram created with the assembled data sets in Session 2A and Session 2B), the correlation coefficients ($R$) of the two parameters in Study 1 and Study 2 were found to be $-0.70$ ($p < 0.001$) and $-0.61$ ($p < 0.001$), respectively. Another correlation between npRQ and total ketone bodies in Study 2 also gave a significant correlation shown in Fig. 3b ($R = -0.68, p < 0.001$).
Correlations between serum ketone bodies and carnitine species

Considering npRQ as an intermediary parameter versus serum acetylcarnitine and versus ketone bodies, the correlations between both metabolites were investigated in Session 2A and Session 2B (Fig. 4). Blood samples were collected in the resting status (time point −1 h and 4 h showing in Fig. 1) and immediately after the exercise (time point 0.5 h). The EEs of the resting status at −1 h and 4 h were 27.6±2.5 kcal/kgBW/d and 28.1±2.5 kcal/kgBW/d, respectively, which were significantly lower than the EE at 0.5 h, 34.4±4.4 kcal/kgBW/d (p<0.01). Thereby, the data set on acetylcarnitine at 0.5 h was separately analyzed from that of the resting phases (−1 h and 4 h) shown in Fig. 4a and Fig. 4b. The obtained correlations between total ketone bodies and acetylcarnitine were $R = 0.85$ ($p < 0.001$) and $R = 0.78$ ($p < 0.001$) immediately after exercise and the resting phases, respectively. Having assembled whole data sets on the three time points into the analysis, a positive correlation was still detected $R = 0.77$ ($p < 0.001$) (Fig. 4c). In contrast, the total ketone bodies showed a significant but much weaker correlation with lower significance with total carnitine ($R = 0.38$, $p < 0.05$) and no
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Serum levels of carnitine species at baseline and at 4 h. At 4 h in the noningestion session, the serum free carnitine decreased from each baseline by 4.4 μM (Study 1) and 3.1 μM (Study 2), whereas acetylcarnitine increased by 5.0 μM (Study 1) and 3.0 μM (Study 2). Consequently, the total carnitine in both studies mostly remained unchanged. +0.6 μM (Study 1) and −0.1 μM (Study 2). l-Carnitine supplementation increased acetylcarnitine by 230% (+10.9 μM) during the 4 h regardless of the dosages; in contrast, it increased free carnitine in a rather dose-dependent manner. On the other hand, in the presence of carbohydrates, l-carnitine ingestion increased mainly free carnitine, +15.3 μM (+42.7%), compared to only +1.8 μM (+19.2%) for acetylcarnitine.

Biochemical parameters and safety

The results of the biochemical examinations performed in Study 2 are shown in Table 4. The average values of total ketone bodies in the noningestion and 500 mg l-carnitine treatments at 4 h were 220.3 ± 201.1 (mg/dL) and 297.3 ± 222.6 (mg/dL), respectively. The maximum value observed was 687 (mg/dL), as shown in Fig. 6; however, no subjects expressed abnormalities in physical or mental conditions throughout the trials.

**DISCUSSION**

To our knowledge, this is the first human study on the acute effect of low-dose l-carnitine. l-Carnitine is endogenously synthesized mainly in the liver, and as much as 90% of its mass is stored in skeletal muscle (11). Due to its critical role in muscular fat oxidation, one of the major foci of practical interest for researchers has been how to enrich l-carnitine tissue content using oral supplementation. To address this long-term issue, a robust method was presented (13, 14). By administering 1.36 g of l-carnitine with 80 g of carbohydrate twice daily for 12 wk, the total carnitine muscular content and whole-body EE increased by 20% and 6%, respectively. The mechanism behind this increase is the stimulation of insulin-mediated carnitine transport (13, 14).

As muscle is a slow equilibration compartment with
L-carnitine, where its turnover takes 105 h (12), an impact from a single intake was unlikely. In fact, some human trials have shown the acute effects of L-carnitine; however, the results seemed to be limited or equivocal under diverse study designs and/or characteristics of the subjects in terms of training experiences, even with higher dosages such as 2–4 g (22, 25).

Despite these theoretical expectations, in this trial a series of enhancements fat utilization in an acute manner was reproducibly observed by the single administration of 500–750 mg L-carnitine. Exogenous L-carnitine is absorbed from the small intestine via OCTN2, a sodium-dependent organic cation transporter specific to carnitines, e.g. free carnitine, acetylcarnitine (9, 10); then, L-carnitine is delivered to the liver through portal circulation. On the other hand, L-carnitine appears in systemic blood flow as early as 30 min after intake and peaks at 2–4 h (26). Unlike muscle, liver has a shorter turnover, 1.3 h (11); it was thus possible to expect the quickest response from the organ within 2–4 h. In Study 2, significant EE reductions by 17–18% were observed immediately after exercise cessation, which was 0.5 h after the supplementation of 500 mg of L-carnitine. The EE reductions were concomitant with the significant decrease in npRQ, suggesting that the phenomenon might be due to energy savings caused by facilitating fat catabolism. A few studies on the acute effect of L-carnitine showed significant reductions in oxygen consumption, pulmonary ventilation, blood lactate (22), heart rate and blood lactate (23). In those cases, subjects received L-carnitine 1 h prior to exercise. One of the authors speculated that it took less oxygen demand to accomplish the same amount of “common” works, the increase in the work yielded by the same oxygen by L-carnitine supplementation (22). The EE reductions brought by L-carnitine supplementation in the current study were in line with the observations reported. As described previously, Stephens et al. proposed that continuous L-carnitine supplementation upregulates EE by facilitating fat oxidation (13, 14). On the contrary, the current study implies that single shots of L-carnitine did not elevate metabolic levels more than real-time energy requirements or work for energy conservation. Hence, the acute response of exogenous L-carnitine presumably caused by a liver-oriented manner should be distin-
guished from the muscle-oriented event based on long-term supplementation (13, 14).

CoQ10 is an essential component of the mitochondrial electron transport chain. CoQ10 has been reported to increase fatty acid oxidation through AMPK-mediated PPARα induction in 3T3-L1 preadipocytes (16). Emami et al. demonstrated a human trial with short-term (2 wk) supplementation of CoQ10 (27). In their study, a significant preventive effect against oxidative stress accompanied by exercise was observed in elite swimmer subjects. In the current case, we organized two of four sessions in Study 2 using 30 mg of CoQ10 and expected some additive and/or synergetic effects with t-carnitine on energy metabolism. CoQ10 alone showed effects on neither npRQ nor EE within 4 h after ingestion (Session 2C) compared with noningestion (Session 2A). Due to its lipophillicity, CoQ10 is deemed to be absorbed largely through abdominal lymphatic vessels, taking more than several hours for systemic distribution. The slower process might be the reason for the silencing of CoQ10 in this case. In Session 2B (500 mg of LC) and 2D (500 mg of LC+CoQ10), very similar responses were observed for npRQ and EE, indicating that LC worked independently from CoQ10.

One of the characteristics of this study was setting semi-starved conditions with overnight fasting followed by the performance of low-intensity aerobic exercise for 30 min. Starvation results in glycogen shortage, which cues hormone-sensitive lipase to release nonesterified fatty acids (NEFAs) from adipose tissue (“fat mobilization”). A recent study revealed that liver glycogen paucity dispatches a signal from a hepatospecific nerve system called the “liver-brain-adipose neural axis,” which plays a crucial role in switching the fuel source from carbon-deficient conditions were deemed to be derived from the combination of overnight fasting + aerobic exercise, where NEFAs are delivered from adipose tissue into hepatocytes followed by flowing into mitochondria through t-carnitine shuttles potentially reinforced by the supplementation.

In the mitochondrial matrix, long-chain fatty acids are broken down by β-oxidation to form acetyl CoA. Under fasting conditions, acetyl CoA accumulates due to the overcapacity of the liver cell mitochondrial electron transfer system to treat excessively produced NADH and FADH2, which results in the occurrence of ketone bodies, 3-hydroxybutyric acid, acetoacetic acid and acetone (32). The ketone bodies, the former two molecules in particular, migrate into the blood flow and are forwarded to energy-requiring extrahepatic distal target organs, such as the skeletal muscle, the heart and the brain.

Downstream of the inner mitochondrial event, acetyl-carnitine is formed from several substrates, acetyl CoA and free carnitine. The process is catalyzed by carnitine acetyltransferase (CrAT), a bidirectional enzyme that exists principally in the mitochondrial matrix. CrAT plays an important role in buffering the acetyl CoA/free CoA ratio to secure free CoA when metabolic inertia arises from aggressive catabolic processes (33). Acetyl-carnitine penetrates the mitochondrial membrane and finally moves to the systemic blood stream similar to ketone bodies to reach a variety of target organs, including the brain.

In this study, sets of correlations were confirmed among the three parameters, npRQ, total ketone bodies and acetyl-carnitine (Figs. 2–4). The highest correlation coefficient (R=0.85, p<0.001) was detected between total ketone bodies and acetyl-carnitine immediately after the exercise (Fig. 4a). These results indicate that the greater the fat utilization the higher the blood acetyl-carnitine level, which agrees well with the aforementioned theoretical sequential events in acute “liver-oriented” mitochondrial fat catabolism.

On the other hand, no relations were observed between total ketone bodies and free carnitine (Fig. 5b). Given that a portion of free carnitine is consumed to form acetyl-carnitine, the remaining quantity of free carnitine depends on the mass of original free carnitine pools, which differs among individuals. This might explain the lack of correlation between total ketone bodies and free carnitine.

Table 3 includes some characteristic points in terms of the change in serum concentrations of carnitines between 0 h and 4 h. First, in noningestion cases of Study 1 and Study 2, pairs of similar levels of changes in free carnitine and acetyl-carnitine occurred within the observed 4 h. Namely, the decrease in free carnitine (−4.4–−3.1 μM) seemed to be counterbalanced by the increase in acetyl-carnitine (+3.0–+5.0 μM), which kept the total carnitine substantially unchanged (−0.1–+0.6 μM). This suggests that free carnitine was converted to acetyl-carnitine in an apparent stoichiometric manner. It is interesting if the precise material balance of serum carnitines reflects the extent of acute fat breakdown in the liver mitochondria. Second, in the t-carnitine-supplemented cases the increased levels of acetyl-carnitine were similar, +10.9 μM for both dosages (Study 1: 500 mg, Study 2: 750 mg), indicating that 500 mg was sufficient to play a role in the metabolic events during the recovery process. In this context, the noningestion cases might be showing that endogenously stored liver t-carnitine was not essentially saturated, and hence exogenous t-carnitine mitigated some bottlenecks in the congested metabolic flow in fat mobilization. Third, in Study 1, in the presence of carbohydrates acetyl-carnitine changed by only +1.8 μM even when 750 mg of t-carnitine was consumed, which conformed with the finding (Table 2: Session 1C) that fat oxidation was conspicuously downregulated as long as glucose was available as a fuel source (carbon surplus status).
In Fig. 7, several possible metabolic pathways are outlined. Figure 7a shows the pathways under a carbon deficit, a fat-mobilizing condition (28–30, 33). Acetyl-carnitine and ketone bodies are the two major molecules that appear in serum in the fat-predominant, carbon deficit status as seen in Fig. 3. The case of the carbon surplus condition is depicted in Fig. 7b. Whenever carbohydrate is available as a fuel source, glycolysis is preferentially selected (28, 33). In addition, the carbon repletion induces reciprocal lipogenesis through a course of metabolic sequences (34); the carbon surplus condition derives a surge in mitochondrial efflux of citric acid to cytosol followed by converting to malonyl-CoA which in turn inhibits the enzyme carnitine palmitoyltransferase (CPT-1), which is responsible for introducing long-chain fatty acid into the mitochondrial matrix and then facilitating β-oxidation (35). When exogenous L-carnitine is supplied in this situation, high levels of free carnitine
and low levels of acetylcarnitine in the blood together with npQR elevation are theoretically expected, which is consistent with the results of the current study (see Study 1 in Tables 2 and 3).

The L-carnitine content in the liver is reported as 500–1,000 μmol/kg wet weight (12, 36). Assuming that the liver weight of the subjects enrolled in this study (approximately 50 kg body weight (BW)) is 1 kg, the endogenous L-carnitine content is estimated to be 500–1,000 μmol (80–160 mg). Because the bioavailability of orally ingested L-carnitine is at least 16% (37), it could be a fair guess to expect that 80 mg of L-carnitine is delivered to the liver from 500 mg of supplementation. In this context, the single ingestion might induce significantly detectable responses within the turnover timeframe of 1.3 h (12).

As shown in Fig. 6, the serum concentration of total ketone bodies was 134.1 ± 55.9 μM, which is ten times higher than that of acetylcarnitine, 13.5 ± 5.8 μM. Despite this big difference with large SDs between the two parameters, the correlations observed were strong (R² = 0.77–0.85: Fig. 4), suggesting that those molecules are formed and secreted into the blood flow in a precise manner in accordance with liver β-oxidation. Given that the ketone body itself is a crucial fuel source in glycogen-depleted conditions (38), a practically considerable amount of the molecules must be provided to distal organs. Acetylcarnitine can also be transformed enzymatically into acetyl CoA to participate in the loop of energy metabolism (33); however, in the present case it is likely that such a low concentration would have little or no contribution as a practicable fuel.

From another point of view, it was reported that acetylcarnitine exhibits some nerve functions passing through the blood-brain barrier (39). A human positron-emission tomography (PET) study showed the cerebral uptake of acetylcarnitine reaching several crucial brain areas: the prefrontal and temporal cortices, anterior cingulate, cerebellum, and then, the acetyl moiety is incorporated into brain metabolism (40, 41). Kuratsune et al. reported that the blood acetylcarnitine concentration of individuals with chronic fatigue syndrome (CFS) is lower than that of the healthy population (42). The concentration variance between CFS patients and normal controls was small, such as Δ 3.7 μM (male) and Δ 6.1 μM (female); nevertheless, the difference was significant (p<0.001) (42). The observation that apparent small differences in blood acetylcarnitine concentration brings critical fatigue sensation implies that this molecule might be under homeostatic control within several micro molar range. As mentioned above, the elevation of serum acetylcarnitine levels observed in the current study were Δ 3.0–Δ 5.0 μM under the glycogen-depleted conditions (Table 3), which are close to those reported in the CFS research case, Δ 3.7–Δ 6.1 μM. In another CFS study (40), Kuratsune and colleagues reported that the acetyl moiety of the acetylcarnitine taken up into the brain is utilized for the biosynthesis of sensory-nerve-related neurotransmitters such as glutamic acid, γ-aminobutyric acid (GABA) and aspartic acid. The course of event was brought by a single injection of tracer dose, 64.3 nmol/200 μL, of [2-14C]acetylcarnitine through a tail vain of a mouse. From those lines of evidence, small amount of acetylcarnitine could be responsible rather for fatigue sensation related cerebral functions than a part of fuel, which is also consistent with the knowledge that the long chain fatty acid does not work as an energy source in the brain (43). In addition, it was shown that the cerebral acetylcarnitine uptake was suppressed by intravenous administration of glucose (39), the pivotal energy source for brain. In our current study, the conversions from L-carnitine to acetylcarnitine shown in Table 3 were particularly suppressed under the glucose surplus metabolic status (Session 1C), which could also be compatible with the CFS study (39).

The uptake mechanism of carnitine from blood into cells via OCTN2 is well understood (9, 10); in contrast, little is known about the efflux-related mechanisms. Concerning this point, Suhre et al. revealed the presence of L-carnitine efflux transporter (44) on the basolateral membrane of absorptive epithelial cells of Xenopus oocytes. The work was in genome-wide association studies (GWAS) through which many risk loci for complex diseases have been identified. The researchers investigated 37 independent loci that reached genome-wide significance in their meta-analysis. The predicted function of one of them, SL16A9 (MCT9), was experimentally validated as a pH-independent carnitine transporter possibly responsible for L-carnitine efflux from the intracellular matrix into the blood. The apparent precise regulation on serum acetylcarnitine level seen in the aforementioned CFS and our current study implies that the migration of the acetylcarnitine from the intracellular environment into the blood stream does not occur through simple passive diffusion but rather by mediation by an unknown acetylcarnitine-efflux transporter. The finding from the GWAS might be suggestive in this context.

From the perspective of clinical implications in relation to metabolic syndrome, it is important to trigger the fat mobilization status. In the present study, performing aerobic exercise for 30 min before breakfast with an intake of 500–750 mg L-carnitine might be a practical protocol to optimize visceral fat. Iwayama et al. presented interesting evidence that prebreakfast exercise was very effective for 24-h fat oxidation compared with any other exercise-timing patterns (45). On the other hand, strenuous exercise under fasting conditions may cause heart problems due to mitochondrial membrane disruption from free fatty acids (46). In this regard, Oyanagi et al. reported in their series of in vitro studies that the membrane damage arising from the surface-active nature of free fatty acids was alleviated in the presence of L-carnitine (47).

As shown in Table 4, in Session 2A and 2B, the blood biochemical parameters other than ketone bodies (https://www.kuhp.kyoto-u.ac.jp/~kensa/referenece/ item_pdf/6195.pdf) were all observed within the standard criteria (https://crc.nnh.go.jp/clinical_trial_services/drug_trials/spec/kensa_nmc/). Recently, a cohort study on periodic fasting was performed with 1,422 sub-
jects (48), and it confirmed that the parameter changes found in the trial including higher levels of ketone bodies were tolerable, safe and even beneficial for health improvement. Likewise, no health problems were found among the subjects in our study. The protocol used in this study may thus serve as a tangible set of instructions to care for patients with metabolic syndrome and/or diabetes mellitus.

We would like to acknowledge a set of potential limitations in this study. First, our pilot experiments were designed as open-label trials. Further studies with large-scale RCT (randomized controlled trials) are warranted. Second, the urinary excretion of neither carnitines nor ketone bodies was measured. With the additional information, more detailed material balance of the issued molecules could be interpretable. Third, the current trial presented no more than the basal acute phenomena on fatty acid oxidation; further studies are therefore warranted to evaluate the long-term systemic health benefits.

While we acknowledge these limitations, our work depicts the acute metabolic effects on fat utilization of a small dosage of L-carnitine under fat-mobilizing conditions.

Disclosure of state of COI
Satoshi Odo is a technical consultant of Lonza Japan Ltd., and Koji Tanabe is an employee of Lonza Japan, Ltd. The analysis of serum carnitine concentrations was supported by Lonza Japan, Ltd.

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