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Original Article

Effect of Dietary L-Carnitine on Lipid Metabolism in Growing Rats

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Summary

Dietary therapy for obesity is considered as the first-choice treatment and is at least as important as medical treatment. Various supplements, such as L-carnitine (LC), are expected to affect loss weight and are already available as supplements worldwide. However, in many cases, young people who do require losing weight also tend to intake LC supplements. In the present study, the effect of dietary LC on lipid metabolism was evaluated in growing rats. Four-week-old male Wistar rats were fed an AIN-93G modified diet containing LC (0.25 % or 1.0 %). As parameters of lipid metabolism, the relative white adipose tissue (WAT) weights, serum and liver lipid contents, lipid metabolizing enzyme activities and fatty acid related enzymes expression levels in the liver were evaluated.

Dietary LC did not affect the relative WAT weights, serum and liver lipid contents, lipid metabolizing enzyme activities or fatty acid related enzymes expression levels in the liver. However, it was confirmed that serum and liver LC contents increased following the intake of LC diets compared with a non-LC-containing diet. Therefore, the growing rats were supplemented unnecessarily with LC, which is required for the β-oxidation of fatty acid by an endogenous biosynthetic pathway. Hence, it was suggested that LC intake by young people was not required.

Introduction

Obesity has become a worldwide problem with a rapid increase in the rate in obesity in various populations and across all age groups[1, 2)]. Obesity often coexists with other cardiovascular risk factors, such as diabetes, dyslipidemia, and hypertension. The cause of increased rates of obesity is attributed in part to changes in dietary and lifestyle habits, such as the increased availability of high-energy foods, and reduced physical activity[3)]. Dietary therapy is the first-choice treatment and is at least as important as medical treatment. For this reason, various supplements, such as L-carnitine (LC), are expected to affect weight and are already available as supplements worldwide.

LC, a natural vitamin-like compound, is a ubiquitous constituent of mammalian plasma and tissues. LC is supplied to the body through dietary sources (e. g., meat, dairy products, and supplements)[4)] and biosynthesis from lysine and methionine by cytosolic-γ-butyrobetanine hydroxylase (BBH). LC functions to transport long-chain fatty acids across the inner mitochondrial membrane into the matrix for β-oxidation and also has effects on the oxidative metabolism of glucose in tissues[5)]. Therefore, LC levels in each tissue, by itself, seem to be an important factor for regulating lipid metabolism. However, recently, young people who have no requirement to lose weight have also tended to intake LC supplement.

In this study, the liver lipid metabolism in dietary LC was evaluated in rats. Heo *et al.* suggested that a significant increase in carnitine palmitoyl transferase (CPT)-1 activity after 10 days of dietary supplementation with LC was detected in the liver but not in skeletal muscle[6)]. Therefore, the liver can be considered as a primary target organ for the effects of dietary LC administration. Consequently, to reveal the effect of dietary LC on lipid metabolism in growing rats, the relative white adipose tissue (WAT) weight, serum and liver lipid contents, lipid-metabolizing enzyme activities and fatty acid related enzymes expression levels in the liver were evaluated.

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Materials and Methods

1. Materials

L-Carnitine was obtained from Ito Life Science Inc (Tokyo Japan). All of the diet constituents were purchased of Oriental Yeast (Tokyo, Japan). All other chemicals were used regent grade.

2. Animal care

Experimental protocols were reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the "Guide for the Care and Use of Experimental Animals" of the Ministry of Education, Culture, Sports, Science and Technology of Japan. Four-week-old male Wistar rats were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). Rats were housed in plastic cages in a temperature-controlled room (22−24℃) under a 12 h light/dark cycle. Rats were given free access to drinking water and fed a semisynthetic diet prepared according to the recommendations of the American Institute of Nutrition (AIN-93G)[8] and modified diet containing 0.25 % or 1.0 % (w/w) LC (LC0.25 and LC1.0 groups). After acclimation for 3 days with an AIN-93G diet, rats were divided into the following three dietary groups of seven rats each ; control group, LC0.25 group, and LC1.0 group. After feeding with the experimental diets for 4 weeks, rats were weighed and sacrificed under anesthesia with sodium pentobarbital. Blood was collected from a descending artery, and serum was prepared by centrifugation at 1,500 g for 15 min and then stored at −80℃ until analysis. Liver and abdominal WAT were removed rapidly and then were weighed, rinsed, frozen in liquid nitrogen, and stored at −80℃ until analysis. In addition, the pinnule of the liver was taken for mRNA expression analysis and stored in RNA-Later Storage Solution (Sigma Chemical Co., St. MO, USA).

3. Analysis of the serum and liver lipid contents

Serum total lipid (TL), triacylglycerol (TG), cholesterol (CHOL), and phospholipid (PL) contents were determined using an Olympus AU5431 automatic analyzer (Olympus Co., Tokyo, Japan).

Liver lipids were extracted by the method of Bligh and Dyer[9]. Liver PL were separated by silica gel column chromatography using chloroform and methanol as elution solvents[10] and were weighted. Liver CHOL contents were determined by gas-liquid chromatography (GC-14B, Shimadzu, Kyoto, Japan) using 5α-cholestane as an internal standard. Liver TG contents were calculated by subtracting the PL and CHOL contents from TL. Liver protein contents were determined using the method of Bradford with bovine serum albumin as a standard[11].

4. Preparation of the liver and enzyme activity measurements

Liver was homogenized in 10 volumes of a 0.25 M sucrose containing 1 mM EDTA-2Na in 3 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 500 g for 10 min at 4℃. The supernatant was recentrifuged at 9,000 g for 10 min at 4℃ to sediment the mitochondria, and the remaining supernatant was collected. Furthermore, the supernatant was ultracentrifuged at 105,000 g for 60 min at 4℃ to collect the remaining supernatant and microsome fractions.

Acyl-CoA oxidase (ACOX, EC 1.3.3.6) activity was measured in the 500 g supernatant fraction of liver homogenate as described previously[12,13]. CPT-2 (EC 2.3.1.21) activity in the mitochondrial fraction was measured as described by Markwell *et al.*[14]. Fatty acid synthase (FAS)[15], acetyl-coenzyme A carboxylase (ACC, EC 6.4.1.2),[16] glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49)[17] in the 105,000 g supernatant fraction, and microsomal phosphatidate phosphatase (PAP, EC 3.1.3.4)[18] in the microsome fraction activities were measured spectrophotometrically. Protein contents were determined according to the method of Bradford.

5. Analysis of liver mRNA

Total RNA was extracted from 100 mg of liver using TRIZOL Reagent (Invitrogen, Tokyo, Japan). cDNA was then synthesized from total RNA using RevarTraAce (TOYOBO CO., Ltd., Osaka, Japan), Real-time quantitative PCR analysis was performed with an automated sequence detection system (DNA Engine opticon 2, Bio-Rad Laboratories, California, USA) using SYBR®GreenER™ qPCR SuperMix Universal (Invitrogen, Tokyo, Japan). The primer sequences used for the detection of FAS, stearoyl-CoA desaturase-1 (SCD-1), CPT-1, ACOX, sterol regulatory element binding protein-1c (SREBP-1c), peroxisome proliferators activated receptor-α (PPAR-α), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were as follows. Forward : 5'-GAAGGCCACTTGTATTCCCA-3' and 5'-TGCAGCTTGGTCTGAACATC-3' ; for FAS, Forward : 5'-TGTTCGTCAGCACCTTCTTG-3' and 5'-GGATGTTCTCCCGAGATTGA-3' ; for SCD-1, Forward : 5'-CAGCTCGCACATTACAAGGA-3' and 5'-TGCACAAAGTTGCAGGACTC-3' ; for CPT-1, Forward : 5'-ACATATGACCCCAAGACCCA-3' and 5'-TGAGCCAGAACTATTGCGTG-3' ; for ACOX, Forward : 5'-GCACAGCAACCAGAAACTCA-3' and 5'-ACCACTTCAGGTTTCATGCC-3' ; for SREBP-1c, Forward : 5'-GCTTCATCACCCGAGAGTTC-3' and 5'-ACTGTCATCCAGTTCGAGGG-3' ; for PPARα, Forward : 5'-ATGACTC

TACCCACGGCAAG-3' and 5'-TACTCAGCACCAGCATC ACC-3' ; for GAPDH. Results were quantified with a comparative method and were expressed as a relative level after normalization to the GAPDH expression level.

6. Analysis of serum and liver L-carnitine contents

Serum and liver LC contents were determined using an L-Carnitine Enzymatic UV test (Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the manufacturer's protocol.

7. Statistical analysis

Data are expressed as means ± SEM of seven rats. The significance of differences between means was determined using Tukey-Kramer's test. Differences with $p < 0.05$ were considered significant.

Results and Discussion

1. Growth parameters and WAT weights

Control diet (AIN93G diet) contained very low level of LC (date not shown). Daily LC intake were 0.34 mg/kg and 0.16 mg/kg (respectively, initial intake and final intake ; LC0.25 group), 1.34 mg/kg and 0.66 mg/kg (respectively, initial intake and final intake ; LC1.0 group).

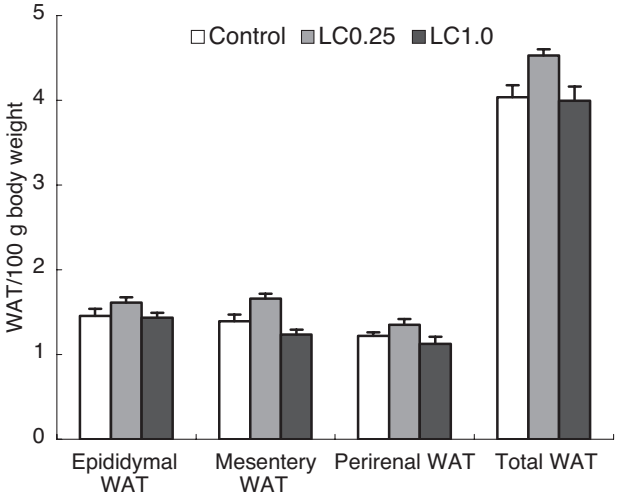

Fig. 1 Relative WAT weights of rats fed the experimental diets for 4 weeks.
Data are means ± SEM (n = 7). WAT, white adipose tissue.
Total WAT = epididymal WAT + mesentery WAT + perirenal WAT.

Table 1 shows growth parameters of rats. Initial body weights were not different among the groups. Final body weight, body weight gain, and energy intake of the LC0.25 group tended to be higher than in the control and LC1.0 groups.

Fig. 1 shows relative epididymal, mesentery, perirenal, and total WAT weights. Each WAT weight tended to be higher in the LC0.25 group than in the control group. However, the relative WAT weights were not different between the control and LC1.0 groups. Therefore, the relative increase in WAT weights in the LC0.25 group were due to the increased energy intake, but reason for the increase in energy intake was not clear.

2. Serum and liver lipid contents

Fig. 2 shows serum (a) and liver (b) lipid contents. Serum TL, TG, CHOL and PL contents (Fig. 2a) were not significant different among the groups. A previous study, high fat diet contained 0.3 % LC decreased serum TG and CHOL contents compared with high fat diet[19]. It was suggested that dietary LC did not affect serum or liver lipid contents in growing rats fed normal fat diets. Furthermore, there were no significant differences in liver TL, TG, CHOL, or PL contents (Fig. 2b) among the groups. From this result, it was suggested that dietary LC did not affect serum or liver lipid contents in growing rats.

3. Liver enzyme activities

Table 2 shows liver enzymes activities related to fatty acid metabolism. FAS, ACC and PAP activities, which are key enzymes in the regulation of fatty acid and TG de novo synthesis, were not significantly different among the groups. In addition, G6PDH, a key enzyme in the production of cellular NADPH activity, which is required for the biosynthesis of fatty acids and CHOL, was not significantly different among the groups.

CPT and ACOX activities, which are key enzymes of fatty acid β-oxidation in mitochondria and peroxisomes, were not significantly different among the groups. LC supplementation had been expected to alter CPT-2 activity in the liver mitochondria, but dietary LC did not affect CPT-2 activity in growing rats.

Table 1 Growth parameters of rats fed the experimental diets for 4 weeks

	Control	LC0.25	LC1.0
Initial body weight (g)	122.3 ± 2.0	124.3 ± 2.7	123.1 ± 2.4
Final body weight (g)	312.6 ± 5.2	321.3 ± 5.0	307.9 ± 3.6
Body weight gain (g/day)	7.05 ± 0.2	7.30 ± 0.1	6.99 ± 0.1
Energy intake (kcal/day)	73.8 ± 2.7	77.3 ± 2.8	72.1 ± 2.8

Data are means ± SEM (n = 7).
Food consumption and body weight were recorded every two days.

4. Liver mRNA expression levels

Enzyme and receptor expression levels related to fatty acid metabolism in the liver were measured to investigate the effect of dietary LC using real-time quantitative PCR (Fig. 3). FAS and SCD-1, key enzymes in the cellular synthesis of monosaturated fatty acid from saturated fatty acids, CPT-1, which mediates the transport of long chain fatty acids across the membrane by binding them to carnitine, and ACOX expression levels tended to be higher in the LC0.25 group than in the control and LC1.0 groups.

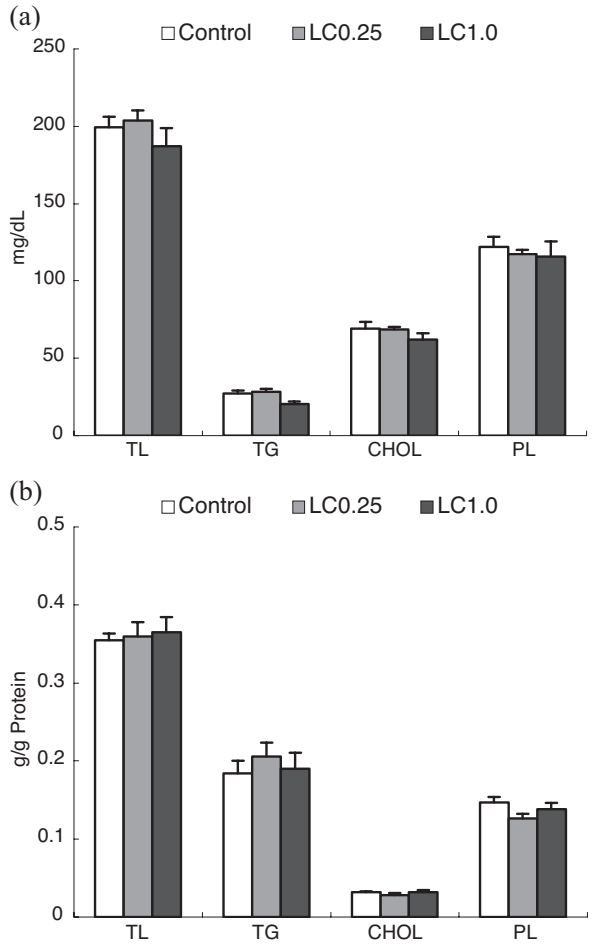

Fig. 2 Serum (a) and liver (b) lipid contents of rats fed the experimental diets for 4 weeks.
Data are means ± SEM (n = 7). TL, total lipid; TG, triacylglycerol; CHOL, cholesterol; PL, phospholipid.

The reason for the increases in FAS, SCD-1, CPT-1, and ACOX expression levels in the LC0.25 group were due to the increase in energy intake. Furthermore, SREBP-1c[19] and PPARα[20], a lipogenic and lipolytic transcriptional factor, expression levels were not significantly different in the control, LC.0.25, and LC1.0 groups. From this result, it was suggested that dietary LC did not affect liver lipid metabolism enzyme or receptor expression levels in growing rats.

5. Serum and liver LC contents

Fig. 4 shows serum and liver LC contents. LC found in the body is either provided by dietary LC or comes from an endogenous biosynthetic pathway. LC biosynthesis is via the activity of BBH on lysine and methionine. In rats, BBH is mainly found in the liver[7]. Serum LC contents were significant higher in the LC0.25 and LC1.0 groups than in the control group. In addition, liver LC contents were significant higher in the LC1.0 group than in the control group. This study did not analyze BBH activity. However, Davis *et al.* suggested that dietary LC decreased liver BBH activity in rats[21]. Therefore, it was predicted that the increased serum and liver LC contents in the LC 0.25 and LC1.0 groups were due to intake of LC from the diets.

In this study, it was confirmed that increased serum and liver LC contents were detected in the dietary LC groups. However, CPT-2 activity and CPT-1 expression level were not altered in rats with or without LC supplementation. Therefore, it was suspected that the growing rats did not require supplementation with LC, which is required for the β-oxidation of fatty acid, due to the presence of the endogenous biosynthetic pathway.

Conclusion

The present study evaluated the effect of LC on lipid metabolism in growing rats. Dietary LC led to increased serum and liver LC contents; however, the relative WAT

Table 2 Fatty acid metabolic enzymes activities in the liver of Wistar rats

	Control	LC0.25	LC1.0
	(nmol/min/mg protein)		
FAS	1.5 ± 0.2	1.6 ± 0.1	1.4 ± 0.1
ACC	49.8 ± 1.4	52.1 ± 3.9	52.9 ± 2.0
PAP	6.3 ± 0.2	6.2 ± 0.1	6.9 ± 0.4
G6PDH	16.8 ± 1.2	17.8 ± 2.2	15.7 ± 1.4
CPT-2	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
ACOX	1.5 ± 0.2	1.4 ± 0.1	1.6 ± 0.1

Data are means ± SEM (n = 7).
FAS, fatty acid synthase; ACC, acetyl-coenzyme A carboxylase; G6PDH, glucose-6-phosphate dehydrogenase; PAP, phosphatidate phosphatase; CPT-2, carnitine palmitoyl transferase-2; ACOX, acyl-coenzyme A oxidase.

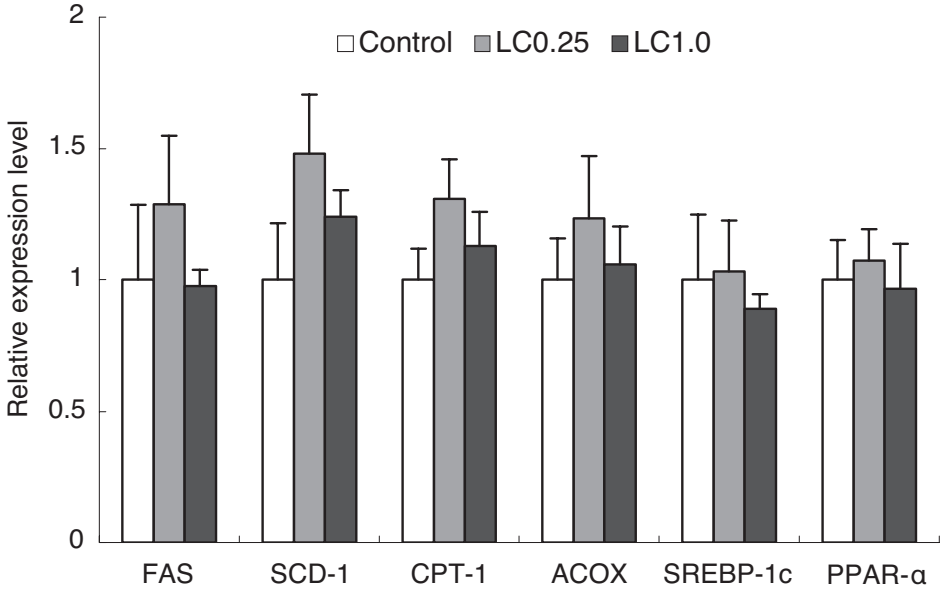

Fig. 3 Fatty acid metabolism enzyme and receptor expression levels in the liver. Rats were fed the experimental diets for 4 weeks. Fatty acid metabolism enzymes expression levels were analyzed by quantitative RT-PCR and expressed relative to the control group. Relative values are presented the ratio of mRNA to GAPDH mRNA.
Data are means ± SEM (n = 7). FAS, fatty acid synthase ; SCD-1, stearoyl-CoA desaturase-1 ; CPT-1, carnitine palmitoyl transferase-1 ; ACOX, acyl-coenzyme A oxidase ; SREBP-1c, sterol regulatory element binding protein-1c ; PPAR-α, peroxisome proliferators activated receptor-α ; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

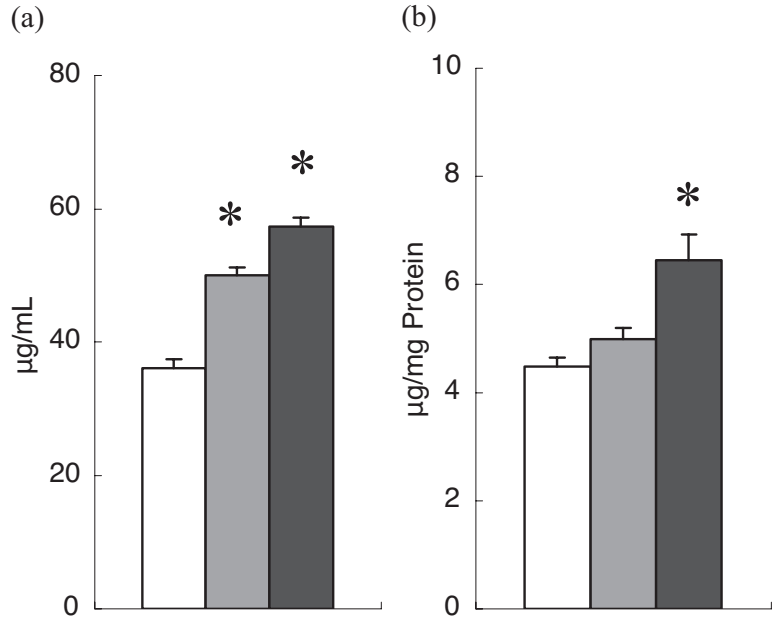

Fig. 4 Serum (a) and liver (b) LC contents of rats fed the experimental diets for 4 weeks.
Control (□), LC0.25 (■) and LC1.0 (■).
Data are means ± SEM (n = 7). * Mean values were significantly difference at $p < 0.05$. LC ; L-carnitine.

weights, serum and liver lipid contents, CPT-2 activity, and CPT-1 expression level did not alter in growing rats. From these results, growing rats did not require supplementation with LC for the β-oxidation of fatty acid. Therefore, it was suggested that LC intake by young people is not required as part of the diet.

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