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L-Carnitine intake prevents irregular feeding-induced obesity and lipid metabolism disorder

Tao Wu [1], Anqi Guo [1], Qingyu Shu, Yangjian Qi, Ying Kong, Zhiping Sun, Shumin Sun, Zhengwei Fu *

College of Biological and Environmental Engineering, Zhejiang University of Technology, China

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ABSTRACT

L-Carnitine supplementation has been used to reduce obesity caused by high-fat diet, which is beneficial for lowering blood and hepatic lipid levels, and for ameliorating fatty liver. However, whether L-carnitine may affect irregular feeding-induced obesity and lipid metabolism disorder is still largely unknown. In the present study, we developed a time-delayed pattern of eating, and investigated the effects of L-carnitine on the irregular eating induced adiposity in mice. After an experimental period of 8 weeks with L-carnitine supplementation, L-carnitine significantly inhibited body weight increase and epididymal fat weight gain induced by the time-delayed feeding. In addition, L-carnitine administration decreased levels of serum alanine aminotransferase (GPT), glutamic oxalacetic transaminase (GOT) and triglyceride (TG), which were significantly elevated by the irregular feeding. Moreover, mice supplemented with L-carnitine did not display glucose intolerance-associated hallmarks, which were found in the irregular feeding-induced obesity. Furthermore, quantitative real-time polymerase chain reaction (qRT-PCR) analysis indicated that L-carnitine counteracted the negative alterations of lipid metabolic gene expression (fatty acid synthase, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, cholesterol 7α-hydroxylase, carnitine/acylcarnitine translocase) in the liver and fat of mice caused by the irregular feeding. Therefore, our results suggest that the time-delayed pattern of eating can induce adiposity and lipid metabolic disorders, while L-carnitine supplementation might prevent these negative symptoms.

1. Introduction

Obesity among children and adults has emerged as a global epidemic, and it is becoming a serious public health issue in the world (Jin et al., 2013). Obesity is one of the risk factors for severe health problems associating with a variety of diseases, such as non-alcoholic fatty liver disease, type 2 diabetes, cardiovascular disease and cancer (Calle and Thun, 2004; Oben et al., 2010; Mokdad et al., 2003; Lavie et al., 2009). Changes in dietary and physical activity patterns are the possible reasons of obesity and overweight. Recent studies indicated that not only a high-fat diet could induce obesity (Hatori et al., 2012; Kohsaka et al., 2007), but also an unexpected feeding time would result in abnormal body weight gain and metabolism disorder (Salgado-Delgado et al., 2010; Yoon et al., 2012). In mice, the "wrong" feeding time (i.e., during the light) caused an increase in body weight, compared with the "right" feeding time (i.e., during the dark) (Arble et al., 2009). Additionally, most of young people tend to eat dinner late in the evening after work, which increased the risk of obesity (Baron et al., 2011). Therefore, eating time may be a vital factor in weight gain and inducing obesity.

L-Carnitine (β-hydroxy-γ-trimethyl ammonium butyrate) was first discovered in 1905 as a constituent of muscle tissue (Rebouche, 2004). The main function of L-carnitine is its role in the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix in which β-oxidation takes place (Hathcock and Shao, 2006; Marcovina et al., 2013). Therefore, most of the dietary lipids cannot be used as an energy source without L-carnitine, which will finally result in accumulated fatty-acids and body obesity. Several studies have been published in recent years suggesting that L-carnitine supplementation could reduce high-fat diet induced obesity (Yang et al., 2006; Amin and Nagy, 2009). Moreover, L-carnitine is beneficial for lowering lipid levels in the blood or liver (Kim et al., 2008) and ameliorating fatty liver (Xia et al., 2011), potentially through carnitine-mediated lipid metabolism. In addition to the role in fatty acid oxidation, L-carnitine is also effective in normalizing insulin sensitivity of type 2 diabetic patients by controlling the synthesis of key glycolytic and gluconeogenic enzymes (Mingrone, 2004).

However, no report can be found in the literatures relating to the effect of L-carnitine on the irregular feeding-induced obesity and lipid

Abbreviations: ET, experimental time; DL, dark–light; GPT, alanine aminotransferase; GOT, glutamic oxalacetic transaminase; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; GTT, glucose tolerance test; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction.

* Corresponding author at: College of Biological and Environmental Engineering, Zhejiang University of Technology, No. 6 District, Zhaohui, Hangzhou, Zhejiang, 310032, China.
E-mail address: azwfu@zjut.edu.cn (Z. Fu).
[1] Authors with equal contributions.

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metabolic disorders. Therefore, in the present study, we first developed an animal model of irregular feeding-induced obesity in mice. Then, we examined the effects of the L-carnitine and irregular feeding on the body weight and visceral fat mass, the lipid profiles in the serum, the glucose tolerance ability, and the expression of multiple lipid metabolic genes in mice.

2. Materials and methods

2.1. Materials

L-Carnitine was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China), and was mixed with normal commercial diet (M01-F, Shanghai Slac Laboratory Animal Co. Ltd.) at 0.5% w/w (L-carnitine containing diet). A feeding of this diet (12.5 mg L-carnitine/mouse/day) which was equivalent to a dosage of about 400 mg of L-carnitine per kg of mouse weight per day was fed to each mouse in the L-carnitine related group. The composition of each diet is listed in Table S1.

2.2. Animals and experimental design

Male ICR mice aged 8 weeks were purchased from China National Laboratory Animal Resource Center (Shanghai, China). They were caged individually in a temperature (22 ± 1 °C) and light (light off at 08:00 and light on at 20:00) controlled room. The onset of darkness was defined as experimental time 0 (ET0) and the onset of light at ET12. Water and food were available ad libitum. Mice were adapted to this lighting and feeding condition for at least 1 week before the following experiments.

To examine the effect of L-carnitine on the irregular feeding induced obesity and lipid metabolism disorder, mice were randomly divided into three groups of the Control group (Con), the Late-Feeding group (LF) and the Late-Feeding + L-carnitine group (LF + LC). The feeding schedule was altered in different groups, but the 12 h:12 h dark–light (DL) cycle was not changed (Fig. S1). In the Con group, mice were fed at ET0–ET12 with 100% of total normal diet. In the LF group, mice were fed at ET4 with 50% of total normal diet and ET12 with another 50% of total normal diet. In the LF + LC group, mice were also fed at ET4 with 50% of total normal diet, ET12 with L-carnitine containing diet (20% of total), and ET13 with 30% of total normal diet. All animals were treated as described above for a period of 8 weeks.

All mice were killed under deep anesthesia with ether. Liver and epididymal fat were removed surgically, weighed and immediately frozen in liquid nitrogen, and kept at −80 °C until the RNA was extracted. Blood was collected from the axillary vessels into centrifuge tubes, kept on ice briefly and centrifuged at 6000 ×*g* for 5 min at 4 °C and stored at −40 °C until biochemical analysis. Every effort was made to minimize animal suffering and the number of rats required for each experiment. All experiments were performed according to institutional guidelines, and the study was approved by the Research Committee of Zhejiang University of Technology.

2.3. Biochemical analysis

The levels of alanine aminotransferase (GPT) and glutamic-oxaloacetic transaminase (GOT), triglycerides, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol in plasma were measured using chemistry analyzer (Achtection c8000; Abbott, North Chicago, Illinois, USA) using commercial kits (Whitman Biotech, Nanjing, China).

2.4. Food intake, body, and tissue weight

Mice of each group were given the same total amount of food every day (5.0 g/mouse/day). To ensure each mouse of the LF + LC group could consume the entire 12.5 mg of L-carnitine every day, mice were fed with 1 g L-carnitine containing diet (20% of total) first at ET12, and then fed with 30% of total normal diet at ET13. Mice were weighed every four days from week 1 to week 8. At the end of this experiment, mice were fasted for 10 h before they were killed, and their livers and epididymal fat were also weighed.

2.5. Glucose tolerance test

During the final week of the experimental period, a glucose tolerance test (GTT) was performed. Mice were fasted for 16 h, and fasted glucose was measured using a Glucometer (Optium Xceed) by tail bleeds. Subsequently, mice were intraperitoneally injected with 2 g D-glucose/kg of body weight (0.2 ml of a glucose solution per mice), and the blood glucose was measured at 15, 30, 60, 90 and 120 min after the D-glucose administration (Bhandari et al., 2008).

2.6. RNA isolation and reverse transcription

The total RNA of livers and epididymal fat were isolated using the TRIzol reagent (Takara Biochemicals, China) and reverse-transcribed by M-MLV reverse transcriptase kit (TOYOBO, Tokyo, Japan) according to the manufacturer's instructions as previously described (Wu et al., 2008).

2.7. Real-time PCR

Real-time polymerase chain reaction (PCR) was performed on an Eppendorf MasterCycler ep RealPlex4 (Wesseling-Berzdorf, Germany), with the SYBR ExScript PCR Kit (TOYOBO, Tokyo, Japan) as described previously (Wu et al., 2008). The sequences of primers used in the Real time PCR were shown in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control for normalization.

2.8. Data analysis

Results are expressed as mean ± SEM of n = 5 animals. The values for mRNA levels are presented as relative values in all experiments. Differences between the different groups were evaluated using the Student–Newman–Keuls test following one-way ANOVA. $^{*}p < 0.05$, the LF group versus the Con group; $^{\#}p < 0.05$, the LF + LC group versus the LF group.

3. Results

3.1. Effects of the L-carnitine and irregular feeding on body weight gain

To test whether L-carnitine can prevent irregular feeding induced obesity, we subjected 8-week-old male ICR mice to three different eating patterns for 8 weeks. As shown in Fig. 1, the body weight of the LF group was increased as compared with that of the Con group ($p > 0.05$). While the body weight of the LF + LC group was clearly decreased as compared with that of the LF group after 2 weeks of the L-carnitine administration, which was shown significantly on days 16, 24, 28, 48, and 52 ($p < 0.05$) and close to significant on day 32 ($p = 0.0516$), day 36 ($p = 0.0579$), day 40 ($p = 0.0546$), day 44 ($p = 0.076$) and day 56 ($p = 0.0806$), respectively.

3.2. Effects of the L-carnitine and irregular feeding on tissue weight

Fig. 2 showed the ratio of the tissue weight relative to the body weight of three different groups. No significant difference was found in the liver weight/body weight ratio between the LF and LF + LC groups (Fig. 2A). As shown in Fig. 2B, the epididymal fat weight/body weight ratio was higher in the LF group than that in the Con group ($p < 0.05$). After the L-carnitine administration, however, the epididymal fat weight/body weight ratio significantly reduced in the LF + LC group as compared to the LF group ($p < 0.05$).

Table 1
Primer sequences used for PCR amplification.

Gene	Accession number	Primer sequence 5′ to 3′
GAPDH	NM_008084.2	Forward, GACCTCAACTACATGGTCTACA Reverse, ACTCCACGACATACTCAGCAC
PPARα	NM_011144.6	Forward, CCTCAGGGTACCACTACGGAGT Reverse, GCCGAATAGTTCGCCGAA
PPARγ	NM_001127330.1	Forward, CCAACTTCGGAACTCAGCTCTG Reverse, AACCTGATGGCATTGTGAGACA
Fasn	NM_007988.3	Forward, GCAGCAAGTGTCCACCAACAA Reverse, CTCATCGGAGCGCAGGATAGA
HMGCoAR	NM_008255.2	Forward, CAGCTTACAGAGCCAATGATGGAG Reverse, AGCCCATAAATGATTCAGTCACCAA
Cyp7a1	NM_007824.2	Forward, ACCTCCGGGCCTTCCTAAA Reverse, TCAAACATCACTCGGTAGCAGAA
CPT1	NM_031559	Forward, CACTGGCCGAATGTCAAG Reverse, TGCAAACATCCAGCCGTG
CPT2	NM_009949	Forward, GACAGCCAGTTCAGGAAGACAG Reverse, TATTCTGTTTATCCTGAGCGAGC
Slc20a25	NM_020520	Forward, GAGAGGGCATCACAGGGCT Reverse, CTTCCCCAGACCAAACCCA

3.3. Effects of the L-carnitine and irregular feeding on the levels of serum GPT, GOT and lipids

As shown in Fig. 3A–B, the levels of serum GPT and GOT, as markers of liver injury, were both elevated in the LF group as compared to those in the Con group ($p > 0.05$). However, the serum GPT and GOT levels were both significantly lower in the LF + LC group than those in the LF group ($p < 0.05$). The effects of L-carnitine supplementation and irregular feeding on serum lipid profiles in serum are also shown in Fig. 3C–F. Serum triglyceride (TG) concentration was significantly higher in the LF group as compared to that in the Con group ($p < 0.05$), while it was significantly lower in the LF + LC group as compared to the LF group ($p < 0.05$). As for the serum total cholesterol (TC) concentrations, similar changes were shown between the LF and Con groups as those described in the serum TG concentrations. However, after L-carnitine administration, the serum TC level of the LF + LC group was clearly decreased as compared with that of the LF group, which was shown close to significant ($p = 0.0586$). In addition, the levels of high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were similar among three groups, respectively (Fig. 3E–F).

3.4. Effects of the L-carnitine and irregular feeding on the glucose tolerance ability

To assess the ability of mice to dispose of a glucose load, we performed glucose tolerance tests following i.p. injection of D-glucose (2 g/kg of body weight) after an overnight fast (Fig. 4). At 15 min after the D-glucose injection, the change in the blood glucose level was significantly higher in the LF group than that in the Con group ($p < 0.05$), while it was significantly lower in the LF + LC group than that in the LF group ($p < 0.05$). And a tendency for lower levels of the blood glucose was shown in the LF + LC group as compared with that in the LF group throughout the experimental period.

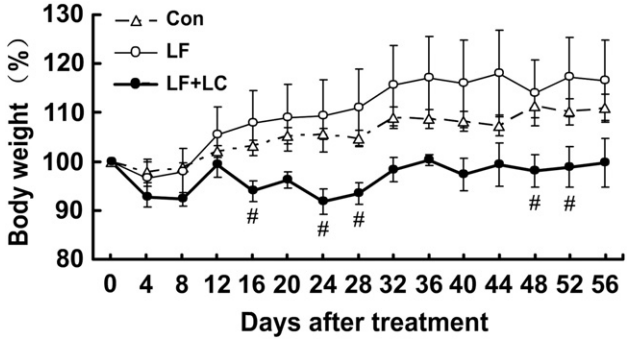

Fig. 1. Effects of the L-carnitine and irregular feeding on body weight. Mice (n = 5/group) were treated with three feeding regimens for 8 weeks. Body weight was measured every four days and expressed as percentage of that in day 0. Values were expressed as mean ± SEM. $^{*}p < 0.05$, the LF group versus the Con group; $^{\#}p < 0.05$, the LF + LC group versus the LF group.

3.5. Effect of L-carnitine on hepatic mRNA levels of genes involved in lipid metabolism

In order to study the molecular events possibly responsible for the observed changes in the lipid metabolism, mRNA levels of some genes related to the lipid metabolism in the liver of mice were determined by real time PCR (Fig. 5). The mRNA level of peroxisome proliferator activated receptor γ (*PPARγ*), which controls fat storage in adipose tissue by promoting differentiation and survival of adipocytes, was not altered among three groups (Fig. 5A). The expression of lipogenic gene such as fatty acid synthase (*Fasn*) was significantly increased in the LF group as compared with that in the Con group ($p < 0.05$). However, the expression of this gene in the LF + LC group was significantly decreased when compared with that in the LF group ($p < 0.05$). Moreover, the transcript level of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (*HMGCoAR*), a key regulator of the cholesterol biosynthesis pathway, was significantly increased in the LF group ($p < 0.05$) while it greatly decreased in the LF + LC group ($p < 0.05$) as compared with that in the Con group. The expression of cholesterol 7α-hydroxylase (*Cyp7a1*), which encodes the rate-limiting step in bile acid production from cholesterol, was especially increased in the LF group as compared with that in the Con group ($p < 0.05$). No significant difference was found in the mRNA level of this gene between the LF and LF + LC groups ($p > 0.05$). To analyze the effect of L-carnitine on the expression of lipolytic genes, the mRNA levels of peroxisome proliferator activated receptor α (*PPARα*), carnitine palmitoyltransferase 1 (*CPT1*), carnitine palmitoyltransferase 2 (*CPT2*) and carnitine/acylcarnitine translocase (*Slc20a25*) were measured in the liver (Fig. 5E–H). The expression of *PPARα* was slightly lower in the LF group than that in the Con group

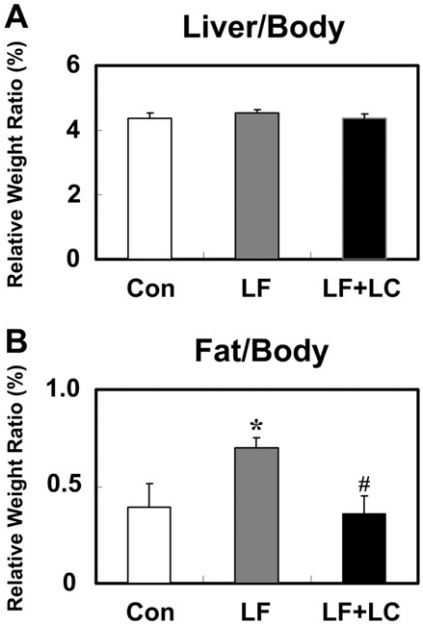

Fig. 2. Effects of the L-carnitine and irregular feeding on tissue weight. Mice (n = 5/group) were treated with three feeding regimens for 8 weeks. At the end of experiment, the liver and epididymal fat were removed and weighed. The ratio of the weight of the respective tissue relative to body weight (the body weight on day 56) is shown in the column (A, B). Values were expressed as mean ± SEM. $^{*}p < 0.05$, the LF group versus the Con group; $^{\#}p < 0.05$, the LF + LC group versus the LF group.

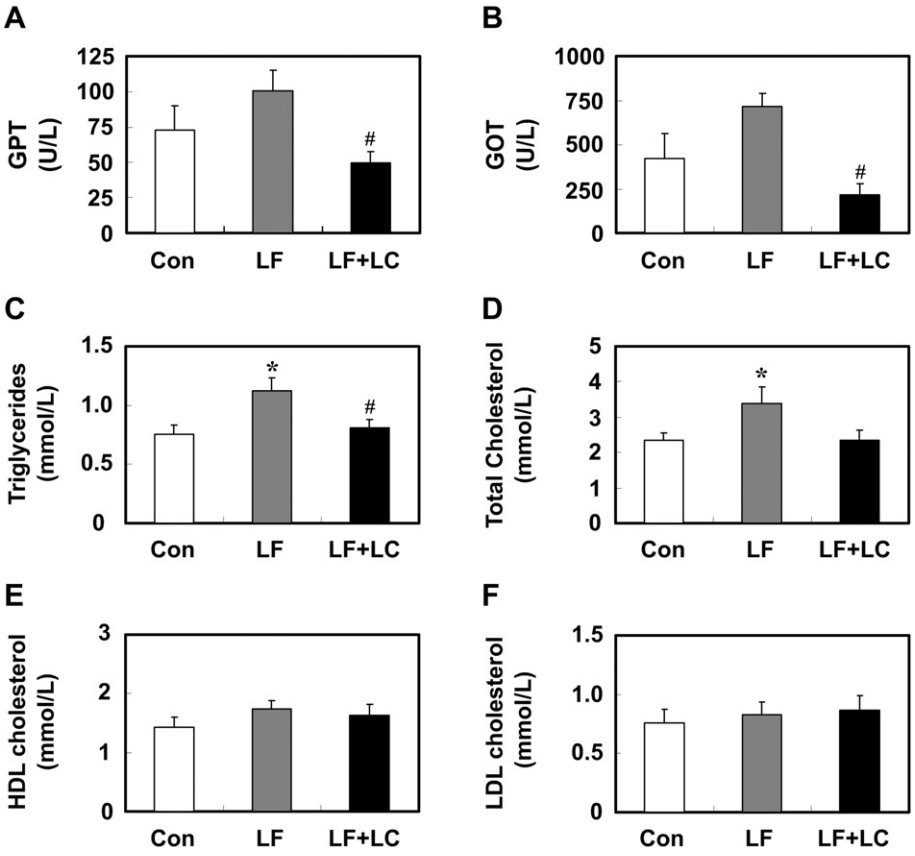

Fig. 3. Effects of the L-carnitine and irregular feeding on serum parameters. Mice (n = 5/group) were treated with three feeding regimens for 8 weeks. At the end of experiment, the serum concentration of GPT (A), GOT (B), TG (C), TC (D), HDL-C (E) and LDL-C (F) were determined. Values were expressed as mean ± SEM. $^{*}p < 0.05$, the LF group versus the Con group; $^{\#}p < 0.05$, the LF + LC group versus the LF group.

($p > 0.05$). In addition, the mRNA level of *CPT1*, the downstream target of *PPARα*, was significantly reduced in the LF group compared with that in the Con group. However, the mRNA level of *CPT2* and *Slc20a25* did not distinctly changed in three different groups.

3.6. Effect of L-carnitine on adipose mRNA levels of genes involved in lipid metabolism

Subsequently, we investigated whether the expression of lipid metabolic genes in the epididymal fat were altered as similar as that in the liver by L-carnitine supplementation (Fig. 6). In Fig. 6A, no significant difference was found in the mRNA level of *PPARγ* in three different groups ($p > 0.05$). The level of *Fasn* mRNA (Fig. 6B) was significantly higher than that in the Con group ($p < 0.05$). However, the expression of this gene in the LF + LC group was significantly lower than that in the LF group ($p < 0.05$). Additionally, the transcript levels of *HMGCoAR* (Fig. 6C) displayed a significant rise in the LF + LC group as compared with that in the LF group ($p < 0.05$). The expression of *Cyp7a1* (Fig. 6D) was especially increased in the LF + LC group as compared with that in the LF group ($p < 0.05$). In Fig. 6E–G, most of the examined lipolytic genes (*PPARα*, *CPT1* and *CPT2*) were not altered among the three groups. However, the expression of *Slc20a25* (Fig. 6H) was significantly increased in the LF + LC group as compared with that in the LF group ($p < 0.05$).

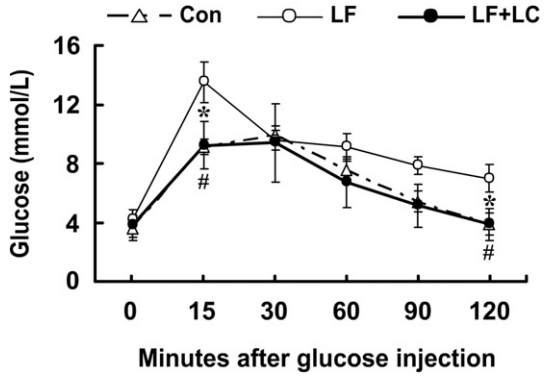

Fig. 4. Effects of the L-carnitine and irregular feeding on the glucose tolerance ability. During the final week of the experimental period, the blood glucose in fasted mice was measured after i.p. injection with 2 g/kg body weight D-glucose (n = 5/group). Values were expressed as mean ± SEM. $^{*}p < 0.05$, the LF group versus the Con group; $^{\#}p < 0.05$, the LF + LC group versus the LF group.

4. Discussion

Obesity is regarded as a disorder of energy balance, which perturbs body weight homeostasis (van Herpen and Schrauwen-Hinderling, 2008). The meal pattern has been identified as a factor influencing body weight (Fabty et al., 1964), which greatly contributes to the increasing prevalence of obesity (Metzner et al., 1977; Toschke et al., 2005; Mota et al., 2008). Night eating syndrome (NES) is described as a time-delayed pattern of eating, which is observed most frequently among lots of overweight and obese individuals (Colles et al., 2007; Tanofsky-Kraff and Yanovski, 2004). To develop a time-delayed feeding model, mice were provided with food during the resting phase in this study (Fig. S1). Results obtained here showed that a time-delayed feeding clearly induced higher body and epididymal fat weight in mice of the LF group compared with those of the Con group. Moreover, the administration of L-carnitine significantly reduced this time-delayed feeding-induced increase of body and epididymal fat weight gain, in spite of the same amount of total food intake. These data are similar with the previous studies reporting that L-carnitine could improve

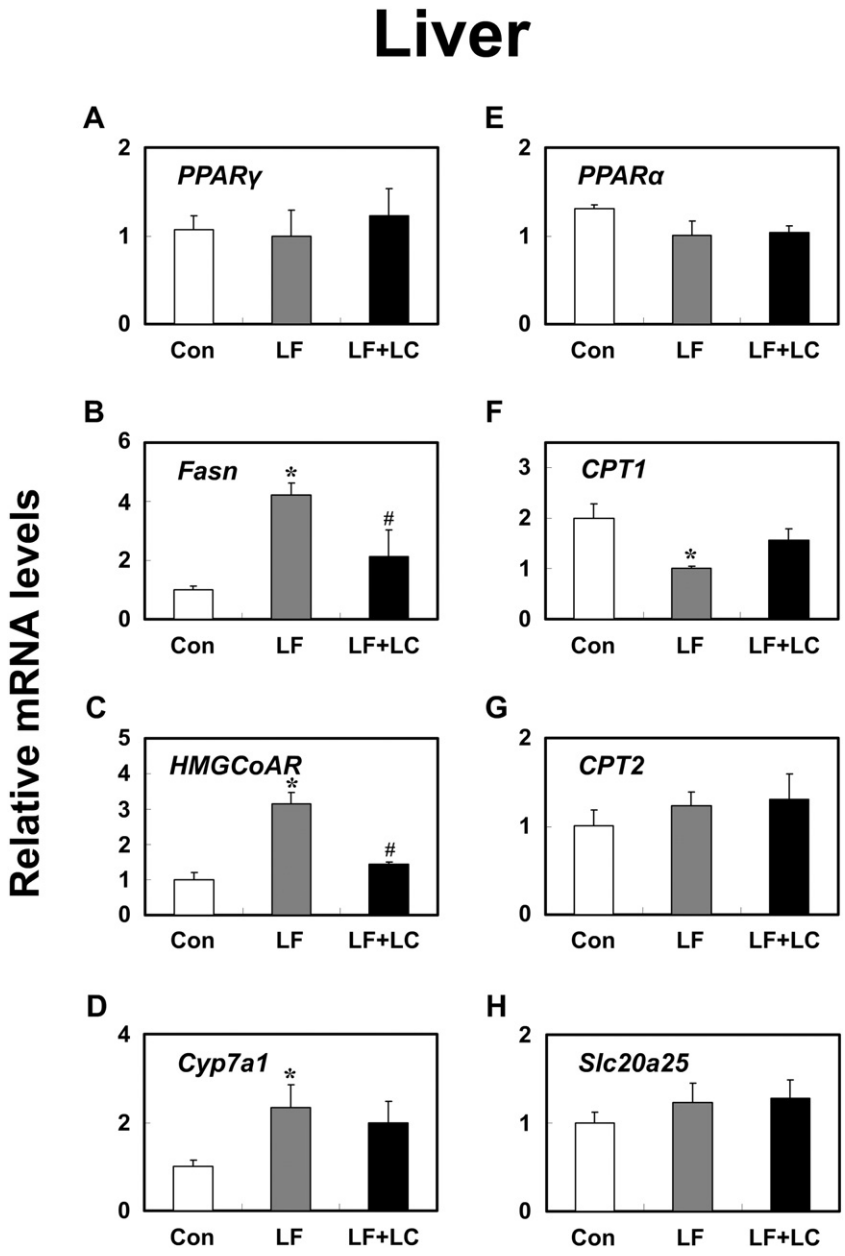

Fig. 5. Effect of L-carnitine on hepatic mRNA levels of genes involved in lipid metabolism. Real time PCR was used to determine gene expression levels of lipid metabolism in the livers of Con, LF and LF + LC mice. The mRNA content was normalized using GAPDH. Values were expressed as mean ± SEM (n = 5/group). $^{*}p < 0.05$, the LF group versus the Con group; $^{\#}p < 0.05$, the LF + LC group versus the LF group.

high-fat-induced obesity and its associated metabolic problems. The anti-obesity effect of L-carnitine might be mediated by the induction of lipolysis and fatty acid oxidation (Yang et al., 2006; Amin and Nagy, 2009).

In order to investigate the effect of L-carnitine on the regulation of lipid metabolism, we measured the serum GPT, GOT and lipids. Previous research reports have shown that the serum levels of GPT and GOT are often increased in high-fat-induced mice (Yoon et al., 2012; Fabty et al., 1964; Yamamoto et al., 2000; Fraulob et al., 2010). For example, Kang et al. (2011) recently found that L-carnitine with other compounds markedly suppressed the elevation of plasma GPT and GOT in mice fed with the high-fat diet. Our results showed that the serum levels of GPT and GOT were elevated in mice by the treatment of the irregular feeding schedule, while they were significantly reduced by the administration of L-carnitine, suggesting that L-carnitine could improve liver function. In addition, the irregular dietary significantly increased the serum TG and TC concentrations, being in agreement with a recent study, which claimed that an irregular meal pattern may be associated with elevated serum TG level in adults (Sierra-Johnson et al., 2008). Dietary supplementation of L-carnitine for 8 weeks reduced the serum cholesterol concentrations in mice fed with the irregular diet. L-Carnitine is necessary for mitochondrial transport metabolism of long-chain fatty acids, thus the dietary supplementation of L-carnitine might improve the utilization of fat, and result in marked reduction in plasma levels of TG and TC (Amin and Nagy, 2009; Kang et al., 2011). The present study failed to find any significant differences in the levels of HDL-C and LDL-C in the conditions of the irregular meal pattern or L-carnitine supplement. However, previous studies reported that some compounds including L-carnitine could significantly reduce the level of HDL cholesterol in high fat diet (HFD)-fed mice (Yang et al., 2006; Kang et al., 2011). The possible explanation may be that our experimental period was not long enough and the normal diet (not HFD) was used,

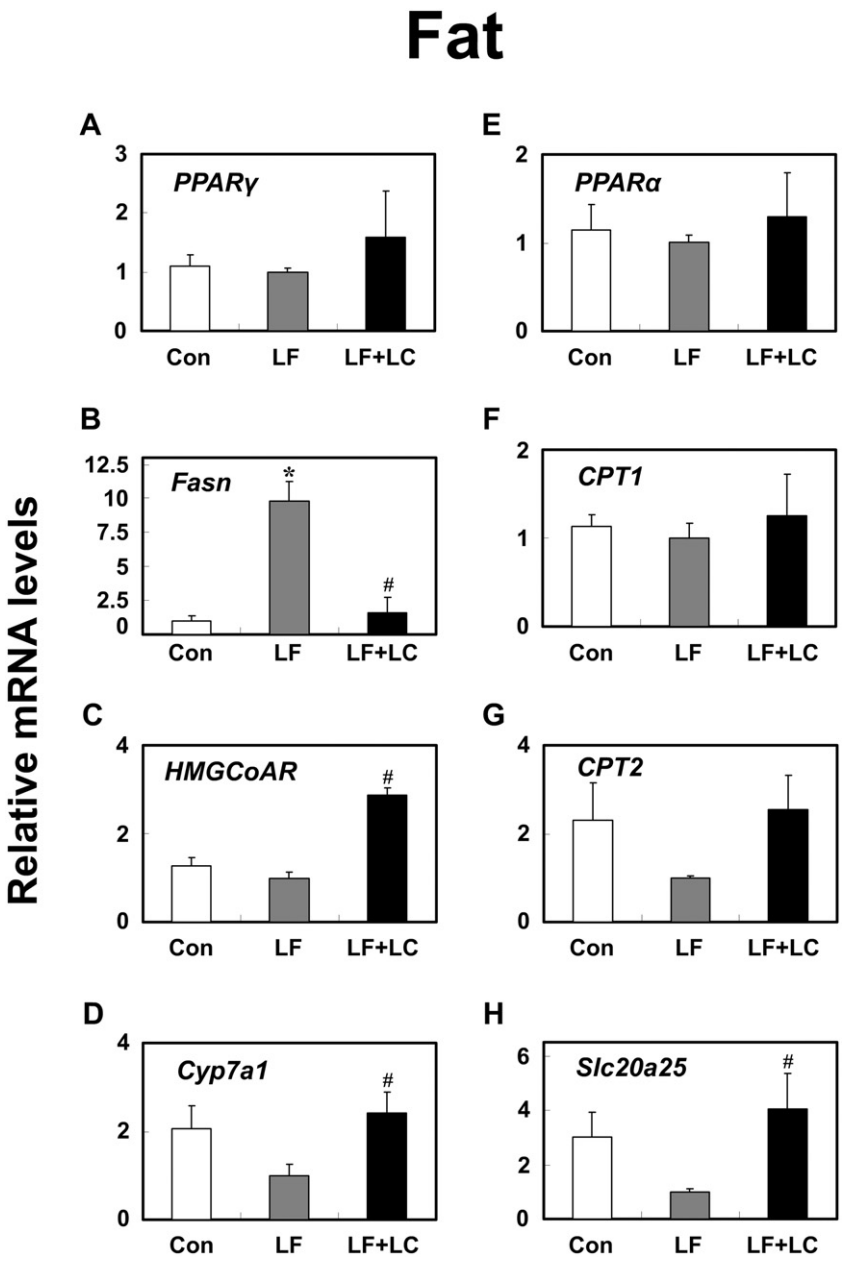

Fig. 6. Effect of L-carnitine on adipose mRNA levels of genes involved in lipid metabolism. The experimental procedures and explanation of each symbol are as described in Fig. 5. Values were expressed as mean ± SEM (n = 5/group). $^{*}p < 0.05$, the LF group versus the Con group; $^{\#}p < 0.05$, the LF + LC group versus the LF group.

resulting in mild metabolic disorders and obesity caused by the irregular feeding. Thus, it failed to find any significant differences in the serum HDL-C between the Con and LF groups.

Irregular eating pattern is considered as a potentially remediable risk factor for disordered glucose metabolism (Jaquiery et al., 2013). In our study, the LF mice displayed higher elevations in blood glucose levels following a glucose injection compared to the Con mice. This change in the LF mice suggests that the irregular eating pattern could lead to the glucose intolerance. However, this glucose intolerance induced by the irregular eating pattern could be alleviated by L-carnitine administration, suggesting that L-carnitine has the role in protecting from the impaired glucose tolerance. Recent studies have reported that L-carnitine supplementation could enhance glucose disposal in diabetic mice. Thus, our experimental data, to some extent, agreed with those of several studies showing that an improvement of the glucose tolerance in response to L-carnitine supplementation, as evidenced by reduced fasting glucose level (Rajasekar and Anuradha, 2007; Power et al., 2007).

To further understand the effect of L-carnitine on the molecular mechanism of lipid metabolism, we examined the expression of lipid metabolic genes in the liver and epididymal fat. The results showed that the irregular eating pattern significantly upregulated the expression of lipogenic gene *Fasn* both in the liver and fat, suggesting a substantial net increase of lipogenesis in adipose tissues coupled with increasing body weight and tissue weight in the LF mice. Interestingly, the administration of L-carnitine prevented the increase of *Fasn* transcript level induced by the irregular feeding. In the liver, the irregular eating pattern also led to the additional expression of *HMGCoAR* mRNA, which has shown the potential in maintaining cholesterol homeostasis and been considered as a susceptible gene for cholesterol de novo biosynthesis (Bonne et al., 2002). However, L-carnitine administration also restored the alteration of *HMGCoAR* transcript

level caused by the irregular feeding. Cholesterol conversion into bile acids in the liver is a key pathway in reducing the serum cholesterol level. Bile acid synthesis and excretion greatly contribute to the removing of cholesterol from the body (Russell, 2003). On the other hand, the irregular feeding led to an increase in the *Cyp7a1* mRNA level. Moreover, our study showed that the gene expression of *Cyp7a1* was slightly decrease by L-carnitine consumption. Interestingly, in the epididymal fat, we found that the gene expression of *HMGCoAR* was increased in mice treated with L-carnitine. This observation might be due to the possibility that L-carnitine initially caused a reduction of cholesterol synthesis and plasma cholesterol levels, which then led to a feedback mechanism causing an upregulation of *HMGCoAR*. Moreover, L-carnitine significantly increased the gene expression of *Cyp7a1* in the epididymal fat, suggesting that the cholesterol-lowering effect of L-carnitine is attributed to its promotion of the cholesterol conversion into the bile acid by up-regulating the gene expression of *Cyp7a1*. Thus, L-carnitine as a food supplement counteracts the negative alteration of lipid metabolic gene expression caused by the irregular eating pattern.

Taken together, the present study provides insight into the effect of L-carnitine on the irregular feeding-induced obesity and lipid metabolism disorder. The evidence indicates that the time-delayed pattern of eating resulted in lipid metabolism disorder and obesity in mice, and L-carnitine treatment could attenuate fat accumulating rate and enhanced glucose disposal ability in the time-delayed feeding mice. Moreover, L-carnitine improved the disturbed serum lipid profile and negative alteration of lipid metabolic gene expression caused by the irregular feeding. Our results therefore suggest that L-carnitine has a beneficial effect on the irregular feeding-induced obesity and lipid metabolism disorder in mice.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2014.10.040.

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