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

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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.

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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 ($p < 0.05$).