Effects of L-Carnitine Supplementation on Growth Performance and Fatty Acid Proportion in Epididymal Adipose Tissue of Rats Fed Diets containing Fat from Different Sources

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Abstract

Two experiments were conducted to evaluate the effects of L-carnitine supplementation on growth performance and epididymal adipose tissue fatty acid composition in rats fed diets containing different sources of fat. In the first study, rats were assigned to four groups receiving dietary treatments to evaluate the effects of supplementation with L-carnitine 0 ppm or L-carnitine 50 ppm in combination with two types of dietary fat: 100% soybean oil and a mixture of 50% soybean oil and 50% beef tallow. In the second study, the animals walked 2 h/day at a speed of 840 m/h. No significant differences were observed in the growth test results of each study. In experiment 1, the total saturated fatty acid (SFA) proportion was significantly higher in the L-carnitine 0 ppm 50% soybean oil and 50% beef tallow group than in the other groups. Dietary L-carnitine suppressed the increase in the SFA proportion observed upon beef tallow consumption. In experiment 2, L-carnitine decreased the SFA proportion and increased the monounsaturated fatty acid (MUFA) proportion. No interaction between the ingested fat type and L-carnitine supplementation on the proportions of individual fatty acids was observed, but L-carnitine supplementation increased the C16:1 proportion and decreased the C18:0 proportion as a main effect in experiment 2. In conclusion, the current results suggest that dietary L-carnitine decreased the SFA proportion in accordance with C16:1 utilization for energy production in walking rats.

Keywords: L-carnitine; Fat type; Rats; Walking; Fatty acid composition

Introduction

The fatty acid composition of pork fat directly reflects the composition of dietary fat sources rich in long-chain triglycerides (LCTs) as well as carbohydrate metabolism. L-carnitine has been proposed to increase the β-oxidation of LCTs across the inner mitochondrial membrane when energy is obtained via an anaerobic pathway. L-carnitine is a quaternary amine that is endogenously synthesized from lysine and methionine in the liver and kidney in mammals, and thus dietary supplementation is not essential [1]. Dietary L-carnitine has a physiological effect on the qualities of pork meat and/or the carcass [2-4]. Apple et al. [4] reported that dietary carnitine increased the intramuscular total saturated fatty acid (SFA) proportion and the total mono-unsaturated fatty acid (MUFA) proportion in the primary and secondary lean layers and decreased total poly-unsaturated fatty acid (PUFA) proportion of intramuscular fat. A relationship between L-carnitine and lipids has been proposed but only a few studies have reported an acceleration of changes in individual fatty acid content due to lipid utilization in response to L-carnitine supplementation (abdominal fatty acid composition of ducks, Arslan et al. [5] jowl fat composition of pigs, Ying et al. [3].

In the current study we chosen young rat as an experimental animal, because the purified diet has no L-carnitine in that composition and juvenile animal is insufficient the endogenous synthesis of L-carnitine. Therefore the effects of L-carnitine supplementation to the diets on the results compared in this study make clear. In nutritional studies in small rodents, fatty acid composition and accumulation volume are evaluated in epididymal adipose tissue. In the current study, the effects of dietary L-carnitine supplementation on growth performance and epididymal adipose tissue fatty acid composition were investigated in rats fed diets containing different types of fat as a model study of mono gastric animal.

Materials and Methods

Animals and diets

A total of 48 3-week-old female Sprague-Dawley strain rats (Japan Clea, Tokyo, Japan) were used in the experiments. The rats were housed individually in dormitory-type cages. A daily 12-h light/dark cycle, environmental temperature of 24 ± 2°, and 60% relative humidity were maintained. AIN-93G was prepared as the basal diet and soybean oil was used as a fat source. The beef tallow used to supplement the experimental group diet was purchased from Fuji Chemical Co., Ltd. (Tokyo, Japan). The animals consumed each diet in powder form under the pair-feeding method.

Experimental design and methodology

Two studies were conducted using different feeding conditions. In the first study (25 rats, mean body weight 73.6 ± 0.2 g), the control group included 7 rats who received L-carnitine 0 ppm with 100% soybean oil. The remaining 18 rats were assigned to three experimental groups: group 1 received L-carnitine 0 ppm with a mixture of 50% soybean oil and 50% beef tallow, group 2 received L-carnitine 50 ppm (50 mg/kg feed) with 100% soybean oil, and group 3 received L-carnitine 50 ppm with a mixture of 50% soybean oil and 50% beef tallow (Table 1). The effects of the various dietary conditions on the fatty acid composition and weight of the epididymal adipose tissue and growth performance, body weight gain, feed consumption, and feeding efficiency were evaluated. The feeding experiment was conducted for 4 weeks. The rats were sacrificed, and the epididymal adipose tissue was collected. Samples for determining the fatty acid composition were stored at -40° until analyzed.
Table 1: Modified composition of AIN-93G used in this study. The remaining diet ingredients were identical in the control and the treatment groups. There were trace levels of L-carnitine in the other ingredients.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Group – 1</th>
<th>Group – 2</th>
<th>Group – 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean L-Car 0 ppm</td>
<td>7.0%</td>
<td>7.0%</td>
<td>3.5%</td>
<td>3.5%</td>
</tr>
<tr>
<td>Soybean L-Car 50 ppm</td>
<td>50</td>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Table: The present study aimed to investigate the relationships between L-carnitine supplementation and growth performance and epididymal adipose tissue fatty acid composition using diets with different sources of fat and concentrations of L-carnitine. The weight of the adipose tissue was not determined in the first study, but no significant difference in adipose tissue weight was observed among the groups in the second experiment. Results of fecal fat level determination showed no significant difference and also there is no statistical difference in the digestibility of fat. These results demonstrate that the different compositions of fatty acids and L-carnitine supplementation had no effect on growth performance (Tables 2 and 3). Tsai et al. [8] were unable to observe a growth-promoting effect of dietary L-carnitine in rats fed a high-fat diet, but growth-promoting effect of dietary L-carnitine was observed in rats fed a low-fat diet with a low methionine content. Tsai et al. reported that L-carnitine promoted growth performance under limited nutritional conditions that were unfavorable to the animals. In the present study, the diets contained normal levels of nutrients, with varying fatty acid compositions. However, the results of the present study are consistent with those of Tsai et al. [8]. In the second study, rats underwent walking exercise, which accelerates the utilization of energy produced by β-oxidation of ingested fat. However, in contrast to the results of previous studies [9,10] no significant difference in growth performance was observed. The effect required for walking may not be sufficient to increase fat consumption.

Results and Discussion

The dry matter and ether extract of the diets and feces were determined by using Association of Official Analytical Chemists (AOAC) methods [6]. The fatty acids in the fat sources and the epididymal adipose tissue were converted to methyl esters by Trans esterification with boron fluoride/methanol reagent [7]. The fatty acid composition was determined by gas chromatography (GC-17A, Shimadzu, Co., Ltd., Kyoto, Japan) under the following conditions: 2.0 m×3.2 mm glass capillary column, column temperature of 180°C, injection temperature of 250°C, and flow rate of 25 mL/min.

Analysis

The results were statistically analyzed by two-way analysis of variance at a significance level of P<0.05. Values are expressed as the means ± standard error.
balance was negative [2]. Dietary (high fat) and environmental (cold
exposure) factors have also been employed to evaluate the role of L-
carnitine. The relationships between L-carnitine and fatty acids have
been investigated under different conditions. Apple et al. [4] reported
that L-carnitine supplementation increased the total SFA proportion in the
intramuscular fat layer and total MUFA proportion in the lean
layers, and decreased the total PUFA proportion in the intramuscular
reported that the total PUFA proportion was lower in low L-carnitine
content meat than in L-carnitine regular content meat. The present
results may be specific to the experimental conditions used, but the
decrease in the SFA proportion suggests that L-carnitine is required to
degradate SFAs as an energy source.

No interaction between the ingested fat type and L-carnitine
supplementation on the individual fatty acid proportion in the
epididymal adipose tissue was observed, as shown in Tables 3 and 5.
The compositions of fatty acids compared in this study, however,
differered markedly depending on the original fat administered, which
lacked C18:0 in the first experiment and C16:0 in the second
experiment. Consistent with the results of the present study, Bertol et
al. [12] concluded that in monogastric animals, dietary fatty acids are
reflected in the body fat composition, because the ingested fatty acids
enter tissues directly. The present results do not support the
observation by Su and Jones [13] that animal fat decreased energy
efficacy compared with plant oil because of increased fecal loss.
Table 4: Results of the growth tests and chemical analysis in the second study Mean ± SE – The number of replicates, n, was as follows: Control group: 5; Group 1, 6; Group: 2, 6; Group 3, 6

Katsumata et al. [11] reported that the L-carnitine content was lower in meat of the low lysine diet group compared to the meat of the control group and that the combination of reduced β-oxidation of fatty acids and enhanced de novo fatty acid synthesis may have contributed to a high intramuscular fatty acid content. Another report proposed that dietary carnitine does not affect the organ carnitine content, although plasma carnitine levels are increased by the carnitine supplementation [14]. By contrast, some reports have proposed that L-carnitine supplementation is significantly efficacious for fat degradation. Apple et al. [4] reported the changes in the total proportions of SFAs, MUFAs, and PUFAs in fresh pork bellies, but did not describe the relationship between L-carnitine supplementation and levels of individual fatty acids. Arslan et al. [5] also proposed that dietary L-carnitine supplementation decreased the total proportion of SFA, with a decrease in C16:0. Ying et al. [3] proposed that the 50mg/kg of L-carnitine reduced C18:2 in the jowl fat of pigs fed DDGS. Although no significant effects of L-carnitine on the proportions of individual fatty acids were observed in the first experiment, in the second experiment, L-carnitine supplementation increased C16:1 and decreased C18:0 as shown in Table 5. The decrease in C18:0 might suggest a preferential utilization of LCTs as an energy source. The C16:0 proportions in the abdominal fat in the L-carnitine group were significantly lower than that reported in an earlier study of ducks [5]. L-carnitine supplementation increased palmitoyltransferase activity, a fatty acid β-oxidation enzyme [15]. This enzyme activation may be related to the decrease in C16:0 fatty acids. In the present study, dietary L-carnitine decreased the SFA proportion in accordance with the consumption of C18:0 for energy production in the walking rats [16,17]. More strenuous exercise may clarify the relationships between L-carnitine and each fatty acid in the β-oxidation pathway for energy metabolism [18,19].


<table>
<thead>
<tr>
<th>Soybean oil</th>
<th>Tallow</th>
<th>Control group</th>
<th>Group – 1</th>
<th>Group – 2</th>
<th>Group – 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean L-Car 0 ppm</td>
<td>Soybean + Tallow L-Car 0 ppm</td>
<td>Soybean L-Car 50 ppm</td>
<td>Soybean + Tallow L-Car 50 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>0.1</td>
<td>1.4</td>
<td>0.8 ± 0.0^a</td>
<td>1.0 ± 0.0^b</td>
<td>0.8 ± 0.0^b</td>
</tr>
<tr>
<td>C16:0</td>
<td>15.7</td>
<td>24.5</td>
<td>27.7 ± 0.4</td>
<td>26.5 ± 0.4</td>
<td>26.4 ± 0.5</td>
</tr>
<tr>
<td>C16:1</td>
<td>tr.</td>
<td>3.2</td>
<td>5.5 ± 0.2^b</td>
<td>6.5 ± 0.3^b</td>
<td>6.7 ± 0.4^a</td>
</tr>
<tr>
<td>C18:0</td>
<td>5.5</td>
<td>15.5</td>
<td>3.4 ± 0.1^a</td>
<td>3.7 ± 0.1^a</td>
<td>2.9 ± 0.1^b</td>
</tr>
<tr>
<td>C18:1</td>
<td>36.2</td>
<td>52.8</td>
<td>39.8 ± 0.3^a</td>
<td>49.6 ± 0.3^b</td>
<td>39.5 ± 0.4^a</td>
</tr>
<tr>
<td>C18:2</td>
<td>36.5</td>
<td>1.9</td>
<td>20.2 ± 0.3^a</td>
<td>11.3 ± 0.6^b</td>
<td>21.1 ± 1.1^a</td>
</tr>
<tr>
<td>C18:3</td>
<td>6</td>
<td>0.7</td>
<td>2.6 ± 0.1^a</td>
<td>1.4 ± 0.1^b</td>
<td>2.6 ± 0.1^a</td>
</tr>
<tr>
<td>Total SFA</td>
<td>21.3</td>
<td>41.4</td>
<td>32.0 ± 0.6^a</td>
<td>31.2 ± 0.3^a</td>
<td>30.1 ± 0.5^b</td>
</tr>
</tbody>
</table>
Table 5: Results of fatty acid composition analysis (%) in the second study Mean ± SE – The number of replicates, n, was as follows: Control group: 5; Group 1, 6; Group: 2, 6; Group 3, 6. In the values of SE, 0.0 means that it is smaller than 0.04. Values with different superscript letters are significantly different (P<0.05)

<table>
<thead>
<tr>
<th></th>
<th>MUFA</th>
<th>PUFA</th>
<th>MUFA</th>
<th>PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MUFA</td>
<td>36.2</td>
<td>56.0</td>
<td>45.2 ± 0.4</td>
<td>56.1 ± 0.2</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>42.5</td>
<td>2.6</td>
<td>22.8 ± 0.3</td>
<td>12.7 ± 0.5</td>
</tr>
</tbody>
</table>

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References