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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 C18:0 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 ± 2º, 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 -40º until analyzed.

	Control group Soybean L-Car 0 ppm	Group – 1 Soybean L-Car 50 ppm	Group – 2 Soybean + Tallow L-Car 0 ppm	Group – 3 Soybean + Tallow L-Car 50 ppm
Soybean (921 Kcal/100g)	7.0%	7.0%	3.5%	3.5%
Tallow (921 Kcal/100g)	0	0	3.5%	3.5%
L-carnitine (ppm)	0	50	0	50

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.

In the second study (23 rats, mean body weight 76.8 ± 0.7 g), the control group included five rats, and the remaining 18 rats were assigned to three groups in the same 2×2 factorial arrangements used in the first study. However, in this study, the animals walked 2 h/day at a speed of 840 m/h in random order during daytime period, using a motor walking wheel, the circumference of which was one meter (Natsume Seisakusho, Tokyo), a condition that required high energy.

The animals used in this study were cared for in accordance with the guidelines of the Animal Welfare Act of Tokyo University of Agriculture.

Analysis

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°, injection temperature of 250°, and flow rate of 25 mL/min.

Statistics

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.

Results and Discussion

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.

	Control group Soybean L-Car 0 ppm	Group – 1 Soybean + Tallow L-Car 0 ppm	Group – 2 Soybean L-Car 50 ppm	Group – 3 Soybean + Tallow L-Car 50 ppm
Growth Tests				
Body Weight Gain (g/head/day)	7.9 ± 0.2	7.7 ± 0.2	7.9 ± 0.2	8.0 ± 0.2
Diet Intake (g/head/day)	19.9 ± 0.1	19.8 ± 0.1	20.0 ± 0.0	19.7 ± 0.1
Feed efficiency (%)	40.7 ± 1.2	40.0 ± 1.0	40.0 ± 0.9	42.0 ± 0.8
Chemical Analysis				
Dry matter of diet (%)	91.0	90.3	90.6	90.5

Ether extract in diet (DM %)	7.3	6.1	7.7	6.5
Dry matter of feces (%)	95.0 ± 0.2	95.0 ± 0.1	94.9 ± 0.3	94.8 ± 0.1
Ether extract in feces (DM %)	2.7 ± 0.4	2.9 ± 0.5	3.3 ± 0.1	3.1 ± 0.4
Digestibility of ether extract (%)	97.7 ± 0.2	96.4 ± 0.7	96.3 ± 0.5	96.3 ± 0.5

Table 2: Results of the growth tests and chemical analysis in the first study Mean ± SE – The number of replicates, n, was as follows: Control group: 7; Group 1, 6; Group: 2; Group 3, 6

	Soybean oil	**Tallow**	**Control group Soybean L-Car 0 ppm**	**Group – 1 Soybean + Tallow L-Car 0 ppm**	**Group – 2 Soybean L-Car 50 ppm**	**Group – 3 Soybean + Tallow L-Car 50 ppm**
C14:0	0.1	2.4	1.2 ± 0.0^b	1.5 ± 0.0^a	1.1 ± 0.0^b	1.4 ± 0.0^a
C16:0	10.5	28.4	20.5 ± 0.4^a	22.2 ± 0.3^b	20.9 ± 0.4^a	21.5 ± 0.5^b
C16:1	tr.	5.0	8.0 ± 0.4^a	10.1 ± 0.4^b	8.1 ± 0.5^a	9.9 ± 0.2^b
C18:0	2.5	10.7	1.3 ± 0.3	1.7 ± 0.3	1.4 ± 0.3	0.9 ± 0.4
C18:1	21.9	49.3	32.0 ± 0.8^a	42.8 ± 0.4^b	32.5 ± 0.9^a	43.6 ± 0.8^b
C18:2	56.8	4.0	33.5 ± 1.0^a	19.8 ± 0.8^b	33.0 ± 1.3^a	21.0 ± 1.3^b
C18:3	8.2	0.3	3.5 ± 0.2^a	1.8 ± 0.1^b	3.1 ± 0.2^a	1.7 ± 0.1^b
Total SFA	13.1	41.5	23.1 ± 0.2^b	25.7 ± 0.3^a	23.4 ± 0.4^a	23.8 ± 0.8^b
Total MUFA	21.9	54.3	40.2 ± 1.0^b	52.3 ± 0.4^a	40.9 ± 1.4^b	53.9 ± 0.8^a
Total PUFA	65.0	4.3	37.2 ± 1.1^a	21.6 ± 0.9^b	36.3 ± 1.3^a	22.8 ± 1.3^b

Table 3: Results of fatty acid composition analysis (%) in the first study Mean ± SE – The number of replicates, n, was as follows: Control group: 7; 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)

In the first study (Table 4), the proportion of total SFAs was significantly higher in group 1 than in the other groups, suggesting an interaction effect between the type of ingested fat and L-carnitine supplementation. Beef tallow increased the SFAs proportion, whereas L-carnitine suppressed the increase in the SFAs proportion induced by beef tallow consumption. The total proportions of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) differed significantly only in response to the main effect of different oil sources. By contrast, the main effect of L-carnitine supplementation decreased the total proportion of SFAs and increased the total proportion of MUFAs in study 2 (Table 5), and the total proportions of PUFA and MUFA revealed the main effect of the different oil sources. In this study, the effect of L-carnitine on the fatty acid proportion depended on the experimental conditions, and the general roles of L-carnitine were also observed in a study of the nursing sows in which the energy 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 fat layer and lean layers of fresh pork bellies. Katsumata et al. [11] 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 degrade 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, differed 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.

	Control group	**Group – 1**	**Group – 2**	**Group – 3**

	Soybean L-Car 0 ppm	Soybean + Tallow L-Car 0 ppm	Soybean L-Car 50 ppm	Soybean + Tallow L-Car 50 ppm
Growth Tests				
Body Weight Gain (g/head/day)	7.4 ± 0.3	7.6 ± 0.2	7.6 ± 0.2	7.8 ± 0.1
Diet Intake (g/head/day)	18.6 ± 0.1	18.3 ± 0.1	18.6 ± 0.1	18.6 ± 0.1
Feed efficiency (%)	41.6 ± 0.6	42.4 ± 0.7	41.8 ± 1.1	42.5 ± 0.9
Adipose tissue weight (g)	2.2 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.4 ± 0.3
Chemical Analysis				
Dry matter of diet (%)	90.6	90.8	90.7	90.7
Ether extract in diet (DM %)	8.2	8.1	7.9	7.8
Dry matter of feces (%)	93.0 ± 0.5	92.3 ± 0.2	91.5 ± 0.3	91.5 ± 0.3
Ether extract in feces (DM %)	1.9 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.8 ± 0.2
Digestibility of ether extract (%)	98.0 ± 0.1	98.1 ± 0.2	97.9 ± 0.1	98.0 ± 0.2

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 of 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].

	Soybean oil	Tallow	Control group Soybean L-Car 0 ppm	Group – 1 Soybean + Tallow L-Car 0 ppm	Group – 2 Soybean L-Car 50 ppm	Group – 3 Soybean + Tallow L-Car 50 ppm
C14:0	0.1	1.4	0.8 ± 0.0^{a}	1.0 ± 0.0^{b}	0.8 ± 0.0^{b}	1.0 ± 0.0^{b}
C16:0	15.7	24.5	27.7 ± 0.4	26.5 ± 0.4	26.4 ± 0.5	26.6 ± 0.3
C16:1	tr.	3.2	5.5 ± 0.2^{b}	6.5 ± 0.3^{b}	6.7 ± 0.4^{a}	7.9 ± 0.3^{a}
C18:0	5.5	15.5	3.4 ± 0.1^{a}	3.7 ± 0.1^{a}	2.9 ± 0.1^{b}	3.2 ± 0.1^{b}
C18:1	36.2	52.8	39.8 ± 0.3^{a}	49.6 ± 0.3^{b}	39.5 ± 0.4^{a}	49.1 ± 0.2^{b}
C18:2	36.5	1.9	20.2 ± 0.3^{a}	11.3 ± 0.6^{b}	21.1 ± 1.1^{a}	10.9 ± 0.3^{b}
C18:3	6	0.7	2.6 ± 0.1^{a}	1.4 ± 0.1^{b}	2.6 ± 0.1^{a}	1.4 ± 0.1^{b}
Total SFA	21.3	41.4	32.0 ± 0.6^{a}	31.2 ± 0.3^{a}	30.1 ± 0.5^{b}	30.8 ± 0.3^{b}

Total MUFA	36.2	56.0	45.2 ± 0.4^{b}	56.1 ± 0.2^{a}	46.3 ± 0.5^{b}	57.0 ± 0.2^{a}
Total PUFA	42.5	2.6	22.8 ± 0.3^{a}	12.7 ± 0.5^{b}	23.6 ± 0.9^{a}	12.2 ± 0.2^{b}

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)

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References

1. Vaz FM, Wanders RJ (2002) Carnitine biosynthesis in mammals. Biochem J 361: 417-429.
2. Owen KQ, Nelssen JL, Goodband RD, Tokach MD, Friesen KG (2001) Effect of dietary L-carnitine on growth performance and body composition in nursery and growing-finishing pigs. J Anim Sci 79: 1509-1515.
3. Ying W, Tokach MD, DeRouchey JM, Houser TE, Dritz SS, et al. (2013) Effects of dietary L-carnitine and dried distillers grains with solubles on growth, carcass characteristics, and loin and fat quality of growing-finishing pigs. J Anim Sci 91: 3211-3219.
4. Apple JK, Sawyer JT, Maxwell CV, Yancey JW, Frank JW, et al. (2011) Effects of L-carnitine supplementation on quality characteristics of fresh pork bellies from pigs fed 3 levels of corn oil. J Anim Sci 89: 2878-2891.
5. Arslan C, Citil M, Saatci M (2003) Effects of L-carnitine administration on growth performance, carcass traits, blood serum parameters and abdominal fatty acid composition of ducks. Arch Anim Nutr 57: 381-388.
6. Association of Official Analytical Chemists (2002) Official methods of analysis. AOAC Arlington VA.
7. Morrison WR, Smith LM (1964) Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride-Methanol. J Lipid Res 5: 600-608.
8. Tsai AC, Romsos DR, Leveille GA (1974) Significance of dietary carnitine for growth and carnitine turnover in rats. J Nutr 104: 782-792.
9. Suzuki S, Sukemori S, Ikeda S, Kurihara Y, Ito S (2002) Effects of walking exercise on body fat weight gain and body composition of rats. 1. Effects of two exercise protocols on body fat deposition. Jpn J Livestock Manage 37: 105-111.
10. Suzuki S, Sukemori S, Ikeda S, Kurihara Y, Ito S (2002) Effects of walking exercise on body fat weight gain and body composition of rats. 2. Effects of walking exercise on body fat deposition under environmental temperature of 30? Jpn J Livestock Manage 38: 83-89.
11. Katsumata M, Kobayashi S, Matsumoto M, Tsuneishi E, Kaji Y (2005) Reduced intake of dietary lysine promotes accumulation of intramuscular fat in the longssimus dorsi muscles of finishing gilts. Anim Sci J 76: 237-244.
12. Bertol TM, de Campos RM, Ludke JV, Terra NN, de Figueiredo EA, et al. (2013) Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. Meat Sci 93: 507-516.
13. Su W, Jones PJ (1993) Dietary fatty acid composition influences energy accretion in rats. J Nutr 123: 2109-2114.
14. Lambert BD, Dobson CM, Cherry NM, Sanderford MG (2009) Chemical form of dietary L-carnitine affects plasma but not tissue carnitine concentrations in male Sprague-Dawley rats. J Anim Physiol Anim Nutr (Berl) 93: 174-180.
15. Lien TF, Horng YM (2001) The effect of supplementary dietary L-carnitine on the growth performance, serum components, carcass traits and enzyme activities in relation to fatty acid beta-oxidation of broiler chickens. Br Poult Sci 42: 92-95.
16. Heo K, Odle J, Han IK, Cho W, Seo S, et al. (2000) Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J Nutr 130: 1809-1814.
17. Ikeda S, Sato M, Doi F, Muraoka K, Watanabe N, et al. (2008) Effects of L-carnitine supplementation 1 week prior to farrowing and during lactation on its concentration in milk, return of estrus and growth performance of piglets. Jpn J Swine Sci 45: 1-9.
18. Ikeda S, Sato M, Watanabe N, Odo S, Sukemori S (2012) Effect of L-carnitine supplementation during the last 2 weeks of gestation and the lactation period on body weight changes in sows experienced many farrowing and on the growth performance of their piglets. Jpn J Swine Sci 49: 31-37.
19. National Research Council (1995) Nutiritional requirements of laboratory animals. Nutritional requirements of rat National Academy of Science Washington DC.