

Effects of L-carnitine on the orchidectomized rats

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Abstract

This was conducted to determine the effects of body weight, organ weight, hematological values and biochemical parameters by L-carnitine (Carn) on the orchidectomized (Orch) rats. The animals were divided into 4 groups. Intact group (n=10) received no treatment and operation. Sham group (n=10) received only sham operation and no treatment. Orch group received operation and no treatment. Orch + Carn received operation and L-carnitine. The body weights of each group increased, but that of the Orch + Carn group were significantly lower than those in all the other groups. There were significant differences ($P < 0.05$, $P < 0.001$) of body weights between Orch + Carn group and all the other groups. Also, organ weights such as heart, liver, spleen and kidney were measured. The heart weights were significantly lower ($P < 0.001$) in the Orch + Carn group than those in Intact and Sham groups, respectively. The weights of liver and kidney in the Orch + Carn group were significantly differences ($P < 0.001$) in comparison with those in all the other groups. Also, the spleen weights were significantly lower ($P < 0.05$) in the Orch + Carn group than those in Intact and Sham groups, respectively. The hematological values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly differences ($P < 0.05$, $P < 0.01$, $P < 0.001$) in comparison with those in all the other groups. On the other hand, the hematological values of white blood cell (WBC), red blood cell (RBC) and mean corpuscular hemoglobin concentration (MCHC) were not significantly different in any other groups. The concentrations of total cholesterol (T-chol), triglyceride (TG) and high density lipoprotein (HDL) decreased significantly ($P < 0.05$) in the Orch + Carn group as compared to those in the Orch group. We conclude that L-carnitine was significantly decreased the body weight in the orchidectomized rats. Our findings suggest that L-carnitine may influence the process of lipid packaging and absorption in the orchidectomized rats.

Key words : Rat, L-carnitine, Orchidectomized

INTRODUCTION

L-carnitine, in addition to its well-established role as an obligate for the mitochondrial oxidation of long chain fatty acids, interacts with other metabolic pathways by generating acylcarnitines from the corresponding acyl-CoAs (Bremer, 1983). L-carnitine is a coenzyme required for the transfer of long chain fatty acids from the site of activation to acyl-CoAs in the cytosol across the inner mitochondrial membrane to the matrix of the mitochondria where they undergo β -oxidation

leading to the production of ATP from oxidative phosphorylation. The cellular transport of long chain fatty acids across the plasma membrane has long been thought to occur via passive diffusion (Bremer, 1983; Holloway et al, 2007).

L-carnitine, a natural vitamin like compound, is a ubiquitous constituent of mammalian plasma and tissues, mainly distributed among skeletal and cardiac muscles. L-carnitine is supplied to the body through dietary sources (meat, dairy products), and by biosynthesis from lysine and methionine. L-carnitine functions to transport long chain fatty acids across the inner mitochondrial membrane into the matrix for β -oxidation and has ef-

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fects on oxidative metabolism of glucose in tissues. However, L-carnitine cannot be synthesized in heart or skeletal muscle in dogs (Rebouche and Engel 1983; Broderick et al, 1992).

Recently, Keene (1992), reporting on dilated cardiomyopathy (DCM) in a wide range of breeds, found that myocardial free L-carnitine deficiency occurred in 50~90% of dogs with DCM, and 20% of these also had low serum carnitine levels, suggesting that myocardial L-carnitine deficiency may be an etiological agent responsible in many cases for what was previously an idiopathic disorder. A familial form of cardiomyopathy in the boxer breed was earlier reported by Keene et al (1991) to show serum and tissue free L-carnitine deficiency. Also, Costa and Labuc (1994) demonstrated that oral carnitine therapy was apparently effective in elevating carnitine concentrations of the tissues assayed beyond normal canine concentrations in the adult boxer after 4 months of supplementation in boxers affected with dilated cardiomyopathy. The age dependence of development of heart disease was studied in many investigators. One of reasons is heart disease that the increased incidence of dilated cardiomyopathy was related to L-carnitine deficiency. Here, we investigated to effects of body weight, organ weight, hematological values and biochemical parameters by L-carnitine administration on the orchidectomized rats.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats, aged 63 days, were purchased from Bio-Safety Research, Chonbuk National University and used for the experiment described below when they were 70 days old. The rats weighing 362~414 g were placed in stainless steel wire bottomed plastic cages and housed in a room maintained at 23±1°C and humidity 55% on 12 hrs light/dark cycles. All rats were allowed free access to a pelleted commercial diet and drinking water. After 1 week of acclimation, Rats

were randomly assigned to 4 groups with 10 rats in each group. Orchidectomy was performed in the Orch and Orch+Carn groups, animals in the Sham group were subjected to sham operation. Intact group had no operation nor treatment. The Orch+Carn group was given the L-carnitine subcutaneously (L-carnitine, 1 g/ml, Ildong Pharm.) 4 g/kg body weight for 3 times per week from 1 week after surgery.

Orchidectomy

After intraperitoneal anesthesia using ketamine (80 mg/kg) and xylazine (10 mg/kg), a midline scrotal incision was made. Testis was surgically resected. Specifically, both spermatic cords were ligated at the level of the upper part of the scrotum and the intact testis was removed. The scrotum of rats, Sham group, were opened and the testis was exposed. The testis was then replaced in the scrotum and the wound was closed.

Sample preparation

The rats were weighted 2 times per week. After 5 weeks of treatment, the rats were killed under anesthesia, blood was collected from the vena cava into heparinized or nonheparinized tubes for hematological values (Scil Vet abcTM, ABX Diagnostics, France) and biological parameters (Spotchem EZTM SP-4430, ARKRAY, Japan). After blood collection to measure the organ weights, heart, liver, spleen and kidney were removed and stored in saline and then weighted.

Statistical analysis

All values were reported as mean and standard deviation (SD). Significant differences between the values were statistically analyzed using a one-way analysis of variance (ANOVA), followed by a two pairs Student's *t* test. *P*<0.05 or less was considered statistically significant.

RESULTS

Effect of L-carnitine on body weights

Table 1 shows the effect of body weights on testis removed rats with L-carnitine administration. All rats increased body weights by the end of the experiment. Intact (385.6±9.08 g) and Orch+Carn groups (385.8±15.18 g) had similar mean body weights at the start of the study. On the other hand, the weights of Sham and Orch groups were slightly higher than those in the Intact and Orch+Carn groups, respectively. The body weights of Intact, Sham and Orch groups increased 78.3±12.03 g, 68.0±24.62 g and 49.6±33.33 g, respectively, whereas it only increased 32.6±24.96 g in the Orch+Carn group. L-carnitine prevented the Orch-induced weight gain. The final body weights of Intact (463.9±8.91 g), Sham (465.1±25.23 g) and Orch group (443.4±29.39 g) were significantly higher ($P<0.05$, $P<0.001$) than that of the Orch+Carn group (418.4±21.94 g).

Effect of L-carnitine on organ weights

The organ weights such as heart, liver, spleen and

kidney were weighted and are shown in Table 2. The hearts of the Orch+Carn group (1.4±0.08 g) were significantly lower than those in the Intact (1.5±0.05 g) and Sham groups (1.6±0.02 g) and higher than those in the Orch group (1.2±0.26 g). There were statistically significant differences ($P<0.001$, $P<0.05$) for these organ weights between Orch+Carn and Intact, Sham and Orch group, respectively. The livers and kidneys of the Orch+Carn group (10.6±0.44 g, 2.2±0.27 g) were significantly lower than those in the Intact (15.1±0.34g, 3.7±0.26 g), Sham (15.0±0.49 g, 3.7±0.45 g) and Orch (13.5±0.58 g, 2.9±0.28 g) groups, respectively. A statistically significant difference ($P<0.001$) in organ weights was observed in the Intact, Sham and Orch groups when compared with those of the Orch+Carn group. Also, the spleens of the Orch+Carn group (0.7±0.03 g) were significantly lower ($P<0.05$) than those in the Intact (0.8±0.04 g) and Sham groups (0.8±0.03 g).

Effect of L-carnitine on hematological values

The hematological values are shown in Table 3. The numbers of WBC and RBC were slightly lower in the

Table 1. Effects of L-carnitine on body weight (BW) of orchidectomized rats

	Intact	Sham	Orch	Orch+Carn
Initial BW (g)	385.6±9.08	397.1±6.74	393.8±9.57	385.8±15.18
After 5 weeks BW (g)	463.9±8.91	465.1±25.23	443.4±29.39	418.4±21.94 ^{c,f,j}
BW gain/loss (g)	78.3±12.03	68.0±24.62	49.6±33.33	32.6±24.96

Changes in body weight treated with L-carnitine on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Carn (treated with L-carnitine on orchidectomized) are shown. ^{c,f,j}Indicate the significant differences in values after 5 weeks of Orch+Carn vs. Intact, Sham and Orch, respectively. Data are mean±standard deviation. ^c $P<0.001$ vs. Intact, ^f $P<0.001$ vs. Sham, ^j $P<0.05$ vs. Orch.

Table 2. Effects of L-carnitine on organ weight of orchidectomized rats

	Intact	Sham	Orch	Orch+Carn
Heart (g)	1.5±0.05	1.6±0.02	1.2±0.26	1.4±0.08 ^{c,f,l}
Liver (g)	15.1±0.34	15.0±0.49	13.5±0.58	10.6±0.44 ^{c,f,l}
Spleen (g)	0.8±0.04	0.8±0.03	0.7±0.04	0.7±0.03 ^{a,d}
Kidney (g)	3.7±0.26	3.7±0.45	2.9±0.28	2.2±0.27 ^{c,f,l}

Changes in organ wet weight treated with L-carnitine on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Carn (treated with L-carnitine on orchidectomized) are shown. ^{a,c,d,f,l}Indicate the significant differences in values after 5 weeks of Orch+Carn vs. Intact, Sham and Orch, respectively. Data are mean±standard deviation. ^a $P<0.05$ vs. Intact, ^c $P<0.001$ vs. Intact, ^d $P<0.05$ vs. Sham, ^f $P<0.001$ vs. Sham, ^l $P<0.001$ vs. Orch.

Table 3. Effects of L-carnitine on hematological values of orchidectomized rats

	Intact	Sham	Orch	Orch + Carn
WBC ($10^3/\text{mm}^3$)	8.0±0.17	8.2±0.23	8.0±0.22	8.1±0.45
RBC ($10^6/\text{mm}^3$)	7.9±0.26	8.0±0.15	7.9±0.25	8.0±0.25
MCV (μm^3)	54.1±2.02	53.8±1.47	54.4±2.01	56.0±1.82 ^{a,e,j}
MCH (pg)	19.0±0.26	19.0±0.28	19.1±0.49	20.1±0.49 ^{e,f,l}
MCHC (g/dl)	35.3±0.57	35.0±0.39	35.1±0.85	35.0±0.62

Changes in hematological values treated with L-carnitine on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Carn (treated with L-carnitine on orchidectomized) are shown. ^{a,e,f,j}Indicate the significant differences in values after 5 weeks of Orch+Carn vs Intact, Sham and Orch, respectively. Data are mean±standard deviation. ^a $P<0.05$ vs. Intact, ^c $P<0.001$ vs. Intact, ^p $P<0.01$ vs. Sham, ⁱ $P<0.001$ vs. Sham, ^j $P<0.05$ vs. Orch, ^l $P<0.001$ vs. Orch.

Table 4. Effects of L-carnitine on biochemical parameters of orchidectomized rats

	Intact	Sham	Orch	Orch + Carn
T-chol (mg/dl)	90.0±9.53	91.4±9.72	101.6±8.82	93.3±8.84 ^l
TG (mg/dl)	138.8±8.62	137.7±11.15	151.7±11.11	140.7±9.74 ^l
HDL (mg/dl)	28.3±3.05	28.2±3.04	31.8±2.78	28.8±2.14 ^l
LDL (mg/dl)	16.2±1.31	16.3±1.63	17.2±1.61	16.6±1.57

Changes in biochemical parameters treated with L-carnitine on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Carn (treated with L-carnitine on orchidectomized) are shown. ^lIndicates the significant differences in values after 5 weeks of Orch+Carn vs Orch. Data are mean±standard deviation. ^j $P<0.05$ vs. Orch.

Intact group ($8.0\pm 0.17 \times 10^3/\text{mm}^3$) and higher in the Orch +Carn group ($8.0\pm 0.25 \times 10^6/\text{mm}^3$) than those in any other group, respectively. However, there was no statistically significant difference in WBC and RBC among groups, respectively. Additionally, there was no statistically significant difference for MCHC among groups. The numbers of WBC and RBC have not significantly influenced by the L-carnitine treatment. The levels of MCV and MCH were significantly ($P<0.05$, $P<0.01$, $P<0.001$) increased in the Orch+Carn group ($56.0\pm 1.82 \mu\text{m}^3$, $20.1\pm 0.49 \text{ pg}$) when compared with those of the Intact ($54.1\pm 2.02 \mu\text{m}^3$, $19.0\pm 0.26 \text{ pg}$), Sham ($53.8\pm 1.47 \mu\text{m}^3$, $19.0\pm 0.28 \text{ pg}$) and Orch groups ($54.4\pm 2.01 \mu\text{m}^3$, $19.1\pm 0.49 \text{ pg}$), respectively.

Effect of L-carnitine on biochemical parameters

The serum concentrations of total cholesterol (T-chol), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) are shown in Table 4. The levels of T-chol, TG and HDL were significantly ($P<0.05$) decreased in the Orch+Carn group ($93.3\pm$

8.84 mg/dl , $140.7\pm 9.74 \text{ mg/dl}$, $28.8\pm 2.14 \text{ mg/dl}$) when compared with those of the Orch group ($101.6\pm 8.82 \text{ mg/dl}$, $151.7\pm 11.11 \text{ mg/dl}$, $31.8\pm 2.78 \text{ mg/dl}$), respectively. On the other hand, in the Intact and Sham groups, the levels of T-chol ($90.0\pm 9.53 \text{ mg/dl}$, $91.4\pm 9.72 \text{ mg/dl}$), TG ($138.8\pm 8.62 \text{ mg/dl}$, $137.7\pm 11.15 \text{ mg/dl}$) and HDL ($28.3\pm 3.05 \text{ mg/dl}$, $28.2\pm 3.04 \text{ mg/dl}$) were moderately decreased as compared to those of the Orch+Carn group, but this decrease was not significant. In the Orch group ($17.2\pm 1.61 \text{ mg/dl}$), the levels of LDL slightly increased compared to those of the Intact ($16.2\pm 1.31 \text{ mg/dl}$), Sham ($16.3\pm 1.63 \text{ mg/dl}$) and Orch+Carn groups ($16.6\pm 1.57 \text{ mg/dl}$), but this was not statistically significant.

DISCUSSION

L-carnitine plays an important role in the transfer of long chain acyl groups into the mitochondrial matrix. Several studies demonstrated that L-carnitine improves the body status of α -tocopherol, a potent lipid soluble antioxidant, protecting cellular membrane and plasma lipoproteins (Dayanandan et al, 2001; Derin et al, 2004).

Lipid consists of cholesterol, triglyceride and phospholipids in addition to free fatty acid. Our data here provide convincing evidence that L-carnitine administration significantly decreases the body weights in orchidectomized rats. This was reasonable because L-carnitine has a physiological role in promoting the lymphatic absorption of α -tocopherol, a potent lipid soluble antioxidant, protecting cellular membrane and plasma lipoproteins (Zou et al, 2005). Also, this result is supported by recent observations of Kim et al (2008), who reported that an antiobesity effect of a mixture composed of the *Garcinia cambogia* extract, soy peptide and L-carnitine was observed in the high fat diet induced obesity rat model. The results from the present study clearly demonstrate that the mixture significantly reduces the accumulation of visceral fat mass and effectively lowers blood and hepatic lipid levels, leading to the improvement of insulin resistance in rats rendered obese by high fat diet. The mixture-induced reduction in the accumulation of visceral fat mass was most significant in the perirenal fat tissue compared with visceral fat tissues located elsewhere (Kim et al, 2008).

Our data on biochemical parameters except LDL support the observations of other investigators that decreases in the levels of serum and hepatic lipids, such as T-chol, very low density lipoprotein (VLDL)+LDL, and free fatty acid, in rats fed control diet+mixture compared to those for control diet rats could be attributed to the inhibition of lipid absorption in the gastrointestinal tract (Kim et al, 2008). Dietary lipids are absorbed into the bloodstream as chylomicron; triglycerides in these chylomicrons are then digested as fatty acids and glycerol by lipoprotein lipase, and are eventually transported and stored in the liver and adipose tissues in the form of triglycerides. The remnants of the chylomicrons are taken up mainly by the liver, and are then transformed into lipoproteins, such as VLDL, which transport triglycerides synthesized in the liver to adipose tissues, and LDL, which transports cholesterol to peripheral tissues (Guyton and Hall, 1996).

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