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Effects of L-Carnitine on Obese Rats Exposed to Swimming Exercise

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KEYWORDS Obesity. Glucose. Cholesterol. AST. ALT

ABSTRACT The aim of the present study is to investigate the effects of L-carnitine usage on weight loss, performance value and plasma biochemical parameters of the obesity induced rats during swimming exercise. A total of 40 male Sprague-Dawley rats were allocated randomly to four treatment groups (negative control, placebo group, Group 3 and Group 4 were fed a diet including 150 and 300 mg/kg/day L-carnitine, respectively). The results showed that glucose, cholesterol, HDL, LDL levels and ALT activity of rats in Group 4 were higher than that of controlled group. These results indicated that the supplementation of 300 mg/kg/day L-carnitine into the diet may increase performance and weight loss of obese rats compared to control group, placebo group and Group 3. Although, the weight loss of this group rats was higher than other group rats, determination of elevation in glucose, cholesterol and ALT activity indicates that 150 mg/kg/day L-carnitine intake is appropriate.

INTRODUCTION

Obesity is a multifactorial disease with an increasing prevalence due to redundant accumulation of fat in the body (WHO 2000). The common feature of these individuals is increased fat tissue accumulation. Obesity is the result of genetic, environmental, behavioral, physiologic, social and cultural factors which yields to deterioration in energy balance and storage of fats. Long time in-balance in energy uptake and utilization causes obesity (Yerlikaya and Akin 2013). Obesity is usually seen along with some metabolic diseases such as hypertension, dyslipidemia, Type 2 diabetes mellitus, coronary heart disease, paralysis, prostate and colon cancer (Islamoglu et al. 2008).

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Carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) is an amino acid like, hydrosoluble molecule which acts as a mediator in mitochondri matrix transfer during the oxidation of long chain fatty acids. If there is shortage of carnitine, β-oxidation of long chain fatty acids and, thus, the energy metabolism of the cell fails (Borum 1983). Simultaneously, impediment in the skeletal muscles leads to a decrease in exercise capacity. During over excretion of fatty acids from the body, carnitine demand increases for the detoxification process (Brass 2000; Kalaycioglu et al. 2010).

Exercise is crucial for weight loss in obesity, which is a major problem of the era. The supplemental supports for longer performance during exercise and oxidation of emerged fats are one of the most interesting research subjects.

Purpose of the Study

The purposes of the present study to investigate the effects of L-carnitine usage on physical performance, endurance, weight loss, blood

lipid profile, liver enzymes and myocard injury of the obesity induced rats during swimming exercise.

MATERIAL AND METHODS

Animal Material

A total of 40 male Sprague-Dawley rats weighing 150-200 gr were used. Rats were provided from Ondokuz Mayis University Medical and Surgical Research Laboratory and were housed at this laboratory during the experimental period. Firstly, these rats were fed a diet including high energy food (35% carbohydrate, 20% protein, 45% fat) and *ad libitum* until they reached 250-300 gr body weight. Secondly, these rats were allocated randomly to four treatment groups (namely Group 1- negative control; Group 2 – Placebo group; Group 3 and Group 4 were fed a diet including 150 and 300 mg/kg/day L-carnitine, respectively) consisted of ten replicates. Negative control group was not exercised. Placebo group was exercised and also was fed a diet including intraperitoneal (IP) physiologic saline solution for 20 days. Carnitine obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany) at a dose of 150 and 300 mg/kg/day was given to Group 3 and Group 4 for 20 days IP.

Carnitine was given 30 minutes before swimming exercise daily. Swimming exercise was performed in a square 50 cm deep water tank. Tank was filled 40 cm deep water at 25 ±1°C temperature and rats were swum until exhausted. Exhaustion criteria were accepted as; starting of uncoordinated motions (small extremity movements which cannot provide the animal afloat) or staying under water for 10 seconds without swimming. At the end of the exercise, rats were taken out of the water, dried with a towel and placed in their cages (Dawson and Horvarth 1970; Siktar 2009). Swimming exercises were continued for 20 days. During the study, exercise durations were recorded daily. Live body weights were recorded at the beginning and end of the study and blood was obtained from the heart for biochemical analyses at the end of the study. Drawing blood was performed under ketalar anesthesia. The blood obtained in the injector was transferred to tubes with anticoagulant, centrifuged at 3000 rpm for 10 minutes; plasma part was separated and stored at -80 °C until analyzed.

Biochemical Analyses

Total cholesterol, HDL, LDL, triglyceride, CK, CK-MB, cTnI, total protein, albumin, glucose, AST, ALT values were determined spectrophotometrically using Audit Diagnostics, (Ireland) kit.

Ethical Considerations

Before the study, the required ethics committee approval by *Ondokuz Mayis University* (2012/37) was obtained.

Statistical Analysis

The Shapiro-Wilk test was used to assess the assumption of normality of the obtained data before the use of parametric tests. The wrong dividing between blood parameters (Plasma cholesterol, HDL, LDL, triglyceride, CK, CK-MB, cTnI, total protein, albumin, glucose, AST, ALT values) values of the control and experimental groups (dose 0, dose 150 and dose 300) were analyzed by using One-way ANOVA. Then, Tukey HSD multiple comparison test was applied to determine any further differences among the groups. The results from feeding treatment diets 0 through 300 were analyzed as an orthogonal polynomial. Linear and quadratic effects were determined by orthogonal polynomial contrasts. At the end of the experiment, the effects of L-carnitine usage on weight loss, performance value (swimming time) of the obesity induced rats during swimming exercise were evaluated by using one-way ANOVA with repeated measures. The results are presented as mean ± standard deviation. The significance was evaluated at $P < 0.05$ for all tests. All the computational work was performed by means of SPSS 11.0 V (SPSS 2002).

RESULTS

The effects of different feeding methods (control, placebo, Group 3 and 4) on body weight and weight loss of the obese rats during swimming exercise were presented in Table 1. In an evaluation regarding weight variation, a statistically significant weight loss is recorded in groups 3 and 4 between the beginning and middle of the study ($P<0.05$). In the second half of the study, weight loss in only group 4 was significant

Table 1: Descriptive statistics for body weight of the rats

Groups	*n*	*Body weight at the beginning of the study*		*Weight loss between the beginning and middle of the study*		*Weight loss between the beginning and end of the study*	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Group 1	10	307.0	28.6	-5.50 [b]	6.43	-8.50 [c]	7.09
Group 2	10	304.5	39.2	3.00 [ab]	12.52	5.20 [bc]	18.21
Group 3	10	308.0	29.6	9.50 [a]	6.43	14.00 [b]	11.50
Group 4	10	314.0	37.3	11.00 [a]	13.90	33.00 [a]	17.03
P-values	0.935	0.004	<0.001				

SD: Standard Deviation; Group 1: Negative control group; Group 2: Placebo Group; Group 3: Dose 150; Group 4: Dose 300; [a,b,c]Means within column with different superscripts differ significantly.

(*P*<0.001). Regarding the entire study, significant weight loss compared to control group at a level of *P*<0.05 in group 3 and *P*<0.001 in group 4 was observed.

The effects of different feeding methods including negative control, placebo group (0), 150 and 300 mg/kg/day L-carnitine on plasma biochemical analyze results of the obese rats at the end of swimming exercise are presented in Table 2.

At 20 day, glucose and total cholesterol values of the obese rats fed dose 300 were higher than negative control, placebo and dose 150 groups (P<0.05). Control fed obese rats (not exercised) resulted in lower HDL, LDL and ALT than the rats fed Dose 0, 150 and 300 which were performed swimming exercise. Negative control group resulted in similar AST, CK, total protein, albumin, cTnI, CK-MB compared with placebo group (dose 0), Group 3 (dose 150) and group 4 (dose 300) (P>0.05). In addition, a significant decrease was determined for triglyceride level in exercised groups (2, 3 and 4th groups) compared to non-exercised group (Group 1) (*P*<0.05).

The supplementation of 300 mg/kg/day L-carnitine into the diet may increase performance and weight loss of obese rats compared to placebo group and Dose 150 group during swimming exercise (Table 3).

DISCUSSION

Continuous energy production is actualized to sustain metabolism functions. During the exercise, depending on the type and duration of the exercise, this energy demand increases (Ersoy 2006). Skeletal muscles perform fatty acids oxidation both during exercise and rest. Carnitine is used as the carrier to entrance of fatty acids into the mitochondria during β- oxidation. For

Table 2: Descriptive statistics (mean ± standard deviation) for serum biochemical parameters of the treatments

Parameters	*Group 1*		*Group 2*		*Group 3*		*Group 4*		*P-values*	*Effects*	
										L	*Q*
Glucose	199.53±	7.49[ab]	167.76±	22.35[b]	192.76±	29.60[ab]	229.0±	19.22[a]	0.002	**	*NS*
Cholesterol	42.28±	7.49[ab]	40.74±	6.39[b]	42.34±	5.11[ab]	49.06±	7.69[a]	0.053	*	*NS*
HDL	18.61±	3.11[b]	19.13±	2.54[ab]	20.21±	2.38[ab]	22.30±	2.88[a]	0.030	*	*NS*
LDL	27.84±	6.69[b]	32.00±	6.10[b]	33.95±	4.11[ab]	39.98±	6.98[a]	0.002	*	*NS*
ALT	49.17±	6.66[b]	57.59±	10.33[ab]	60.95±	12.16[ab]	64.3±	17.65[a]	0.047	*	*NS*
AST	123.96±	50.17	130.04±	25.37	143.62±	83.79	142.6±	44.0	0.828	*NS*	*NS*
CK	968.92±	704.6	526.33±	384.6	852.9±	1175.0	485.7±	327.7	0.397	*NS*	*NS*
Total Prot.	6.54±	0.35	6.46±	0.37	6.44±	0.33	6.57±	0.24	0.788	*NS*	*NS*
Albumin	2.55±	0.16[b]	2.80±	0.16[a]	2.82±	0.20[a]	2.77±	0.13[a]	0.003	*NS*	*NS*
cTnI	7.78±	9.15	4.05±	4.28	6.91±	12.86	6.64±	8.33	0.822	*NS*	*NS*
CK-MB	0.1±	0.00	0.1±	0.00	0.1±	0.00	0.1±	0.00	1.00	*NS*	*NS*
Triglyceride	51.81±	15.40[a]	24.55±	5.93 b	21.72±	5.32[b]	23.08±	8.27[b]	0.001	*NS*	*NS*

[a,b]Means within row with different superscripts differ significantly. L: linear effect; Q: Quadratic effect; * *P*<0.05; ** *P*<0.01; NS: Not significant

Table 3: Descriptive statistics (Mean ± Standard Deviation) for swimming time and weight loss (pre and post experiment time) of the experimental groups

Parameters	*Group 2*	*Group 3*	*Group 4*	*P-values*	*Effects*	
					L	*Q*
Swimming time	4475± 1026.1[a]	6603± 868.2[a]	6769± 847.3[a]	0.001	**	*NS*
Weight loss	5.20± 18.20[b]	14.00± 11.49[b]	33.00± 17.02[a]	0.002	**	*NS*

[a,b] Means within row with different superscripts differ significantly. L: linear effect; Q: Quadratic effect; ** $P<0.01$; NS: Not significant

this reason, carnitine deficiency may cause skeletal muscle function impairment, thereby, decrease in exercise capacity (Brass 2000). L- carnitine increases fatty acids oxidation and decreases glukogen utilization in muscles during exercise, thus decreases lactate accumulation and alleviates muscle weakness (Stephen et al. 2007).

Different opinions were reported in the previous studies concerning the effect of carnitine on muscle performance. Brass (2000), Malaguarnera et al. (2003) and Stuessi et al. (2005) suggested that carnitine had no effect. On the other hand, Heinonen et al and Karlic et al reported that carnitine augmented muscle performance (Heinonen and Takala 1994; Karlic and Lohninger 2004). Gultuk et al. (2007) observed a performance increase in the endurance duration of swimming exercise in L-carnitine given rats. They suggested that this effect of carnitine may be due to the elevation in maximum oxygen intake and/or mitochondrial Ca^{+2} secretion. In the present study, performance elevation in swimming exercise was observed in the L-carnitine given group ($P<0.05$).

Serum indicators widely used in the determination of myocardial injury are, cardiac troponin I, creatin kinase (CK) and creatin kinase myokard band (CK-MB). Troponin is a preventive protein located in the actine fiber of all striated muscles (Hunkeler et al. 1991; Bertinchant et al. 1996). Heart sourced Troponin I (cTn I) is only present in the heart and used as the sensitive and specific indicator of heart muscle injury (Caliskan et al. 2010). Additionally, must be evaluated together with CK-MB and cTn I which has a higher space in heart diseases that elevates in serum in a late period and is stable high for a longer time (Apple et al. 1999). Obesity is a main risk factor for cardiovascular disease and a major problem around the world (Lango et al. 2001). Many previous experimental and clinical trial studies have shown that L-carnitine exerts a protective effect against ischemia/reperfusion injuries in cardiomyopathies, reduction of infarct size and prevention of arrhythmias in patients with myocardial infarction, increased exercise tolerance in angina and protection from the cardio toxicity of the anthracycline antineoplastics (Ferrari et al. 2004; Sharma et al. 2004). cTn I, inhibits cardiac ATPase activity in the absence of human myocardium Ca^{+2}, and is present in blood following myocardial injury (Hunkeler et al. 1991; Bertinchant et al. 1996). L- carnitine has a positive effect on Troponin I due to its effect in increasing mitochondrial Ca^{+2} secretions (Gultuk et al. 2007). Najafi and Garjani (2014) suggested in their study that long time usage of L-carnitine is beneficial against cardiovascular diseases. Magoulas and El-Hattab (2012) reported that CK concentration, liver transaminases and fasting glucose levels must be evaluated in the diagnosis of primary L-carnitine deficiency. In contrast to the literature, this study showed that no variations were determined in serum cTn I, CK, CK-MB values ($P>0.05$).

Liver transaminases pass from hepatocytes to serum due to different etiologies such as pharmacologic, exposition to chemical agents and liver diseases and are known as liver function tests. ALT is more specific in liver diseases compared to AST (Laker 1998). In this study AST and ALT analyses were performed to investigate the effects of weight loss in obese and supplemental L-carnitene. Results revealed no difference in serum AST activity ($P>0.05$), but a significant increase was determined in serum ALT activity between group 1 and group 4 ($P<0.05$). The previous studies indicated that L-carnitine is preventive in liver injury, but in anyway liver injury were created in these studies (Uygun et al. 2000; Atila et al. 2002; Aktas et al. 2013). In the present study there is no previous liver injury history. Thus, the researcher suggested that further studies may be indicated for this increase in ALT activity, which is a liver specific enzyme.

In plasma total cholesterol, HDL and LDL cholesterol, no alterations were observed in group 3 ($P>0.05$), an increase was observed in

group 4 ($P<0.05$). Emerged fats due to weight loss in group 3 ($P<0.05$) are balanced via β-oxidation. Higher weight loss during swimming exercise in group 4 obese rats may be due to more decrease in adipose tissue, and part of these fats are undergoing β-oxidation, while the other part remains free in blood.

CONCLUSION

Today, obesity is one of mankind's most serious problems. The most effective way to combat obesity is diet and exercise. In the present study, it is presented that the supplementation of L-carnitine in different doses into the diet may increase performance and weight loss of obese rats during swimming exercise, but glucose, cholesterol, HDL, LDL levels and ALT activity of group 4 (300 mg/kg/day) rats significantly increased while no changes were observed in these parameters of group 3 (150 mg/kg/day) rats. The high values for dose 300 may cause liver dysfunction. So, it can be said that150 mg/kg/day L-carnitine intake is appropriate.

RECOMMENDATIONS

Further studies are needed to 1- investigate the effects of different feeding methods including L-carnitine (0, 50, 100, 150, 200 and 250 mg/kg/day) usage on weight loss, performance value and plasma biochemical parameters of obese rats with different exercise applications (running); 2- compare the effect of different exercise applications (swimming and running) in terms of the parameters for obese rats.

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