Australian Journal of Basic and Applied Sciences, 6(2): 80-84, 2012
ISSN 1991-8178

Effect of Dietary L-carnitine Supplementation on the Concentration of Circulating Serum Metabolites in Growing New Zealand Rabbits

[1]Usama B. Elgazzar, [2]Ismael I. A.Ghanema and [3]Z.M.kalaba

[1]Department of Biochemistry, Faculty of Medicine, Al-Azhar University.
[2]Department of Physiology, Faculty of Veterinary Medicine, Damnhour University
[3]Department of Animal Production, Poultry Physiology, Faculty of Agriculture, Mansuora University.

Abstract: The present study was conducted to evaluate the effect of three dietary L-carnitine levels on serum metabolites and hormones in growing New Zealand rabbits. Twenty 4-week-old growing New Zealand rabbits were assigned to four groups, each with five replicates. Four experimental diets were formulated by adding four levels (0, 25, 50, and 100 mg/dl) of supplemental L-carnitine to a basal diet in pellet form from 4 to 8 weeks of age. At the end of the 8th week, rabbits were slaughtered to measure serum parameters. L-carnitine administration induced a marked dose-dependent reduction of serum cholesterol, triglycerides, LDL-c, VLDL and elevation of HDL-c. Circulating serum aminotransferases (AST and ALT), alkaline phosphatase enzymes and cortisol were decreased while glucose and serum thyroid hormones (T3 and T4) were significantly increased. Serum creatinine was significantly increased, and serum electrolytes (Na+, K+ and Cl-) were altered. These alterations directly proportional to the dose of L-carnitine. In conclusion, L-carnitine supplementation induces improved serum lipid and lipoprotein metabolism but alters other circulating serum metabolites and hormones.

Key words: L-carnitine; Serum Metabolites; Hormones; Rabbits.

INTRODUCTION

L-carnitine(β-hydr oxy-γ-N-trimethylamino butyrate) is a water-soluble quaternary amine that is synthesised in vivo from lysine and methionine in the kidney and liver of all mammals. L-carnitine is metabolically indispensable for the transport of long chain fatty acids from the cytosol into the mitochondrial matrix for β-oxidation, where the activity of carnitine palmitoyl transferase, the fatty acid β-oxidation enzyme, is significantly increased by supplementary L-carnitine (Arslan and Saatci 2003). L-carnitine has been described as a conditionally essential nutrient for humans and animals; about 75% of the carnitine used by the body comes from the diet. In humans, the liver and the kidneys synthesise the remaining 25% from the immediate precursor gamma butyrobetain. Carnitine in the blood is much less concentrated than in tissue. Consequently, carnitine introduced in the diet or synthesised de novo in the liver and kidneys must be actively concentrated from the blood into fatty acid metabolising organs (Maritza *et al*., 2006).

Owen *et al*. (2001) reported that in newborn rats, plasma levels of carnitine increased rapidly after birth and decreased only when the pups were weaned and fed only dry diets.

L-carnitine has been considered a hypolipidemic agent. Supplementation studies show that exogenous carnitine reduces the levels of plasma very low-density lipoprotein cholesterol (VLDL-C) and VLDL-triglycerides in hyperlipidemic rabbits and plasma lipoprotein levels in type 2 diabetic patients with hypercholesterolemia (Rajasekar *et al*., 2005). Plasma glucose concentration is increased by carnitine supplementation, which is attributed to increased fatty acid oxidation and the subsequently reduced oxidation of gluconeogenic precursors. Also, carnitine supplementation tends to increase the plasma urea concentration (Greenwood *et al*., 2001).

Moreover, it was previously reported that L-carnitine supplementation elevates plasma triiodothyronine (T3) levels (Buyse *et al*., 2001), while another study concluded that carnitine was a peripheral antagonist of thyroid hormone action not an inhibitor of the thyroid gland function (DeFelice and Gilgore 1996).

Corresponding Author: Usama B. Elgazzar, Department of Biochemistry, Faculty of Medicine, Al-Azhar University. Tel; +2 0127925258.; E-mail: usamagaz@yahoo.com

Another study reported that carnitine inhibits thyroid hormone entry into the nuclei of human and animal cells (fibroblasts, hepatocytes, neurons) (Salvatore *et al*., 2001). The aim of the present study was to evaluate the effects of dietary supplementation with various doses of L-carnitine on the concentration of circulating metabolites and hormones in growing rabbits.

MATERIALS AND METHODS

A- Experimental Animals and Diets:

The fieldwork of the present study was conducted on the rabbit farm in Zian. Twenty 4-week-old New Zealand rabbits were assigned to four equal experimental groups of 5 rabbits each. All rabbits were kept in community battery cages (five rabbits per cage) set up in an open-sided rabbit house and managed similarly.

Table I: Compositions of the experimental diet.

Ingredient	g
Yellow Corn	6.22
Soybean meal, 44%	22.33
Wheat bran	23.33
Barley	15
Alfalfa hay	30.12
Ground limestone	1.0
Dicalcium phosphate	1.2
Common salt	0.5
Vit.+Min. Premix†	0.3
Total	100 g

†: Each 3 Kg premix contains: 12,000,000 IU Vit. A, 3,000,000 IU Vit. D3, 10,000 mg Vit. E, 3,000 mg Vit. K3, 200 mg Vit. B1, 5,000 mg Vit. B2, 3,000 mg Vit. B6, 15 mg Vit. B12, 50 mg Biotin, 1,000 mg Folic acid, 35,000 mg Nicotinic acid, 10,000 mg Pantothenic acid, 80 g Mn, 8.8 g Cu, 70 g Zn, 35 g Fe, 1 g I, 0.15 g Co and 0.3 g Se.

Experimental diets were formulated with a partial composition of 19% crude protein, 2% crude fat, 10% crude staple and 40% free nitrogen extract and water ad libitum. L-carnitine (300 mg/ml; MEPACO, Egypt) was added to different diet formulas such that Diet 1 (which served as a control) contained no L-carnitine, and Diets 2, 3 and 4 contained 25, 50 and 100 mg/kg supplemental L-carnitine, respectively. All groups of rabbits were fed their respective diets (in pellet form) from 4 to 8 weeks of age. The are shown in the table (1).

B. Criteria of Response:

These included biochemical changes in the levels of certain blood constituents, metabolites, electrolytes, hormones and the activities of certain plasma enzymes.

C. Sampling:

At the end of the 8th week, rabbits were slaughtered for measurement of blood parameters. Individual blood samples were collected from the jugular veins of rabbits into centrifuge tubes without anticoagulant and used for separation of clear serum for biochemical analysis. The serum was separated by centrifugation of the blood samples at 4000 r.p.m. for 15 min and stored at -20°C until assayed.

D. Biochemical Analysis:

The serum samples were used for determination of total cholesterol (TC) and triglycerides (TG). HDL-c was determined enzymatically after precipitation of VLDL and LDL-c with phosphotungestic acid; HDL-c was calculated according to the Friedwald equation: LDL-c = (TC-HDL-c-TG/5) glucose. Creatinine and activities of plasma alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were examined using commercial kits according to previously described methods (Frings and Dunn 1970; Trinder, 1969; Allain *et al*., 1974; Fossati and Prencipe 1982; Houto, 1985): Kind and King 1954; Reitman and Frankel 1957). Simultaneously, the levels of Na, Cl and K (Tietz, 1987) were also estimated using commercial kits. Concentrations of corticol, triiodothyronine (T3) and thyroxine (T4) were determined using commercial kits according to previously described methods (Sainio *et al*., 1988; Sterling, 1975 and Liewendahl, 1990).

E. Experimental Design and Statistical Analysis:

A completely randomised design with four levels of supplemental dietary L-carnitine was used in the present study. Statistical analyses for various variables were performed using the SAS program, (1987). The differences were considered significant at P£0.05.

RESULTS AND DISCUSSION

The results of this investigation showed that L-carnitine supplementation induced a marked decrease of serum TC, TG, LDL-c and VLDL and increasing in HDL-c levels as compared to those of control group (Table 2), and this decrease was directly proportional to the dose of L-carnitine. Similarly, several studies conducted on different animal species were in agreement with these results (Arslan *et al*., 2003; Maritza *et al*., 2006). Although we did not investigate the mechanism of these reductions, they are likely not secondary to any major modification of circulating plasma lipoproteins. It was previously suggested that L-carnitine supplementation prevents increases in plasma triacylglycerol levels by increasing the removal of VLDL-triacylglycerol through the activation of lipoprotein lipase and fatty acid oxidation (Tanaka *et al*., 2004), and that the reduction of plasma total cholesterol with L-carnitine treatment was attained mostly via a decrease of cholesteryl esters rather than by a decrease in free cholesterol. Moreover, it was previously reported that the reduced activity of L-carnitine on serum cholesterol resulted from an increase in biliary sterol excretion or an increase in the conversion of cholesterol to bile acids (Seccombe *et al*., 1987). The L-carnitine-mediated decrease of cholesterol esters in the modified VLDL+LDL-c fraction suggests changes in fatty acid composition that favour the catabolism of this serum fraction (Maritza *et al*., 2006).

Regarding the effect of L-carnitine supplementation on thyroid hormones, we found a dose dependant significant increase in T3 and T4 levels in comparison to the control group (Table 2). These results are in agreement with previous findings (Buyse *et al*., 2001) showing that L-carnitine supplementation significantly increased the T3 concentration in chickens. These results may be explained by (Benvenga *et al*., 2004) who reported that L-carnitine is a peripheral antagonist of thyroid hormone action. In particular L-carnitine inhibits both T3 and T4 entry into the cell so leading to accumulation of them in the peripheral blood. Also (Nikolas Hedberg) mentioned that L-carnitine was acting in thyroid hormone target tissue, and not at the level of thyroid gland as an inhibitor of thyroid hormone synthesis. It was previously found that serum FT3 was in the upper range in L-carnitine-treated women after one month (Salvatore *et al*., 2001), whereas serum FT4 was above the normal upper limit and serum TSH was consistently suppressed.. In our experiment, as well as in previous reports (Lucilla *et al*., 1990; Yoshikazu *et al*., 2008). Serum cortisol level was significantly decreased and inversely proportional to the dose of L-carnitine(Table 2). Control of cortisol levels is one of the most important issues for promoting longevity and avoiding chronic diseases, as well as enhancing performance.

Dietary L-carnitine significantly decreased the serum concentration of AST, ALT and alkaline phosphatase enzymes (Table 3). This decrease was confirmed by a previous study Sanjay and Singh (2010). These findings also coincide with another study reporting that L-carnitine supplimintation significantly decreases serum AST; ALT and Alkaline phosphatase (Ahmed *et al*., 2010).

Table 2: Effect of L-carnitine supplementation on serum, cholesterol, triglycerides, HDL, LDL, VLDL, Cortisone, T3 and T4.

Groups	Cholesterol, mg/dl	Triglycerides, mg/dl	HDL, mg/dl	LDL, mg/dl	VLDL, mg/dl	Cortisol, ng/l	T3, ng/ml	T4, ng/ml
Control	71.8 ± 1.24a	135.4 ± 3.72a	11.8 ± 0.71c	33.74 ± 1.08b	27.04 ± 0.75a	13.08 ± 0.37a	0.77±0.05d	5.71± 0.23d
25 mg carnitine	69.6±0.93a	118.0±1.95b	14.22±1.07ab	31.78±1.36a	23.60±0.39b	10.49±0.5b	1.49±0.04c	7.75±0.13c
50 mg carnitine	54.6±0.93b	105.4±1.91c	15.5±0.93b	18.72±0.37c	21.08±0.38c	9.18±0.45c	3.37±0.31b	11.12±0.16b
100 mg carnitine	53.8±1.28b	87.4±3.08d	19.0±0.71A	17.32±1.35b	17.48±0.62d	5.31±0.14d	5.05±0.13a	14.97±0.39a

Means within the columns that carry different superscripts a-d are significantly different at the level P<0.01.

Concerning the effect of L-carnitine supplementation on serum glucose, we found that the serum glucose level was significantly increased compared to the control (Table 3). This increase may conceivably be due to the hyperglycemic effect of elevated thyroid hormones. These finding coincide with previous findings (Greenwood *et al*., 2001) indicating that the plasma glucose concentration is increased in L-carnitine-supplemented steers. Accordingly, this elevation could be attributed to increased fatty acid oxidation and the subsequently reduced oxidation of gluconeogenic precursors. Conversely, our findings are in disagreement with a previous study (Buyse *et al*., 2001) reporting that L-carnitine supplementation significantly reduced plasma glucose in broiler chickens.

Serum creatinine was significantly increased, and serum electrolytes (Na+, K+ and Cl-) were altered (Table 3). This alteration was directly proportional to the dose of L-carnitine and may be attributed to the nephrotoxic effect of L-carnitine during urinary excretion. A previous study (Greenwood *et al*., 2001) reported that urinary excretion increases as the supplementation level increases, and this was observed in terms of the

elevated plasma urea concentration when intermediate levels of supplemental carnitine were provided. These results are also in agreement with previous findings (Salvatore *et al*., 2001).

Table 3: Effect of L-carnitine supplementation on serum AST, ALT, alkaline phosphatase, creatinine, Na, K, Cl and glucose.

Groups	Ast, U/l	ALT, U/l	Alk.P, U/l	Creatinine, mg/dl	Na, mEq/l	K, mEq/l	Chloride, mEq/l	Glucose, mg/dl
Control	21.20±1.28c	20.20±1.28a	43.60±3.74 a	0.78±0.04c	169.56±1.39a	4.96±0.09b	83.00±0.88d	114.20±2.92c
25 mg carnitine	18.40±1.21b	19.70±1.22a	40.12±0.98b	1.23±0.02b	163.66±1.81b	5.24±0.09b	91.68±1.03c	109.80±2.44c
50 mg carnitine	12.20±1.28a	14.60±1.21b	34.3±1.33c	1.54±0.04a	162.02±1.32b	5.16±0.06b	103.84±1.96b	134.00±2.21b
100 mg carnitine	9.00±1.41a	11.20±1.77c	30.40±2.98d	1.45±0.05a	160.28±1.16b	7.46±0.17a	111.68±1.94a	164.80±2.65a

Means within the columns that carry different superscripts a-d are significantly different at the level P<0.01

In conclusion, with this experimental design, L-carnitine supplementation improved peripheral and hepatic fatty acid β-oxidation with an increase in lipoprotein metabolism. However, supplementation had an adverse effect on other circulating metabolites and hormones (e.g., serum AST, ALT, alkaline phosphatase, glucose, creatinine, Na, K, Cl, cortisol, T3 and T4 hormones). Thus we do not recommend L-carnitine supplementation.

REFERENCES

Ahmed, M.G., E.Y. Heba and H.I. Hala, 2010. Protective effect of mushroom and their ethyl extract on aging compared with L-carnitine. International Journal of nutrition and Metabolism, 2(4).: 63-69.

Allain, C.A., L.S. Poon, C.S.G. Chang, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clinical Chemistry, 20: 470-475.

Arslan, C., M. Citil and M. Saatci, 2003. Effect of L-carnitine administration on growth performance, carcass traits, Blood serum parameters and abdominal fatty acid composition of ducks. Arch. Anim. Nut., 57(5): 381-388.

Benvenga, S., A. Amato, M. Calvani, F. Trimarchi, 2004. Effects of carnitine on thyroid hormone action. Ann N.Y. Acad Sci., 1033: 158-67.

Buyse, J., G.P.J. Janssens and E. Decuypere, 2001. Te effect of dietary L-carnitine supplementation on the performance, organ weights and circulating hormone and metabolite concentrations of broiler chickens reared under a normal or low temperature schedule. British Poultry Science, 42: 230-241.

De Felice, S.L. and S.G. Gilgore, 1996. The antagonistic effect of carnitine in hyperthyroidism. Preliminary report. J New Drugs, 6: 351-353.

Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry, 28: 2077-2080.

Frings, C.S. and R.T. Dunn, 1970. A colorimetric method for determination of total serum lipids based on the sulfo-phosphovanillin reaction. Amer. J. Clin. Pathol., 53: 89-91.

Greenwood, R.H., E.C. Titgemeyer, G.L. Stokka, J.S. Drouillard and C.A. Loest 2001. Effect of L-carnitine on nitrogen retention and blood metabolites of growing steers and performance of finishing steers. J Anim. Sci., 79: 254-260.

Houto, O., 1985. Colorimetric determination of serum creatinine interpretation of clinical laboratory test, 220-234.

Kind, P.R.N. and E.J. King, 1954. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. J. Clin. Pathol., 7: 322-326.

Liewendahl, K., 1990. Assessment of thyroid status by laboratory methods: developments and perspectives. Scand. J. Clin. Lab. Invest., 201: 83-92.

Lucilla, P., G. Alberto, M. Patrizia, C.G. Gottfrie, C. Santucci, G.P. Reboldi and S. Umberto, 1990. Effect of Acetyl-L-Carnitine on Serum Levels of Cortisol and Adrenocorticotrophic Hormone and Its Clinical Effect in Patients with Senile Dementia of Alzheimer Type. Karger AG, Basel; 1: 165-168.

Maritza, F.D., A.U. Julio, L. Flor and H.R. Frank, 2006. L-carnitine-induced modulation of plasma fatty acids metabolism in hyperlipidemic rabbits. Rev Electron Biomed/Electron J Biomed., 1: 33-41.

Nikolas, R., D.C. Hedberg, D.A.B.C.I.: Autoimmune thyroid disease. Board Certified Chiropractic internist www.drhedberg.com.

Owen, K.Q., J.L. Nelssen, R.D. Goodband, M.D. Tokach and K.G. Friesen 2001. Effect of dietary L-carnitine on growth performance and body composition in nursery and growing-finishing pigs. J.Anim. Sci., 79: 1509-1515.

Rajasekar, P., M.K. Ravichandran and C.V. Anuradha, 2005. Interaperitoneal L-carnitine regulates lipid metabolism and reduces oxidative stress in fructose-induced hyperlipidemic rats. Diabetologia Croatica, 34-3.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol., 28: 56-63.

Sainio, E.L., T. Lehtola and P. Roininen, 1988. Radioimmunoassay of total and free corticosterone in rat plasma: measurement of the effect of different doses of corticosterone. Steroids, 51(5-6): 609-622.

Salvatore, B., M. Rosaria, Ruggeri, R. Antonia, L. Daniela, C. Alfredo and T. Francesco, 2001. Usefulness of L-carnitine, A Naturally Occurring Peripheral Antagonist of thyroid Hormone Action, in Iatrogenic Hyperthyroidism: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. J. Clin. Endocrinol. Metab., 86(8): 3579-3594.

Sanjay, M. and R.B. Singh, 2010. Effect of Mushroom on lipid profile, Lipid peroxidation and Liver function of Aging Swiss Albino Rats. The open Nutraceuticals Journal, 3: 248-253.

Seccombe, D.W., L. James, P. Hahn and E. Jones, 1987. L-carnitine treatment in the hyperlipidemic rabbit. Metabolism, 36: 1192-1196.

Statistical Analysis System, (SAS)., 1987. User's Guid "SAS" Institue Carry , North Carolina.

Sterling, L., 1975. Diagnosis and Treatment of Thyroid Disease, Cleveland, CRC Press.

Tanaka, Y., S. Rorie, F. Fumiko, W. Hatsue, K. Terue, O. Mitsuyo, H. Kyoko and A. Susumu, 2004. Acetyl-L-carnitine supplementation restores decreased tissue carnitine levels and impaired lipid metabolism in aged rats. J. Lipid Research, 45.

Tietz, N.W., 1987. Fundamentals of Clinical Chemistry, 3rd edition, published by W.B. Saunders Co., P.A. Philadelphia.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem, 6: 24-27.

Yoshikazu, Y., Y. Takahashi, H.1. Sawako, W. Miwako and Y. Toshito, 2008. Effects on the Human Body of a Dietary Supplement Containing L-Carnitine and Garcinia cambogia Extract: A Study using Double-blind Tests. J Clin Biochem Nutr., 42(2): 89-103.