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

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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-hydroxy-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).
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 1: Compositions of the experimental diet.

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

†: 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 phosphotungastic 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 supllimintation significantly decreases serum AST; ALT and Alkaline phosphatase (Ahmed et al., 2010).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol, mg/dl</th>
<th>Triglycerides, mg/dl</th>
<th>HDL, mg/dl</th>
<th>LDL, mg/dl</th>
<th>VLDL, mg/dl</th>
<th>Cortisol, ng/l</th>
<th>T3, mg/ml</th>
<th>T4, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.8 ± 1.24a</td>
<td>135.4 ± 3.72a</td>
<td>11.8 ± 0.71a</td>
<td>33.74 ± 1.08b</td>
<td>27.04 ± 0.75a</td>
<td>13.08 ± 0.37a</td>
<td>0.77 ± 0.05d</td>
<td>5.71 ± 0.23d</td>
</tr>
<tr>
<td>25 mg</td>
<td>69.6±0.93a</td>
<td>118.0±1.95b</td>
<td>14.22±1.07ab</td>
<td>31.78±1.36a</td>
<td>23.60±0.39b</td>
<td>10.49±0.5b</td>
<td>1.49±0.04c</td>
<td>7.57±0.13c</td>
</tr>
<tr>
<td>50 mg</td>
<td>54.6±0.93b</td>
<td>105.4±1.91c</td>
<td>15.5±0.93b</td>
<td>18.72±0.37c</td>
<td>21.08±0.38c</td>
<td>9.18±0.45c</td>
<td>3.37±0.31b</td>
<td>11.12±0.16b</td>
</tr>
<tr>
<td>100 mg</td>
<td>53.8±1.28b</td>
<td>87.4±3.08d</td>
<td>19.0±0.71A</td>
<td>17.32±1.35b</td>
<td>17.48±0.62d</td>
<td>5.31±0.14b</td>
<td>5.05±0.13a</td>
<td>14.97±0.39a</td>
</tr>
</tbody>
</table>

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.

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

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


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


