

L-CARNITINE ADMINISTRATION PREVENTS OXIDATIVE STRESS IN HIGH FRUCTOSE-FED INSULIN RESISTANT RATS

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SUMMARY

Rats fed a high fructose diet display disturbances in insulin action and form an animal model of insulin resistance. We investigated the impact of L-carnitine administration (300 mg/kg body weight/day, i.p. in 0.89% saline) on lipid peroxidation and antioxidant potential in blood of fructose-induced insulin resistant rats. Oral glucose tolerance test was performed before sacrifice and biochemical analyses were done in blood. Fructose-fed rats showed increased levels of peroxidation markers ($p < 0.05$) such as thiobarbituric acid reactive substances, lipid hydroperoxides, and conjugated dienes in plasma. Increased levels of glucose, fructose, insulin, free fatty acids and triglycerides ($p < 0.05$) were observed in circulation. Homeostatic model assessment (HOMA) values were higher as compared to control rats. Inadequate enzymatic (superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase) and non-enzymatic (reduced glutathione, vitamin C and vitamin E) antioxidant status was observed in fructose-fed rats. The observed biochemical abnormalities associated with fructose feeding were brought to near-normal when

simultaneously treated with L-carnitine. The effect of L-carnitine on the oxidant-antioxidant balance can be attributed to the metabolic as well as antioxidant effects of L-carnitine.

INTRODUCTION

Rats fed a high fructose diet provide an animal model of insulin resistance. Studies have shown that high dosage of fructose induces hyperinsulinemia, hypertriglyceridemia, impaired glucose tolerance and hypertension in rats (1,2). In addition, fructose consumption in rats has prooxidant effects (3). Fructose-fed rats display oxidative stress, an imbalance between free radical production and antioxidant defense in several tissues (4,5).

L-Carnitine (CA) (β -hydroxy- γ -trimethyl ammonium butyric acid) is a vitamin like compound (6) obtained from the diet that is also synthesized in the body from the essential amino acids lysine and methionine. It is essential for the transport of long chain fatty acids into the mitochondrial matrix through the action of specialized acyl transferases. CA is also reported to possess antioxidant properties (7). The antioxidant benefits have been implicated in several forms of toxicity (8,9). Furthermore, CA is reported to have effects on insulin action. Clinically, CA has been shown to improve insulin sensitivity in uremic (10) and diabetic (11) patients. However, no experimental or

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clinical data are available on the influence of CA on the oxidant-antioxidant imbalance in the insulin resistant state.

Considering the above findings, we examined the effects of CA on lipid peroxides, the products of oxidative modification of lipids and antioxidants in blood of rats fed a high fructose diet, which show the characteristic features of insulin resistance.

MATERIALS AND METHODS

Chemicals

L-Carnitine was purchased from Sisco Research Laboratories, Mumbai, India. All other chemicals used in the study were obtained from Sd fine Chemicals (Mumbai, India) and were of analytical grade.

Animals and diets

Male adult Wistar rats weighing 150-160 g were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai Nagar. They were housed in an animal room under controlled conditions on a 12-h light/12-h dark cycle. They all received a standard pellet diet (Karnataka State Agro Corporation, Agro Feed Division, Bangalore, Karnataka, India) and water *ad libitum*. The animals used in the present study were cared for as *per* the principles and guidelines of the Institutional Animal Ethics Committee. All the procedures were approved by the Committee.

After acclimatization, the animals were divided into the following groups consisting of six rats each: group 1, CON received control diet and water *ad libitum*; group 2, FRU received fructose-enriched diet and water *ad libitum*; group 3 received the fructose diet and were administered CA (300 mg/kg body weight/day, i.p. in 0.89% saline); and group 4 received the control diet and were given CA (300 mg/kg body weight/day, i.p. in 0.89% saline). Initial and final body weights of animals were measured during the experimental period. The compositions of control and fructose diets are given in Table 1. The diets were prepared fresh daily. The animals were maintained in their respective groups for 30 days. An oral glucose tolerance test (OGTT) was

Table 1. **Composition of diet (g/100 g)**

Ingredients	Control diet	High fructose diet
Corn starch	60.0	–
Fructose	–	60.0
Caseine (fat free)	20.0	20.0
Methionine	0.7	0.7
Ground nut oil	5.0	5.0
Wheat bran	10.6	10.6
Salt mixture *	3.5	3.5
Vitamin mixture *	0.2	0.2

* The composition of mineral mix (g/kg) -

MgSO₄·7H₂O-30.5; NaCl-65.2; KCl-105.7;

KH₂PO₄-200.2; 3MgCO₃, Mg(OH)₂·3H₂O-38.8;

FeC₆H₅O₇·5H₂O-40.0; CaCO₃-512.4; KI-0.8; NaF-0.9;

CuSO₄·5H₂O-1.4; MnSO₄-0.4 and CONH₃-0.05.

* One kilogram of vitamin mix contained thiamine mononitrate, 3 g;

riboflavin, 3 g; pyridoxine HCl, 3.5 g; nicotinamide, 15 g; d-calcium

pantothenate, 8 g; folic acid, 1 g; d-biotin, 0.1 g; cyanocobalamin, 5

mg; vitamin A acetate, 0.6 g; α-tocopherol acetate, 25 g; and choline

chloride, 10 g.

carried out on day 29. For this, the rats were fasted overnight (12 h) and were orally administered glucose (2 g/kg body weight) after collecting fasting blood samples. Additional blood samples were drawn every one hour up to 120 minutes and were immediately analyzed for glucose and insulin. The area under the curve (AUC) for glucose and insulin was calculated. Fasting blood samples were collected on day 30 and processed for the preparation of plasma and hemolysate. Assays were carried in plasma for insulin, fructose, lipid hydroperoxides (LHP), thiobarbituric acid reactive substances (TBARS), conjugated diens (CD), glutathione (GSH), vitamins C and E, free fatty acids (FFA) and triglycerides (TG). The assays for antioxidant enzymes, i.e. superoxide dismutase (SOD) (E.C.1.15.1.1), catalase (CAT) (E.C.1.11.1.6), glutathione peroxidase (GPx) (E.C.1.15.1.9), and glutathione-S-transferase (GST) (E.C.2.5.1.14) were done in the hemolysate.

Analytical procedures

Blood glucose was analyzed by the method of Sasaki *et al.* (12), and fructose by the method of Rao (13). Plasma insulin was estimated by the micro particle enzyme immunoassay method, with a kit obtained from Boehringer Mannheim, Germany. Insulin levels were expressed as μU/ml. Homeostatic model assessment (HOMA) as a measure of insulin resistance was

calculated by the formula: (insulin $\mu\text{U}/\text{mL} \times \text{glucose mmol}/\text{L} / 22.5$) (14). Plasma levels of FFA (15) and TG (16) were determined. TBARS in plasma were quantified by the method of Niehaus and Samuelsson (17), while CD content was measured by the method of Rao and Recknagel (18). The methods for the analysis of LHP, GSH, vitamins C and E, SOD, CAT, GPx, and GST are given elsewhere (19).

Statistical Analysis

Values are expressed as mean \pm SD. Data within the groups were analyzed using one-way analysis of variance followed by Duncan's multiple range test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Initial and final body weights of animals are shown in Figure 1. Weight gain was observed in all groups and did not significantly vary between the groups.

Figures 2a, 2b and 2c show the levels of blood glucose, fructose and insulin, respectively, in control and experimental animals. The values were significantly elevated in fructose-fed rats as compared with control rats. Fructose rats treated with CA showed significant reduction in the levels as compared with rats fed only fructose.

Figure 1. Initial and final body weight of animals. Values are means \pm SD (n=6 in each group); CON, control rats; FRU, fructose-fed rats; CA, carnitine.

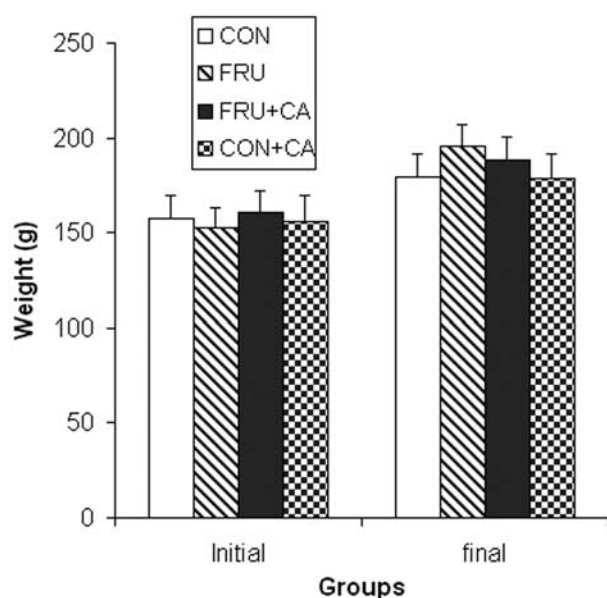
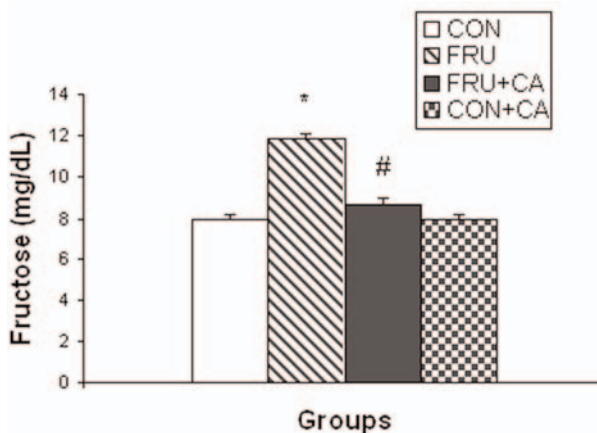
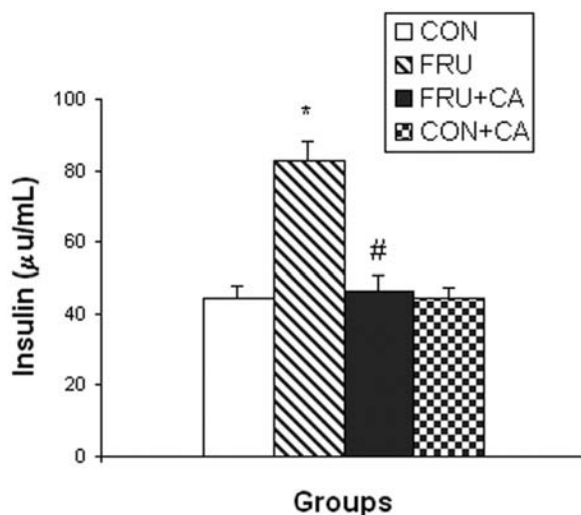
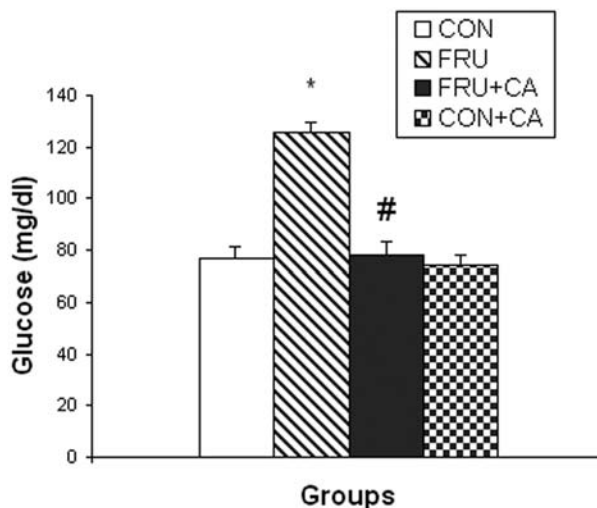


Figure 2a, 2b and 2c. Glucose, insulin and fructose concentrations in experimental animals. Values are means \pm SD (n=6 in each group); * $p < 0.05$ as compared to control; # $p < 0.05$ as compared to fructose; CON, control rats; FRU, fructose-fed rats; CA, carnitine.



The degree of insulin resistance as calculated by HOMA was higher in fructose-fed rats (Fig. 3a). The HOMA values were not significantly different between CA treated rats and control rats. The levels of plasma FFA and TG were also significantly elevated in fructose-fed rats (Figs. 3b and 3c), which also signify insulin resistance. Lipid levels were significantly reduced in CA-treated fructose-fed rats as compared with rats fed fructose alone.

Figure 3a, 3b, and 3c. HOMA values and concentrations of FFA and TG in plasma of experimental animals. Values are means \pm SD (n=6 in each group); *p<0.05 as compared to control; #p<0.05 as compared to fructose; CON, control rats; FRU, fructose-fed rats; CA, carnitine; HOMA, homeostatic model assessment; FFA, free fatty acids; TG, triglycerides.

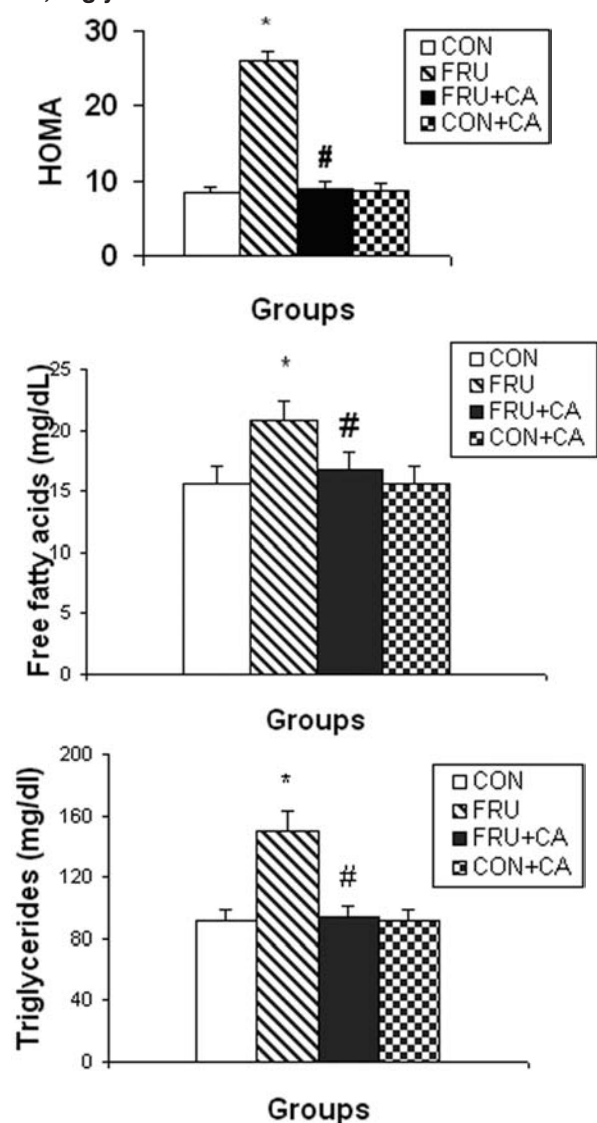
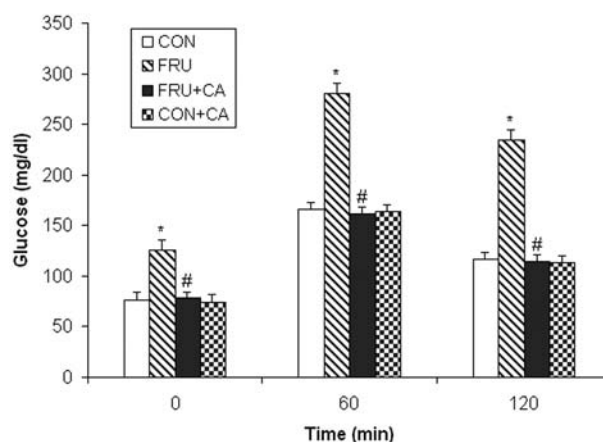


Figure 4 depicts the results of OGTT in control and experimental animals. The fasting glucose levels in fructose-fed rats were higher than those in other experimental groups. Significant elevations in the glucose levels after the oral glucose load was noted in fructose-fed rats at all time points. All other experimental groups showed a response similar to that of control rats.

Figure 4. Oral glucose tolerance test (OGTT) in experimental animals. Values are means \pm SD (n=6 in each group); *p<0.05 as compared to control; #p<0.05 as compared to fructose; CON, control rats; FRU, fructose-fed rats; CA, carnitine.



The $AUC_{glucose}$ and $AUC_{insulin}$ levels in control and experimental animals are given in Table 2. Both $AUC_{glucose}$ and $AUC_{insulin}$ levels were significantly higher in fructose-fed rats than in control rats. CA treated fructose-fed rats showed significantly reduced values of $AUC_{glucose}$ and $AUC_{insulin}$ as compared to those of untreated fructose-fed rats.

Table 3 summarizes the levels of LHP, TBARS and CD in plasma of control and experimental animals. Fructose rats showed significantly higher levels of peroxidation products as compared to control rats. In CA treated fructose rats, the levels of LHP, TBARS and CD were near normal and were significantly lower as compared with untreated fructose rats. In rats fed the control diet, the administration of CA did not produce significant alterations as compared to the control group.

Table 4 summarizes the activities of enzymatic antioxidants SOD, CAT, GPx and GST in the hemolysate of control and experimental animals. The activities were significantly lower ($p<0.05$) in fructose-

Table 2. Area under the curve (AUC) values for glucose and insulin on oral glucose tolerance test

	CON	FRU	FRU + L-CA	CON + L-CA
AUC _{glucose} (mg/mL/min)	157.59±11.03	276.10 ±22.64 ^a	155.20±10.08 ^b	153.77±10.67
AUC _{insulin} (μU/mL/min)	28.36± 2.13	52.70 ±3.42 ^a	28.73±2.21 ^b	28.43±2.23

Values are means ± SD of 6 rats from each group. CON = control rats; FRU = fructose-fed rats; FRU + CA = fructose fed rats treated with L-carnitine; CON + CA= control rats treated with L-carnitine; ^asignificant as compared with control rats (p<0.05) (DMRT); ^b significant as compared with fructose fed rats (p<0.05) (DMRT).

Table 3. Levels of lipid peroxidation indices such as thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LHP) and conjugated diens (CD) in plasma (μmol/L)

	CON	FRU	FRU + L-CA	CON + L-CA
TBARS	2.77 ± 0.22	5.25 ± 0.36 ^a	3.12 ± 0.24 ^b	2.54 ± 0.18
LHP	0.93 ± 0.08	1.33 ± 0.11 ^a	0.99 ± 0.08 ^b	0.82 ± 0.06
CD	0.77 ± 0.06	1.10 ± 0.09 ^a	0.72 ± 0.05 ^b	0.76 ± 0.04

Values are means ± SD of 6 rats from each group. CON = control rats; FRU = fructose-fed rats; FRU + CA = fructose fed rats treated with L-carnitine; CON + CA= control rats treated with L-carnitine; ^asignificant as compared with control rats (p<0.05) (DMRT); ^b significant as compared with fructose fed rats (p<0.05) (DMRT).

Table 4. Activities of enzymatic antioxidants in the hemolysate

	CON	FRU	FRU + L-CA	CON + L-CA
SOD (units/ mgHb)	3.02 ± 0.17	2.33 ± 0.14 ^a	2.91 ± 0.10 ^b	3.42 ± 0.21
CAT (μmoles/min/mgHb)	172.08 ± 14.37	139.44 ± 12.45 ^a	168.84 ± 12.95 ^b	174.44 ± 14.52
GPx (μg/min/mgHb)	9.07 ± 0.96	6.72 ± 0.45 ^a	8.45 ± 0.70 ^b	9.32 ± 0.78
GST (μmoles/min/mgHb)	6.27 ± 0.48	4.79 ± 0.28 ^a	5.97 ± 0.34 ^b	6.48 ± 0.51

Values are means ± SD of 6 rats from each group. CON = control rats; FRU = fructose-fed rats; FRU + CA = fructose fed rats treated with L-carnitine; CON + CA= control rats treated with L-carnitine; ^asignificant as compared with control rats (p<0.05) (DMRT); ^bsignificant as compared with fructose fed rats (p<0.05) (DMRT). Unit-amount of enzyme that gave 50% inhibition of NBT reduction/milligram of hemoglobin.

Table 5. Activities of non-enzymatic antioxidants in plasma (μmol/L)

	CON	FRU	FRU + L-CA	CON + L-CA
GSH	938.76 ± 64.27	652.67 ± 53.98	878.10 ± 75.43	941.69 ± 71.12
Vitamin C	164.68 ± 11.14	103.94 ± 8.86	157.06 ± 13.17	169.64 ± 11.09
α-Tocopherol	23.32 ± 1.85	15.88 ± 1.25	20.86 ± 1.23	25.46 ± 2.12

Values are means ± SD of 6 rats from each group. CON = control rats; FRU = fructose-fed rats; FRU + CA = fructose fed rats treated with L-carnitine; CON + CA= control rats treated with L-carnitine; ^asignificant as compared with control rats (p<0.05) (DMRT); ^b significant as compared with fructose fed rats (p<0.05) (DMRT).

fed rats than in control rats. In fructose rats treated with CA (groups 3 and 4) the activities were significantly higher as compared to fructose rats.

Significant decreases in the concentration of non-enzymatic antioxidants were observed in fructose-fed rats (Table 5). Simultaneous treatment of fructose-fed rats with carnitine brought the levels to near control values.

DISCUSSION

High fructose feeding for four weeks resulted in hyperglycemia, hypertriglyceridemia and hyperinsulinemia. An abnormal response to oral glucose load was also observed. These findings have already been

reported by us (4,5,19) and are consistent with those of other investigators (1,2). The presence of insulin resistance is indicated by higher values of HOMA.

Insulin resistance may occur due to a defect in insulin binding caused by decreased receptor number or affinity, or defects at the level of effector molecules such as glucose transporters and enzymes involved in glucose metabolism (20-22).

Suga *et al.* (23) suggest that fructose feeding decreases the efficacy of insulin extraction by the liver, which retards insulin clearance from the circulation. Hepatic metabolism of fructose leads to alterations in the activities of key enzymes of glucose metabolism (24) and activation of stress sensitive pathways (25) that may desensitize insulin signaling.

Enhanced lipid peroxidation in fructose-fed rats could be associated with high circulating glucose. Hyperglycemia is well known to increase reactive oxygen species (ROS) generation and subsequent lipid peroxidation. Hypertriglyceridemia is another factor that could enhance the formation of lipid peroxides. It has been reported that lipid peroxide levels correlate with hypertriglyceridemia in diabetic patients (26). In addition, fructose itself enhances the reactive oxygen formation *in vitro* (27).

The increase in plasma lipid peroxides could also have resulted from a decline in cellular, non-enzymatic and enzymatic antioxidant potential in fructose-fed rats. The catalytic actions of antioxidant enzymes are important for the effective removal of oxygen radicals. Free radical damage decreases the activities of antioxidant enzymes such as CAT, GPx (28) and SOD (29). Modification of Cu-Zn SOD by glycation at specific lysine residue leads to inactivation and fragmentation of the enzyme in the diabetic condition (30). Recently, Cavarape *et al.* (31) have reported that catalase gene expression is reduced in liver and heart of rats fed fructose. These findings suggest a higher susceptibility to oxidative stress in fructose-fed rats.

The administration of CA to the rats mitigated the adverse effects of fructose load on glucose and insulin levels. Glucose tolerance was improved and the other components of insulin resistance syndrome, FFA and TG, were brought to normal levels in CA treated rats as compared to fructose rats.

CA is suggested to enhance the oxidative utilization of glucose by stimulation of the activity of pyruvate dehydrogenase by decreasing the acetyl coA/coA ratio (32). In addition, stimulation of glycolysis by CA in the diabetic rat heart has been shown as a result of increased flux of glucose through the activation of phosphofructokinase, the rate-limiting enzyme of the glycolytic pathway (33).

CA treatment reduces the plasma TBARS levels. This may be due to the enhancement of the fatty acid transport by carnitine into mitochondria for energy production, thereby lowering the availability of lipids

for peroxidation. CA inhibits the microsomal peroxidation (34), and CA and its esters have been shown to partially inhibit iron-induced lipid peroxidation in liposome by chelating free iron (35).

CA by virtue of its ability to enhance ATP production (36) might have improved the overall protein and thus antioxidant enzyme synthesis in the cells. Moreover, CA can protect these enzymes from further peroxidative damage by its ability to reduce peroxidation reactions.

The enzyme GPx requires GSH as the substrate. Elevations in GSH levels have been observed upon carnitine supplementation in rats, and this in turn could increase the activity of GPX.

The administration of CA improves glutathione status in fructose-fed animals. The synthesis of the antioxidant glutathione is regulated in two steps, which are an ATP dependent process. The energy enhancing action of CA may be responsible for the increase in glutathione status after CA supplementation. Several authors have demonstrated the glutathione promoting action of CA in rats (34,37).

Vitamin C is one of the cofactors in CA biosynthesis (38). Supplementation of CA may spare vitamin C, thereby elevating its level. As vitamin C has the ability to regenerate vitamin E, normalization of vitamin E concentration by CA administration may be attributed to the synergistic effect of the antioxidants.

The impaired oxidant-antioxidant balance can be attributed, at least in part, to the elevation of TG and glucose in fructose-fed rats. The ability to bring a favorable metabolic environment and antioxidant role could be suggested for the observed benefits of CA in fructose-fed rats.

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