

INTRAPERITONEAL L-CARNITINE REGULATES LIPID METABOLISM AND REDUCES OXIDATIVE STRESS IN FRUCTOSE-INDUCED HYPERLIPIDEMIC RATS

Panchamoorthy Rajasekar¹, Mambakkam Katchapeswaran Ravichandran²,
Carani Venkatraman Anuradha¹

*Key words: insulin resistance, lipids, lipoproteins,
lipid peroxidation, antioxidants, carnitine*

SUMMARY

The role of nutritional substances in the management of insulin resistance is of interest. The present study was aimed to explore the effect of administration of L-carnitine on lipid metabolism and oxidative stress in a high fructose-fed, hyperlipidemic, insulin resistant rat model. Rats fed fructose showed hyperlipidemia, significant alterations in lipoprotein profile, and accumulation of triglycerides in the liver. High density lipoprotein cholesterol was significantly reduced, while very low density lipoprotein cholesterol and low density lipoprotein cholesterol were significantly elevated ($p < 0.05$). The activities of lipoprotein lipase and lecithin cholesterol acyl transferase in plasma and liver were significantly reduced. Alterations in the activities of plasma and liver cholesterol ester hydrolase, cholesterol ester synthetase and hydroxymethyl glutaryl-coenzyme A reductase were observed. Fructose-fed rats displayed reduction in plasma nitrite, accumulation of lipid peroxidation end products and depletion of antioxidants in the liver. Simultaneous administration of carnitine along with fructose diet alleviated the effects of fructose. These rats showed near-normal levels of the parameters studied. The results suggest that carnitine supplementation may have some benefits in patients with insulin resistance.

Correspondence to: Carani Venkatraman Anuradha, MD, PhD,
Department of Biochemistry, Annamalai University,
Annamalai Nagar - 608002, Tamil Nadu, India
E-mail: cvradha@hotmail.com or cvardha@yahoo.com

INTRODUCTION

Diet high in fructose induces insulin resistance (IR) in rats (1), hamsters (2) and dogs (3). Investigators have produced IR by administering fructose in drinking water (5-10%) or by feeding a diet in which fructose contributes to more than 50% of total calories. Rats develop IR, hyperlipidemia, and hypertension as early as 2 weeks of fructose diet initiation (4). The lipid changes reported include increased levels of triglycerides (TG), free fatty acids (FFA) and low density lipoprotein cholesterol (LDL-C) (5). Fructose has also been shown to have pro-oxidant effects. Increased formation of lipid peroxidation end products (6,7) and defects in free radical defense system (8) have been documented in high fructose-fed rats. The abnormalities induced in the fructose-fed rat are secondary to a primary defect in insulin action, and the model has been validated to parallel syndrome X, a human multi-metabolic disease (9).

Carnitine (CA, β -hydroxy- γ -trimethyl ammonium butyric acid) is present in foods, especially in beef, egg, cow's milk, wheat germ, barley and oat seeds. CA is synthesized in tissues such as the liver, kidney and brain. Essential amino acids, e.g., lysine and methionine, and vitamins such as niacin, pyridoxine and ascorbic acid are required for its biosynthesis. Physiologically, CA is important in the transformation of long chain free fatty acids into acylcarnitine and in

their transport across the inner mitochondrial membrane into the mitochondrial matrix for β -oxidation.

CA has received consideration as a hypolipidemic agent. Supplementation studies show that exogenous CA reduces the levels of plasma very low density lipoprotein cholesterol (VLDL-C) and very low density lipoprotein TG (VLDL-TG) in hyperlipidemic rabbits (10) and plasma lipoprotein(a) levels in type 2 diabetic patients with hypercholesterolemia (11). CA is reported to exert antioxidant action in experimental animals (12). Clinical trials have shown that CA facilitates aerobic metabolism of glucose and improves insulin sensitivity in insulin resistant diabetic patients (13).

In our previous study, we observed that CA could improve insulin action in this model (14). We hypothesize that CA, by improving insulin action, can regulate lipid metabolism and through its antioxidative properties can ameliorate oxidative stress. In view of this, a study was undertaken to investigate the hypolipidemic and antioxidative effects of CA in a well-characterized hyperlipidemic, insulin resistant rat model.

MATERIALS AND METHODS

Animals and diet

Male adult Wistar rats of body weight 150-160 g were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. 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 Ltd., Agro Feeds Division, Bangalore, India) and water *ad libitum*.

L-Carnitine was obtained from the Sisco Research Laboratories (P) Ltd, Mumbai, India. All other chemicals and solvents were of analytical grade and were from Himedia Laboratories Pvt. Ltd, Mumbai, India. The animals used in the present study were cared for as *per* the principles and guidelines of the Ethics Committee of Animal Care of Annamalai University. All the procedures were approved by Institutional Animal Ethics 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 (FRU + CA) received fructose diet and were administered CA (300 mg/kg body weight/day, i.p. in 0.89% saline); and group 4 (CON + CA) received control diet and were given CA (300 mg/kg body weight/day, i.p. in 0.89% saline). Body weight and food intake of animals were measured during the experimental period. The composition of control and fructose diet is presented in Table 1. The diets were prepared fresh daily. The animals were maintained in their respective groups for 30 days.

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

Ingredient	Control diet	High fructose diet
Corn starch	60.0	-
Fructose	-	60.0
Casein (fat free)	20.0	20.0
Methionine	0.7	0.7
Groundnut oil	5.0	5.0
Wheat bran	10.6	10.6
Salt mixture #	3.5	3.5
Vitamin mixture *	0.2	0.2

Mineral mix composition (g/kg): MgSO₄·7H₂O-30.5; NaCl-65.2; KCl-105.7; KH₂PO₄-200.2; MgCO₃-3.65; Mg(OH)₂·3H₂O-38.8; FeC₂H₃O₇·5H₂O-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.

At the end of the experimental period the rats were sacrificed by cervical decapitation. Blood samples were collected and plasma was separated by centrifugation. The liver tissue was removed immediately, cleaned of blood and transferred to containers containing ice-cold saline. Liver homogenate was prepared with 0.1M Tris-HCl buffer, pH 7.4.

Lipid analysis

The extraction of lipids from plasma and liver was carried out according to the procedure of Folch et al. (15). Total cholesterol (16), phospholipids (17), triglycerides (18) and free fatty acids (19) were analyzed. Free cholesterol was assayed in plasma by the method of Leffler and McDougald (20) after precipitation as cholesterol digitonide. Ester cholesterol content was arrived at by subtracting the free cholesterol concentration from total cholesterol. Cholesterol in the lipoprotein fractions was also

determined. HDL-C was analyzed in the supernatant obtained after precipitation of plasma with phosphotungstic acid/Mg²⁺ LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) were calculated as follows:

$$\text{VLDL-C} = \text{triglycerides}/5$$

$$\text{LDL-C} = \text{total cholesterol} - (\text{HDL-C} + \text{VLDL-C})$$

Lipoprotein lipase (LPL) was assayed in plasma and liver by the method of Korn (21). Lecithin cholesterol acyl transferase (LCAT) was assayed in plasma and liver by the method of Hitz *et al.* (22). Cholesterol ester synthetase (CES) and cholesterol ester hydrolase (CEH) were determined in plasma and liver tissue

Figure 1. Food intake was not significantly altered in control and experimental animals. Values are means \pm SD (n=6 in each group); CON=control rats; FRU=fructose-fed rats; CA=carnitine.

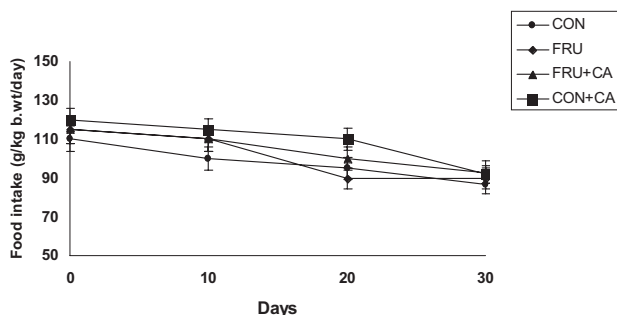
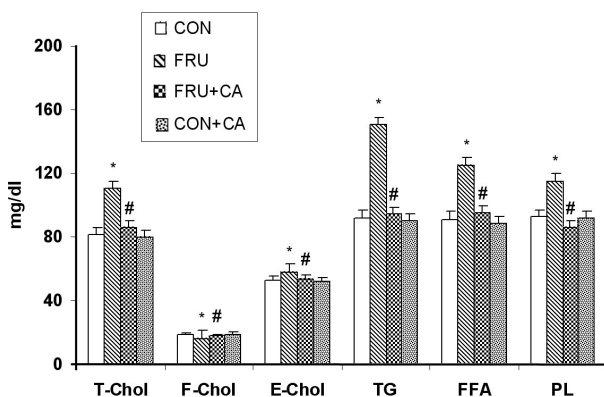


Figure 2. Concentrations of total, ester and free cholesterol, TG, FFA and PL in plasma of experimental animals. Values are means \pm SD (n=6 in each group); *p<0.05 as compared to control; #p<0.05 as compared to fructose; CON=control rats; FRU=fructose-fed rats; CA=carnitine; T-Chol=total cholesterol; E-Chol=ester cholesterol; F-Chol=free cholesterol; TG=triglycerides; FFA=free fatty acids; PL=phospholipids.



homogenates by the method of Kritechevsky *et al.* (23). Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase activity was measured by the method of Rao and Ramakrishnan (24).

Peroxidation indices and antioxidant analysis

The content of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LHP), and conjugated dienes (CD), and the activities of 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), glutathione S-transferase (GST) (E.C.2.5.1.14), and vitamins C and E were measured in the liver. The methods for the above analysis are given elsewhere (14). Protein carbonyls (25) and aldehydes (26) in liver, and nitrite content in plasma (27) were determined. Protein content in the liver was measured by the method of Lowry *et al.* (28) and that of total (T-SH), non-protein (NP-SH) and protein bound (P-SH) sulfhydryl groups (29) by standard methods.

Statistical analysis

Values are expressed as mean \pm SD. Data within 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

The final body weights of animals were as follows: CON: 179.5 g \pm 4.65; FRU: 195.7 g \pm 5.47; FRU + CA: 188.66 g \pm 4.19; and CON + CA: 178.35 g \pm 5.38. Weight gain was observed in all groups and did not vary significantly between the groups.

Figure 1 shows food intake of the animals during the experimental period. At the end of the experimental period the food intake was 90.2 \pm 10.1 g/kg b.w./day in rats fed high fructose diet and 87.8 \pm 11.2 g/kg b.w./day in rats fed control diet. Food intake was not significantly different between the groups throughout the experiment.

The plasma lipid concentrations in control and experimental animals are shown in Figure 2. Fructose-fed rats had elevated concentrations of plasma total cholesterol (35%) and ester cholesterol (11%), and decreased concentrations of free cholesterol (14%) as

compared to control rats. TG was significantly elevated (63%) in fructose-fed rats as compared to control rats. CA treated rats showed a significant reduction in lipid levels as compared to fructose-fed rats. Phospholipids were elevated by 24% and FFA by 38% in fructose-fed rats as compared to control rats, and were near-normal in CA-treated fructose rats.

Lipid concentrations in the liver of control and experimental animals are shown in Figure 3. The concentrations of cholesterol, TG and FFA were significantly increased, by 10%, 77% and 46%, respectively, in fructose-fed rats as compared to control rats. CA administration to fructose-fed rats resulted in a significant decrease ($p < 0.05$) in cholesterol, TG and FFA compared to fructose-fed rats. The level of phospholipids was significantly lower ($p < 0.05$; 28%) in fructose-fed rats as compared to control-diet fed rats. CA administration brought the concentrations of lipid constituents to near-normal in CA treated fructose rats.

The distribution of cholesterol in lipoprotein fractions is given in Table 2. Significant increases in VLDL-C and LDL-C, and a significant decrease in HDL-C were observed in fructose-fed rats. These alterations were reversed and the values were near-normal in CA-treated rats.

The activities of LPL, LCAT, CEH and CES in plasma and LPL, LCAT, CEH, CES and HMG-CoA reductase in the liver are presented in Tables 3 and 4. LPL and LCAT activities were lowered in the plasma

and liver of fructose-fed rats. CEH activity was appreciably lowered and that of CES elevated in the plasma and liver of fructose-fed rats. There was an increased activity of liver HMG-CoA reductase in the fructose-fed group. The activities of these enzymes were restored to baseline control values in fructose-fed rats treated with CA.

Table 5 shows the levels of TBARS, LHP, CD, protein carbonyl groups, and aldehydes in the liver and nitrite levels in the plasma of control and experimental animals. Fructose-fed rats showed significantly higher

Figure 3. Concentrations of cholesterol, TG, FFA and PL in the liver 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; Chol=cholesterol; TG=triglycerides; FFA=free fatty acids; PL=phospholipids.

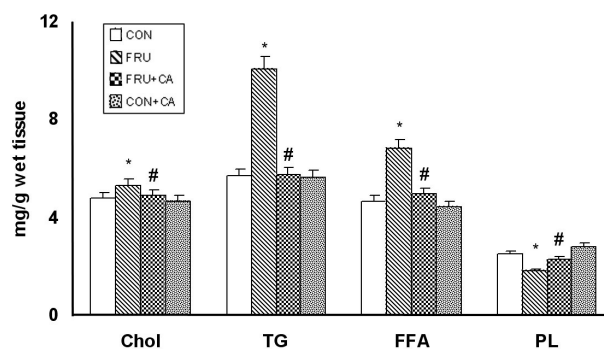


Table 2. Distribution of cholesterol in lipoprotein fractions (mg/dL)

Parameter	CON	FRU	FRU + CA	CON + CA
HDL-cholesterol	34.30 \pm 2.46	29.98 \pm 1.88 ^a	32.82 \pm 1.67 ^b	34.89 \pm 2.79
LDL-cholesterol	29.10 \pm 5.12	50.24 \pm 11.52 ^a	33.98 \pm 6.02 ^b	26.22 \pm 6.05
VLDL-cholesterol	18.41 \pm 1.30	30.03 \pm 2.62 ^a	20.12 \pm 1.84 ^b	18.81 \pm 1.54

Values are means \pm SD of 6 rats from each group; CON=control rats; FRU=fructose-fed rats; FRU+CA=fructose fed rats treated with carnitine; CON + CA=control rats treated with carnitine; ^a significant as compared to control rats ($p < 0.05$; ANOVA followed by DMRT); ^b significant as compared to fructose fed rats ($p < 0.05$; ANOVA followed by DMRT).

Table 3. Activities of lecithin cholesterol acyl transferase (LCAT), lipoprotein lipase (LPL), cholesterol ester hydrolase (CEH) and cholesterol ester synthetase (CES) in plasma

Parameter	CON	FRU	FRU + CA	CON + CA
LCAT (μ moles of cholesterol/h/L)	75.33 \pm 5.41	64.08 \pm 4.77 ^a	71.81 \pm 5.59 ^b	76.18 \pm 6.40
LPL (μ moles of glycerol liberated/h/L)	5.72 \pm 0.44	4.75 \pm 0.33 ^a	5.41 \pm 0.51 ^b	5.96 \pm 0.47
CEH (n moles of cholesterol liberated/h/L)	20.37 \pm 1.68	18.45 \pm 1.45 ^a	19.23 \pm 1.33 ^b	23.84 \pm 2.04
CES (n moles of cholesterol esterified/h/L)	15.75 \pm 1.12	17.56 \pm 1.51 ^a	15.83 \pm 1.47 ^b	14.92 \pm 1.51

Values are means \pm SD of 6 rats from each group; CON=control rats; FRU=fructose-fed rats; FRU+CA=fructose fed rats treated with carnitine; CON + CA=control rats treated with carnitine; ^a significant as compared to control rats ($p < 0.05$; ANOVA followed by DMRT); ^b significant as compared to fructose fed rats ($p < 0.05$; ANOVA followed by DMRT).

Table 4. **Activities of lecithin cholesterol acyl transferase (LCAT), lipoprotein lipase (LPL), cholesterol ester hydrolase (CEH) and cholesterol ester synthetase (CES) in liver**

Parameter	CON	FRU	FRU + CA	CON + CA
LCAT (μ moles of cholesterol/h/mg protein)	22.15 \pm 2.12	19.66 \pm 1.26 ^a	21.79 \pm 1.39 ^b	22.74 \pm 2.11
LPL (μ moles of glycerol liberated/h/50 mg acetone powder)	11.53 \pm 0.94	10.51 \pm 0.57 ^a	10.42 \pm 0.81 ^b	11.81 \pm 0.54
CEH (n moles of cholesterol liberated/h/mg protein)	18.82 \pm 1.15	15.95 \pm 1.36 ^a	18.65 \pm 1.42 ^b	19.19 \pm 1.41
CES (n moles of cholesterol esterified/h/mg protein)	19.94 \pm 1.03	23.40 \pm 2.07 ^a	20.53 \pm 1.58 ^b	18.70 \pm 1.47
HMG-CoA reductase (ratio of HMG-CoA to mevalonate) ^c	3.20 \pm 0.30	2.03 \pm 0.16 ^a	2.98 \pm 0.29 ^b	3.24 \pm 0.25

Values are means \pm SD of 6 rats from each group; CON=control rats; FRU=fructose-fed rats; FRU+CA=fructose fed rats treated with carnitine; CON + CA=control rats treated with carnitine; ^a significant as compared to control rats ($p < 0.05$; ANOVA followed by DMRT); ^b significant as compared to fructose fed rats ($p < 0.05$; ANOVA followed by DMRT); ^c lower ratio indicates higher enzyme activity and vice versa.

Table 5. **Levels of LHP, TBARS, CD, protein carbonyl groups and aldehyde in the liver, and nitrite levels in plasma of control and experimental animals**

Parameter	CON	FRU	FRU + CA	CON + CA
LHP (μ mol/mg protein)	1.48 \pm 0.13	2.08 \pm 0.17 ^a	1.61 \pm 0.14 ^b	1.42 \pm 0.10
TBARS (μ mol/mg protein)	1.57 \pm 0.12	2.65 \pm 0.18 ^a	1.65 \pm 0.14 ^b	1.49 \pm 0.08
CD (A 233)	0.68 \pm 0.03	0.84 \pm 0.08 ^a	0.71 \pm 0.06 ^b	0.65 \pm 0.05
Protein carbonyl groups (μ mol/mg protein)	1.84 \pm 0.17	2.14 \pm 0.20 ^a	1.92 \pm 0.16 ^b	1.79 \pm 0.12
Aldehyde (aldehyde conjugates, μ mol QS e.q/g tissue)	0.899 \pm 0.13	1.09 \pm 0.95	0.938 \pm 0.71	0.838 \pm 0.37
Nitrite (μ mol/L)	11.07 \pm 0.68	9.63 \pm 0.92	11.38 \pm 0.61	11.46 \pm 0.88

Values are means \pm SD of 6 rats from each group; CON=control rats; FRU=fructose-fed rats; FRU+CA=fructose fed rats treated with carnitine; CON + CA=control rats treated with carnitine; ^a significant as compared to control rats ($p < 0.05$; ANOVA followed by DMRT); ^b significant as compared to fructose fed rats ($p < 0.05$; ANOVA followed by DMRT).

Table 6. **Concentrations of enzymatic and non-enzymatic antioxidants in the liver of control and experimental animals**

Parameter	CON	FRU	FRU + CA	CON + CA
<i>Enzymatic antioxidants</i>				
SOD (units A)	4.05 \pm 0.26	2.75 \pm 0.19 ^a	3.87 \pm 0.29 ^b	4.18 \pm 0.20
CAT (μ mol/min/mg protein)	59.88 \pm 4.54	41.33 \pm 3.62 ^a	55.89 \pm 4.32 ^b	61.23 \pm 5.15
GPx (μ mol/min/mg protein)	5.37 \pm 0.47	4.14 \pm 0.33 ^a	5.13 \pm 0.41 ^b	5.45 \pm 0.39
GST (μ mol/min/mg protein)	5.39 \pm 0.47	4.19 \pm 0.34 ^a	4.99 \pm 0.43 ^b	5.43 \pm 0.42
<i>Non-enzymatic antioxidants</i>				
GSH (NP-SH) (μ mol/mg protein)	5.63 \pm 0.30	5.00 \pm 0.47 ^a	5.80 \pm 0.52 ^b	6.05 \pm 0.54
TSH (μ g/mg protein)	15.27 \pm 1.25	14.17 \pm 1.04 ^a	15.17 \pm 1.11 ^b	16.77 \pm 1.36
PSH (μ g/mg protein)	9.69 \pm 0.89	9.11 \pm 0.75 ^a	10.01 \pm 0.90 ^b	11.06 \pm 0.92
Vitamin C (μ mol/mg protein)	50.00 \pm 4.17	26.67 \pm 2.04 ^a	47.50 \pm 3.53 ^b	52.33 \pm 5.10
Vitamin E (μ mol/mg protein)	19.89 \pm 1.39	14.89 \pm 1.05 ^a	17.58 \pm 1.25 ^b	19.94 \pm 1.59

Values are means \pm SD of 6 rats from each group; CON=control rats; FRU=fructose-fed rats; FRU+CA=fructose fed rats treated with carnitine; CON + CA=control rats treated with carnitine; ^a significant as compared to control rats ($p < 0.05$; ANOVA followed by DMRT); ^b significant as compared to fructose fed rats ($p < 0.05$; ANOVA followed by DMRT).

levels of peroxidation markers and decreased plasma nitrite levels as compared to control rats. In CA treated fructose-fed rats, the levels of peroxidation markers were significantly lower while the plasma nitrite level was higher as compared to untreated fructose-fed rats.

The enzymatic antioxidants SOD, CAT, GPx and GST, and non-enzymatic antioxidants such as total (T-SH), non-protein (NP-SH) and protein thiols (P-SH), and vitamin C and E levels were significantly lower in fructose-fed rats than in normal rats (Table 6). In fructose-fed rats treated with CA, the activities of both

enzymatic and non-enzymatic antioxidant activities were significantly higher as compared to untreated fructose rats.

Control animals treated with CA showed no alterations in the lipid levels and lipid peroxidation indices. We found increased antioxidant levels in these rats which, however, were not significant.

DISCUSSION

High fructose-feeding causes hyperlipidemia, hyperinsulinemia and the development of whole body insulin resistance in the rat. This has been reported from our laboratory (14,30) and by other investigators (1,4,5).

The accumulation of TG could occur due to increased lipogenesis, decreased clearance or reduced fatty acid oxidation. Increased availability of precursor molecules glycerol-3-phosphate and excess free fatty acids in fructose-fed rats may increase TG content while the diminished activity of LPL results in impaired clearance from circulation. The increase in HMG-CoA reductase signifies an increase in cholesterol synthesis. The addition of fructose to cultured rat hepatocytes increases HMG-CoA reductase approximately 3-fold (31). Elevation in both VLDL and LDL fractions contributes to a rise in total plasma cholesterol. The increase in ester cholesterol and reduction in free cholesterol may be attributed to the elevated activity of the enzyme CES and decreased activity of the enzyme CEH, while a decreased activity of LCAT indicates impairment in HDL-C synthesis and metabolism.

Upregulation of lipogenic enzymes such as fatty acid synthase and acyl CoA carboxylase have been reported in insulin resistance (32). An increase in diacyl glycerol acyl transporter activity was shown by Casaschi *et al.* in the liver of a similar model system, fructose-induced hyperlipidemic hamster (33).

The dyslipidemia observed in insulin resistance is thought to be initiated by the resistance of fat storing cells. The inability to store TG results in its mobilization to plasma and also to non-fat storing tissues such as liver. Overaccumulation of TG in the liver and muscle produces excess of metabolites such as fatty acids, ceramides and diacyl glycerol. These may enter deleterious non-oxidative pathways and induce a

state of lipotoxicity (34). Further, these metabolites induce a cascade of serine/thyrosine phosphorylation reactions that diminish the glucose transport activity and other events that desensitize insulin receptor signaling (35).

The increase in FFA could be yet another mechanism by which fructose could produce IR. Increased delivery of FFA to muscle interferes with glucose utilization, through the principles of Randle cycle (36). This can attenuate insulin signaling and exacerbate IR. Conversely, diminished insulin-stimulated glucose disposal could lead to impaired FFA reesterification and thereby to higher circulating FFA concentrations.

The development of oxidative stress, an imbalance between pro- and anti-oxidant status, has been shown to play an important role in mediating insulin resistance, and therefore we studied the extent of peroxidation and antioxidant potential. Peroxidative deterioration of lipids in fructose-fed rats is evident from the increased levels of TBARS, LHP and aldehydes, while the increased protein carbonyl content and diminished protein-SH group signify protein damage.

Increased peroxidation in fructose-fed rats could be due to hyperglycemia reported in these rats (14). Besides this, fructose feeding itself can induce oxidative stress by a number of mechanisms. The increased catabolism of fructose could be associated with the cellular energy depletion that can increase the susceptibility of cells to lipid peroxidation. Further, down-regulation of HMP shunt enzymes in the presence of fructose (37) could lead to decreased generation of reducing equivalents (NADPH). Furthermore, it has been postulated that fructose can accelerate free radical production similar to glucose. For example, Suzuki *et al.* (38) have observed an increased production of H₂O₂ and formation of hydroxyl radicals by hamster pancreatic cells incubated with fructose in the presence of a metal ion catalyst.

Abnormalities in nitric oxide (NO)/superoxide radicals would reduce vascular tone with a secondary decline in insulin action. Diminished plasma nitrite is suggestive of reduced NO production or its inactivation by superoxide. A correlation between IR and defects in NO system has been reported (39).

Shinozaki et al. (40) have observed a reduction of NO in fructose-fed rats and relate this to the rise in blood pressure observed in these rats.

The major targets of damaging free radicals are the cellular and membrane phospholipids. Oxidative damage to cell membranes cannot only release the membrane phospholipids into blood but also cause change in the physicochemical state of the plasma membrane by mechanical disruption, release of lipid oxidative products that may cause inhibition of enzyme systems. Oxidative stress may have implications in insulin signaling and insulin-mediated glucose uptake since free radicals can impair insulin action through membrane structural changes (41). A significant positive correlation between insulin resistance and TBARS formation was observed in fructose-fed rats (30).

Exogenous CA mitigated lipid abnormalities in fructose-fed rats. One obvious mechanism of the TG lowering effect is the role of CA to increase the influx of fatty acids as acylcarnitine into mitochondria. This reduces the substrate availability for the synthesis of TG in the liver. The effects of CA on lipid metabolism may also be related to its effect on glucose utilization and improvement of insulin action. CA increases the utilization of glucose by activating pyruvate dehydrogenase and by decreasing the intra-mitochondrial acetyl CoA/CoA ratio (42). Improved insulin action by exogenous CA, reported in our previous study (14), could regulate the key enzymes of lipid metabolism and normalize lipid levels in the circulation.

Several mechanisms may be attributed to the antioxidative effect of CA. CA plays a role in chelating free Fe²⁺ ions and hence could reduce free radical generation (43). Further, CA, by virtue of its ability to enhance ATP production (44), could improve the overall protein and thereby antioxidant enzyme levels in the cells. In addition, the vitamin C and methionine sparing activity of CA (45) can reduce lipid peroxidation by maintaining the levels of antioxidants like vitamin E and thiol groups, predominantly GSH. Elevation of GSH, in turn, could increase the activities of GSH-dependent enzymes in CA-treated rats. Efficient expression of the insulin receptor gene required certain transcription factors activated by GSH (46). CA has been shown to reduce lipid peroxidation in the heart and liver in experimental atherosclerosis (47) and myocardial infarction (48). Further, an increased plasma concentration of NO in CA-treated rats indicates the potential role of NO in the effects of CA. In 1997, Cameron and Cotter (49) reported that CA could alter nerve conduction and nerve blood flow in diabetic rats through activation of the NO system.

The role of nutritional substances in the management of IR has been the focus of intensive research in the past few years. Deficiency of plasma total and free CA levels has been observed in type 2 diabetic patients and is related to specific diabetic complications (50). The results of the study could be important considering the rise in the prevalence of IR and multi-metabolic syndrome in the general population. Further research on the clinical utility of CA to combat hyperlipidemia and oxidative stress associated with IR is necessary.

REFERENCES

1. Thorburn AW, Storlein LH, Jenkins AB, Khouri S, Kraegen EW. Fructose induced *in vivo* insulin resistance and elevated plasma triglyceride levels in rats. *Am J Clin Nutr* 1989;49:1155-1163.
2. Kasim Karakas SE, Vriend H, Almario R, Chow LC, Goodman MN. Effects of dietary carbohydrates on glucose and lipid metabolism in golden Syrian hamsters. *J Lab Clin Med* 1996;128:208-213.
3. Martinez FJ, Rizza RA, Romero JC. High fructose feeding elicits insulin resistance, hyperinsulinemia and hypertension in normal mongrel dog. *Hypertension* 1994;23:456-463.
4. Dai S, McNeill JH. Fructose induced hypertension is concentration and duration dependent. *J Pharmacol Toxicol Methods* 1995;33:101-107.
5. Kelley GL, Allan G, Azhar S. High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. *Endocrinology* 2004;145:548-555.
6. Vasdev S, Ford CA, Longerich L, Gadag V, Wadhawan S. Role of aldehydes in fructose-induced hypertension. *Mol Cell Biochem* 1998;181:1-9.

7. Busserolles J, Gueux E, Demigne C, Mazur A, Rayssiguier Y. Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. *Endocrinology* 2004;145:548-555.
8. Faure P, Rossini E, Lafond JL, Richard MJ, Favier A, Halimi SJ. Vitamin E improves the free radical defense system potential and insulin sensitivity of rats fed high fructose diets. *Nutrition* 2004;127:103-107.
9. Reaven GM. Insulin resistance, hyperinsulinemia, hypertriglyceridemia and hypertension: parallels between human disease and rodent models. *Diabetes Care Rev* 1991;14:195-202.
10. Leighton James AKM, Bhuiyan J, Foster D, Secombe D. Effect of L-carnitine treatment on very low density lipoprotein kinetics in the hyperlipidemic rabbit. *Clin Biochem* 1995;28:451-458.
11. Derosa G, Cicero AFG, Gaddi A, Mugellini A, Ciccarelli L, Fogari R. The effect of carnitine on plasma lipoprotein(a) levels in hypercholesterolemic patients with type 2 diabetes mellitus. *Clin Ther* 2003;25:1429-1439.
12. Izgut-Uysal VN, Agac A, Derin N. Effect of carnitine on stress-induced lipid peroxidation in rat gastric mucosa. *J Gastroenterol* 2001;36:231-236.
13. Migrone G, Greco AV, Capristo E, *et al.* AL-carnitine improves glucose disposal in type 2 diabetic patients. *J Am Coll Nutr* 1999;18:77-82.
14. Rajasekar P, Kaviarasan S, Anuradha CV. L-carnitine administration prevents oxidative stress in high fructose-fed insulin resistant rats. *Diabetol Croat* 2005;34:21-28.
15. Folch J, Lees M, Stanley GSH. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 1951;126:497-509.
16. Zlatki A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. *J Lab Clin Med* 1953;45:486.
17. Zilversmit DB, Davis AK. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. *J Lab Clin Invest* 1950;35:155.
18. Foster CS, Dunn O. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clin Chem* 1973;19:338-340.
19. Falholt K, Falholt W, Lund B. An easy colorimetric method for routine determination of free fatty acids in plasma. *Clin Chim Acta* 1973;46:105.
20. Leffler HH, Mc Dougald GH. Estimation of cholesterol in serum by means of improved techniques. *Am J Clin Pathol* 1963;39:311-315.
21. Korn ED. Clearing factors: a heparin activated lipoprotein lipase. Isolation and characterization of enzyme from normal rats. *J Biol Chem* 1955;215:1-26.
22. Hitz J, Sterinmetz J, Surt G. Plasma LCAT-reference values and effects of xenobiotics. *Clin Chim Acta* 1983;133:85-96.
23. Kritchevsky D, Kothari HV. Aortic cholesterol esterase studies in white carneau and show Racer pigeons. *Biochem Biophys Acta* 1973;326:489-491.
24. Rao V, Ramakrishnan S. Indirect assessment of hydroxy methyl glutaryl CoA reductase activity in liver tissue. *J Clin Chem* 1975;21:1523-1527.
25. Levine RL, Garland D, Oliver CN, *et al.* Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990;186:464-478.
26. Vasdev S, Ford CA, Longerich L, Gadag V, Wadhawan S. Role of aldehydes in fructose induced hypertension. *Mol Cell Biochem* 1998;181:1-9.
27. Rock E, Astier C, Lab C, *et al.* Magnesium deficiency in rats induces a rise in plasma nitric oxide. *Magnes Res* 1995;8:237-242.
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's-Phenol reagent. *J Biol Chem* 1951;193:265-275.
29. Sedlack J, Lindsay RH. Estimation of total protein sulfhydryl groups in tissues with Ellman's reagent. *Anal Biochem* 1968;25:192-205.
30. Thirunavukkarasu V, Anuradha CV. Influence of α -lipoic acid on lipid peroxidation and antioxidant defense system in blood of insulin resistant rats. *Diab Obes Metabol* 2004;6:200-207.

31. Spence JT, Koudelka AP, Tseng-Crank JC. Role of protein synthesis in the carbohydrate-induced changes in the activities of acetyl coA-carboxylase and hydroxymethylglutaryl CoA reductase in cultured rat hepatocytes. *Biochem J* 1985;227:939-947.
32. Lee Y, Hirose H, Zhou YT, Esser V, McGarry JD, Unger RH. Increased lipogenic capacity of the islets of obese rats: a role in pathogenesis of NIDDM. *Diabetes* 1997;46:408-413.
33. Casaschi A, Maiyoh GK, Adeli K, Theriquat AG. Increased diacylglycerol acyltransferase activity is associated with triglyceride accumulation in tissue of diet-induced insulin-resistant hyperlipidemic hamsters. *Metabol Clin Exp* 2005;54:403-409.
34. Unger RH. Lipotoxic diseases. *Annu Rev Med* 2002;53:319-336.
35. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000;103:17-22.
36. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 1998;14:263-283.
37. Fields M, Lavis CG, Lore M, Antholine WS. The influence of gender on developing copper deficiency and on free radical generation of rats fed a fructose diet. *Metabolism* 1992;44:989-994.
38. Suzuki K, Islam KN, Kaneto H, Ookawara T, Taniguchi N. The contribution of fructose and nitric oxide to oxidative stress in hamster islet tumor (HIT) cells through the inactivation of glutathione peroxidase. *Electrophoresis* 2000;21:285-288.
39. Qiu C, Muchant D, Beierwattes WH, Racusen L, Baylis C. Evolution of chronic nitric oxide inhibition hypertension: relationship to renal function. *Hypertension* 1998;31:21-26.
40. Shinozaki K, Kashiwagi A, Nishio Y, *et al.* Abnormal biopterin metabolism is a major cause of impaired endothelium dependent relaxation through nitric oxide/O₂- imbalance in insulin resistant rat aorta. *Diabetes* 1999;48:2437-2445.
41. Rudich A, Tirosh A, Potashnik R, Hemi R, Kanety H, Bashan N. Prolonged oxidative stress impairs insulin induced GLUT 4 translocation in 3T3-L1 adipocytes. *Diabetes* 1998;47:1562-1569.
42. Ferannini E, Buzzigoli G, Bevilacqua S, *et al.* Interaction of carnitine with insulin-stimulated metabolism in humans. *Am J Physiol* 1988;255:946-952.
43. Reznick AZ, Kagan VE, Ramsey R, *et al.* Antiradical effects in L-propionyl carnitine, protection of the heart against ischemia-reperfusion injury: the possible role of iron chelation. *Arch Biochem Biophys* 1992;296:394-401.
44. Conti P, Reale M, Stuard S, *et al.* Reduced human lymphocyte blastogenesis and enhancement of adenosine triphosphate by L-carnitine. *Mol Cell Biochem* 1994;131:1-8.
45. Khairallah EA, Wolf G. Growth-promoting and lipotropic effect of carnitine in rats fed diet limited in protein and methionine. *J Nutr* 1984;87:469-476.
46. Araki E, Murakami T, Shirotani T, *et al.* A cluster of four SP1 binding sites required for efficient expression of the human insulin receptor gene. *J Biol Chem* 1991;6:3944-3948.
47. Dayanandan A, Kumar P, Panneerselvam C. Protective role of L-carnitine on liver and heart lipid peroxidation in atherosclerotic rats. *J Nutr Biochem* 2001;12:254-257.
48. Sushamakumari S, Jaydeep A, Suresh Kumar JS, Venugopal PM. Effect of carnitine on malondialdehyde, taurine and glutathione levels in the heart of rats subjected to myocardial stress by isoproterenol. *Indian J Exp Biol* 1989;27:134-137.
49. Cameron NE, Cotter MA. Neurovascular effects of L-carnitine treatment in diabetic rats. *Eur J Pharmacol* 1997;29:239-244.
50. Tamamogullari N, Silig Y, Icagasioglu S, Atalay A. Carnitine deficiency in diabetes mellitus complications. *J Diabetes Complications* 1999;13:251-253.