PROTECTIVE EFFECT OF L-CARNITINE ON METABOLIC DISORDERS, OXIDATIVE STRESS, ANTIOXIDANT STATUS AND INFLAMMATION IN A RAT MODEL OF INSULIN RESISTANCE

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A B S T R A C T

The high-fructose diet induces insulin resistance, hyperinsulinaemia, hyperglycemia, alterations in lipid metabolism, and oxidative stress in rat tissues which Oxidative stress plays a vital role in pathology associated with insulin resistance. The present study was aimed to explore the effect of L-carnitine (CAR) on insulin resistance, inflammation, oxidative stress, antioxidant status, and lipid metabolism in male rats fed with high fructose diet. Insulin resistance was induced by feeding high fructose diet (60 g/100 g). sixty male albino rats were divided into four groups containing 15 rats each. Group I:(Control group) rats received the control diets. Group II (fructose-fed group) rats received fructose- enriched diet (60 g /100g). Group III: (fructose + CAR group) animals received high fructose diet and were administered CAR (300 mg/Kg body weight /day,orally). Group IV: (control +CAR group) rats received the control diet and were administered CAR. After 45 and 60 days of treatment blood samples and liver tissue were collected for determination of serum glucose, insulin, insulin resistance, pyruvate, lactate, leptin, total cholesterol, triacylglycerols, phospholipids, free fatty acids, sialic acid, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and nitric oxide in addition to L- malondialdehyde (L-MDA). Moreover, antioxidant enzymes (SOD,CAT and GPx) in liver tissues were also determined. The obtained results revealed that, high fructose diet induce a significant increased in serum glucose, insulin, insulin resistance, lipid profiles, pyruvate, lactate, lipten, TNF-α, IL-6, sialic acid, MDA and nitric oxide concentrations and decrease serum phospholipids with marked reduction in CAT, SOD and GpX concentrations in liver tissues compared to rats fed normal diet. L-carnitine treatment to high fructose fed rats reduced the effects of fructose and associated with significant normalization of all serum parameters level and was able to improve dyslipidemia, inflammation and insulin resistance, attenuated the increased MDA and enhanced antioxidant status in liver tissues. These results suggest that, L-carnitine is effective in improving the high fructose induced oxidative stress, inflammation and insulin resistance in male rats. Also, the administration of of L-carnitine to rats fed a high fructose diet prevents the development of oxidative stress and its associated complications include hyperglycemia , hyperinsulinemia and dyslipidemia.

Keywords: L-carnitine; High fructose diet; insulin resistance; oxidative stress; inflammatory markers.

(BVMJ-25 [2]:99 -112, 2013)

1. INTRODUCTION

The "high-fructose diet" experimentally induces insulin resistance accompanied by deleterious metabolic consequences including hyperinsulinemia, hyperglycemia, glucose intolerance, hypertriglyceridemia and hypertension and these metabolic effects are similar to those observed in the human multi metabolic syndrome X and in which a cluster of disorders are described. Hyperglycemia and insulin resistance could also promote inflammation by increased oxidative stress and alteration in lipid metabolism in rat tissues (1).

Fructose metabolism occurs in the liver, which has a great capacity to uptake and phosphorylates it. This nutrient can be

transformed into glucose and glycogen, but this pathway is very "inefficient". So, the liver choice is to produce pyruvate, which is transferred to mitochondria and is transformed in fatty acids. These fatty acids are used as the mainly liver energy source, stored as triglycerides depots or released in the blood stream as VLDL and NEFA. This characteristic makes fructose a highly lipogenic nutrient (2). The influx of triglycerides into hepatocytes leads to an overproduction of reactive oxygen species by beta-oxidation, which causes an anti-oxidant/oxidant imbalance. The elevation of pro-oxidant species causes membrane and DNA damage and the inactivation of some regulatory proteins, which causes tissue inflammation and induces insulin resistance, apoptosis, cellular mutations and other effects (3).

Moreover, insulin resistance in rat feeding high fructose diet is associated with an over abundance and higher ectopic lipid deposition of lipids especially FFA and TG in liver, muscle and adipose tissue which may generate toxic lipid-derived metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides. The presence of these metabolites in the intracellular environment leads to a higher serine/thre onine phosphorylation of insulin receptor substrate-1 (IRS-1), which has been shown to reduce insulin signaling (4).

Carnitine (β-hydroxy-γ-trimethyl amino butyrate), has been described as a conditionally essential nutrient for humans. CAR may acts as antioxidant either having a primary antioxidant activity (inhibiting free radical generation, scavenging the initiating free radicals, and terminating the radical propagation reactions) or more likely, functioning as a secondary antioxidant (repairing oxidized polyunsaturated fatty acids esterified in membrane phospholipids(5). Moreover, CAR transport long-chain fatty acids across the inner mitochondrial membrane into the matrix for β-oxidation and has effects on oxidative metabolism of glucose in tissues(6). Accordingly, this study was performed to investigate the ameliorative effect of L-carnitine on glucose, insulin resistance, lipid metabolism, biomarkers of oxidative stress, antioxidant status, and some inflammatory markers in rats fed high-fructose diet for 60 days.

2. MATERIALS AND METHODS

2.1. Experimental animals:

sixty white male albino rats of 8-10 weeks old and weighting 150- 200 gm were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied ad- libitum.

2.2. Chemicals and drugs used:

All chemicals were of analytical grade and obtained from standard commercial suppliers. The drug and chemicals used in the present study were:

a- Fructose was obtained as bottle contains (D (+)fructose) 250 g in the Crystalline form. It was manufactured by El Nasr Pharmaceutical company and purchased from El-Gomhouria Co. For Trading Chemicals, Medicines And Medical Appliances, Egypt. Rats fed fructose- enriched diet daily (60 g /100g)for 60 days (7*)*.

b- L-carnitine was obtained as a capsule form (one capsule contains 350 g L-carnitine) and manufactured by MEPACO (Arab Co. For Pharmaceuticals & Medicinal Plant). Carnitine was dissolved in Propylene glycol and administered to rats at a dose of (300 mg/Kg body weight /day,orally) for 60 days.

Insulin resistance was induced in rats by feeding high-fructose diet (60 g/100 g of control diet). The diet composition was given in Table 1.

2.3. Experimental design:

After acclimatization, the animals were divided into four groups containing 15 rats

each, placed in individual cages and classified as follows:

Group I (control group): Rats received control diet, served as control non-treated for all experimental groups.

Group II (fructose-fed group): Rats received fructose-enriched diet (60 g fructose /100g) for 60 days.

Group III (fructose +L-carnitine group): Rats received daily fructose enriched diet (60 g fructose /100g of diet) and were administered L-carnitine (300mg/Kg body weight /day, orally) for 60 days.

Group IV (control + L-carnitine group): Rats received the control diet and were administered L-carnitine for 60 days.

Table (A): Composition of control diet (g/100 g) (8)

Ingredient	*Control diet*	*High fructose diet*
Fructose	-----	60
soya bean meal (44%C.P. or 49%C.P.)	24	24
ground yellow corn	36.3	36.3
ground whole wheat	22	22
wheat bran	10	10
soya bean oil	3	3
Ca. carbonate	0.5	0.5
salt NaCl	1	1
dry yeast	1	1
mineral & vit.mixture *	2	2
methionine	0.2	0.2

* The composition of mineral mix (g/kg) contained 30.5 g MgSO4·7H2O, 65.2 g NaCl, 105.7 g KCl, 200.2 g KH2PO4, 3.65 g MgCO3, 38.8 g Mg(OH)2·3H2O, 40.0 g FeC6H5O7·5H2O, 512.4 g CaCO3, 0.8 g KI, 0.9 g NaF, 1.4 g CuSO4·5H2O, 0.4 g MnSO4 andm0.05 g CONH3. One kilogram of vitamin mix contained 3.0 g thiamine mononitrate, 3.0 g riboflavin, 3.5 g pyridoxine HCl, 15 g nicotinamide, 8.0 g d-calcium pantothenat, 1.0 g folic acid, 0.1 g d-biotin, 5 mg cyanocobalamin, 0.6 g vitamin A acetate, 25 g α-tocopherol acetate and 10 g choline chloride.

1- Blood samples:

On day 45, 60 th, rats were fasted overnight, Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental periods to processed serum who used directly for glucose determination and then kept in a deep freeze at-20° C until used for subsequent biochemical analysis.

2- Liver tissue samples:

At the end of the each experimental period, rats were sacrificed by cervical decapitation. The liver specimen was quickly removed and weighted, then perfused with cold saline to exclude the blood cells and then blotted on filter paper; and stored at -20°C. Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 5,000 r.p.m for 15 minutes at 4°C then the supernatant was used for the determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

2.4. Biochemical analysis:

Serum glucose, insulin, insulin resistance, total cholesterol, triacylglycerols (TG), phospholipids, free fatty acids (FFA), pyruvate, lactate, sialic acid, L-Malondialdehyde (L-MDA) ,Leptin, Tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), and Nitric oxide (NO), liver superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined using methods described by (9); (10) ; (11); (12); (13); (14); (15); (16); (17); (18); (19); (20); DRG® Interleukin-6 (rat) (EIA-NO.4845); (21); (22); (23); (24) and (25) .

2.5. Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of $P<0.05$ were considered to be significant.

3. RESULTS

The obtained data in table (1) revealed a significant increase in serum glucose, insulin, insulin resistance index, pyruvate, lactate and leptin concentrations in rats feeding High-fructose diet all over the periods of the experiments compared to rats fed control diet. Administration of L-carnitine in rats fed high fructose diet resulted in a significant decrease in the concentrations of serum glucose, insulin, insulin resistance index, pyruvate and lactate with a non-significant decrease in serum leptin level all over the experimental periods as compared to untreated fructose-fed rats.

The obtained data presented in table (2) revealed a significant increased in the concentrations of serum total cholesterol, triacylglycerols, NEFA, sialic acid, IL-6 and TNF-α with significant decrease in phospholipids concentration in rats feeding High-fructose diet all over the periods of the experiments compared to rats fed control diet. L-carnitine treatment to rats fed high fructose diet resulted in a significant decrease in serum total cholesterol, triacylglycerols, NEFA, sialic acid, IL-6 and TNF- α concentrations with a significant increase in serum phospholipids all over the periods of the experiment as compared to untreated fructose-fed rats.

The obtained results in table (3) revealed that a significant increased in serum L-malondialdehyde and nitric oxide concentrations and associated with significant decrease in liver tissues catalase, superoxide dismutase and glutatione peroxidase concentration in rats feeding High-fructose diet all over the periods of the experiments when compared to rats fed control diet. L-carnitine administration to rats fed a high fructose diet resulted in a significant decrease in serum L-malondialdehyde and nitric oxide levels with significant increase liver superoxide dismutase and glutatione peroxidase activities and resulted in a non-significant increase in liver catalase activity all over the periods of the experiment as compared to untreated fructose-fed rats.

4. DISCUSSION

Consumption of a high-fructose diet promotes development of three pathological characteristics associated with metabolic syndrome, visceral adiposity, dyslipidemia, and insulin resistance (26). Insulin resistance in rat feeding high fructose diet may be associated with an overabundance and higher ectopic lipid deposition especially FFA and TG in liver, muscle and adipose tissue, which may generate toxic lipid-derived metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides. The presence of these metabolites in the intracellular environment leads to a higher serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1), which has been shown to reduce insulin signaling (4). Also, fructose feeding decreases the efficacy of insulin extraction by the liver which retards insulin clearance from the circulation (27). L-carnitine (L-CA) treatment to the high fructose–fed rats resulted in reduction of serum glucose, insulin and insulin resistance levels due to mitigated lipid abnormalities in fructose-fed rats which one of causes of insulin resistance by removing toxic lipid-derived metabolites which reduce insulin signaling, also L-CA increases the utilization of glucose by activating pyruvate dehydrogenase

Table (1): Effect of L-carnitine administration on some serum biochemical parameters in high fructose fed rats and their control.

Parameters	Glucose mg/dl		Insulin (µU/mL)		IR		pyruvate (mmol/dl)		lactate (mmol/dl)		Leptin (pg/ml)	
Animal groups	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days
Control group	90.00 ± 1.81 ^{c}	89.20 ± 2.42 ^{b}	25.39 ±0.80 ^{b}	25.46 ± 0.49 ^{c}	5.65 ± 0.25 ^{b}	5.62 ± 0.23 ^{b}	78.68 ± 4.40 ^{b}	69.09 ± 2.25 ^{c}	4.27 ± 0.03 ^{a}	4.20 ± 0.09 ^{b}	134.46 ± 3.81 ^{c}	133.98 ± 14.12 ^{b}
Fructose group	113.80 ± 4.57 ^{a}	117.80 ± 7.36 ^{a}	33.11 ± 1.42 ^{a}	31.91 ± 2.12 ^{a}	9.37 ± 0.66 ^{a}	9.41 ± 1.13 ^{a}	94.86 ± 1.34 ^{a}	94.99 ± 6.20 ^{a}	4.57 ± 0.05 ^{a}	4.64 ± 0.06 ^{a}	181.24 ± 6.40 ^{a}	178.34 ± 5.87 ^{a}
Fructose + L-CAR group	95.30 ± 3.46 ^{bc}	95.40 ± 2.80 ^{b}	30.84 ± 1.90 ^{a}	26.27 ± 1.61 ^{bc}	7.29 ± 0.58 ^{ab}	6.23 ± 0.54 ^{b}	76.28 ± 3.01 ^{b}	78.80 ± 2.31 ^{bc}	3.62± 0.37 ^{b}	4.31 ± 0.04 ^{b}	161.94 ± 6.02 ^{ab}	169.75 ± 2.80 ^{a}
Control+ L-CAR group	110.20 ± 8.87 ^{ab}	98.20 ± 1.24 ^{b}	29.94 ± 2.03 ^{ab}	30.82 ±1.75 ^{ab}	8.31 ± 1.15 ^{a}	7.47 ± 0.41 ^{ab}	83.92± 5.88 ^{ab}	85.61± 4.49 ^{ab}	4.45± 0.05 ^{a}	4.60 ± 0.03 ^{a}	143.41± 12.19 ^{bc}	168.84 ± 6.73 ^{a}

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P<0.05).

Table (2): Effect of L-carnitine administration on serum lipid profile and inflammatory markers in high fructose fed rats and their control.

Parameters	T.Cholestrol (mg/dl)		Triacylglycerols (mg/dl)		phospholipids (mg/dl)		FFA (MmEq/L)		Sialic acid (mg/dl)		IL-6 (pg/ml		TNF- α (pg/ml)	
Animal groups	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days
Control group	108.60 ± 2.18 b	108.40 ±1.21 bc	110.00 ±4.02 b	108. ±2.35 b	102.77 ±5.36 a b	123.02 ± 6.42 ab	264.04 ±14.14 c	264.69 ± 4.81 c	26.56 ± 1.27 b	26.89 ± 1.57 b	62.53 ± 4.40 c	78.58 ± 3.07 ab	54.83 ± 3.60 b	46.38 ± 1.67 c
Fructose group	124.20 ± 3.44 a	123.40 ±1.08 a	137.80 ±6.37 a	160.0 ±7.36 a	91.57 ± 6.01 b	91.91 ± 3.51 c	336.20 ±10.37 a	337.30 ± 11.60 a	39.75 ± 1.17 a	35.68 ± 1.78 a	105.22 ± 3.58 a	97.53 ± 7.84 a	73.72 ±3.62 a	65.56 ± 1.30 a
Fructose + L-CAR group	111.60 ± 2.01 b	105.00 ± 1.70 c	108.00 ±2.0 bc	111.0 ±2.83 b	111.24 ± 1.73 a	127.95 ± 2.10 a	284.28 ±12.85 bc	301.03 ± 5.99 b	28.19 ± 2.26 b	28.95 ± 1.27 b	81.73 ± 5.16 b	66.37 ± 9.89 b	65.19 ±2.92 ab	57.34 ± 0.71 b
Control+ L-CAR group	111.00 ± 2.83 b	111.60 ±1.83 b	95.90 ±3.43 c	109.0 ±2.21 b	105.16 ± 8.27 ab	110.03 ± 4.66 b	318.00 ±7.49 ab	319.60 ±10.08 a b	32.12 ± 2.13 b	38.32 ± 1.70 a	75.43 ± 5.34 bc	72.19 ± 2.26 b	57.95 ± 7.13 b	69.04 ± 2.18 a

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P<0.05).

Table 3: Effect of L-carnitine administration on liver CAT, SOD and GPx activities, serum MDA and NO levels in high fructose fed rats and their control.

Parameters	CAT (mmol/ g. tissue)		SOD (U/ g. tissue)		GPX (nmol/ g. tissue)		MDA (nmol/mL)		NO (μmol/l)	
Animal groups	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days	45 Days	60 Days
Control group	55.54 ± 1.94 ^{a}	57.76 ± 2.47 ^{a}	54.77 ± 0.98 ^{b}	58.21 ± 1.50 ^{a}	29.13 ± 0.83 ^{a}	25.67 ± 0.93 ^{a}	64.76 ± 4.57 ^{c}	87.20 ± 3.48 ^{b}	8.25 ± 0.49 ^{b}	9.33 ± 0.31 ^{bc}
Fructose group	39.30 ± 2.31 ^{b}	37.48 ± 3.00 ^{c}	49.60 ± 0.92 ^{c}	50.40 ± 0.39 ^{c}	25.81 ± 0.51 ^{b}	16.77 ± 1.10 ^{b}	95.30 ± 3.05 ^{a}	113.79 ± 1.36 ^{a}	12.52 ± 0.98 ^{a}	13.79 ± 0.98 ^{a}
Fructose + L-CAR group	42.54 ± 2.55 ^{b}	44.25 ± 3.20 ^{bc}	59.97 ± 1.38 ^{a}	56.49 ± 0.81 ^{ab}	23.74 ± 0.83 ^{b}	23.14 ± 0.32 ^{a}	79.53 ± 2.98 ^{b}	105.66 ± 1.05 ^{a}	8.84 ± 0.41 ^{b}	8.02 ± 0.69 ^{c}
Control+ L-CAR group	47.43 ± 3.51 ^{b}	47.91 ± 4.20 ^{b}	49.79 ± 1.18 ^{c}	53.88 ± 0.52 ^{b}	17.31 ± 0.76 ^{c}	23.72 ± 1.18 ^{a}	72.67 ± 6.59 ^{bc}	82.089 ± 4.23 ^{b}	9.43 ± 0.94 ^{b}	10.45 ± 0.62 ^{b}

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P<0.05).

and by decreasing the intra-mitochondrial acetyl CoA/CoA ratio, so promotes oxidative glucose utilization, lowers intracellular glucose levels, and improves insulin sensitivity (1).

Serum lactate and pyruvate concentrations were significantly increased in fructose fed rats due to fructose metabolism bypasses the regulatory step catalyzed by Phospho-fructokinase (PFK). Thus fructose continuously enters the glycolytic pathway at the level of glyceraldehydes and dihydroxyacetone phosphate and produces glucose, lactate and pyruvate continuously in an unregulated manner. The elevated circulating levels of gluconeogenic substrates pyruvate, lactate and glycerol in the fructose-fed animals confirm impaired glycolysis. Increased gluconeogenic substrate availability could be suggested to be the major cause of the fasting hyperglycemia in fructose-fed rats (28). L-carnitine showed a significant decrease in serum lactate and pyruvate levels because of suppression of gluconeogenic enzyme activity and return gluconeogenesis in fructose-fed rats to normal as seen in the control rats (28).Also, L -Carnitine can remove of short and medium-chain fatty acids formed as a consequence of normal metabolism, preventing a toxic accumulation of these compounds in the mitochondria and leading to an increase of free CoA. An increase in free CoA results in activation of the pyruvate dehydrogenase complex and subsequently improves coupling between glycolysis and glucose oxidation (29) .

A significant increase in serum leptin concentration was observed in fructose fed rats due to high consumption of fructose causes lipid abnormalities and high triacylglycerols promote leptin resistance by preventing leptin from crossing the blood brain barrier, because the main target of leptin action is the leptin receptor that is located in the hypothalamus. In order for circulating leptin to reach its receptor in the hypothalamus, it must first cross the blood-brain barrier (30). Treatment with L-carnitine to high fructose–fed rats exhibited a non significant decrease in serum leptin level as compared to fructose-fed rats. Leptin secretion was independent of the L carnitine administration and was not exclusively regulated by the adiposity degree. Because of exogenous L-carnitine mitigated lipid abnormalities in fructose-fed rats, therefore lowering triacylglycerols which allow circulating leptin to reach its receptor in the hypothalamus, and cross the blood-brain barrier (1) .

Regarding serum lipid profile, the unregulated fructose metabolism generates both glycerol and acyl portions of acyl-glycerol molecules, the substrates for triglycerides(TG) synthesis. Increase in acyl CoA carboxylase and sterol regulatory element binding protein, binds to sterol responsive elements found on multiple genes, and activates a cascade of enzymes involved in lipid biosynthesis pathway such as HMG-CoA reductase and fatty acid synthase. The activity of this protein in liver is reported to be enhanced in insulin resistant fructose fed mice (31), and this explains the increased levels of cholesterol and fatty acids during fructose feeding. FFA could directly increase reactive oxygen species via peroxidation reactions and via mitochondrial production (32). The major targets of damaging free radicals are the cellular and membrane phospholipids. The oxidative tissue damage can release the membrane lipids such as free fatty acids (FFA) and phospholipids into blood. Also, fructose feeding can lead to a decrease in the ability of insulin to stimulate the activity of lipoprotein lipase (LPL). This reduction in the activity of LPL; can be ascribed to the insulin resistance induced by fructose. Besides, it could be possible that the activity of hepatic lipase, that hydrolyses triglycerides and phospholipids from lipoprotein, is blocked in these rats (33). L-carnitine treatment to fructose–fed rats showed a significant reduction in lipid profile due to the role of L-carnitine in 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 L-carnitine on lipid metabolism may also be related to its effect on glucose utilization and improvement of insulin action. L-carnitine increases the utilization of glucose by activating pyruvate dehydrogenase and by decreasing the intra-mitochondrial acetyl CoA/CoA ratio (34). L-carnitine increases the synthesis of phospholipids required for membrane formation and integrity and plays a role in the membrane repair by reacylation of phospholipids (35).
Regarding the pro-inflammatory cytokines, high fructose diet consumption may lead to Steatosis or fat overload in the hepatocytes which results in activation of stress/inflammatory pathways that trigger the resident macrophages in the liver (Kupffer cells) for inflammatory reactions (36). the rise in TNF- α and IL-6 in fructose-fed rats could be related to NF-KB activation which in turn is attributed to the rise in reactive oxygen species (ROS) levels and oxidative stress. NF-KB activation increased the expression of a large number of pro-inflammatory cytokines and stimulation of inflammatory cascade have been observed in fructose-fed rats (37). Treatment with L-carnitine to fructose–fed rats resulted in significant reduction in serum TNF- α and IL-6 levels. The real mechanism underlying this is not clear, but we can assume that L-carnitine can interfere with processes involved in β -oxidation and accumulation of lipotoxic metabolites that might contribute to mitochondrial dysfunction and insulin resistance. L-carnitine could act through mechanisms that are independent of the putative detoxifying role and use it as antioxidants to protect cellular structures against damage from oxygen free radicals and from reactive products of lipid peroxidation. (38).
A significant increase in serum sialic acid concentration was observed in fructose fed rats which sialic acid is a component of cell membranes and elevated levels may indicate excessive cell membrane damage but more specifically to the cells of vascular tissue. Also, sialic acid can be used as a measurement of acute phase response because many of the proteins of the immune response are actually glycoproteins which have sialic acid as the terminal sugar of their oligosaccharide chain (39). Treatment with L-carnitine to high fructose–fed rats resulted in significant decrease in serum sialic acid level. These results might be attributed to the Protection of cellular membranes through L-carnitine that it may exert a direct effect on the membrane. It may prevent cell damage by stabilizing the membrane against free radical-induced injury, and also may prevent mitochondrial injury, thus increasing energy production and decreasing the leakage of free radicals (40).In addition to increases the synthesis of phospholipids required for membrane formation and integrity and plays a role in the membrane repair by reacylation of phospholipids (35).
The obtained results revealed that, high fructose fed rats resulted in significant increase in serum malondialdehyde (MDA) concentration compared with control group. Similarly, Polyunsaturated fatty acids in membrane phospholipids are the major targets for free radicals (formed on account of increased blood pressure and/or glycaemia) which are capable of inducing a chain reaction of lipid peroxidation. These reactions in lipid membranes give rise to the formation of end products which are used to detect free radical damage. One of the important end products measured as an indicator of lipid peroxidation is known to be MDA (41). Administration of L-carnitine to fructose–fed rats resulted in significant decrease in serum malondialdehyde concentration due to L-carnitine increases the synthesis of phospholipids required for membrane formation and integrity and plays a role in the membrane repair by reacylation of phospholipids (35). It has been suggested that, L-carnitine may exert a direct effect on the membrane. It may prevent cell damage by stabilizing the membrane against free

radical-induced injury, and also may prevent mitochondrial injury, thus increasing energy production and decreasing the leakage of free radicals (40).

Feeding high fructose diet to rats induced elevation in the expression of inducible nitric Oxide Synthase (iNOS) is assumed to be one of the candidates that mediate inflammation involved insulin resistance. This report elucidate the elevation of nitric oxide which result of increasing the iNOS expression associated with insulin resistance which induced by feeding high fructose diet to rats. Inducible nitric oxide synthase has been implicated in many human diseases associated with inflammation. iNOS deficiency was shown to prevent insulin resistance. The role of iNOS in hyperglycemia and hepatic insulin resistance remains to be investigated (42). Administration of L-carnitine to rats fed a high fructose diet resulted in significant decrease in serum nitric oxide level. These results could be attributed to that, L-Carnitine reduced the activity of angiotensin-converting enzyme which might result in lower Ang II and NADPH oxidase dependent ROS production, thus improving NO bioavailability owing to a lower depletion by ROS. Also, L-Carnitine increased the activity of antioxidant which SOD scavenges superoxide ions and improves NO activity(43). So, L-Carnitine is antioxidant that interferes with the chain reaction of lipid peroxidation and stabilizes the cell membrane. Its use has proven to be beneficial to reduce oxidative stress that has been shown to damage endothelial relaxation and reduce the level of NO (44).

Regarding, antioxidant enzymes the obtained results showed significant decrease in catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in liver tissues of rats fed on a high fructose diet (HFD). feeding of fructose rich diet presented bigger triacylglycerols content. High concentrations of liver triglycerides lead to an increase of the activity of fatty-acyl-coA oxidase activity and also stimulate the liver to produce energy through beta-oxidation. NEFA can induce insulin secretion by pancreatic islets, leading to a hyperinsulinemic state, as presented by the fructose fed group. High insulin levels can downregulate the Malonil-CoA activity, reducing the mitochondrial NEFA transport, leading to an excessive NEFA oxidation at peroxisomes and endoplasmic reticulum (45). Fatty oxidation in these cytoplasmic organelles areas releases high amounts of reactive oxygen species, which can damage mitochondrial membranes (rich in polyunsaturated fat), producing lipid peroxidation metabolites. These metabolites are extremely toxic to the cells and are released into the blood stream, as observed in the fructose group (46). The chronic imbalance of reactive oxygen species production can impair the ability of the antioxidant system to reduce the levels of these radicals, which attenuates its protective function (47). Reactive oxygen species (ROS) can themselves reduce the activity of antioxidant enzymes such as CAT, SOD and GPx (48). Administration of L-carnitine to rats fed a high fructose diet resulted in significant significant increase in liver catalase, glutathione peroxidase and superoxide dismutase concentrations compared to fructose-fed rats. Carnitine plays a role in chelating free Fe^{2+} ions and hence could reduce free radical generation. Furthermore, Carnitine by virtue of its ability to enhance ATP production could reduce susceptibility to oxidative damage. Scavenging of ROS is determined by antioxidant enzymes such as SOD and CAT. The enhancement of β-oxidation induced by L-carnitine would generate ATP, thereby reversing H_2O_2 initiated depletion of ATP in cells and attenuating cell injury. ATP was considered to be a critical event in lethal cell injury produced by oxygen radicals (49).

Conclusion:

This study demonstrates that, the administration of L-carnitine to rats fed a high fructose diet enhanced the levels of

antioxidants status and prevents the development of oxidative stress and its associated complications include hyperglycemia and hyperinsulinemia. Also, the results indicate that, L-carnitine is effective in improving the dyslipidemia, inflammation and insulin resistance induced in high fructose-fed rats and may have implications in the treatment of insulin resistance and its metabolic complications.

5. REFERENCES

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مجلة بنها للعلوم الطبية البيطرية

عدد 25 (2): 99-112 ديسمبر 2013

التأثير الوقائي لإل – كارنيتين على اضطرابات الأيض، الإجهاد التأكسدي، وحالة مضادات الأكسدة والالتهاب في نموذج مقاومة الأنسولين في الفئران

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الملخص العربي

في هذه الدراسة تم تقييم التأثير الواقي لإل – كارنيتين على التغيرات في مستوى سكر ودهون الدم ، الأكسدة الفوقية للدهون ، دلائل للالتهاب والإنزيمات المضادة للأكسدة في أنسجة الفئران المغذاة على عليقه عالية الفركتوز . هذا وقد أستخدم لأجراء هذه الدراسة عدد 60 من ذكور الفئران البيضاء تتراوح أعمارهم من 8-10 أسبوع وأوزانها من 150-200 جرام وقد قسمت إلي أربعة مجموعات متساوية اشتملت كل مجموعة على عدد خمسة عشر فأر وتم توزيعها كالآتي: المجموعة الأولى: (المجموعة الضابطة): لم تعطى أي أدوية وتتغذى على العليقة الكنترول فقط واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية: (مجموعة إل- كارنيتين الطبيعية): تم تغذيتهم على العليقة الكنترول فقط عن طريق الفم ب لإل- كارنيتين بجرعة مقدارها 300 ميللي جرام لكل كيلوجرام من وزن الجسم يوميا لمدة 60 يوم . المجموعة الثالثة:(مجموعة المغذاة على عليقه عالية الفركتوز): تم تغذيتهم على عليقة عالية الفركتوز يومياً بجرعة مقدراها 60 جرام لكل 100جرام من العليقة الكنترول طوال فترة التجربة وهى 60 يوم. المجموعة الرابعة:(المجموعة المغذاة على العليقة عالية الفركتوز والمعالجة ب إل - كارنيتين): تم تغذيتهم على عليقه عالية الفركتوز يومياً بجرعة مقدراها 60 جرام لكل 100جرام من العليقة الكنترول مع تجريعهم ب إل-كارنيتين يومياً عن طريق الفم بجرعة مقدراها 300 ملليجرام لكل كيلوجرام من وزن الجسم طوال فترة التجربة وهى 60 يوم. وقد تم تجميع عينات الدم على فترات بعد 45 و60 يوم من بدء التجربة وقد أوضحت الدراسة ما يلى: وجود زيادة فى كلا من سكر الدم، أنسولين ، مقاومة الأنسولين ،البيروفيت ،حامض اللاكتيك ،الليبتن، الكولسترول الكلى ،الدهون الثلاثية ،الدهون الحرة ،انترلوكين 6 وعامل النخرالورمى- ألفا بالإضافة إلى الأكسدة الفوقية للدهون ، وحامض السياليك وأكسيد النيتريك بينما حدث انخفاض معنوي في الدهون الفوسفاتية وأيضا في نسيج الكبد حدث انخفاض معنوي فى مستوى نشاط إنزيم سوبر أكسيد ديسميوتيز ،الكتاليز والجلوتاثايون بروكسيديز. خلصت الدراسة أن إل –كارنيتين يعمل على خفض مستوي السكر والدهون في الدم و يزيد من حساسية الأنسولين، يقلل من الإجهاد ألتأكسدي ومقاومة الليبتين الناجمين عن الاستهلاك المفرط للفركتوز . كما يتمتع إل -كار نيتين بخصائص مضادات الأكسدة حيث لديه القدرة للحد من دلائل الالتهاب وأيضا الحد من زيادة الأكسدة الفوقية للدهون و وإعادة نشاط بعض الإنزيمات المضادة للأكسدة لطبيعتها ثانية .لذلك ينصح بتناول إل -كارنيتين للمرضى الذين يعانون من ارتفاع الدهون و السكر في الدم، الأكسدة الفوقية للدهون والإجهاد التأكسدي في حدود الجرعة الطبية الموصى بها وذلك للوقاية من الأمراض والتغيرات المصاحبة لمضاعفات استمرار زيادتها .

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):99-112, ديسمبر 2013)