

## The use of L-carnitine in diabetes: a novel strategy for combating its complications

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**Abstract:** Diabetes mellitus is a severe, chronic form of diabetes caused by insufficient production of insulin and resulting in abnormal metabolism of carbohydrates, fats, and proteins. The present study was designed to investigate the effect of diabetes mellitus and L-carnitine on albino rats, to achieve this target, forty adult albino male rats were classified into 4 equal group. The 1<sup>st</sup> group serve as control, while 2<sup>nd</sup> group received Streptozotocin for induction diabetes. The 3<sup>rd</sup> group was treated with L-carnitine and the 4<sup>th</sup> group administrated with Streptozotocin and L-carnitine and regard as diabetic - L-carnitine treated group. Blood samples were collected to determine blood glucose, IL2, IL6, TNF- $\alpha$ , Troponin T, CRP, VEGF and LT index in. Diabetic group showed significant elevations of blood glucose level, IL2, IL6, TNF- $\alpha$ , Troponin T and CRP. On the other hand, VEGF and LT index display significant reductions in compare with control group. L-carnitine treatment of diabetic group induced significant decreases of blood glucose level, IL2, IL6, TNF- $\alpha$ , Troponin T and CRP in compare with diabetic group. Conversely, VEGF and LT index recoded remarkable elevations in compare with diabetic group. The counter effect of L-carnitine may be attributed to its antioxidant mechanism. The current investigation presents L-carnitine as a solution to overcome the deleterious effects of diabetes mellitus in albino rats.

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**Keywords:** L-carnitine; diabetes; combating; complication

### 1. Introduction:

Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Over time, diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage are the result of damage to small vessels, referred to as microvascular disease. Diabetes is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel diseases. Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Streptozotocin (STZ; *N*-nitro derivative of glucosamine) is a naturally occurring, broad spectrum antibiotic and cyto-toxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals. Streptozotocin injection leads to the degeneration of the Langerhans islets beta cells (Abu Abeeleh, 2009).

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine (Steiber et al., 2004). In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy. It is often used as a nutritional supplement. Carnitine was originally found as a growth factor for mealworms and labeled *vitamin B*. Carnitine exists in two stereoisomers: Its biologically active form is L-carnitine, whereas its enantiomer, *D*-carnitine, is biologically inactive. In animals, carnitine is biosynthesized primarily in the liver and kidneys from the amino acids lysine (via trimethyllysine) or methionine. Vitamin C (ascorbic acid) is essential to the synthesis of carnitine. During growth or pregnancy, the requirement of carnitine might exceed its natural production (Liedtke et al., 1982). The carnitines exert a substantial antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress induced at the myocardial and endothelial cell level (Cederblad et al., 2008).

The present study is conducted to cast the light on the different biochemical immunological alterations due to Diabetes mellitus. In addition, using Carnitine to reduce the previous deleterious effects of diabetes mellitus.

## 2. Material and Methods

### Experimental design:

Forty adult, male albino rats (average body weight  $275 \pm 25$ g). Animals were housed in cages at controlled temperature ( $22^{\circ}$  C) with a 12:12-h light: dark cycle and had free access to water and chow diet over a 2 weeks adaptation period.

Rats were fasted for 12-h before diabetes was induced using STZ. Twenty rats received a single intraperitoneal injection of 150mg/kg of STZ (Sigma, St. Louis, MO, USA). STZ was freshly dissolved in 0.05 M citrate buffer, pH 4.5. For the i.p. injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using povidone-iodine solution and the designated amount of STZ was injected in the caudal abdominal cavity using sterile needle. Severity of the induced diabetic state was assessed by daily monitoring of blood glucose levels. For the determination of blood glucose using Glucocheck (Biotest Medical Corp., Tortola, VI, USA), whole blood was from the tail vein from all rats immediately before STZ injection and daily until euthanized. STZ was dissolved in citrate buffer (pH 4.5) and injected intraperitoneally within 10min after preparation. Animals whose blood glucose level exceeded 200 mg/dl at 24 h after treatment were considered diabetic (Abu Abeeleh, 2009).

STZ received group subdivided into two equal groups, one serve as a diabetic group and other diabetic group administered L-carnitine (CAR) (Sisco Research Laboratories (P) Ltd., Mumbai, India.) with a dose 300 mg/Kg/day, intraperitoneally for one week.

Ten rats were administered L-carnitine (CAR) only. Control, ten, rats were injected citrate buffer.

Blood samples were collected 3 weeks post treatments. Serum was separated using a centrifuge (ALC Centrifuge 4206, Milano, Italy) at 1500 g for 5 minutes. Serum was then placed in labeled plastic tubes and stored at  $-20^{\circ}$ C until testing (Mingrone, 2004).

### Measurement of IL-6, TNF- $\alpha$ and IL-2 levels by enzyme linked immunosorbent assay (ELISA) :

Serum IL-6, TNF- $\alpha$  and IL-2 levels were measured by using a polyclonal ELISA kits (RapidBio Lab., Calabasas, California, USA) following the manufacturer's instructions. Briefly, the anti-IL-6 capture polyclonal antibody was absorbed on a polystyrene 96-well plate and the IL-6 present in the sample was bound to the antibody coated wells. The biotinylated anti-IL-6 detecting pAb was added to bind the IL-6 captured by the first antibody. After washing, avidin-peroxidase (Sigma, USA) was added to the wells to detect the

biotinylated detecting antibody and finally 2,2'-azino-bis(3-thylbenthiiazoline-6-sulfonic acid) (ABTS; Sigma, USA) substrate was added and a colored product was formed in proportion to the amount of IL-6 present in the sample, which was measured at optical density 405 nm ( $OD_{405}$ ) with an ELISA microplate reader (model 450, Bio-Rad, Chicago, Illinois, USA). A standard curve was generated, and the IL-6 concentration (pg/ml) of the samples was calculated. The measurement of TNF- $\alpha$  and IL-2 are similar to that of IL-6. All determinations were performed by full-time technical personnel.

### Lymphocyte Transformation assay (blastogenesis)

Estimation of lymphocyte transformation index was carried out to evaluate the cellular immune response, using concanavaline -A (Con-A) cell mitogen. Isolated Lymphocytes were setting into a 96 well tissue culture plate. The 1<sup>st</sup> row of wells was left free as blank (culture medium only), while each of the other wells received 100 $\mu$ l of treated lymphocyte culture suspension ( $10^6$  lymphocytes/ml) with 50 $\mu$ l of Con -A. The culture plate was incubated for 48 hours at  $37^{\circ}$  C in humid incubator (5 %  $CO_2$ ). After 48 hours, MTT dye [3-(4, 5-dimethylthiazol-2)-2,5-diphenyl tetrazolium bromide] was added in a ratio of 1:10 of the total sample then incubated at  $37^{\circ}$ C for 4 hours. After incubation 50 $\mu$ l of lysing buffer (2.5ml sulfuric acid and 25g sodium dodecyl sulfate /250 ml Distilled water) were added and incubated overnight. Optical density was recorded at 470 nm using ELISA reader (DynaLink-USA) to estimate lymphocyte transformation index (LT). The difference of optical density readings between the treated and control samples were subjected to statistical analysis [Brulles and Wells (1977) & Rai-el-Balhaa et al., 1987].

### Serum Troponin T; C-reactive protein and VEGF Measurements:

Troponin T levels were determined by means of a third-generation troponin T assay (Elecsys, Roche Diagnostics) that uses recombinant cardiac troponin T as standard material (Hallermayer et al., 1999).

C-reactive protein (CRP) levels were determined using ELISA commercial kit (*Quantikine M murine; R&D Systems, Minneapolis, MN*). Serum levels of C-reactive protein (CRP) were quantified by a highly sensitive ELISA kit (Life Diagnostics, West Chester, UK). Serum levels of insulin were quantified by an ultrasensitive ELISA kit (Mercodia, Uppsala, Sweden). The assays were performed in triplicate.

The serum samples were processed in a single batch using a commercially available sandwich VEGF ELISA detection kit (R&D Systems,

Minneapolis, MN) according to manufacturer instructions. The sensitivity of the assay was 9 pg/ml and the intraassay variability was 10%.

### 3. Results:

Table(1) demonstrates the effect of diabetes mellitus and L-carnitine treatment on plasma glucose, IL2, IL6, TNF- $\alpha$ , Troponin T, CRP, VEGF and LT index in albino rats.

Diabetic group revealed significant elevations of blood glucose level, IL2, IL6, TNF- $\alpha$ , Troponin T and CRP. On the other hand, VEGF and

LT index displayed significant reductions compared with control group. L-carnitine treatment evoked insignificant alterations of all tested parameters except LT index that recorded a significant increase.

L-carnitine treatment of diabetic group induced significant decreases of blood glucose level, IL2, IL6, TNF- $\alpha$ , Troponin T and CRP compared with diabetic group. Conversely, VEGF and LT index recorded remarkable elevations in comparison with diabetic group.

**Table(1): Effect of diabetes mellitus and L-carnitine treatment on glucose, IL2, IL6, TNF- $\alpha$ , Troponin T, CRP, VEGF and LT index in albino rats.**

Parameters	Groups			
	Control	Diabetic	L-carnitine	Diabetic + L-carnitine
Glucose( mg/dl)	135.28 $\pm$ 7.57	371.22 $\pm$ 21.37*	121.89 $\pm$ 9.88*	284.25 $\pm$ 15.31*
IL 2 (Pg/ml)	126.27 $\pm$ 10.59	241.39 $\pm$ 19.52*	133.95 $\pm$ 15.34	166.52 $\pm$ 11.31*
IL6 (Pg/ml)	166.31 $\pm$ 10.22	200.68 $\pm$ 13.64*	151.37 $\pm$ 12.51	192.39 $\pm$ 8.54*
TNF- $\alpha$ (Pg/ml)	230.52 $\pm$ 20.55	308.82 $\pm$ 25.41*	205.62 $\pm$ 12.36	271.64 $\pm$ 10.22*
Troponin T( Pg/ml)	24.05 $\pm$ 2.49	46.27 $\pm$ 2.54*	22.58 $\pm$ 2.08	30.39 $\pm$ 2.37*
CRP (ng/ml)	3.75 $\pm$ 0.39	6.89 $\pm$ 1.69*	3.52 $\pm$ 0.85	4.95 $\pm$ 0.38*
VEGF ( Pg/ml)	164.21 $\pm$ 12.35	125.91 $\pm$ 5.93*	155.69 $\pm$ 8.26	142.34 $\pm$ 5.34*
(LT) index	1.87 $\pm$ 0.21	1.22 $\pm$ 0.31*	2.24 $\pm$ 0.25*	1.75 $\pm$ 0.26

\*: Significant at P< 0.05

### 4. Discussion:

In the present study, we demonstrated that diabetes induced significant elevations of blood glucose level, IL2, IL6, TNF- $\alpha$ , Troponin T and CRP. On the other hand, VEGF and LT index display significant reductions.

These findings are on the same line of that reported by Cherney et al. (2011). They recorded that diabetes increased IL-2 production from NKT cells. Moreover, hyperglycemia was associated with significant increases in urinary eotaxin, fibroblast growth factor-2, granulocyte-macrophage colony-stimulating factor, interferon- $\alpha$ , interleukin-2 (Buschard et al., 2011).

On the similar ground, Navarro-Gonzalez et al. (2010) reported that TNF-alpha and IL-6 have stronger predictive value in diabetic atherosclerosis development. These may be attributed to hyperglycemia activating inflammatory cells and relieves of inflammatory factors. Hyperglycemia can trigger inflammation, which is a quality-effect relationship reaction (Wu et al., 2010). Moreover, Serum concentrations levels of TNF-alpha and IL-6 are significantly elevated in hypertensive type 2 diabetic patients.

Vascular endothelial growth factor (VEGF)-mediated stimulation of endothelial cell proliferation and migration are key events in angiogenesis. Manipulation of VEGF signaling is seen as a

promising therapeutic target for a number of disorders in which angiogenesis is inappropriate, yet the molecular mechanisms of action of VEGF in the endothelium are incompletely understood (Fischer et al., 2008). VEGF-A protein content is also reduced in diabetic muscles. In line with the reduced levels of VEGF-A and other angiogenic factors, and increased levels of angiogenesis inhibitors, capillary-to-muscle fiber ratio is lower in diabetic mice compared to healthy controls (Riikka et al., 2006). This may be due to diabetes Impaired VEGF-induced proliferation response in EPC as well as an increase in negative myocardial protein expression (Mieno et al., 2010).

Our results indicate that diabetes induced a significant elevation of Troponin. Troponin is a complex of three regulatory proteins that is integral to muscle contraction in skeletal and cardiac muscle, but not smooth muscle. Discussions of troponin often pertain to its functional characteristics and/or to its usefulness as a diagnostic marker for various heart disorders. results suggest that diabetes significantly enhances endotoxin-induced cardiac toxicity, possibly through mechanisms that involve inflammatory/acute-phase cytokines (Song et al., 2003). Moreover, Diabetes mellitus trigger ongoing myocardial damage detected as elevated serum cardiac troponin T *indicates increased risk for future cardiac events in patients with chronic heart failure* (Setsuta et al., 2011).

C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation. CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. Elevated C-reactive protein (CRP) is known to be a marker of inflammation. Increased CRP levels have been described in people with type 2 diabetes, gestational diabetes, and the metabolic syndrome. Obesity has also been associated with elevated CRP and is associated with type 2 diabetes and the metabolic syndrome. Elevated CRP levels have also been described in adults with type 1 diabetes. CRP is not usually present in detectable levels in young children (Pieroni et al., 2003 and Wolf et al., 2003).

The present study elucidates the fact that diabetes reduce LT. This finding supported by that of Rosemari et al.(2002)lymphocyte proliferation from diabetic rats to Con A and LPS stimuli is decreased. The previous findings may be attributed to the role of insulin, which was able to promote a significant proliferative effect on these cells. Also, high glycemia in addition to the lack of insulin participates in the reduced proliferation capacity of lymphocytes from diabetic rats (Nichols et al, 2007).

Our recorded data put on view the that L-carnitine treatment of Diabetic group induced a significant decrease of blood glucose level , IL2, IL6, TNF- $\alpha$ , Troponin T and CRP in compare with diabetic group. Whereas, VEGF and LT index recoded remarkable elevations compared with diabetic group.

These findings may be due to carnitine covers an important role in lipid metabolism, acting as an obligatory cofactor for beta-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial membrane as acylcarnitine esters. Furthermore, since carnitine behaves as a shuttle for acetyl groups from inside to outside the mitochondrial membrane, it covers also a key role in glucose metabolism and assists in fuel-sensing. A reduction of the fatty acid transport inside the mitochondria results in the cytosolic accumulation of triglycerides, which is implicated in the pathogenesis of insulin resistance. Acute hypercarnitinemia stimulates nonoxidative glucose disposal during euglycemic hyperinsulinemic clamp in healthy volunteers. L-carnitine reduces oxidized LDL cholesterol reduce the fasting blood glucose levels in patients with type 2 diabetes (Mingrone, 2004 & Malaguarnera et al., 2009).

Our obtained results are agreed with that recoded by Dionyssopoulou et al. (2005). They indicated that L-carnitine treatment induced a significant increase of macrophages and T-cells,

while non remarkable elevated levels of il2, Il6 and TNF- $\alpha$ .

Also, our data were supported by findings of Cavazza (2002) and Judith et al. (2010). They reported that carnitines exert a substantial antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress induced at the myocardial and endothelial cell level. This may be refer to that carnitine plays an important role in the metabolism of both carbohydrates and lipids, leading to an increase of ATP generation. carnitine supplementation studies in rodents provide "proof-of-concept" that carnitine is effective at improving insulin-stimulated glucose utilization and in reversing abnormalities of fuel metabolism associated with type 2 diabetes (Mynatt, 2009).

The current investigation indicated that LCAR augment LT. This finding agrees that recorded by De Simone et al. (1982), they that L-carnitine increases the proliferative responses of both murine and human lymphocyte following mitogenic stimulation and increase polymorphonuclear chemotaxis.

Evidently and rightfully, the current study advocates the clinical use of L-carnitine, concurrently with antidiabetics in diabetes to alleviate its immunological consequences and cardiovascular complications.

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