

# LEVO-CARNITINE NORMALIZES BLOOD GLUCOSE AND LIPID PROFILE IN INSULIN RESISTANT TYPE II DIABETIC RATS

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## Abstract

**Objective:** This study was planned to determine the effect of levo-carnitine on blood glucose and lipid profile in high fat diet fed, streptozotocin induced insulin resistant type II diabetic rats.

**Study Design:** Randomized controlled trial (RCT)

**Place and Duration of the Study:** Department of Physiology, Army Medical College, Rawalpindi from 20th May 2008 to 15 Jan 2009.

**Material and Methods:** Ninety Sprague-Dawley rats were divided into three groups; each having 30 rats. Group I (control) was fed on normal diet while in Group II (Diabetic) diabetes was induced by feeding high fat diet and giving the injection of streptozotocin. Group III (Carnitine) was treated like group II to induce diabetes mellitus and later Levo-carnitine 200 mg/kg body weight/day was administered intraperitoneally for 6 days.

**Results:** The blood glucose and lipid profile were significantly deranged in the diabetic and carnitine groups as compared to the control. After levo-carnitine treatment the blood glucose level decreased significantly ( $p < 0.001$ ) in carnitine group (6.2 mmol/l) as compared to the diabetic group (22.7 mmol/l). The levels of triglycerides, cholesterol and LDL were significantly decreased ( $P < 0.001$ ) in carnitine group 0.5 mmol/l, 1.3 mmol/l and 0.2 mmol/l as compared to the diabetic group 1.9 mmol/l, 4.4 mmol/l and 3.1 mmol/l respectively. HDL level was significantly raised ( $p < 0.001$ ) in the carnitine group (0.9 mmol/l) as compared to the diabetic group (0.4 mmol/l).

**Conclusion:** It is concluded that Levo-carnitine administration tends to normalize glycemic control and lipid profile in type II diabetes mellitus. However, human trial is recommended to ascertain its efficacy as an antidiabetic and antidysepidemic agent for therapeutic use.

**Keywords :** Levo-carnitine, type II diabetes mellitus, insulin resistance, blood glucose, lipid profile

## Article

### INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic diseases worldwide due to its chronic nature, and serious complications including blindness, kidney failure and cardiovascular disease. It is one of the most expensive diseases with regard to the total health care cost per patient<sup>1</sup>. Since prevalence of DM is increasing and newer complications and clinical conditions are emerging, it will account for immense medical, social and economic burden. The top 10 countries with respect to diabetic population are: India, China, USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh. In this part of the world (Asia) about 46 million people are suffering from DM. In Pakistan, the current burden of DM is 5.2 million which is expected to increase to 13 million by the year 2030<sup>2</sup>.

Levo-carnitine ( $\beta$ -hydroxy -  $\gamma$ -trimethyl-L-homocarnitine) is a natural vitamin-like compound present in both intracellular and extracellular environment. It is synthesized in liver, kidney and brain from two essential amino acids; lysine and methionine. It functions as a transporter of short, medium and long-chain fatty acids across the inner mitochondrial membrane, thereby facilitating  $\beta$ -oxidation. It participates in cellular energy production, maintenance and repair processes of neurons and buffers potentially toxic acyl-CoA metabolites. It modulates the ratio of acyl-CoA/CoA and improves mitochondrial energetics. It also stabilizes membranes of intracellular organelles<sup>1,3</sup>.

Levo-carnitine possesses antidiabetic properties<sup>3,4</sup>. It regulates hepatic glucose metabolism and

suppresses glucose overproduction in high fructose fed animals<sup>5</sup>. In normal subjects, higher amount of glucose is utilized for oxidation in place of non esterified fatty acids (NEFA)<sup>6</sup>. Levo-carnitine also lowers the levels of triglycerides, free fatty acids and cholesterol in high fructose fed animal model and long-chain acyl-CoA induced free radicals production in cardiac ischemia<sup>7</sup>.

The glucose utilizing and lipid lowering effects of levo-carnitine have been evaluated in type 1 diabetes mellitus (DM-1) and in high fructose fed insulin resistant rats<sup>7</sup>. To our knowledge no study has so far been documented to reveal the effect of levo-carnitine on high fat diet fed, streptozotocin induced insulin resistant type II diabetic rats. This is a recently developed rodent model of type 2 diabetes mellitus which closely reflects the natural history and metabolic characteristics of human type 2 diabetes mellitus and is considered as an ideal type 2 diabetic model for drug testing<sup>8</sup>. Therefore we hypothesized that Levo-Carnitine can normalize blood glucose and lipid profile in insulin resistant Type II diabetic rats. This study was planned to determine the effect of levo-carnitine on blood glucose and lipid profile in high fat diet fed, streptozotocin induced insulin resistant type II diabetic rats.

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## **MATERIAL AND METHODS**

### **Animals**

These randomized controlled trials (RCT) were conducted at the Dept of Physiology, Army Medical College, Rawalpindi from 20th May 2008 to 15 Jan 2009.

Ninety healthy Sprague-Dawley rats, weighing  $220 \pm 30$  grams were taken from National Institute of Health (NIH) Islamabad, Pakistan and kept under standard conditions for four weeks with a daily photo period of 12 hours light and 12 hours dark at  $23 \pm 2^\circ\text{C}$ . Rats were randomly divided into three groups, each having 30 rats.

**Group I:** (Control group; n = 30)

Rats were fed on normal pelleted diet (NPD) ad libitum for 04 weeks. This NPD was prepared at NIH according to the standard approved by the Universities Federation for Animals Welfare. The composition of NPD has been presented in table 1.

**Table 1: Composition of normal pelleted diet (NPD) for rats**

<b>S. No</b>	<b>Ingredients</b>	<b>Weight g/kg</b>
1	Wheat Flour	285
2	Wheat Brawn	285
3	Dried skimmed milk Powder	200
4	Soybean Oil	0.050
5	Mollasen	15
6	Fish me at	150
7	Salt (common).	5
8	Vitamin and Mineral mix	10

Rats were given intra-peritoneal injection of 0.1ml normal saline daily for 6 days during 4th week of the study.

**Group II:** (Diabetic group; n = 30)

Rats were fed high fat diet (table 2)

**Table 2: Composition of high fat diet**

<b>S. No</b>	<b>Ingredients</b>	<b>Weight g/kg</b>
1	Powdered NPD	365
2	Animal fat	310
3	Casein	250
4	Cholesterol	10
5	Vitamin and Mineral mix	60
6	DL-Methionine	03
7	Yeast powder	01
8	Sodium chloride	01

for four weeks specially prepared at NIH according to the laid down standard. A single intra-peritoneal injection of streptozotocin (Alexis Biochemicals, USA) 35 mg/kg was given at the beginning of the 3rd week. Intra peritoneal injection of 0.1 ml normal saline was given daily for 06 days during 4th week of the study.

**Group III:**(Carnitine group; n = 30)

Rats underwent similar preparation as group II till the end of 3rd week. However, intra peritoneal injections of L-carnitine (Sigma, USA) 200mg/kg/day were given daily for 06 days during the 4th week of study instead of normal saline.

After three weeks, 1.5 ml of tail blood was drawn and serum was separated. Blood glucose and TG/HDL ratio was measured to confirm diabetes mellitus and insulin resistance respectively.

Terminal sample (4-5ml blood) was drawn by a single intra cardiac puncture. The serum was separated for the estimation of glucose and lipid profile.

#### **Analytical Procedure**

##### **Glucose and Lipid analysis**

Serum glucose was estimated by glucose oxidase method. It is an enzymatic colorimetric method and a kit supplied by Linear Chemicals, Spain (Cat No. 30234) was used for assay. Enzymatic colorimetric method was used to estimate TG and HDL cholesterol by lab kits of Linear Chemicals, Spain (Cat No. 1155010 and Cat No. 1133010 respectively). Total cholesterol was measured by cholesterol esterase method by using the kit of Pioneer Diagnostic, USA (Cat No. PD2802). All assays were done by using automated chemistry analyzer (Vitalab Selectra E), while LDL was calculated by using Friedewald formula.

##### **Statistical analysis**

The data was analyzed on SPSS version 15. Descriptive statistics were used to describe the data. Analysis of Variance (ANOVA) had been used to compare the levels of blood glucose and lipid profile between the groups followed by Tukey's HSD (Honestly Significant Differences) Test for pair wise comparison. P-value < 0.05 was considered statistically significant.

#### **RESULTS**

The development of diabetes mellitus and insulin resistance in diabetic and carnitine groups has been presented as blood glucose and TG/HDL ratio in table 3.

**Table 3: The blood glucose and TG/HDL ratio in control diabetic and carnitine groups**

<b>Parameter</b>	<b>Control</b>	<b>Diabetic</b>	<b>Carnitine</b>
Glucose (mmol/l)	4.79±0.9	18.76±1.31	17.91±1.25
TG/HDL ratio	1.125±0.19	9.72±2.01	9.81±2.09

Diabetes mellitus: blood glucose >11.1mmol/liter

Insulin resistance: TG/HDL ratio >1.8

The data represents the gross increase (P<0.001) in blood glucose level and insulin resistance on experimental induction of type- 2 diabetes mellitus in group II and III as compared to healthy controls.

The comparison of weight, blood glucose and lipid profile of control, diabetic and carnitine groups are presented in table 4.

**Table 4: Comparison of blood glucose and lipid profile of control, diabetic and carnitine groups at the end of 4<sup>th</sup> week of study.**

<b>Parameter</b>	<b>Control (n=30)</b>	<b>Diabetic (n=30)</b>	<b>l-Carnitine (n=30)</b>
Weight (gm)	269.70±12.55	318.96±17.41**	309.90±9.72 <sup>@@</sup> <sup>⊗</sup>
Glucose (mmol/L)	4.8 ± 0.8	22.7 ± 1.5**	6.2 ± 1.4 <sup>@@</sup> <sup>⊗</sup>
Triglyceride (mmol/L)	0.4 ± 0.0	1.9 ± 0.2**	0.5 ± 0.1 <sup>⊗</sup> <sup>⊗</sup>
Cholesterol (mmol/L)	1.2 ± 0.1	4.4 ± 0.3**	1.3 ± 0.2 <sup>⊗</sup> <sup>⊗</sup>
HDL (mmol/L)	0.8 ± 0.1	0.4 ± 0.1**	0.9 ± 0.1 <sup>@@</sup> <sup>⊗</sup>
LDL (mmol/L)	0.3 ± 0.1	3.1 ± 0.3**	0.2 ± 0.2 <sup>⊗</sup> <sup>⊗</sup>

Values are expressed as mean ± standard deviation.

(1) Comparison between control and diabetic group (\*\* p<0.001)

(2) Comparison between carnitine and control group (<sup>⊗</sup> p<0.001, <sup>⊗</sup> p<0.05)

(3) Comparison between diabetic and carnitine group (<sup>@@</sup> p<0.001, <sup>⊗</sup> p<0.05)

The blood glucose and lipid profile were significantly (p<0.001) deranged in the diabetic group as compared to the control. The body weight of rats increased both in the diabetic and the carnitine groups and levo-carnitine had brought significant (p<0.05) change in body weight of the carnitine group.

The effect of levo-carnitine was evident from the significantly reduced blood glucose levels in the carnitine group (6.2 mmol/lit) compared to the diabetic group (22.7 mmol/lit), although these levels were higher than the control group (4.8mmol/lit). Levo-carnitine administration normalized the lipid profile in the carnitine group with significantly (p<0.001) low levels of TG's, cholesterol and LDL as compared to the diabetic group. The levels of HDL in the carnitine group were increased more than the control group. The values of blood cholesterol were higher in the carnitine group as compared to control and LDL levels were found lower in the carnitine group when compared to the control.

## DISCUSSION

Diabetes mellitus is a worldwide metabolic disorder affecting a large population. Numerous treatment regimens and animal models are emerging for research on diabetes mellitus. Rodents are the most appropriate and often used model for antidiabetic drug testing because of their easy handling, low price and resemblance with human metabolic characteristics<sup>8</sup>.

The high fat diet increased intramyocellular lipids, caused insulin resistance and burdened pancreatic β cells. We used injection streptozotocin to induce DM-2 that works by causing β cell death and reduction in β cell mass of islet of langerhans in pancreas. It led to the development of frank hyperglycemia and removal of dietary stress like high fat diet (HFD) or high fructose diet (HSD) did not reverse the diabetic state of animal because β cell mass was reduced. Srinivasan and his colleagues used a single injection of streptozotocin (35mg/kg body weight) for producing oxidative damage to β cells of pancreas and the development of DM-2. This protocol reduced the cost and duration of the study by decreasing the time span for giving the diet to the animal, hitherto developed diabetes mellitus in 3 weeks.

Development of insulin resistance and diabetes mellitus in diabetic and carnitine groups were confirmed by estimating TG/HDL ratio and serum glucose. TG/HDL ratio was more than 1.8 which is the cut off value for insulin resistance as criteria laid down by McLaughin et al<sup>9</sup>. The blood glucose

level in both the groups was  $>11.11$  mmol/liter which confirmed DM in sprague dawley rats. Hyperglycemia produced in diabetic and carnitine groups is believed to be due to hepatic insulin resistance leading to increased gluconeogenesis and decreased glucose utilization along with its diminished use in the peripheral tissue following insulin resistance<sup>10</sup>. Similarly the derangement in lipid profile of diabetic and carnitine groups is suggested to be the outcome of hepatic insulin resistance leading to more production of VLDL which in turn leads to increased levels of LDL and decreased HDL and peripheral sequel of insulin resistance manifested by the increased lipolysis and increased blood lipid levels<sup>10</sup>. Depletion of plasma, liver and muscle carnitine was observed in diabetes mellitus by Rajasekar et al in two different studies<sup>11</sup> which was thought to be consequent to excess utilization of carnitine to form acylcarnitine due to increased FFA availability. In our study levo-carnitine was injected intraperitoneally to avoid the first pass effect of liver to ensure effective plasma levels and optimal bioavailability as documented by Brass et al<sup>12</sup>. 200mg/kg levo-carnitine was administered daily because this concentration was safe and effective in causing metabolic effects in rats. This led to normalized blood glucose levels in carnitine group as compared to the diabetic group. Similar results were obtained by Paulson et al in which levo-carnitine decreased the glucose levels in inbred strain of rats<sup>13</sup>. Rodrigues et al used a very high dose (3 gm/kg day) of levo-carnitine in type 1 diabetic model of Wistar rats for lowering blood glucose<sup>14</sup>. Mingrone in his study observed the glucose lowering effect of levo-carnitine in humans by giving intravenous acetyl-levo-carnitine (ALC) (3.8 to 5.2 mg/kg/min) to produce hypercarnitinemia during euglycemic hyperinsulinemic clamp<sup>15</sup>.

The studies by Balasaraswathi et al<sup>16</sup> and Rajasekar<sup>17</sup> have documented that levo-carnitine 300mg/kg/day intraperitoneal was able to reduce blood glucose in insulin resistance model of high fructose fed Wistar rats. High fructose model differs from natural DM-2 as it decreases the insulin extraction by the liver, alters the activity of key hepatic enzymes involved in glucose metabolism and activates stress sensitive pathways to desensitize insulin signaling<sup>18</sup>.

There are few studies which have documented that levo-carnitine administration did not lower the glucose level. Patel J et al<sup>19</sup> worked on neonatal streptozotocin (STZ) induced diabetic rats and treated them with levo-carnitine (600 mg/kg/day) orally for six weeks but there was no beneficial effect against hyperglycemia. It could be due to the difference in animal model of their study. The effect of levo-carnitine (3 gm/ day) in human type 2 diabetic subjects had resulted in no change in insulin sensitivity, possibly due to the first pass effect in humans after oral intake of levo-carnitine. Uysal N et al<sup>20</sup> studied the effects of levo-carnitine in a dose of 500 mg/kg i.p and 600mg/kg/day i.p respectively in STZ induced diabetic rats. In spite of the high dose of levo-carnitine there was no significant reduction in blood glucose. It might be due to the use of high dose of STZ ( $>40$ mg/kg) which had resulted in advanced  $\beta$ -cell damage beyond the level of DM-2, i.e. in the range of DM-1. The effect of levo-carnitine on glucose metabolism has been associated to its ability to stimulate fatty acid oxidation in mitochondria. This restores the intramitochondrial acyl-CoA/CoA ratio and stimulate pyruvate dehydrogenase activity and glucose oxidation. In addition, enhanced oxidative utilization of glucose by carnitine might have occurred as a result of increased flux of glucose through phosphofructokinase(PFK). The PFK has been reported to be inhibited in the insulin resistant rats. Levo-carnitine has also been suggested to act as CoA buffer that maintains acyl CoA/CoA ratio in cells and plays role in many metabolic processes<sup>21</sup>.

In our study the lipid profile was also normalized in the carnitine group. Levo-carnitine reduced the levels of TGs, cholesterol and LDL. The beneficial effect of levo-carnitine on HDL was particularly marked in the carnitine group and considered as protective action of levo-carnitine against atherosclerosis. The work of Giovanni et al on non diabetic elderly subjects with rapid muscle fatigue revealed the beneficial effect of levo-carnitine on total fat mass, total muscle mass, TGs, total cholesterol, HDL and LDL<sup>22</sup>. It has been documented that the blood glucose, FFA and TGs were significantly lowered in a similar study but there was no significant increase in body weight<sup>19</sup>. Contrary to our results Rahbar and colleagues worked on type 2 diabetic patients and administered 3 gm/day levo-carnitine orally. After 12 weeks of treatment there were significant increase in TGs while no significant change was observed in levels of LDL, HDL, HbA1C, or total cholesterol<sup>23</sup>. It might be due to the first pass effect in humans that occurred after oral administration of levo-carnitine

and resulted in less plasma levels and bioavailability of carnitine<sup>13</sup>.

## **CONCLUSION**

Levo-carnitine administration has blood glucose lowering and lipid profile normalizing actions in type II diabetic rats. A human trial is recommended to ascertain its efficacy as antidiabetic and anti-dyslipidemic agent for therapeutic use.

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## **Reference**

1. Jo W.M. Höppener, and Cees J.M. Lips. Review: Role of islet amyloid in type 2 diabetes mellitus. *Int J Biochem Cell Biol* 2006; 38(5-6): 726-736.
2. Department of chronic diseases and health promotion (CHP) Diabetes Unit: World Health Organization Switzerland. Available from: URL: <http://www.who.int/diabetes/en/index.html>
3. Calabrese V, Maria A, Stella G, Calvani M, Butterfield DA. Acetylcarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes. *J Nutr Biochem* 2006; 17 (2): 73-88.
4. Calò LA, Pagnin E, Davis PA, Semplicini A, Nicolai R., Calvani M, Pessina AC. Antioxidant effect of L-carnitine and its short chain esters. Relevance for the protection from oxidative stress related cardiovascular damage. *Int J Cardiol* 2006; 8: 54-60.
5. Rajasekar P, Viswanathan P, Anuradha CV. Beneficial impact of L-carnitine in liver: a study in a rat model of syndrome X. *Amino Acids* 2008; 35 (2): 475-83.
6. Gaetano AD, Mingrone G, Castagneto M, Calvani M. Carnitine increases glucose disposal in humans. *J Am Coll Nutr* 1999; 18(4):289–95.
7. Rajasekar P, Anuradha CV. Effect of L-carnitine on skeletal muscle lipids and oxidative stress in rats fed high-fructose diet. *Exp Diabetes Res* 2007; 72741: 1-8.
8. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: An overview. *Indian J Med Res* 2007; 125, pp 451-72.
9. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 2003; 139: 802-9.
10. Champe PC, Harvey RA, Ferrier DR. *Illustrated Biochemistry, Diabetes Mellitus*. 3rd ed. USA: Lippincott Williams and Wilkins, 2005; P 342-51.
11. Rajasekar P, Kaviarasan S and Anuradha CV. Levocarnitine administration prevents oxidative stress in high fructose fed insulin resistant rats. *Diabetologia Croatica* 2005; 34(1):21-28.
12. Brass EP, Hoppel CL, Hiatt WR. Effect of intravenous L-carnitine on carnitine homeostasis and fuel metabolism during exercise in humans. *Clin Pharmacol Ther* 1994; 55:681–92.
13. Paulson DJ, Schmidt MJ, Traxler JS, et al. Improvement of myocardial function in diabetic rats after treatment with L-carnitine. *Metabolism* 1984; 33: 358-63.
14. Rodrigues B, Xiang H, McNeill JH. Effect of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. *Diabetes* 1988; 37: 1358-64.
15. Mingrone G. Carnitine in type 2 diabetes. *Ann N Y Acad Sci* 2004;1033:99-107.
16. Balasaraswathi K, Rajasekar P and Anuradha CV. Changes in redox ratio and protein glycation in precataractous lens from fructose-fed rats: Effects of exogenous L-carnitine. *Clin Exp Pharmacol Physiol* 2008;35:168–73.
17. Rajasekar P and Anuradha CV. L-Carnitine inhibits protein glycation in vitro and in vivo: evidence for a role in diabetic management. *Acta Diabetol* 2007; 44:83–90.
18. Kelly GL, Allan G, Azhar S. High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. *Endocrinology* 2004; 145:548-55.
19. Patel JK, Goyal R, Bhatt P. Beneficial effects of levo-carnitine on lipid metabolism and cardiac function in neonatal streptozotocin rat model of diabetes *Int J Diabetes and Metabolism* 2008: 16:

29-34.

20.Uysal N, Yalaz G, Acikgoz O, Gonenc S, Kayatekin BM. Effect of levo-carnitine on diabetogenic action of streptozotocin in rats. *Neuro Endocrinol Lett* 2005; 26(4):419-22.

21.Rajasekar P, Anuradha CV. Fructose-induced hepatic gluconeogenesis: Effect of levo-carnitine. *Life Sci* 2007; 80:1176–83.

22.Giovanni P, Angela DM, Carmelo L, Simona D, Giovanna F, Mariano M. Levo-carnitine administration in elderly subjects with rapid muscle fatigue: effect on body composition, lipid profile and fatigue. *Drugs and Aging* 2003; 20(10):761-67.

23.Rahbar AR, R Shakerhosseini, N Saadat, F Taleban, A Pordal and B Gollestan. Effect of L-carnitine on plasma glycemic and lipidemic profile in patients with type II diabetes mellitus *Eur J Clin Nutr* 2005; 59:592–6.