

L-carnitine Increases Skeletal Muscle GLUT4 Expression and Improves Metabolic Control in Type 2 Diabetes

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Keywords

- L-carnitine
- GLUT4
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Abstract

Background: L-carnitine is known to play a beneficial role in diabetes; however, there are no studies on the effect of L-carnitine on glucose transporter 4 (GLUT4) expression in high glucose condition. So the aim of this in vivo study was to investigate whether L-carnitine can improve insulin resistance in type 2 diabetes (T2DM) through increasing the expression of GLUT 4 in skeletal muscles. **Methods:** Thirty healthy male Sprague Dawely rats were randomly divided into: Control negative, control positive (Diabetic) and Carnitine groups. For induction of T2DM the control positive and carnitine groups' rats were fed high fat diet (HFD) for about 2 month, while control negative rats were fed on normal diet. Diabetic and carnitine groups received 35 mg/kg streptozocin (STZ) intraperitoneal. After confirming the development of T2DM in diabetic and carnitine groups L-carnitine was administered orally to the carnitine group in a dose of 3 gm carnitine / kg once daily for 4 weeks. At the end of treatment period, rats were sacrificed and blood samples were collected directly from the heart and the hindlimb soleus muscles were removed, snap- frozen and stored at -80°C until analysis. **Results:** L-carnitine administration resulted in significant decrease ($p < 0.05$) in serum glucose, serum insulin, and triglycerides: High Density Lipoprotein Cholesterol (TG:HDL) ratio of the carnitine group as compared to diabetic group. Carnitine administration showed also significant increase AMP-activated protein kinase (AMPK) activity and in total skeletal muscle GLUT-4 protein concentration. **Conclusion:** The results of this study demonstrate that carnitine supplementation ameliorates insulin resistance in type 2 diabetic rats and up-regulating GLUT4 protein expression in the skeletal muscles through increasing the activity of AMPK. So, carnitine administration may be a possible candidate for the treatment of T2DM.

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INTRODUCTION

Type 2 diabetes (T2DM) is a metabolic disease characterized by hyperglycemia, peripheral insulin resistance (IR), and impaired metabolic function (1). Although there are several medications currently available to help manage T2DM, there is an increasing need for more effective treatments than those currently available. T2DM is associated with a number of complications and co-morbidities, including diabetic retinopathy, loss of vision, diabetic nephropathy, lower limb amputation and cardiovascular disease mortality (2). It was estimated that diabetes alone caused 5.1 million deaths in 2013 (1).

Skeletal muscle glucose uptake is the rate limiting step of glucose utilization (3). Several evidences indicate that the levels of GLUT4 expression in skeletal muscle are crucial for the regulation of total body glucose homeostasis (4, 5). Skeletal muscle is a major site of impaired insulin action in type 2 diabetes mellitus. Many studies showed that decreased muscular lipid oxidation capacity results in the accumulation of toxic lipid intermediates, such as diacylglycerol, acyl-coenzyme A (acyl-CoA) and ceramides, and activates atypical protein kinase C isoforms. This is followed by serine phosphorylation of insulin receptor substrates and, eventually, impaired insulin signaling (6, 7). It has been established that insulin-stimulated rate of glycogen synthesis in muscles is markedly reduced in T2DM (8). Furthermore, in T2DM patients, insulin-dependent pyruvate dehydrogenase (PDH) activity appears to be reduced which is considered to be the cause of reduced insulin-stimulated glucose oxidation in such patients (9). Also, in type 2 diabetes mellitus,

muscular glucose uptake is decreased; therefore, skeletal muscle can be a major therapeutic target for the daily control of hyperglycemia and improvement in insulin sensitivity.

L-carnitine (L-b-hydroxy-g-N-trimethylaminobutyric acid) is a non-protein amino acid which main sources in humans include both diet and endogenous synthesis. It is present in the free or acyl-carnitine form in the plasma (10). L-carnitine is widely found in animal foods such as meat, fish, milk and dairy products, and it can be synthesized in liver and kidney from the amino acids lysine and methionine. Carnitine plays an important role in lipid metabolism. It regulates fatty acid transport between the cytosol and the mitochondria (11). Carnitine supplementation has been effective for reducing IR in fat rich diet fed mice without changing intake or weight (12). In studies with healthy humans, carnitine infusions improved glucose metabolism measured with hyperinsulinaemic euglycaemic clamp, mainly by a non-oxidative mechanism that results in the accumulation of glycogen (13-16). In type 2 diabetes mellitus, carnitine infusion improved glucose oxidation and glycogen storage (17, 18). The aim of this experimental study was to investigate the effect of L-carnitine on metabolic control in T2DM and also to investigate its effect on GLUT 4 expression in the skeletal muscles of type 2 diabetic rats.

MATERIALS AND METHODS

Thirty healthy male Sprague Dawely rats, 80 ± 5 days old with average weight of 250 ± 50 gram sold were used in the study. They were obtained from Medical Experimental Research Center of Mansoura University, Egypt. The experimental

protocol was approved by the Institutional Animal Ethics Committee of Mansura University. Rats were housed in polycarbon cages and were exposed to a 12 h light-dark cycle at a room temperature of 21-24°C and 50 - 60% relative humidity. Rats were randomly divided into: control negative, control positive (Diabetic) and Carnitine+T2DM groups (10 rats each). For induction of T2DM control positive and carnitine groups rats were fed high fat diet (HFD) (58%fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) for about 2 month, while control negative rats were fed on normal diet ad libitum (60% CHO/10% fat/30% protein, as a percentage of total kcal). The composition of food given to the groups is presented in table 1 (19). After 2 month of HFD, 35 mg/kg streptozocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 10 mM citrate buffer (pH 4.5) was given intraperitoneal in the lower right quadrant of the abdomen to the diabetic and carnitine groups, while the control negative rats were injected with citrate buffer alone (20). After one week of streptozocin injection, serum glucose and insulin and also triglyceride to high density lipoproteins (TG:HDL) ratio of all rats were measured by retro-orbital plexus sampling to confirm the development of T2DM in diabetic and carnitine groups (21). Development of T2DM and insulin resistance (IR) was confirmed according to the criteria laid down by **Yassin and Mwafy** for Sprague-Dawley rats, plasma glucose level of > 200 mg/dl was used as the “cut off value” of hyperglycemia for confirming T2DM (22). Since insulin resistance cannot be measured directly, therefore TG: HDL ratio was used as surrogate marker. The cut off value of ratio as 1.8 was used to mark insulin

resistance (23). According to **Olson et al** (2012), TG: HDL ratio produces similar results to other methods used to measure insulin resistance such as the QUICKI and HOMA_{IR} (21). Afterwards L-carnitine was administered orally to the carnitine+T2DM group in the dose of 3 gm carnitine / kg once daily for 4 weeks (24). Body weight was measured every week all over the period of experiment.

At the end of treatment period, rats were anesthetized with ketamine hydrochloride (110mg/kg), sacrificed, blood samples were collected directly from the heart and the hind limbsoleus muscles were removed, snap-frozen and stored at -80°C until analysis.

Glucose and Insulin Serum Levels' Measurements:

Non- fasting blood samples for glucose and insulin assays were obtained from the retro-orbital plexus. Retro-orbital blood was drawn in the morning, every week, promptly centrifuged, and serum was stored at -80°C until analysis. Serum glucose concentration was measured by a glucose oxidase method, the absorbance of the testes samples were read using spectrophotometer adjusted at 450 nm. The sensitivity of glucose kit was the kit can detect glucose at a concentration of > 0.02 mM. Serum insulin concentration was determined using a mouse insulin ELISA kit (Sigma - Aldrich, St Louis, MO, USA). The sensitivity of insulin kit was 0.1 ng/ml.

Lipid profile and lipoprotein cholesterol assay:

Free fatty acids (FFA) concentrations were determined by using colorimetric kits (BioVision, Inc. USA) and spectrophotometer apparatus adjust at 570 nm. Triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol

(HDL-C) concentrations were determined using enzymatic methods as described in the instructions provided with the kits (Analyticon, Biotechnologies AG, Germany). The absorbance of the testes samples were read using spectrophotometer adjusted at 546 nm for TG, TC and 500 nm for HDL-C. Low density lipoprotein cholesterol (LDL-C) concentration was calculated by using formula of Friedwald et al. (25):

$$\text{LDL-C} = [\text{Total chol}] - [\text{HDL-C}] - [\text{TG}/5]$$

Very Low Density Lipoprotein Cholesterol (VLDL-C) was calculated using the following equation:

$$\text{VLDL-C (mg/dl)} = \text{TG}/5$$

TG: HDL ratio was also measured as an index of insulin resistance.

Analytical sensitivity (lower detection limit): The kits can detect TG at a concentration of 0.3 mg/dl, TC at a concentration of 2.8 mg/dl, HDL at a concentration of 1 mg/dl, and FFA at a concentration of 2 μM .

Measurement of AMP-activated protein kinase (AMPK) activity:

- **Sample preparation:**

For measuring the AMPK activity, the muscles were weighed and then homogenized in buffer 1:19wt/vol containing 20mM Tris-HCl (pH7.4), 250mM sucrose, 50mM NaF, 1mM EDTA, 5mM sodium pyrophosphate, 2mM DTT, 1mM benzamide, 4mg/L leupeptin, 1mg/L aprotinin, 1mg/L pepstatin, 50mg/L trypsin inhibitor, and 0.6mM PMSF. The homogenates were centrifuged at 48,000g for 30min and the resulted supernatant was used to measure AMPK activity. All chemicals were purchased from Sigma - Aldrich, St Louis, MO, USA.

- **Assay of AMPK activity:**

AMPK activity was determined by using colorimetric kit (Abcam Co. Cambridge, UK) according to the instruction provided in the kit and spectrophotometer apparatus adjust at 450 nm. The kit can detect AMPK concentration in the range of 3 $\mu\text{g/ml}$ - 500 $\mu\text{g/ml}$

Measurement of GLUT-4 expression by Quantitative real-time PCR:

- **Skeletal muscle GLUT4 mRNA expression:**

GLUT4 mRNA content in soleus skeletal muscle was measured according to the method reported by Buhl et al. (26). The specific primers were GLUT-4 sense: TTC TGG CTC TCA CAG TAC TC; GLUT4 reverse: CAT TGA TGC CTG AGA GCTGT; β -actin sense: TGG AAT CCT GTG GCA TCC ATG AAA C; β -actin reverse: TAA AAC GCA GCT CAG TAA CAG TCC G. The PCR products were stained with ethidium bromide loaded on agarose gel for electrophoresis, and visualized at UV light.

- **Skeletal muscle GLUT4 protein content:**

Total GLUT4 protein content in hind limb skeletal muscle extracts was measured by western blotting technique, using specific monoclonal antibodies. The primary antibody was a rabbit affinity purified polyclonal anti-glut-4 (Catalog number PA5-19333 from Thermo Scientific).

- **Protein preparation:**

Muscles were weighed and homogenized in 10mL of ice-cold buffer containing 100mM HEPES pH7.6, 150mM NaCl, 5mM EDTA, 5mM MgCl₂, 1% Triton X-100, protease inhibitors (2mM phenyl- methylsulphonyl fluoride), and phosphatase inhibitor (100mM sodium orthovanadate). The homogenate was centrifuged

at 10000g for 20min at 4°C, and the resulting supernatant was centrifuged at 9000g for 20min at 4°C.

Determination of GLUT4 by Western Blot Analysis:

Protein samples (40µg) were denatured in reducing buffer (62mM Tris pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.0035 bromophenol blue) and separated by electrophoresis on an SDS (12%) polyacrylamide gel. The separated proteins were transferred on to a nitrocellulose membrane using the transfer buffer (39mM glycine, 48mM Tris pH 8.3, 20% methanol) at 200mA for 1h. The membranes were blocked with 5% non-fat dry milk in TBS-0.1% Tween for 1h at room temperature, washed three times for 10min each in TBS-0.1% Tween, and incubated with a primary GLUT-4 antibody (Catalog number PA5-19333 from Thermo Scientific) in TBS-0.1% Tween overnight at 4°C. After being washed three times for 10min each in TBS-0.1% Tween, the membranes were incubated with a second antibody peroxidase conjugated goat anti-rabbit immunoglobulin G (Pierce) for 1h at room temperature. After three times washing for 10min each in TBS-0.15%, the membranes were analyzed by the enhanced chemiluminescence system according to the manufacture's protocol (Amersham). The GLUT4 protein signal was quantified by scanning densitometry using a bio image analysis system (Bio-Profil). The results from each experimental group were expressed as relative integrated intensity compared with control normal muscles measured with the same batch.

Statistical Analysis:

Statistical analysis was performed using the Statistical Package for Social Science program

(SPSS, version 10.0, Chicago, IL, USA). The data were expressed as mean ± standard deviation. The significance of differences between mean values was determined using Student's t-test. The level for statistical significance was set at $P \leq 0.05$.

RESULTS

The initial body weight, plasma glucose levels of the rats were within normal range.

In tables (3, 4, 5): STZ injection resulted in significant increase ($p < 0.05$) in all the biochemical parameters (serum glucose, serum insulin, and TG:HDL ratio). While carnitine administration resulted in significant decrease ($p < 0.05$) in serum glucose, serum insulin, and TG:HDL ratio of the carnitine+T2DM group as compared to diabetic group [from 424.6 ± 26.2 mg/dl, 171 ± 14 pmol/l, and 2.40 ± 0.8 to 154.2 ± 15.7 mg/dl, 95 ± 11 pmol/l, and 1.56 ± 0.3 respectively].

The body weight of each rat was measured every week (table 2) and was found to be increased in diabetic and carnitine+T2DM groups at the end of 8th week, due to high fat diet, as compared to the control negative group. After STZ injection there is no significant increase in body weight of diabetic group in spite of continuation of HFD. While in carnitine+T2DM group body weight shows insignificant change as compared to diabetic group.

Figures (1, 2, 3) show lipid profile in different groups at the end of the study. Diabetic group showed significant increase ($p < 0.05$) in serum level of FFA, TG, TC, and LDL-C and insignificant change in HDL-C and VLDL-C as compared to control negative group. While carnitine administration resulted in significant decrease ($p < 0.05$) in serum level of FFA, TG, TC,

and LDL-C and insignificant change in HDL-C and VLDL-C as compared to diabetic group. **Figure (4)** shows AMPK activity in the soleus muscle of different groups. Diabetic group showed significant decrease in AMPK activity as compared to control negative group. While carnitine+T2DM group showed significant increase AMPK activity as compared to diabetic group ($p < 0.05$).

Total GLUT4 mRNA levels in different groups are shown in figure 5. Skeletal muscle total GLUT4 mRNA levels were significantly

decreased in diabetic group. Carnitine administration was associated with a 2-fold increase in GLUT4 mRNA content as compared to diabetic group ($P < 0.05$). As shown in Fig. (6), total GLUT4 protein content in soleus muscle was significantly lower in diabetic group ($P < 0.05$) as compared to control negative group, while carnitine administration resulted in ~2-folds increase in total skeletal muscle GLUT-4 protein concentration as compared to diabetic group. Fig. 7 shows the correlation between measured parameters in different groups.

Table (1): Food composition given to the groups

Normal diet		HFD	
Ingredients	Diet (g/kg)	Ingredients	Diet (g/kg)
Wheat flour	285	Casein	254
Wheat brawn	285	Corn starch	169
Salt (common)	5	Sucrose	85
Mollasen	15	Wheat bran	51
Soybean oil (ml/kg)	50	Safflower oil (ml/kg)	339
Fish meat	150	Gelatin	19
Vitamins/ minerals	10	Salt mix	67
Dried skimmed milk	200	Vitamin e acetate (500 iu/g)	0.31
		VITAMIN MIX	13
		DL-Methionine/L-cystine	3

Table (2): Body weight (mg) all over the period of the experiment in the three groups of rats

	1 st week	8 th week	1 week after STZ	1 week after carnitine	4 week after carnitine
C-ve					
Mean \pm SD	256 \pm 7.4	270 \pm 5.1	275 \pm 4.1	278 \pm 7.9	285 \pm 4.3
P1	-	NS	NS	NS	NS
P2	-	-	-	NS	NS
C+ve					
Mean \pm SD	260 \pm 4.5	335 \pm 7.1	334 \pm 7.4	336 \pm 4.3	339 \pm 5.4
P1	-	0.001	0.01	0.000	0.000
P2	-	-	-	NS	NS
Carnitine+T2DM					
Mean \pm SD	261 \pm 4.3	330 \pm 5.3	330 \pm 5.9	325 \pm 5.5	315 \pm 7.1
P1	-	0.01	0.01	0.2	0.2
P2	-	-	-	NS	NS

All values have been expressed as Mean \pm SD. Control negative group (C-ve; n=10), control positive group (C+ve; n=10), and carnitine+T2DM group (n=10). P1 \leq 0.05 significant as compared with 1st week, and P2 \leq 0.05 significant as compared with 9th week (1 week after STZ).

Table (3): Non fasting glucose serum levels (mg/dl) all over the period of the experiment in the three groups of rats

	1st week	8th week	1 week after STZ	1 week after carnitine	4 week after carnitine
C-ve					
Mean ± SD	105.2 ±15.7	110.3 ± 4.2	104.4 ±14.7	112.55±1 4.1	113.34±18.1
P1	-	NS	NS	NS	NS
P2	-	-	-	NS	NS
C+ve					
Mean ± SD	100.2 ±15.1	102.2 ±16.1	416.8 ±27.3	430.6 ± 28.5	424.6 ± 26.2
P1	-	NS	0.001	0.001	0.001
P2	-	-	-	NS	NS
Carnitine+T2DM					
Mean ± SD	111.3 ±16.3	110.7 ±16.3	412.6 ±27.5	280.2 ± 13.5	154.2 ± 15.7
P1	-	-	0.01	0.01	NS
P2	-	-	-	0.01	0.01

All values have been expressed as Mean ± SD. Control negative group(C-ve; n=10), control positive group (C+ve; n=10), and carnitine+T2DM group (n=10). P1 ≤ 0.05 significant as compared with 1st week, and P2 ≤ 0.05 significant as compared with 9th week (1 week after STZ).

Table (4): Non fasting insulin serum levels (pmol/L) all over the period of the experiment in the three groups of rats

	1st week	8th week	1 week after STZ	1 week after carnitine	4 week after carnitine
C-ve					
Mean ± SD	68±5	60±6	68±6	65±4	68±7
P1	-	NS	NS	NS	NS
P2	-	-	-	NS	NS
C+ve					
Mean ± SD	64±9	73±9	165±18	168±11	171±14
P1	-	-	0.001	0.001	0.001
P2	-	-	-	NS	NS
Carnitine+T2DM					
Mean ± SD	65±7	75±7	169±15	125±15	95±11
P1	-	NS	0.001	0.02	NS
P2	-	-	-	0.01	0.03

All values have been expressed as Mean ± SD. Control negative group(C-ve; n=10), control positive group (C+ve; n=10), and carnitine+T2DM group (n=10). P1 ≤ 0.05 significant as compared with 1st week, and P2 ≤ 0.05 significant as compared with 9th week (1 week after STZ).

Table (5): TG: HDL ratio all over the period of the experiment in the three groups of rats

	1 st week	8 th week	1 week after STZ	1 week after carnitine	4 week after carnitine
C-ve					
Mean ± SD	1.27 ±	1.28 ± 0.4	1.30 ± 0.6	1.29 ± 0.5	1.27 ± 0.5
P1	0.6	NS	NS	NS	NS
P2	-	-	-	NS	NS
C+ve					
Mean ± SD	1.31 ±	1.57 ± 0.8	2.38 ± 0.8	2.40 ± 0.8	2.40 ± 0.8
P1	0.6	NS	0.000	0.000	0.000
P2	-	-	-	NS	NS
Carnitine+T2DM					
Mean ± SD	1.29 ±	1.60 ± 0.7	2.26 ± 0.9	1.95 ± 0.8	1.56 ± 0.3
P1	0.5	NS	0.000	0.001	NS
P2	-	-	-	-	0.000

All values have been expressed as Mean ± SD. Control negative group(C-ve; n=10), control positive group (C+ve; n=10), and carnitine+T2DM group (n=10).P1 ≤ 0.05significant as compared with 1st week, and P2≤ 0.05significant as compared with 9thweek (1 week after STZ).

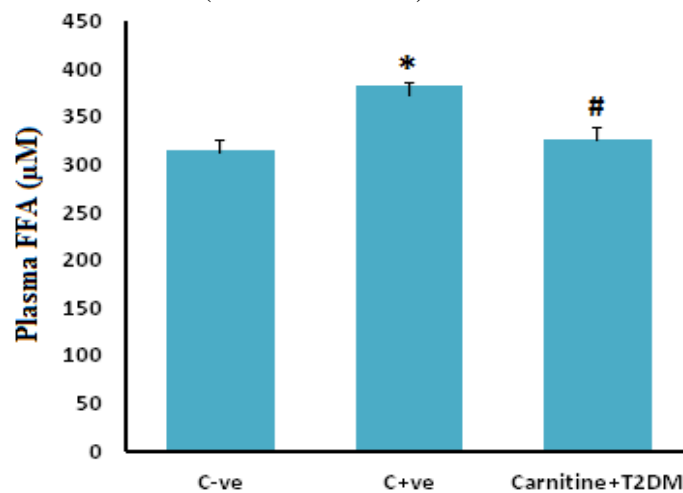


Fig. (1): Effect of carnitine on the plasma FFA level of diabetic rat. * indicates significant differences from the value obtained in control negative group and # indicates significant differences from the value obtained in control positive group at the level of p ≤ 0:05. Values have been expressed as Mean ± SD.

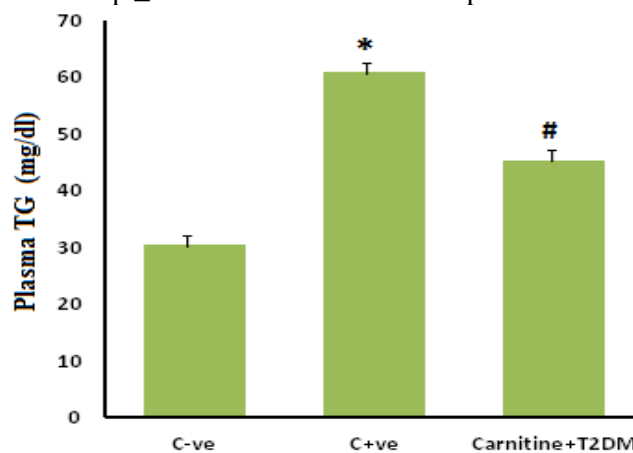


Fig. (2): Effect of carnitine on the plasma triglyceride level of diabetic rat. * indicates significant differences from the value obtained in control negative group and # indicates significant differences from the value obtained in control positive group at the level of p ≤ 0:05. Values have been expressed as Mean ± SD.

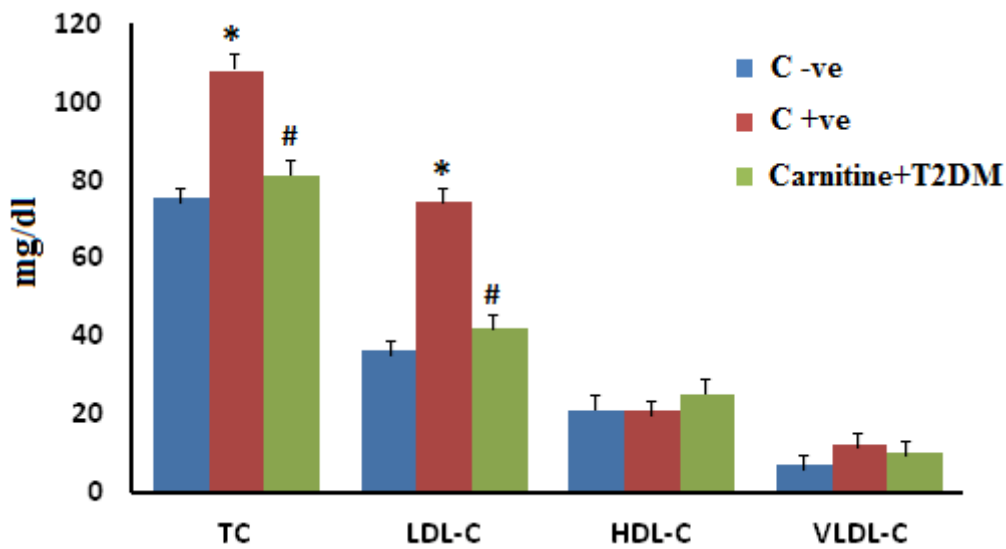


Fig. (3): Effect of carnitine on the serum level of TC, LDL-C, HDL-C, and VLDL-C of diabetic rat. * indicates significant differences from the value obtained in control negative group and # indicates significant differences from the value obtained in control positive group at the level of $p \leq 0.05$. Values have been expressed as Mean \pm SD.

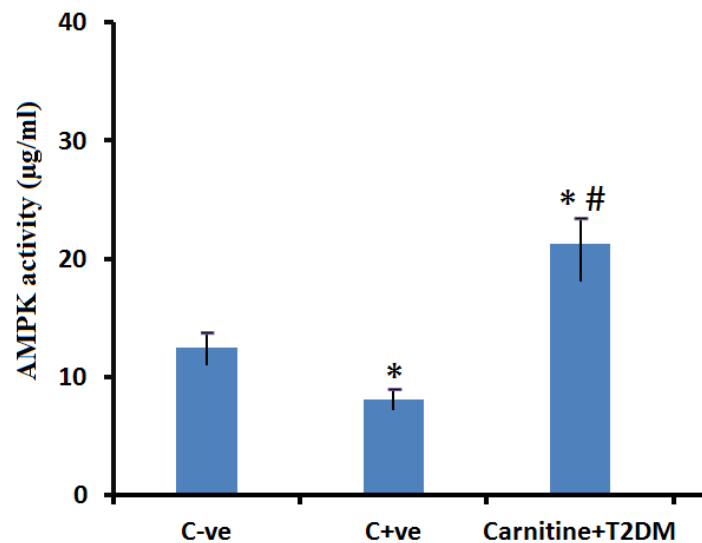


Fig. (4): Effect of carnitine on the AMPK activity in the soleus muscle of diabetic rat. * indicates significant differences from the value obtained in control negative group and # indicates significant differences from the value obtained in control positive group at the level of $p \leq 0.05$. Values have been expressed as Mean \pm SD.

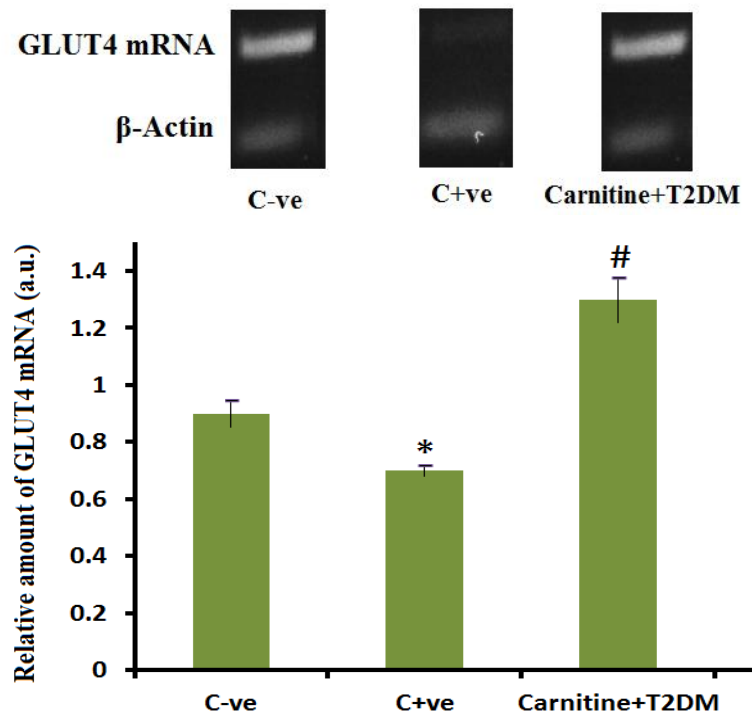


Fig. (5): Effect of carnitine on the relative amount of GLUT4 mRNA in the soleus muscle of diabetic rat. * indicates significant differences from the value obtained in control negative group and # indicates significant differences from the value obtained in control positive group at the level of $p \leq 0:05$. Values have been expressed as Mean \pm SD.

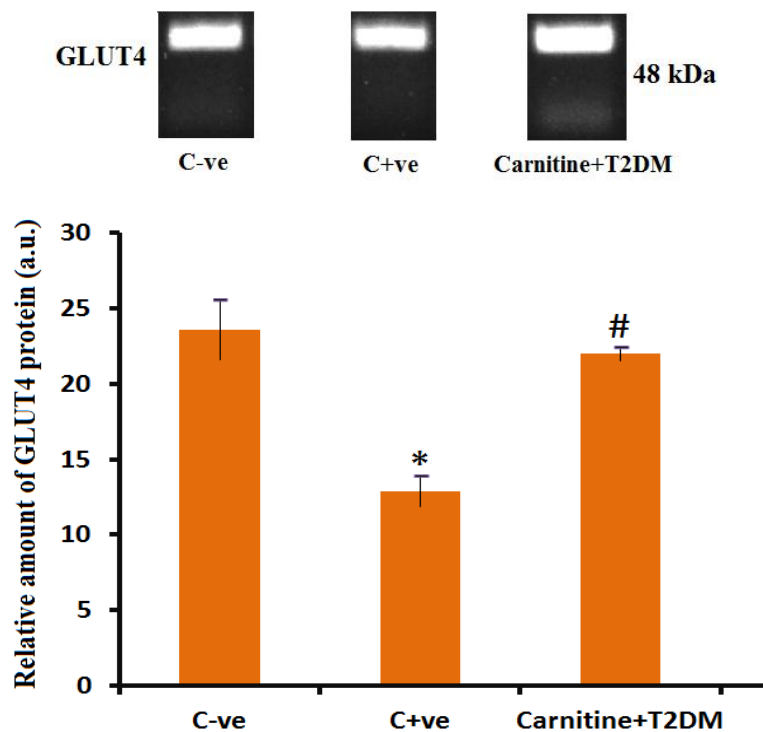


Fig. (6): Effect of carnitine on the relative amount of GLUT4 protein in the soleus muscle of diabetic rat. * indicates significant differences from the value obtained in control negative group and # indicates significant differences from the value obtained in control positive group at the level of $p \leq 0:05$. Values have been expressed as Mean \pm SD.

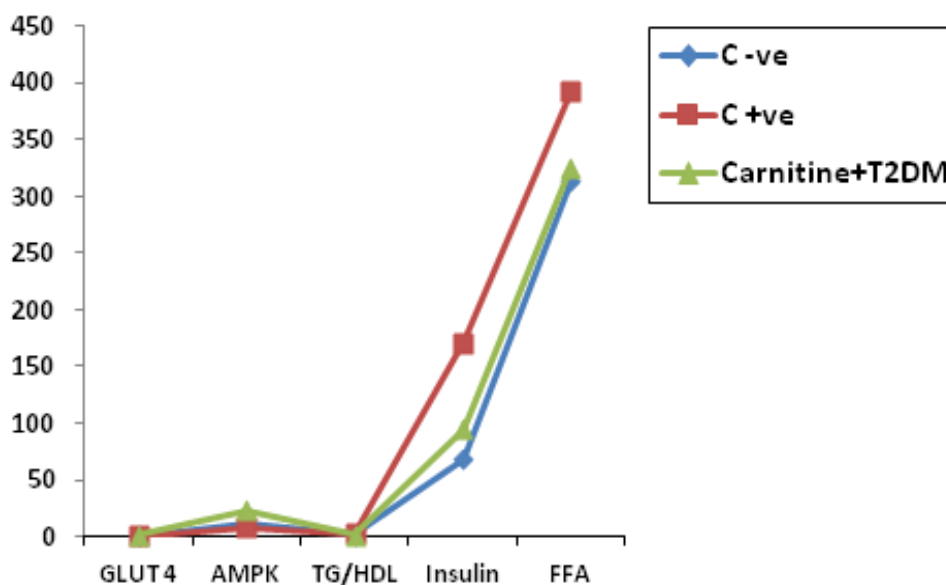


Fig. (7): Correlation between measured parameters in different groups

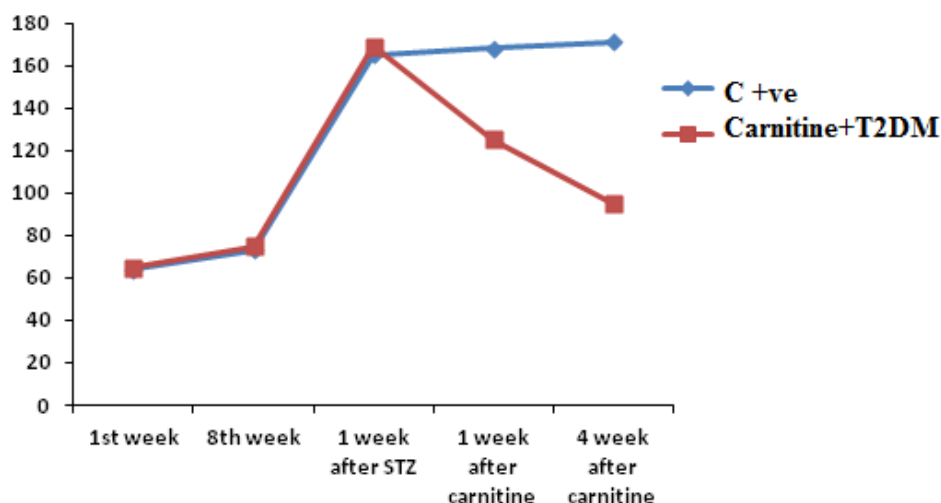


Fig. (8): Non fasting insulin serum levels (pmol/L) all over the period of the experiment in the three groups of rats

DISCUSSION:

In the present study we tested the hypothesis that administration of L-carnitine would increase skeletal muscle GLUT-4 expression and improve the metabolic control in type 2 diabetic rats. To study these effects of carnitine we used the animal model of T2DM developed by **Srinivasan et al.** (20). This model is better than the other models that were based on feeding high sucrose or fructose diet because it is closely resembled the

natural history and metabolic characteristics of human T2DM. These rats, also, gained weight that was greater than the control group thus making it an ideal model of obesity induced T2DM similar to human model with increased body weight, plasma glucose and plasma triglycerides. In this model high fat diet was formulated in such a way that 58% of calories were provided through fat for inducing insulin resistance in rats over a period of two month (27). Then, β -cell destruction by low

dose streptozocin resulted in frank hyperglycemia (20).

Several researches reported that the level of GLUT4 expression in skeletal muscle regulates total body glucose homeostasis (28,29) and the impairment of glucose uptake by the skeletal muscle GLUT4 is a primary defect in type 2 diabetes (30-32). **Koranyiet al.** reported that total GLUT4 concentrations in skeletal muscle have been demonstrated to correlate with muscle glucose uptake capacity and whole body glucose disposal (33). Also, **Russo et al.** demonstrated that selective disruption of GLUT4 transporter in mouse muscle results in a severe insulin resistance and glucose intolerance (32), whereas its over expression ameliorates both glucose and lipid metabolism in transgenic mice.

This research observed that skeletal muscle GLUT4 mRNA levels and GLUT4 protein were significantly decreased in diabetic group. The decrease of GLUT4 expression was associated with a marked increase of both glucose, insulin concentrations and TG: HDL ratio, confirming that the level of expression of this transporter in skeletal muscle may be crucial for the regulation of total body glucose homeostasis and insulin resistance. Carnitine administration resulted in ~2-fold increase in GLUT4 mRNA levels and GLUT4 protein and significant reduction in serum glucose, serum insulin, and TG: HDL ratio. These evidences suggest that the manipulation of GLUT4 expression by different means might be useful for ameliorating overall metabolic control in diabetes.

This research observed also significant decrease in AMPK activity in the soleus muscle of diabetic group as compared to control negative group. While carnitine administration showed significant

increase AMPK activity as compared to diabetic group.

AMPK is an enzyme that has come to be known as a master regulator of metabolism (34) which is activated when cellular energy levels are low (i.e., the intracellular AMP: adenosine triphosphate [ATP] ratio is high). The regulation of AMPK is of great interest in the study of T2DM and metabolic syndrome due to accumulating evidence suggesting that the dysregulation of AMPK plays an important role in the development of IR and T2DM, and that AMPK activation (either physiological or pharmacological) can prevent and/or ameliorate some of the pathologies of IR and T2DM (35). Multiple animal models with a metabolic syndrome phenotype such as ob/ob mice, fa/fa rats, and rats fed a high-fat diet have exhibited decreased AMPK activity in muscle (34) and evidence exists that AMPK activity is diminished in the skeletal muscle (36) or adipose tissue of humans with T2DM or obesity (37). Moreover, long term activation of AMPK-dependent pathways, by endurance exercise or administration of 5-aminoimidazole-4-carboxamide ribonucleoside, a well-known activator of AMPK, has been shown to increase GLUT4 expression, both at the protein and the mRNA levels (38, 39). Our results, demonstrating that carnitine is able to increase GLUT4 expression and to improve glucose metabolism in diabetic animals through activation of AMPK, are in line with previous researches. AMPK can alleviate insulin resistance not only through increasing the expression of GLUT4 but also through regulation of skeletal muscle fatty acid metabolism. AMPK activation results in increasing rates of skeletal muscle fatty acid oxidation by

phosphorylating acetyl-coA carboxylase, leading to reduced malonyl- COA and increased long chain fatty acyl COA flux into the mitochondria via carnitine palmitoyl transferase-1 (40). Studies in human insulin resistance have revealed a clear association between the activation of pro-inflammatory signaling pathways and decreased insulin sensitivity (41, 42). Tumor necrosis factor- α has been implicated in the pathogenesis of insulin resistance because it is elevated in the circulation, skeletal muscle, and adipose tissue of patients with type 2 diabetes (43). High fat diets elevate adipose tissue-derived tumor necrosis factor- α activity and induce increased tissue expression of TNF- α (43, 44). A study has demonstrated that TNF- α can induce skeletal muscle insulin resistance via suppressing AMPK signaling (45). **Zang et al.** have demonstrated that the exposure of rat skeletal muscle cells to TNF- α for 24 hours resulted in phosphorylation of insulin receptor substrate-1 which affects the insulin action. They also displayed that carnitine treatments significantly reversed the TNF- α effects in a dose dependent way through up regulation of insulin receptor substrate-1 activity (46). Also, the anti-inflammatory properties of carnitine may have helped to ameliorate the severity of diabetic pathology, given the known involvement of inflammatory cytokines such as hsCRP, IL-6 in the development of insulin resistance, type 2 diabetes, and its long-term cardiovascular complications (47, 48). Carnitine can modulate the expression of these cytokines (49), and recent studies suggest that it may also have a beneficial effect on cardiovascular mortality associated with diabetes (50, 51).

Moreover, carnitine may improve glucose levels and insulin resistance also through reducing lipid overload. The results of this study showed that T2DM resulted in significant increase in serum FFA, TG, TC, and LDL-C and carnitine administration resulted in significant reduction in all these parameters.

Carnitine plays a key role in the metabolism of FA by regulating its transport between the cytosol and mitochondria (11). It also acts as a cofactor in beta-oxidation by facilitating long chain FA entrance into mitochondria in the form of acyl carnitine esters (carnitine-palmitoyl transferase system CPT-I and II) and the exit of acetyl groups from mitochondria to the cytosol (carnitine acyl transferase system). A reduction in FA transportation to mitochondria facilitates triglyceride accumulation in the cytosol, which is related with the pathogenesis of insulin resistance (52). In carnitine acyl transferase deficient muscle, acetyl-CoA was not converted to its permeable form and was not excreted from mitochondria or cells (53). This led to the over accumulation of acetyl-CoA, which exerted an allosteric inhibiting effect on pyruvate dehydrogenase, a rate-limiting enzyme for pyruvate entry into the tricarboxylic acid cycle, and subsequently impaired glucose utilization. In a clinical study by **Muoio et al.** (53), L-carnitine supplementation in a dose of 2 g/day orally for 6 month resulted in increased serum free carnitine and acetyl- carnitine concentrations, decreased plasma glucose and insulin levels, and decreased the homeostasis model assessment of insulin resistance (HOMA-IR) index. Muscle pyruvate dehydrogenase activity was also increased, and metabolic inflexibility was partially ameliorated by carnitine supplementation.

In conclusion, the results of this study demonstrate that carnitine supplementation improves the metabolic control and ameliorates insulin resistance in T2DM. Also, this study demonstrates that carnitine administration up-regulates GLUT4 protein expression in the skeletal muscles of diabetic rats through increasing the activity of AMPK. So, carnitine may be a possible candidate for the treatment of T2DM and its associated complications especially cardiovascular complications.

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Author contribution

Abeer F. Mostafa and Mohamed Adel designed and performed research.

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