ORIGINAL ARTICLE

EFFECTS OF L-CARNITINE THERAPY ON FAT AND GLUCOSE METABOLISM AMONG MELDONIUM INDUCED CARNITINE DEPLETION IN ALBINO WISTAR RATS

Sumayya Qazi, Mumtaz Ali Qureshi, Abroo Fatima Qazi*, Amin Fahim, Ghulam Shah Nizamani**, Bakhtawar Qureshi*****

Department of Biochemistry, *Physiology, **Pathology, ***Community Medicine, Isra University, Hyderabad, Pakistan

Background: L-carnitine therapy has a potential role in lipid metabolism and glucose homeostasis. Despite its promising effects, further investigation is warranted. This study aimed to determine the impact of L-carnitine therapy of low and high doses on fat and glucose metabolism of albino Wistar rats after Meldonium induced carnitine depletion. **Methods:** A total number of 48 albino Wistar rats were recruited and divided into four groups 12 rats in each group. The bedding material was changed every second day and all animals were given Kaytee Supreme Fortified Daily Diet rat food and clean distal water *ad libitum* at room temperature. The experiment was commenced after 10 days of acclimatization. **Results:** Both high (300 mg/Kg) and low-dose (500 mg/Kg) L-carnitine significantly improved (p<0.05) blood glucose levels, TC, LDL, and HDL after four weeks of L-carnitine therapy. However, high-dose therapy showed significantly higher HDL improvements and decreased TC and LDL levels than low-dose therapy (p<0.05) among Meldonium carnitine-depleted rats. **Conclusion:** L-carnitine high dose therapy (500 mg/Kg of body weight) had a more potent effect on lipid profile in comparison to low dose (300 mg/Kg of body weight).

Keywords: Blood glucose, Carnitine, Cholesterol, lipoproteins, HDL, LDL

Pak J Physiol 2024;20(1):26–9

INTRODUCTION

L-carnitine (LC) is a quaternary amine (3-hydroxy-4-N-trimethylaminobutyrate) that is mainly responsible for the transportation of long-chain fatty acid across the inner membrane of mitochondria for β-oxidation and adenosine triphosphate (ATP) synthesis.[1,2] Besides that, L-carnitine also prevents the pooling of acetyl-CoA that is generated during the process of β-oxidation, thereby preventing the accumulation of fatty acids, protecting the cellular membrane, controlling glycogenesis and ketogenesis and toxic metabolites elimination.[3,4] Evidence is also found in the literature that L-carnitine's effects have improved insulin sensitivity and glucose tolerance.[5,6] Various mechanisms have been put forward in which the desirable impact of L-carnitine on glucose metabolism is established in the literature that includes: 1) mitochondrial oxidation of long-chain acyl-CoA as its accumulation causes insulin resistance 2) improving acety L-CoA to CoA ratio that is essential for pyruvate dehydrogenase complex (PDHC) activity 3) increasing the expression of essential enzymes responsible for gluconeogenesis and glycolysis 4) increasing insulin signalling cascade by improving associated related gene expression and 5) improving insulin growth factor (IGF-1) axis and IGF-1.[7–9] Hence, in this way, L-carnitine is considered an essential factor in regulating glucose metabolism and improving insulin resistance. Multiple studies are also available on data search in which the therapeutic effects of L-carnitine drugs have been discussed. The authors have concluded that various metabolic disorders and carnitine deficiency can effectively be treated by administering exogenous carnitine as a mode of treatment.[10,11] Extensive research has been conducted in recent times in which the positive role of L-carnitine therapy on conditions like chronic kidney disease[12], male and female fertility[13], premature neonates[14], pregnancy[15], fatty liver diseases[16] and myalgic encephalomyelitis[17] has been performed. However, literature still recommends further evidence, particularly its role in metabolic conditions like hyperglycaemia and high blood cholesterol levels. In a study that was performed on mice to whom the non-alcoholic fatty liver disease has been induced through high-fat diet (HFD), the effects of L-carnitine versus nicotinamide adenine nucleotide (NAD^+) versus combination therapy including both LC+NAD and the authors have observed that combination therapy produced better results in improving glucose and fat metabolism in comparison to LC and NAD+ therapy alone.[18] In another study that has been a systematic review and meta-analysis performed on 16 trial studies, it has been concluded by the authors that L-carnitine has a potential role as an adjunctive therapy in diabetes; however, further researches are required for more potent evidence.[19] In order to elucidate the role of L-carnitine in glucose metabolism and insulin resistance, this study aims to determine the effect of effects of low and high L-carnitine therapy doses on fat and glucose metabolism in Wistar rats after Meldonium-induced L-carnitine depletion.

METHODOLOGY

The Comparative Experimental study was conducted in the Department of Biochemistry in collaboration with the Diagnostic and Research Laboratory of Isra University Hospital, Hyderabad from January 2019 to August 2019.

The Animals weighing 200–250 gm were taken and kept at the animal house of Agricultural University, Tando Jam. The animals were housed in polypropylene cages of 43×27×15 cm. Laboratory conditions were maintained at a temperature of 20–25 °C, and they were exposed to 12 hours of light and 12 hours of dark cycle. The bedding material was changed every second day, and all animals were given Kaytee Supreme Fortified Daily Diet rat food and clean distal water ad libitum at room temperature. The experiment was commenced after ten days, and the animals were kept in the lab environment for acclimatization.

A total number of n=48 albino Wistar rats were recruited and divided into four groups, 12 rats in each group. Group A was kept under control, whereas animals in the remaining three groups were administered meldonium mixed with food in the form of powder. Meldonium was administered 100 mg/Kg of body weight for L-carnitine depletion for ten days. No treatment was given after meldonium induction to animals in group B. In contrast, animals in groups C and D were given L-carnitine at a low dose of 300 mg/Kg of body weight and LC, a high dose of 500 mg/Kg daily as a therapeutic agent for four weeks. Blood samples were collected twice through the tail prick method after cleaning it with an alcohol swab at baseline and after four weeks of treatment. A baseline measurement was taken after meldonium induction; post values were measured after four weeks of LC treatment; blood samples were taken after 5 hours of fasting.

Collected blood samples were analyzed for the levels of glucose by using an Accu-Check active glucose meter kit, and levels of cholesterol, including total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were measured using a Cholesterol ELISA assay kit ab285242 (BioVision an Abcam Company).

RESULTS

A total number of n=48 rats were divided into four groups. Each group contain n=12 rats. The weight of rats in each group was measured and compared with another group to maintain the homogeneity of samples between groups. The description is provided in Table-1.

After ten days of keeping animals in the laboratory for acclimatization, meldonium at 100 mg/Kg of body weight was given by mixing with food in the form of powder for ten days (except on animals in the control group) and afterwards, blood test was performed to record the values of fasting blood glucose and lipids levels (TC, HDL and LDL) at baseline followed by therapeutic administration of LC at low dose (300 mg/Kg of body weight) and high dose (500 mg/Kg of body weight) for four weeks to animals in group C and D after which final blood readings were taken to compare the levels of fasting blood sugar (FBS) and lipids from baseline.

Analysis of the findings revealed that both low and high doses of L-carnitine therapeutic drugs were effective in improving fasting blood sugar levels after four weeks of treatment. The values of blood glucose after inducing carnitine depletion through meldonium reduced to 75.6±4.8 mg/dL in group B, 76.3±2.7 mg/dL in low dose carnitine group (group C) and 76.28±2.5 mg/dL in high dose LC group (group D) that had remained non-significant altered I=0.025 in group B with a mean of 76.6±4.9 mg/dL, improved significantly $p<0.05$ in low dose LC group with a mean of 85.62±2.98 mg/dL and in high dose LC group the mean value of 86.1±5.1 mg/dL was found that was significantly different $p<0.001$ from baseline readings (Table-2).

Further, to determine efficacy between low and high-dose LC therapy groups, t-tests were performed that revealed a significant mean difference $p<0.05$ between the control, medium and low and high-dose LC therapy groups. In contrast, no significant ($p=0.16$) mean difference between low and high-dose LC therapy group was found on FBS levels (Table-3).

Further lipid profile analysis was performed at baseline and after four weeks of treatment. Analysis revealed a significant mean difference at $p<0.05$ in the TC, LDL and HDL levels in both low and high doses of L-carnitine treatment (Table-4).

Further, between-group analysis determined the difference between low-dose and high-dose carnitine treatments. Results revealed that high-dose carnitine therapy was significantly effective at $p\leq0.05$ in improving HDL levels and reducing TC and LDL levels compared to low-dose L-carnitine therapy (Table 5).

Table-1: Mean weight of animals allocated in groups

Variables	Number of Samples	Mean±SD	*F*	*F*-critical	*p*
(Group A) Control	12	218.5±5.8	0.619	2.816	0.606
Group B	12	219.9±6.57			
Group C	12	220.9±6.8			
Group D	12	217.8±5.15			

Table-2: Within group analysis of FBS levels mg/dL

Variables	Baseline±SD	Post±SD	*t*-stat	*P*
(Group A) Control	95.8±2.7	96.1±2.1	-0.34	0.36
Group B	75.6±4.8	76.6±4.9	0.68	0.25
Group C	76.3±2.7	85.62±2.98	16.04	0.0001
Group D	76.28±2.5	86.1±5.1	11.72	0.0001

Group A=Control; Group B=Meldonium; Group C=Meldonium+LC 300 mg/Kg; Group D=Meldonium+LC 500 mg/Kg

Table-3: Within group analysis of FBS levels mg/dL

Variables	Groups	Mean±SD	F-ratio	F-critical	*p*	Factors Groups	*p*
Control Group A	B	76.6±4.9	499.07	2.814	0.0001	B	<0.001
	C	85.62±2.98				C	<0.001
	D	86.1±5.1				D	<0.001
Group B	A	96.1±2.1				A	<0.001
	C	85.62±2.98				C	<0.001
	D	86.1±5.1				D	<0.001
Group C	A	96.1±2.1				A	<0.001
	B	76.6±4.9				B	<0.001
	D	86.1±5.1				D	0.16
Group D	A	96.1±2.1				A	<0.001
	B	76.6±4.9				B	<0.001
	C	85.62±2.98				C	0.16

Group A=Control; Group B=Meldonium; Group C=Meldonium+LC 300 mg/Kg; Group D=Meldonium+LC 500 mg/Kg

Table-4: Lipid profile values at baseline and after four weeks of LC treatment

Variables	Group A	Group B	Group C	Group D
TC mg/dL				
Baseline	134.91±13.41	252.55±6.39	252.36±3.47	252.09±11.44
Post	134.36±12.84	252.82±5.42	202.09±7.5	190.45±4.61
***t*-stat**	0.76	-0.31	18.59	16.98
***t*-critical**	1.81	1.81	1.81	1.81
p	0.23	0.37	0.001	0.001
LDL mg/dL				
Baseline	123.27±4.45	161.18±4.69	161.09±2.98	160.36±4.11
Post	123.18±4.98	159.55±2.42	139.18±5.55	129.64±4.39
***t*-stat**	0.09	1.61	14.53	15.33
***t*-critical**	1.81	1.81	1.81	1.81
p	0.46	0.06	0.001	0.001
HDL mg/dL				
Baseline	57.82±2.71	30.09±14.19	33.91±3.36	32.82±3.84
Post	58.27±2.69	31.36±10.05	42.18±2.48	57±4.36
***t*-stat**	-1.11	-0.95	-7.07	-11.48
***t*-critical**	1.81	1.81	1.81	1.81
p	0.29	0.18	0.001	0.001

Group A=Control; Group B=Meldonium; Group C=Meldonium+LC 300 mg/Kg; Group D=Meldonium+LC 500 mg/Kg

Table-5: Comparing effectiveness of low dose and high dose LC therapy on lipid profile

Variables	Group C	Group D
TC (mg/dL) at week four	202.09±7.5	190.45±4.61
***t*-stat**	5.93	
***t*-critical**	1.81	
p	0.0001	
LDL (mg/dL) at week four	139.18±5.55	129.64±4.39
***t*-stat**	3.61	
***t*-critical**	1.81	
p	0.002	
HDL (mg/dL) at week four	42.18±2.48	57±4.36
***t*-stat**	-13.69	
***t*-critical**	1.81	
p	0.0001	

DISCUSSION

The findings of this study revealed that L-carnitine low dose and high dose treatment, i.e., 300 mg/Kg and 500 mg/Kg of body weight respectively, was found to be equally effective in improving the fasting blood sugar levels among meldonium-induced carnitine depletion rats. However, on lipid profile levels that include TC, HDL and LDL, high-dose carnitine treatment was found to be significantly $p<0.05$ more effective in increasing HDL levels and reducing TC and LDL levels compared to the dose treatment approach. The findings of this study were according to the findings of another conducted study in which L-carnitine supplementation therapy of 50 mg/Kg of body weight recommended to children diagnosed with chronic kidney disease (CKD) and were going through haemodialysis turned out to be significantly effective ($p<0.05$) in improving the levels of c-reactive protein and fasting blood sugar levels.[20]

In another study, an association between L-carnitine levels and various cardiovascular disease biomarkers was identified, and it concluded that a significant ($p=0.042$) negative correlation exists between L-carnitine and triacylglycerol levels and blood glucose levels ($p=0.048$). In contrast, a significant positive correlation ($p=0.049$) exists between LC and HDL levels.[21] The findings of that study reflect our findings that LC treatment effectively maintained blood glucose levels and controlled cholesterol and LDL levels. Besides that, it also had a crucial impact on increasing HDL levels in blood.

A study was conducted to determine the effects of three different doses of L-carnitine that were 50, 100, 200 and 300 mg/Kg of body weight, being given to streptozotocin (STZ) induced diabetic rats to determine its impact on the histopathology of the pancreas of STZ induced diabetic rats and compared the same with diabetic control group it was found that LC of 300 mg/Kg of body weight found to be better than other low dose LC treatment and concluded that LC treatment approach had a significant role in improving histopathology of the pancreas and had an antioxidant action as well.[22]

Thus, based on the literature, it was evident that LC treatment significantly improved blood sugar, TC and LDL levels and increased HDL levels. However, for more robust findings based on its effectiveness in response to different dosages, further studies are required to gather more conclusive evidence.

CONCLUSION

The study has provided evidence that L-carnitine treatment decreased fasting blood sugar levels, total cholesterol, and LDL levels, as well as improved HDL levels among meldonium-induced carnitine depletion rats. L-carnitine high-dose therapy (500 mg/Kg of body weight) had a more pronounced effect on lipid profile than low-dose therapy (300 mg/Kg). The impact of L-carnitine on blood glucose levels in both high and low-dose treatment approaches is found to be similar.

REFERENCES

1. Thakur Y, Bharti R, Sharma R. Myths and reality of L-carnitine (3-Hydroxy 4-N trimethylammonium butyrate) supplementation and its chemistry: A systematic review. SCPINM-2021. Materials Today: Proceedings 2022;48:1277–82.
2. Delgado CA, Balbueno Guerreiro GB, Diaz Jacques CE, de Moura Coelho D, Sitta A, Manfredini V, *et al*. Prevention by L-carnitine of DNA damage induced by 3-hydroxy-3-methylglutaric and 3-methylglutaric acids and experimental evidence of lipid and DNA damage in patients with 3-hydroxy-3-methylglutaric aciduria. Arch Biochem Biophys 2019;668:16–22.
3. Yamada K, Taketani T. Management and diagnosis of mitochondrial fatty acid oxidation disorders: focus on very-long-chain acyL-CoA dehydrogenase deficiency. J Hum Genet 2019;64(2):73–85.
4. Macchione F, Salviati L, Bordugo A, Vincenzi M, Camilot M, Teofoli F, *et al*. Multiple acyl-COA dehydrogenase deficiency in elderly carriers. J Neurol 2020;267(5):1414–9.
5. EL-Sheikh HM, EL-Haggar SM, Elbedewy TA. Comparative study to evaluate the effect of L-carnitine plus glimepiride versus glimepiride alone on insulin resistance in type 2 diabetic patients. Diabetes Metab Syndr 2019;13(1):167–73.
6. Sangouni AA, Pakravanfar F, Ghadiri-Anari A, Nadjarzadeh A, Fallahzadeh H, Hosseinzadeh M. The effect of L-carnitine supplementation on insulin resistance, sex hormone-binding globulin and lipid profile in overweight/obese women with polycystic ovary syndrome: A randomized clinical trial. Eur J Nutr 2022;61(3):1199–207.
7. Li LY, Limbu SM, Ma Q, Chen LQ, Zhang ML, Du ZY. The metabolic regulation of dietary L-carnitine in aquaculture nutrition: Present status and future research strategies. Rev Aquac 2019 Nov;11(4):1228–57.
8. Bene J, Hadzsiev K, Melegh B. Role of carnitine and its derivatives in the development and management of type 2 diabetes. Nutr Diabetes 2018;8(1):8.
9. Li N, Zhao H. Role of carnitine in non-alcoholic fatty liver disease and other related diseases: An update. Front Med (Lausanne) 2021;8:689042.
10. Gao X, Tian Y, Randell E, Zhou H, Sun G. Unfavorable associations between serum trimethylamine N-oxide and L-carnitine levels with components of metabolic syndrome in the new foundland population. Front Endocrinol (Lausanne) 2019;10:168.
11. Wang W, Bai M, Jiang T, Li C, Li P, Zhou H, *et al*. Clozapine-induced reduction of L-carnitine reabsorption via inhibition/down-regulation of renal carnitine/organic cation transporter 2 contributes to liver lipid metabolic disorder in mice. Toxicol Appl Pharmacol 2019;363:47–56.
12. Chen YC, Cheng CY, Liu CT, Sue YM, Chen TH, Hsu YH, *et al*. Combined protective effects of oligo-fucoidan, fucoxanthin, and L-carnitine on the kidneys of chronic kidney disease mice. Eur J Pharmacol 2021;892:173708.
13. Agarwal A, Sengupta P, Durairajanayagam D. Role of L-carnitine in female infertility. Reprod Biol Endocrinol 2018;16(1):5.
14. Beshir SA, Zannoun MA, El Samanoudy MI, AL-Samee SH. Evaluation of the Effect of L-Carnitine Supplementation in Preterm Neonates Suffering from Respiratory Distress Syndrome. Int J Med Arts 2022;4(6):2400–6.
15. Kim MK, Park JK, Paek SK, Kim JW, Kwak IP, Lee HJ, *et al*. Effects and pregnancy outcomes of L-carnitine supplementation in culture media for human embryo development from in vitro fertilization. J Obstet Gynaecol Res 2018;44(11):2059–66.
16. Thiagarajan P, Chalmers J, Ban L, Grindlay D, Aithal GP. L-carnitine supplementation in non-alcoholic fatty liver disease: A systematic review and meta-analysis. World J Meta-Anal 2020;8(1):4–14.
17. Virmani MA, Cirulli M. The Role of L-Carnitine in Mitochondria, Prevention of Metabolic Inflexibility and Disease Initiation. Int J Mol Sci 2022;23(5):2717.
18. Salic K, Gart E, Seidel F, Verschuren L, Caspers M, van Duyvenvoorde W, *et al*. Combined treatment with L-carnitine and nicotinamide riboside improves hepatic metabolism and attenuates obesity and liver steatosis. Int J Mol Sci 2019;20(18):4359.
19. Ko J, Wong EY, Tran HN, Tran RJ, Cao DX. The glycemic, cholesterol, and weight effects of L-carnitine in diabetes: a systematic review and meta-analysis of randomized controlled trials. Diabetes Epidemiol Manage 2022;10(1):100122.
20. Hamedi-Kalajahi F, Imani H, Mojtahedi S, Shabbidar S. Effect of L-carnitine supplementation on inflammatory markers and serum glucose in hemodialysis children: a randomized, placebo-controlled clinical trial. J Ren Nutr 2022;32(2):144–51.
21. Lin TJ, Tang SC, Liao PY, Dongoran RA, Yang JH, Liu CH. A comparison of L-carnitine and several cardiovascular-related biomarkers between healthy vegetarians and omnivores. Nutrition 2019;66:29–37.
22. Masoumi-Ardakani Y, Aminizadeh S, Fallah H, Shahouzehi B. L-carnitine different doses affect serum and pancreas tissue Antioxidative defense and histopathology in STZ-induced diabetic rats. Biologia 2020;75(9):1415–23.

Address for Correspondence:

Dr Sumayya Qazi, Department of Biochemistry, Isra University, Hyderabad, Pakistan. **Cell:** +92-334-2166554
Email: drsumayyaqazi@gmail.com

Received: 28 Nov 2023 **Reviewed:** 12 Mar 2024 **Accepted:** 18 Mar 2024

Contribution of Authors:

SQ: Research concept and design, writing of article and final approval
MAQ: Research concept and design, Critical revision of article and final approval of article
AFQ: Critical revision of article and final approval
AF: Data analysis and interpretation and writing of article
GSN: Collection and assembly of data, Critical revision of article
BQ: Collection and assembly of data, Critical revision of article

Conflict of Interest: None
Funding: None