Journal of Pakistan Association of Dermatologists. 2023;**33(4)**:1359-1364.

Original Article

Effects of L-Carnitine supplementation on relative gene expression of OCTN1, OCTN2, OCTN3 in mitochondria and skin in meldonium induced carnitine depleted male albino Wistar Rats

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Abstract

Background L-carnitine supplementation may have a positive impact on the expression of genes involved in fatty acid metabolism, including the OCTN genes that are responsible for L-carnitine uptake into cells. However, further research is needed to fully understand the molecular mechanisms underlying these effects and their implications for human health.

Methods A total number of n=48 Albino Wistar rats were recruited and divided into four groups 12rats in each group. Group A was kept control whereas animals in remaining three groups were administered meldonium mixed with food in the form of powder. Meldonium was administered at 100mg/kg of body weight for the purpose of L-carnitine depletion for 10 days.

Results Relative OCTN 1 gene expression in group A was noted as 1.2 ± 0.01 in contrast to the experimental groups B 2.1±0.1, C 4.6±0.7, D 6.2±0.8 and E as 8.0±0.9. Low and high doses of L-carnitine upregulated the expression of OCTN2 gene (P= 0.0001). The skin of rats was found to be loose on touch during experimental animal handling in Meldonium induced group, however, firmness was raised in groups which were given L-carnitine supplementation in low and high doses respectively.

Conclusion Overall, findings suggest that L-carnitine supplementation has a positive impact on the expression of genes involved in fatty acid metabolism, including the OCTN genes that are responsible for L-carnitine uptake into cells. The firmness of skin increased with low and high doses of L-carnitine supplementation in different groups of rats respectively.

Key words
Organic cation transporter OCTN protein; Rat; Gene expression; Metabolism.

Introduction

L-carnitine is an amino acid-like compound that plays a crucial role in the metabolism of fatty acids. It is synthesized in the liver and kidneys from the amino acids lysine and methionine and is found in many foods, particularly in red meat.[1] Organic Cation Transporter Novel (OCTN) is a family of membrane transporters that are involved in the uptake of organic cations into cells. These transporters are expressed in a wide range of tissues, including the liver, kidney, intestine, and skeletal muscle. There are three known members of the OCTN family: OCTN1, OCTN2, and OCTN3. OCTN1 and OCTN2 are the most widely studied members of

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the family, and are involved in the transport of a variety of endogenous and exogenous compounds, including L-carnitine, acetyl-L-carnitine, ergothioneine, and several drugs.[2] OCTN1 is primarily expressed in the intestine, liver, and kidney, and is involved in the uptake of organic cations into these tissues. OCTN2, on the other hand, is expressed in a wider range of tissues, including skeletal muscle, heart, and placenta, and is the main transporter responsible for the uptake of L-carnitine into cells. Mutations in the gene encoding OCTN2 have been associated with primary carnitine deficiency, a rare genetic disorder characterized by a reduced ability to transport L-carnitine into cells.[3] This can lead to a variety of symptoms, including muscle weakness, hypoglycemia, and cardiomyopathy. L-carnitine is known to play an important role in regulating the expression of genes involved in fatty acid metabolism, including the OCTN genes that encode the transporters responsible for L-carnitine uptake into cells. Several studies have investigated the effects of L-carnitine supplementation on OCTN gene expression in various cell types and tissues.[4-6] In a study of rat skeletal muscle, L-carnitine supplementation was found to increase the expression of both OCTN1 and OCTN2, as well as several other genes involved in fatty acid metabolism.[7-8] In other studies in human skeletal muscle cells found that L-carnitine supplementation increased the expression of OCTN2, as well as genes involved in mitochondrial function and energy metabolism.[9-11] Overall, these findings suggest that L-carnitine supplementation may have a positive impact on the expression of genes involved in fatty acid metabolism, including the OCTN genes that are responsible for L-carnitine uptake into cells. However, further research is needed to fully understand the molecular mechanisms underlying these effects and their implications for human health. Hence the present study is aimed to identify the effects of L-carnitine on relative gene expression of OCTN gene among Wistar rats.

Methods

This is a comparative experimental study and was conducted in the department of Biochemistry in collaboration with Diagnostic and Research Laboratory of Isra University Hospital, Hyderabad

Animals Caging and Laboratory Conditions The Animals weighing 200-250gm were taken at kept at animal house of Agricultural University, Tando Jam. The animals were housed in polypropylene cages of 43x27x15 cm in length. Laboratory condition was maintained at temperature of 20 to 25^{0}C and 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 10 days and the animal were kept in the lab environment for acclimatization.

Study Protocol A total number of n=48 Albino Wistar rats were recruited and divided into four groups 12rats in each group. Group A was kept control whereas animals in remaining three groups were administered meldonium mixed with food in the form of powder. Meldonium was administered at 100mg/kg of body weight for the purpose of L-carnitine depletion for 10 days. No treatment was given after meldonium induction to animals in group B whereas animals in group C and D were given L-carnitine low dose 300mg/kg of body weight and LC high dose 500mg/kg of body weight daily as a therapeutic agent for 4 weeks. The gene analysis was performed on OCTN1, OCTN2, OCTN3 axon by the following primer sequence. OCTN1 primers were designed (antisense:

ACTGCCCATGAGGATGTAGG in exon 9; sense: TC-GTGCAAGTTGAGGAGAGGCGGGAAGC) exon 6. OCTN2 primers were designed (antisense: CAAGCTGTGTGTGG-TTAGCCTTGG; sense: TCTCCCGCTCTGCGGTATCC) based on the published sequence of the rat small intestine OCTN2 Cdna. OCTN3 primers were designed (antisense: TTCGGGATACATTTCCTTTAGA-CTCC; sense: CTGCCGACTCTATGCTTGACTACG) based on the published sequence of mouse OCTN3 cDNA.[12]

Results

A total number of n=60 Albino Wistar rats were used for the purpose of this study, animals were allocated into one of the five groups n=12 in each group by using a convenient method of randomization technique. Animals in group A were controlled to whom neither meldonium was administered nor was L-carnitine therapy given. Group B was experimental control to whom meldonium was administered at a dose of 100mg/kg. Animals in group C were also experimental control group to whom meldonium reversal was administered orally for 10 days on a same dose as set for animals in group B. The next two groups were experimental group, group D animals were initially induced with meldonium followed by low dose L-carnitine therapy and group E after meldonium induction was treated with high dose L-carnitine therapy.

Table 1 Demonstrates the average weight and standard deviation of rats in groups

Variables	*N*	*Weight*	*Standard Deviation*
Group A		210	5.4
Group B		225	6.5
Group C	15	214	4.9
Group D		235	8.2
Group E		239	8.9

The mean weight of animals divided in five groups was illustrated in **Table 1**.

Effects of treatment and the comparison with the controlled group was evaluated by applying inferential statistical test one-way analysis of variance and results obtained were illustrated as under:

Relative OCTN 1 Gene Expression in control and experimental groups of rats

Relative OCTN 1 gene expression in group A was noted as 1.2±0.01 in contrast to the experimental groups B 2.1±0.1, C 4.6±0.7, D 6.2±0.8 and E as 8.0±0.9. Low and high doses of L-carnitine upregulated the expression of OCTN2 gene (P= 0.0001) **(Table 2)**.

Relative OCTN 2 Gene Expression in control and experimental groups of rats

Relative OCTN 2 gene expression in group A was noted as 1.1±0.1 in contrast to the experimental groups B 1.2±0.1, C 10±0.9, D 14±1.8 and E as 27±2.1. Low and high doses of L-carnitine upregulated the expression of OCTN2 gene (P= 0.0001) **(Table 3)**.

Table 2 Relative OCTN 1 Gene Expression in control and experimental groups of rats.

Groups	*Induction*	*Mean*	*SD*	*F value*	*P value*
A (Control)	N/A	1.2	0.01	32.1	0.001
B (Experimental control)	Meldonium	2.1	0.1		
C (Experimental control)	100kg/wt	4.6	0.7		
D Low Dose	Meldonium				
(L-carnitine)	100kg/wt +L-carnetine 100kg/wt	6.2	0.8		
E High Dose(Carnetine)	Meldonium				
	100kg/wt +L-carnetine 500kg/wt	8.0	0.9		

Table 3 Relative OCTN 2 Gene Expression in control and experimental groups of rats.

Groups	*Induction*	*Mean*	*SD*	*F value*	*P value*
A (Control)	N/A	1.1	0.1	81.4	0.001
B (Experimental control)	Meldonium	1.2	0.1		
C (Experimental control)	100kg/wt	10	0.9		
D Low Dose	Meldonium				
(L-carnitine)	100kg/wt +L-carnitine 100kg/wt	14	1.8		
E High Dose	Meldonium				
(L-carnitine)	100kg/wt +L-carnitine 500kg/wt	27	2.1		

Table 4 Relative OCTN 3 Gene Expression in control and experimental groups of rats.

Groups	*Induction*	*Mean*	*SD*	*F value*	*P value*
A (Control)	N/A	0.2	0.01	29.77	0.001
B (Experimental control)	Meldonium	0.06	0.1		
C (Experimental control)	100kg/wt	0.02	0.1		
D Low Dose	Meldonium				
(L-carnitine)	100kg/wt +L-carnitine 100kg/wt	0.06	0.002		
E High Dose	Meldonium				
(L-carnitine)	100kg/wt +L-carnetine 500kg/wt	0.20	0.03		

Relative OCTN 3 Gene Expression in control and experimental groups of rats

Relative OCTN 3gene expression in group A was noted as 0.2±0.01 in contrast to the experimental groups B 0.06±0.1, C 0.02±0.1, D 0.06±0.002 and E as 0.20±0.03. Low and high doses of L-carnitine did not up regulated the expression of OCTN 3 gene **(Table 4)**.

Further incidental finding on examination of texture of skin revealed that in control group no change in texture was observed whereas in experimental group induced with Meldonium 100kg/wt skin get loosed whereas in Low Dose and High dose Carnitine group, firm skins were observed. Whereas no change in skin of rats in group C were observed as shown in **Table 5**.

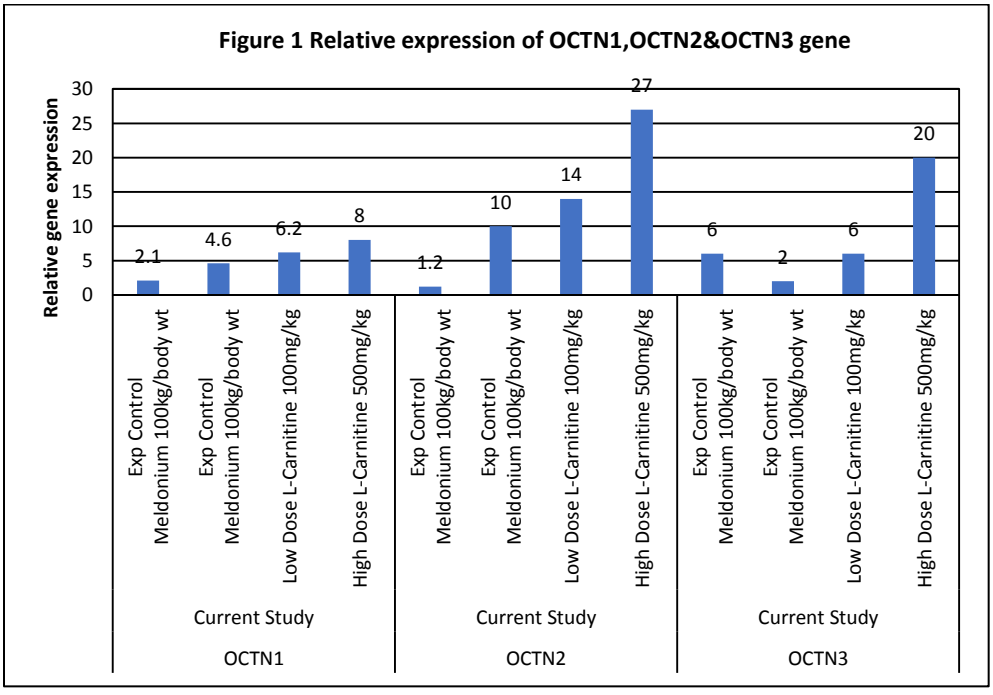
Discussion

The analysis of the results of the present study had revealed that L-Carnitine had significantly up regulated the expression of OCTN1 and OCTN2 gene, however no such differential up regulation in the expression of OCTN3 gene was noted. The electronic data search on various search engine such as Google scholar, Pedro and Cohcrane had not provided any such literature in which the effects of low and high dose of L-Carnitine therapy was determined on the relative gene expression of the above mention genes, however in a such study the effects of carnitine therapy on mRNA expression of OCTN2, mitochondrial OCTN1 and peroxisomal OCTN3 were determined on quadriceps, gluteus and myocardium of adult mdx mice and it was reported in the study that carnitine induction had up regulated the mRNA of OCTN1 and OCTN2 in the quadriceps muscle and up regulation of OCTN1 and OCTN3 in the myocardium but no such increase in the protein expression was noted.[13] Hence suggesting the fact that mRNA expression of all three gene increased with the administration of L-carnitine. The findings of this study supported the finding of present study as the authors of present study had noted a significant up regulation of OCTN1 and OCTN2 gene after the administration of low and high dose of L-carnitine of 100mg/kg and 500mg/kg

Table 5 Texture of skin on touch.

Groups	Induction	Texture of skin on Observation (incidental finding) in different groups with ingestion of carnitine (Oral) and Meldonium induced blockage of carnitine shuttle in mitochondria
A (Control)	N/A	Normal texture
B (Experimental Control)	Meldonium 100kg/wt	Loose skin
D (Low Dose Carnitine)	Meldonium 100kg/wt +L-carnitine 100kg/wt	Firm skin (+)
E (High dose Carnitine)	Meldonium 100kg/wt +L-carnitine 500kg/wt	Firm skin (++)

of body weight, whereas no such up regulation was noted in the relative gene expression of OCTN3. There is limited scientific evidence to support the notion that L-carnitine has a direct effect on skin texture. However, L-carnitine has been shown to have potential benefits for overall skin health and appearance, particularly in combination with other ingredients. In terms of skin health, L-carnitine has been shown to have antioxidant properties, which may help to protect the skin from damage caused by free radicals. Free radicals are unstable molecules that can damage cells and contribute to aging and disease. By reducing oxidative stress, L-carnitine may help to improve the overall health and appearance of the skin. Furthermore, L-carnitine has been found to have potential benefits for wound healing and skin regeneration. A study published in the Journal of Cosmetic Dermatology found that a combination of L-carnitine and panthenol (a form of vitamin B5) improved the healing of skin wounds in rats. While this research was conducted on animals, it suggests that L-carnitine may have potential benefits for skin healing in humans.[14]



Conclusion

Overall, findings suggest that L-carnitine supplementation may have a positive impact on the expression of genes involved in fatty acid metabolism, including the OCTN genes that are responsible for L-carnitine uptake into cells. However, further researches are needed to fully understand the molecular mechanisms underlying these effects and their implications for human health. The skin was found to be loose in Meldonium induced group and firm in rats which were treated with low and high doses of L Carnitine.

Moreover, it has also been suggested by the authors that the upcoming researches must be based on comparative analysis of various therapeutic regimes in order to determine the efficacy of treatment approaches for better estimation of dose response analysis.

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