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Original Article

The Effect of Genistein, L-carnitine and their combination on gene expression of hepatocyte *PPAR-α* and *CPT-1* in experimental nephrotic syndrome

Abbas Yousefinejad[1], *Fereydoon Siassi*[2], *Abbas Mirshafiey*[3], *Fariba Koohdani*[1], *Mohammad-Hassan Javanbakht*[1], *Reza Sedaghat*[4], *Atena Ramezani*[5], *Hamed Mohammadi*[1], *Mahnaz Zarei*[2], *Mahmoud Djalali*[1]

[1]Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran; [3]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [4]Department of Anatomy and Pathology, School of Medicine, Shahed University, Tehran, Iran; [5]Department of Nutrition, School of Public Health, Mazandaran University of Medical Sciences, sari, Iran; Student Research Committee, Department of Clinical Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran.

Summary. *Objective:* One of the disorders that occur in nephrotic syndrome would be hyperlipidemia. L-carnitine and genistein have the capability to control this syndrome by changing in lipid metabolism. In the present study, we delved into the effects of Genistein and L-carnitine on the *PPAR-α* and *CPT-1* gene expressions in experimental nephrotic syndrome. *Methods:* In controlled experimental study, 50 male Sprague–Dawley rats were randomly divided into five groups consisting of 10 animals each with similar mean body weights (300±50 g): NC (normal-control), PC (patient-control), LC (L-carnitine), G (genistein), and LCG (L-carnitine-genistein). The spot urine samples were collected, and urine protein-to-creatinine ratio was measured. Hepatocytic RNA was extracted and real-time PCR was used for *PPAR-α* and *CPT-1* gene Expression measurement. *Results:* At the end of the study, final weight of the patient groups was considerably lower than the NC group (P=0.001), and weight gain of the NC group was higher than the other groups (P<0.05). The urine protein and urine protein-to-creatinine ratio were significantly lower in LC, G, and LCG groups in comparison with PC group, at week 7 (P<0.001). The expression of *PPAR-α* and *CPT-1* mRNA were not significantly higher in LC,G, and LCG groups in comparison with PC group, but *ΔCT* of these genes showed significant differences between the LC,G, and LCG groups and the PC group (P<0.001). *Conclusion:* Our study showed an increasing trend in *PPAR-α* and *CPT-1* gene expressions, and synergistic effect of L-carnitine and genistein in experimental nephrotic syndrome.

Key words: CPT-1; Gene; Genistein; L-carnitine; Nephrotic syndrome; PPAR-α

Introduction

Nephrotic syndrome is a disorder arising from renal damage, and causes extreme protein leakage into the urine (1). One of the complications that occur alongside this disease is hyperlipidemia (2). It leads to high levels of cholesterol (hypercholesterolemia): specifically, there is a considerable increase in low density lipoprotein-cholesterol (LDL-C) along with increases in the serum of very low density lipoprotein-cholesterol (VLDL-C) concentration. Consequently, the risk of cardiovascular diseases is higher. Therefore, it reflects the importance of lipid metabolism control in this syndrome (3). Food phyto-estrogens are, in point

of fact, a subgroup of flavonoids that have extended benefits for human health: for this reason, many studies focus on their effects (3). This group comprises several subgroups of non-steroid estrogens, including isoflavones and lignans widely distributed among plants (3). Isoflavones (genistein and daidzein) are the most significant groups being widely studied. However, these isoflavones exist only in soy beans and several other types of legumes (3). Despite the fact that soy functionality mechanism in bringing down the level of serum cholesterol has not been made clear to the full extent, soy protein has been applied for purposes of clinical treatment of hypercholesterolemia for many decades (4). The substances, such as phyto-estrogens, have the capacity to control cardiovascular diseases through bringing about changes in lipid metabolism (5). The profitable effects of dietary soy protein on the serum lipid concentrations have been highlighted in a wide range of different investigations that have been conducted both on animal and human cells (5-7). The key topic of whether any response to soy protein would be possibly mediated through the presence of isoflavones such as genistein,is important.

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine (8). This compound is actually needed in living cells for purposes of transporting fatty acids from cytosol to mitochondria during the process of lipid degradation and procreating the needed energy (9). Lately, the role played by carnitine in energy metabolism and in gene expression involved in lipid metabolism is taken into consideration. It has been proven that L-carnitine (a chemical structure of carnitine) is able to statistically significantly bring down the levels of triglycerides within rats' tissues and plasma (10).

Peroxisome Proliferator-Activated Receptor-alpha (PPAR-α) is a ligand-dependent transcription factor, a type of nuclear receptors (11). This factor can control fatty acid oxidative metabolism by means of the inducing transcription of carnitine-palmitoyltransferase-1 (CPT-1) in addition to several other enzymes intended for manipulating and/or controlling lipid oxidation (12-13). Genistein can cause some increase in PPAR-α (14, 15) and CPT-1 (16) gene expression. L-carnitine is also able to bring about an increase in the gene expression of PPAR-α (17) and CPT-1 (18, 19). Notwithstanding this fact, the conducted investigations concerning the twin-effects of genistein and L-carnitine on the expressivity of these two mentioned genes are very limited. The lack of comprehensive studies in this regard has given rise to the design and conducting of present study, bearing in mind as the goal: the investigation of genistein and L-carnitine effects both singularly (separately) and in conjunction with one another as for the gene expression of PPAR-α and CPT-1 in experiential nephrotic syndrome.

Methods

Animals and experimental diets

In this controlled experimental study, male Sprague–Dawley rats at 8 weeks of age were housed individually in a room with controlled temperature (20–22°C), humidity (55–65%), and lighting (from 0700 to 1900 h), and fed assigned experimental diet (AIN 93 M diet, table 1) (20). After 7 days of acclimatization to the conditions, the rats were randomly divided into five groups, consisting of 10 animals each with similar mean body weights (300±50 g): Normal-control (NC), Patient-control (PC), L-carnitine (LC), genestein (G), and L-carnitine-genistein (LCG). We used CMC (Carboxymethyl cellulose dissolved in distilled water) as a suspending agent for genistein and L-carnitine.

All groups of rats received the experimental diet during the study (8 weeks). NC group received daily 50 mg/kg body weight CMC suspension (concentration: 0.5 mg/ml) by gavage for 8 weeks. PC group received 50 mg/kg body weight daily CMC by gavage for 8 weeks and 7.5 mg/kg body weight single dose Adriamycin (an agent that induces nephrotic syndrome), through tail vein at the end of week 2 (21), and then, we kept on gavage feeding of rats with CMC for 6 weeks. LC, G, and LCG groups were similar to PC group, but just with a difference; instead of only CMC, they received daily 50 mg/kg body weight L-carnitine, 50 mg/kg body weight genistein, and 50 mg/kg body weight L-carnitine plus 50 mg/kg body weight genistein, respectively. For reaching this dosage, 15 mg genistein or L-carnitine was dissolved in

0.5 cc CMC (concentration: 0.5 mg/ml) for reaching the final solution (concentration: 26 mg/ml).

Animals were maintained ad libitum on assigned experimental diet and water during the experiment,t and dietary intake and body weight were recorded every day and weekly, respectively, and weight change was measured by the difference recorded at the end of the weeks and expressed change per day. This study was approved by the review board of animal ethics of Tehran University of Medical Sciences (TUMS), and we followed the institute's guidelines in the care and use of laboratory animals.

Sample preparation

During the study, we collected urine samples at the end of weeks 2, 3 and 7, and measured urine protein-to-creatinine ratio from spot urine samples. After 8 weeks, the animals were sacrificed after overnight fasting for 12 hours. Blood was collected from abdominal aorta, and livers were surgically excised. The blood was allowed to coagulate and centrifuged at 1,100 g for 15 minutes, and serum was stored at -20˚C until the assays. Liver tissues were collected and immediately frozen in liquid nitrogen, placed in 1.5-mL Eppendorf tubes, and stored at -80˚C until analysis.

Table1. Composition of experimental diet (AIN-93 modified diet for rodents)

Ingredient	g/kg diet
Cornstarch	465.692
Casein (>85% protein)	140.000
Extrinized cornstarch (90-94% tetrasaccharides)[1]	155.000
Sucrose	100.000
Soybean oil (no additives)	40.000
Fiber[2]	50.000
Mineral mix	35.000
Vitamin mix	10.000
L-Cystine	1.800
Choline bitartrate (41.1% choline)[3]	2.500
Tert-butylhydroquinone	0.008
	u/kg diet
Total energy[4] kcal	3601.0
protein%	14.1
CHO%	75.9
fat%	10.0

[1]Dyetrose (Dyets, Bethlehem, PA) and Lo-Dex 10 (American Maize, Hammond, IN) meet these specifications. An equivalent product may also be used.
[2]Solka-Floc®,200 FCC (FS&D, St. Louis, MO) or its equivalent is recommended.
[3]Based on the molecular weight of the free base.
[4]The estimate of caloric content was based on the standard physiological fuel values for protein, fat, and carbohydrate of 4, 9 and 4, respectively.

RNA Extraction from Liver

Hepatocytic RNA was extracted and purified using RNeasy plus Mini Kit (Qiagen, Valencia, Calif., USA) according to the manufacturer's protocol. Quantity and purity of extracted RNA was checked by NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Del., USA). A ratio of A 260/280 between 1.9 and2.1 was taken into account as pure RNA. Single-strand cDNA was synthesized using QuantiTect Reverse Transcription Kit (Qiagen).

Real-time PCR for Gene Expression

PCR primers for PPAR-alpha and CPT-1 genes and 18-S gene (as housekeeping) were designed by Primer express 3 software (Applied Biosystem, Foster city, CA, USA) (Table 2).

PCR reactions were briefly as follows: PCR proceeded in special optical tubes in 48- reaction plates (MicroAmp Optical, ABI) with 20 µl reaction mixture containing 10µl Power SYBR® Green PCR Master Mix (Applied Biosystem, Foster city, CA, USA), 7µl DEPC treated water, 0.5µl forward primer, 0.5µl reverse primer ,and 2µl cDNA as template. The wells were sealed with optical adhesive film (Applied Biosystem, Foster city, CA, USA), and the plate was centrifuged for a few seconds at high speed. Amplification conditions were performed using the standard two-step run protocol; step 1:10s at 95˚C, step 2: 40 cycles of 15s at 95˚C plus 1s at 60˚C. After completion of amplification cycles, melt curve was generated to verify if a single gene product had been amplified. For this study, duplicate reactions of the same sample were run.

For each gene, mRNA expression level was normalized to the level of 18-S. The fold changes of genes

Table2. Primer sequences for real-time PCR

Gene name	Sequence	Length	TM	CG%
PPAR-alpha	Forward 5´-TGTATGAAGCCATCTTCACG-3´	20	50.96	45
	Reverse 5´-GGCATTGAACTTCATAGCGA-3´	20	51.57	45
CPT-1	Forward 5´-TCAACCTCGGACCCAAATTG-3´	20	51.55	50
	Reverse 5´-GCCCCGCAGGTAGATATATTC-3´	21	52.92	52
18-S	Forward 5´-CCATCCAAT CGGTAGTAGC-3´	19	49.61	53
	Reverse 5´-GTAACCCGT TGAACCCCATT-3´	20	50.05	50

expression were computed using the comparative Ct ($2^{-\Delta\Delta Ct}$) method (Equation 1). Ct (Threshold cycle) number is first plotted against cDNA input (or logarithm cDNA input), and the slope of the plot was calculated to determine the amplification efficiency (E). ΔCt for each gene (target or reference) was then calculated by subtracting the Ct number of target sample from that of control sample (22).

Equation 1: $2^{-\Delta\Delta Ct}$

Whereas $\Delta\Delta Ct = \Delta Ct_{reference} - \Delta Ct_{target}$

Whereas $\Delta Ct_{target} = Ct_{control\ (18S)} - Ct_{treatment\ (PC,\ LC,\ G,\ LCG)}$ and $\Delta Ct_{reference} = Ct_{control\ (18S)} - Ct_{treatment\ (NC)}$

2.5. Statistical analysis

Statistical analysis was done using SPSS 18.0 for windows. Normality of all data was checked by Kolmogorov-Smirnov test. Data was expressed as mean ± SD. Comparison of quantitative variables was evaluated by one-way ANOVA, followed by post hoc Scheffé test. P-value < 0.05 was considered statistically significant.

Results

Body weight and food intake

As shown in Table 3, initial body weights between groups were not significant. However final weight and weight gain of the patient groups were significantly less than NC group (P<0.05). The PC group didn't show any statistically significant differences compared with LC, G, and LCG groups in all these variables (Table 3).

3.2. Serum albumin, urine protein, and protein-to-creatinine ratio

There were not any statistically significant differences between groups in urine protein and urine protein-to-creatinine ratio at week 2. As shown in table 4, in PC group, before NS induction (week 2), urine protein and protein to creatinine was 111.85±5.92 and 3.96±0.71, respectively, but one week after NS induction, these values had an increasing (671.14±180.28 and 29.98±7.41). At the end of week 7, these values

Table3. Body weight and food intake in rats fed experimental diets for 8 weeks

Variable	NC group	PC group	LC group	G group	LCG group	p value[a]
Initial weight (g)	296.50±25.50	291.50±28.19	294.77±23.62	303.22±34.23	312.00±26.25	0.501
Final weight (g)	355.30±18.43	283.30±34.13[b]	277.88±48.73[b]	283.44±47.47[b]	263.14±67.88[b]	0.001
Weight change (g/day)	1.04±0.45	-0.14±0.50[b]	-0.36±0.83[b]	-0.34±0.58[b]	-0.87±0.77[b]	< 0.001
Food intake (g/day)	20.36±0.73	16.73±0.49[b]	16.36±1.16[b]	15.33±1.10[b]	15.16±0.40[b]	< 0.001
Protein Intake (g/day)	2.55±0.09	2.10±0.06[b]	2.05±0.14[b]	1.92±0.14[b]	1.90±.05[b]	< 0.001
Energy intake (Kcal/day)	73.34±2.65	60.25±1.77[b]	58.93±4.20[b]	55.21±3.98[b]	54.61±1.45[b]	< 0.001

Values are means±SD.

[a]One-way ANOVA between groups.

[b]Values are significantly different compared with NC group by post hoc Scheffé test at P<0.05.

Table 4. Urine protein, protein-to-creatinine, and serum albumin in experimental groups at weeks 2, 3 and 7 and serum albumin.

Variable	NC group	PC group	LC group	G group	LCG group	p value[a]
Week 2: Urine protein (mg/dl)	107.70±4.92	111.85±5.92	102.00±48.74	105.11±28.94	98.00±9.38	0.877
Week 3: Urine protein (mg/dl)	107.50±6.36	671.14±180.28[b]	273.00±99.73[c]	384.66±237.18[b]	210.25±131.77[c]	< 0.001
Week 7: Urine protein (mg/dl)	103.90±22.65	1864.00±298.27[b]	328.50±27.87[c]	680.88±348.82[bc]	400.25±120.13[c]	< 0.001
Week 2: Urine protein-to-creatinine ratio	2.96±1.26	3.96±0.71	1.85±0.28	3.74±1.82	3.67±1.47	0.104
Week 3: Urine protein-to-creatinine ratio	3.39±1.12	29.98±7.41[b]	18.70±30.71	14.38±7.92	5.88±.3.71[c]	0.001
Week 7: Urine protein-to-creatinine ratio	3.87±1.30	71.48±17.20[b]	22.97±3.19[c]	38.11±19.59[bc]	20.67±21.28[c]	< 0.001
Serum albumin (g/dl)	3.63±0.25	1.32±0.34[b]	2.73±0.23[bc]	1.47±0.63[b]	2.86±1.35[c]	< 0.001

Values are means±SD.
[a]One-way ANOVA between groups.
[b]Values are significantly different compared with NC group by post hoc Scheffé test at P<0.05.
[c]Values are significantly different compared with PC group by post hoc Scheffé test at P<0.05.

had a massive increasing again; 1864.00±298.27 and 71.48±17.20, respectively. The differences were significant between PC group and LC, G, and LCG groups at week 7 (p<0.001); these patient groups had eventually lower urine protein in comparison with PC group, but there was not any significant difference between LC, G. and LCG groups. However LC and LCG groups had lower urine protein in comparison with G group (Table 4). Also, serum albumin only in LC and LCG groups were significantly higher than PC group (p<0.001) (Table 4, Graph 1, 2).

PPAR-alpha and CPT-1 Expression in hepatocyte-Extracted mRNA

The PPAR-alpha mRNA expression in LC (p value=0.12), G (p value=0.29), and LCG (p value=0.05) groups were not significantly different than PC group (Table 5), but ΔCT of the gene expression had statistically significant differences between the LC, G, and LCG groups and the PC group (P<0.001) (Table 5). According to the relative CPT-1 gene expression, PC and G groups had lower gene expression than NC group (p=0.012, p = 0.017). Also there were not any signifi-

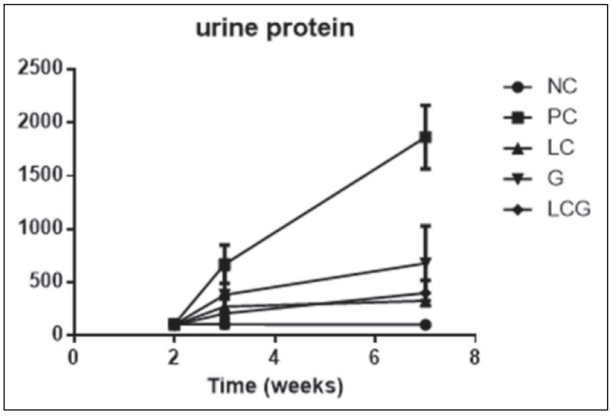

Figure 1. Urine protein in the groups at the end of weeks 2, 3, 7

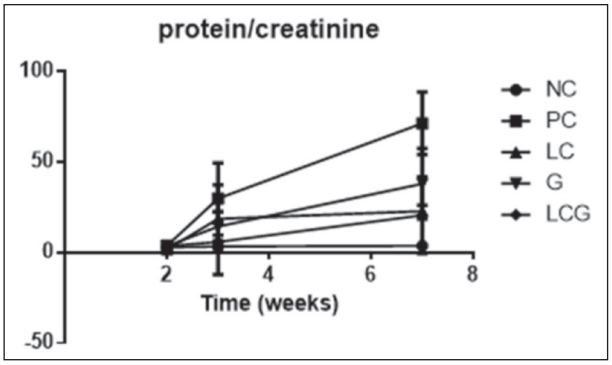

Figure 2. Urine protein to creatinine in the groups at the end of weeks 2, 3, 7

cant differences between LC (p value=0.79), G (p value=0.96), and LCG (p value=0.90) groups in comparison with PC group. However, these patients groups had insignificantly higher gene expression compared with PC group (Table 5). ΔCT of CPT-1 gene had statistically significant differences between the LC (p=0.006), G, and LCG (P<0.001) groups and the PC group (Table 5).

Discussion

Over the separate effects of genistein and L-carnitine, we studied their twin effects in the rats. As shown in Table 3, all patient groups exhibited significant weight loss compared to the NC group. The reasonable cause for this phenomenon was rats' affliction by the nephrotic syndrome which has consequently followed by reductions in food and energy intakes among these groups. Several studies have shown that weight loss, is accompanied by an important antiproteinuric effect (23). So lower calorie intake as a consequence of nephrotic syndrome maybe can explain these outcomes. In fact, NS leads to weight loss (21), and this effect is not reversed by any of the treatments undertaken in this study, as shown by the body weight values presented in Table 3. Also, NS can lead to lower food intake (21). Our results showed that NS induced a decrease in food intake, whereas none of the treatments improved the NS effect. Moreover, G and LCG treatments further reduced the food intake.

In spite of receiving less protein in the patient groups as compared with the NC group, the urine protein and the urine protein-to-creatinine ratio have been higher in the patient groups versus the NC group. This indicates that they have been exhibit nephrotic syndrome syndrome and the tissue protein breakdown leading to higher weight loss among these groups, although such increase amongst a number of patient groups has not been statistically significant.

On the other hand, adriamycin-induction of NS leads to increased levels of protein in urine. Treatment with LC, G, or both in rats with nephrotic syndrome reduced the symptoms of NS, as suggested by a lower level of urine protein.

The Genistein Effect on PPAR-α and CPT-1 Gene Expression

The consumption of soy protein with its isoflavones (genistein and daidzein) prevents further accumulation of liver triglycerides, itself leading to some reduction in the noxious pernicious effects of lipotoxicity (23). The soy protein diet compared with casein diet leads to some increase in the PPAR-α gene expression within the liver, which cannot have any relationship with the CPT-1 mRNA increase (24). This gene expression pattern through the lipid and carbohydrate oxidation increase is remarkably in correlation with the consumed energy increase among type 2 diabetic mice (25).

In our study, receiving genistein has caused some increase in the trend of PPAR-alpha gene expression in the rats' livers compared with the PC group, although these increases have not been statistically significant (table 5).

Takahashi et al. (2008) showed that PPAR-α mRNA levels among those rats had been fed with soy protein was higher against those rats fed with casein,

Table 5. PPAR-alpha and CPT-1 genes expressions in hepatocytes

Variable	NC group	PC group	LC group	G group	LCG group	p value[a]
Δ-CT of PPAR-alpha	11.85±2.04	14.88±0.47	9.85±1.96[c]	8.49±2.25[bc]	7.82±1.30[bc]	< 0.001
Gene expression of PPAR-alpha	1.42±1.07	0.11±0.03[b]	1.18±0.73	0.97±0.61	1.39±0.78	0.043
Δ-CT of CPT-1	8.39±2.37	12.28±0.83[b]	7.64±1.84[c]	6.60±2.32[c]	5.83±1.7[c]	< 0.001
Gene expression of CPT-1	1.47±1.29	0.07±0.03[b]	0.53±0.54	0.33±0.33[b]	0.45±0.46	0.007

Data are reported as means ± SD. ΔCT = CT of 18S – CT of target gene.
[a]One-way ANOVA between groups.
[b]Values are significantly different compared with NC group by post hoc Scheffé test at P<0.05.
[c]Values are significantly different compared with PC group by post hoc Scheffé test at P<0.05.

while the group fed with soy protein plus isoflavones dose-dependently had higher PPAR-α gene expression compared with the other two groups (14). Mezei et al., in a study on male and female Obese Zucker Rats (OZR) afflicted by diabetes type 2, showed the consumption of some sort of diet rich in soy protein isoflavones is able to give rise to the betterment of lipid metabolism in these animals; the liver cholesterol and triglyceride concentrations in all OZRs having fed upon this diet has actually been lower compared with rats dieted on soy protein with low isoflavones and with casein diet. Also, the effect on the part of soy containing genistein and daidzein on the cellular PPAR-α models was studied, and it was factually made clear that both types of isoflavones have been able to redouble the gene expressivity of PPAR-α (15).

Receiving genistein increased CPT-1 gene expression trend in our investigation; however, this increase did not turn out to be statistically significant, either (table 5).

Another study showed in rats dieted on Soy Protein Isolate (SPI+), fatty acid oxidation and PPAR-α mRNA gene expression did increase statistically significantly in comparison with rats dieted on reduced soy protein isolate isoflavones (SPI-), and those on casein (26). Kim et al. (2004), , were also able to prove that genistein could actually increase the expression of genes that involved in lipid catabolism, for instance, the liver CPT-1 (CPT-1L) within hepatic cells (HepG2). In their study, increases in levels of CPT-1L mRNA after treatment with genistein in the presence of ICI1782780 – which is an estrogen receptor controller – showed no fluctuations, leading to the propounding of the hypothesis that such an effect on the part of genistein would factually be independent of the estrogen receptors. Moreover, the study indicated that genistein has had the capacity to bring about some rise in PPAR-α gene expression both in the mRNA level and at the protein level. Additionally, genistein would have the propensity to activate PPAR-α transcription activity which fact brought forth isoflavones as a probable potential ligand (16).

All the same, another study on rats indicated that within the group receiving genistein and daidzein, CPT activity in addition to β oxidation had been lower than those of the control group (27). Choi et al. (2008) also came to realize – through studying non-diabetic female rats with complementary dietary genistein for a time period of 9 weeks – that genistein would have the capacity to statistically significantly reduce the levels of fatty acids β oxidation in addition to CPT-1 activity among the observed rats; yet, some improvement was seen – as compared with the control group – in the level of triglyceride and the serum free fatty acids (28).

The L-carnitine Effect on the CPT-1 and PPAR-α Gene Expression

The effect of L-carnitine on the gene expressivity of CPT-1 and PPAR-α was also brought under scrutiny in our present investigation. It has been indicated that the treatment with L-carnitine could actually give rise to the PPAR-α increase within the kidney tubular cells (29). Not only this, but the production of prostaglandins specially prostacyclin (PGI_2) which are produced from arachidonic acids, in fact, is dependent on the presence of carnitine (30). Prostacyclin is a type of ligand for PPAR-α and PPAR-δ (31). Ingrid et al. (32) have reported that PGI_2 production would increase after the short term (4 days) consumption of L-carnitine among rats. Therefore, it could be considered as a strongly positive hypothesis that L-carnitine – through its effect on PGI_2 production could, indirectly, activate PPAR-α. In fact, L-carnitine itself hugely bringing up the level of CPT-II and CPT-1 transcription; in addition, it has been able to statistically significantly increase the CPT-1 activity within young adult animals' livers (18, 19).

In our investigation, the group receiving L-carnitine has also shown higher levels of CPT-1 and PPAR-α gene expression levels as contrasted against the PC group, and even as contrasted against the group receiving genistein. It should, however, be noted that such differences were not statistically significant (table 5). NS reduced the PPAR-alpha expression and that treatment with LC, G, or both helped resume the PPAR-alpha normal expression. However, the addition of both LC and G does not improve the response seen with any of the two compounds.

Chen et al. (2009) have also showed that L-carnitine can be the cause of increase in the level of prostacyclin (PGI_2) production and PPAR-α activity in the rat's tubular cells

(NRK-52E) (29). Li et al. (2012) have also indicated in their study on human liver cells (HL7702) that the decreased PPAR-α gene expression by H_2O_2 would be moderated in case contacted with L-carnitine (17).

The Genistein's and L-Carnitine's Twin-effects on CPT-1 and PPAR-α Gene Expression

Very few studies have been performed in this field. Observation was made in our investigation over the synergistic effect of the twin-reception of L-carnitine and genistein's effect upon the gene expressivity of both types of proteins: CPT-1 and PPAR-α, although, yet again, the distinctive differences were not statistically significant. In an investigation concerning the enzyme activity and the gene expression of CPT-1A in the hepatocytes (HepG2) after a period of incubation with 10 mg of genistein and 1 mmol of L-carnitine and some combination of them both, full evaluation was made for determining the CPT-1A activity and gene expression cells that had been co-incubated simultaneously with genistein and L-carnitine, gene expression and activity were two to three times more than those cells which had undergone the mentioned incubation period only with one of these two substances (33).

Another study on C57B1/6J mice (34) showed the CPT-1 gene expression in hepatic cells in the group receiving high fat diet alongside genistein and carnitine (HD+G+C) was factually 40 percent higher as compared with the group receiving high fat diet alongside genistein (HD+G): thus, the synergistic metabolic effect of genistein and carnitine was shown forth.

Such synergistic impressive effects could well be indicative of both in-common and non-in-common metabolic routes between L-carnitine and genistein, of course, deeper studies are needed. For the specific mechanism of genistein effect on the CPT-1 and PPAR-α gene expression, the gene expression activation of PPAR-α could well be deemed prior to CPT-1, because genistein could cause gene expression and activity on the part of SREBP-2 (35) with any increase in the insulin-to-glucagon ratio (27); thus bringing up the level of PPAR-α gene expression to, consequently, increase the CPT-1 gene expressivity, although we can, in no way, disregard the hypothesis of the direct effect of genistein on the way of expression of both these proteins. The mechanism of L-carnitine could also be through some way of production of PGI2 (30), as was mentioned earlier. Also, its direct/indirect influence on PPAR-α gene expression to be followed by CPT-1 expressivity might also be somehow justifiable, although the hypothesis of the direct effect of L-carnitine on CPT-1 expressivity could never be brushed aside.

In present investigation, as a result of limitations, we have had to apply 10 rats in each group while the time period for conducting the study was also set at 8 weeks; in case the sample size was larger and the time period for conducting the investigation was longer. There would certainly be higher likelihood of gaining at some statistically significant outcome in gene expression, although ΔCT within all patient groups receiving the supplements was significantly different from that of the PC group.

Conclusion

The results of this study showed that a 6-week L-carnitine and genistein increased the hepatic PPAR-α and CPT-1 gene expression, and had synergistic effects in rats with nephrotic syndrome; however the changes were not statistically significant. These finding could warrant future studies to determine the therapeutic effects of these supplements on nephrotic syndrome and lipid metabolism management.

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Correspondence:
Mahmoud Djalali
Department of Cellular and Molecular Nutrition, School of Nutrition Sciences and Dietetics, Tehran University of Medical Sciences, Poorsina Street, Enghelab Avenue,Tehran, Iran
Telephone: +98-21-88622706
Fax: +98-21-88622707
E-mail: mjalali87@yahoo.com