Research Article [Araştırma Makalesi]

Effect of Protein Re-Feeding with or without L-Carnitine on Mitochondrial Electron Transport Chain in Hypoenergetic and Hypoprotein Feeding Rats

[Hipoenerjetik Hipoprotein Diyetin Takibinde Uygulanan L-Karnitin İçeren veya İçermeyen Protein ile Beslenmenin Sıçan Mitokondriyel Elektron Taşıma Zinciri Üzerindeki Etkisi]

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ABSTRACT

Aim: Supplementation of L-carnitine may effect on mitochondrial function and general metabolic activity. The objective of this study was to evaluate the effect of hypoenergetic hypoprotein diet and protein re-feeding with or without L-carnitine on mitochondrial electron transport chain of rats.

Methodology: This study was carried out on 40 male albino rats divided into four major groups: control group, hypoenergetic hypoprotein, hypoenergetic hypoprotein + protein and hypoenergetic hypoprotein + protein + L-carnitine. Gastrocnemius glycogen and the activities of mitochondrial electron transport chain (complex I–IV) was determined.

Results: Muscle glycogen increased especially in rats refed with protein plus L-carnitine. The activities of complex I and II significantly decreased in hypoenergetic and protein refed groups. On the other hand, rats refed with protein and L-carnitine showed increase in the activity of complex I, while the activity of complex II decreased. Complex III showed a decrease in all groups, but the activity of complex IV decreased in protein refed group. There was a positive correlation between all parameters except between complex IV with glycogen and with complex I.

Conclusion: L-carnitine may improve the activity of electron transport chain. The protein feeding with L-carnitine ameliorates the undesirable effects of hypoenergetic hypoprotein diet on the muscle glycogen and mitochondrial oxidative phosphorylation enzymes.

Key Words: L-carnitine, hypoenergetic, hypoprotein, malnutrition, protein, rats, respiratory chain.

ÖZET

Amaç: L-karnitin desteğinin mitokondriyel fonksiyon ve genel metabolik aktivite üzerinde etkileri olabilir. Bu çalışmanın amacı hipoenerjetik hipoprotein diyetin ve takibinde uygulanan L-karnitin içeren ya da içermeyen protein ile beslenmenin sıçan mitokondriyel elektron taşıma zinciri üzerindeki etkisinin incelenmesidir.

Metod: Bu çalışma dört ana gruba ayrılan 40 erkek albino sıçan üzerinde gerçekleştirilmiştir: kontrol grubu, hipoenerjetik hipoprotein, hipoenerjetik hipoprotein + protein ve hipoenerjetik hipoprotein + protein+ L-karnitin. Gastroknemius glikojen düzeyi ve mitokondriyel elektron taşıma zincirinin (kompleks I-IV) aktiviteleri belirlenmiştir.

Bulgular: Kas glikojen düzeyinin özellikle tekrar protein ve L-karnitin ile beslenen sıçanlarda arttığı saptanmıştır. Tekrar hipoenerjetik ve protein ile beslenen grupta kompleks I ve II aktiviteleri belirgin şekilde azalmıştır. Diğer taraftan tekrar protein ve L-karnitin ile beslenen rat grubunda kompleks I in aktivitesi artarken kompleks II'nin aktivitesi azalmıştır. Kompleks III aktivitesi bütün gruplarda, kompleks IV aktivitesi ise tekrar protein ile beslenen grupta azalmıştır. Kompleks IV ile glikojen ve kompleks I hariç bütün parametreler arasında pozitif korelasyon saptanmıştır.

Sonuç: L-karnitin elektron taşıma zincirinin aktivitesini arttırabilir. L-karnitin ile protein bazlı beslenme, hipoenerjetik hipoprotein diyetin kas glikojeni ve mitokondriyel oksidatif fosforilasyon enzimleri üzerindeki istenmeyen etkilerini ortadan kaldırır.

Anahtar Kelimeler: L-karnitin, hipoenerjetik, hipoprotein, malnütrisyon, protein, sıçanlar, solunum zincir

Introduction

Malnutrition is the result of disturbance in the equilibrium between dietary intake and nutrient needs. The sequential changes of malnutrition are altered cellular metabolism, impaired physiologic function and finally loss of body tissues (1). Malnourished animal exhibit low glucose values, which may be due to decrease gluconeogenesis, depletion of liver glycogen and increased utilization of glucose by tissue. In severe malnutrition atrophy of the heart, cardiac wasting may occur and mitochondrial function reduced in the failing heart (2).

It has been suggested that hypoenergetic feeding is associated with significant changes in the fatigability of skeletal muscle and with a reduction in the activities of some key enzymes of the glycolytic pathway and Krebs cycle (3). Malnutrition probably limits mitochondrial respiration, mainly by decreasing the activities of complex I (NADH-CoQ reductase, EC 1.6.99.5), complex II (succinate-CoQ reductase, EC 1.3.5.1), complex III (ubiquinol-cytochrome c reductase, EC 1.10.2.2), and complexes IV (cytochrome c oxidase, EC 1.9.3.1) (4).

L-Carnitine (4-N-trimethylammonium-3-hydroxybutric acid) is a non-essential organic nutrient, required for entry of long-chain fatty acids (as acylcarnitine esters) into the mitochondria for energy production (5). It synthesized from the essential amino acids lysine and methionine and stored in skeletal muscles, heart and brain. Supplementation of L-carnitine with an antioxidant may have the salutary effect of increasing mitochondrial function and general metabolic activity without a concomitant increase in oxidative stress (6, 7).

The goal of this work was to evaluate supplementation of L-carnitine that may affect the mitochondrial function and general metabolic activity to rats hypoenergetic hypoprotein diet. Also to clarify the role of L-carnitine on mitochondrial electron transport chain on rats protein re-feed diet.

Materials and Methods

Animals and Experimental Design:

Forty male Wistar rats, aged about 15-20 weeks and weighing 150-190 g. Rats were kept for 2 weeks for acclimatization in the same conditions at room temperature, with the same light/dark cycle before starting the experiments. Rats were kept on chow and free access of tap water *ad libitum*. All rats were weighted every 2 days during the experiment (8). Rats were randomly divided into 4 groups, each group included ten rats.

Group I (Control): in which rats received control diet (364 kJ/kg) *ad libitum* for two weeks. Group II (Hypoenergetic-Hypoprotein): rats of this group received hypoenergetic (92 kJ/kg) hypoprotein diet for one week. Group III (Hypoenergetic + Protein): rats received hypoenergetic diet for one week followed by feeding on hypoenergetic diet supplemented with protein for an

other one week. This hypoenergetic diet had the same amount of protein as received by control group (129 kJ/kg). Group IV (Hypoenergetic + Protein + L-Carnitine): in which rats received the same diet regimen as Group III plus oral administration with L-Carnitine (750 mg/kg bw/day x 7 d) (6).

Diet Regimen:

Diet regimen consumed by the four rat groups in kJ/kg bw/ day according to Briet & Jeejeebhoy (3) are illustrated in Table 1. All the diets contained the same amount of electrolytes, vitamins, and trace elements but of different amounts of carbohydrates, fats and protein (9).

At the end of the feeding period; 2, 2 and 1 rat were died from group II, III and IV, respectively.

Blood Collection and Storage:

At the end of experiment, rats were sacrificed under anesthesia with diethyl-ether. All experiments comply with current Egyptian animal laws. Blood was collected *via* heart puncture; serum was separated after coagulation at room temperature for 20 min, by low speed centrifugation (3000 rpm) for 15 min at 4°C. Obtained serum was divided into aliquots and these aliquots were stored at -70°C until analyses.

A) Determination of Some Liver Enzyme Activities:

1) Alanine Aminotransferase (ALT), Aspartat Aminotransferase (AST) activities were determined by using commercial kits supplied from Randox, Egypt (10)

2) *Alkaline phosphatase (ALP)* activity was determined by using commercial kit that was supplied by Bio Mérieux Co, from France according to **Belfield & Goldberge** (11).

Table 1. Energy Content and Composition of the Different Diet Regimen Consumed by the Different Groups of Rats (Briet & Jeejeebhoy, 2001) and (NRC, 1995)

Group IV	Group III	Group II	Group I	Energy (g/kg/d)
65.0	65.0	66.0	262.8	Glucose (kJ)
49.7	49.7	12.5	48.8	Casein (kJ)
13.4	13.4	13.5	52.4	Corn Oil (kJ)
129.0	129.0	129.0	364.0	Total Energy (kJ)
750.0	--	--	--	L-Carnitine (mg/kg)
2.6	2.6	2.6	2.6	$CaCO_3$ (g/kg/d)
0.255	0.255	0.255	0.255	NaCl (g/kg/d)
1.548	1.548	1.548	1.548	Vitamins (g/kg/d)
0.138	0.138	0.138	0.138	Minerals (g/kg/d)

B) Preparation of Skeletal Muscle Homogenate and Isolation of Mitochondrial Fraction: Determination of Muscle Glycogen:

Rats were decapitated and gastronomies muscles were excised and divided into specimens and wrapped in a foil and stored at -70 ºC until used. Muscle glycogen was evaluated as described by Scifter *et al.* (12) that depends on the formation of a green color by reaction of the sugars with antherone reagent under acidic conditions

C) Mitochondrial isolation:

Muscle was disrupted with 20 volume of its weight (W/V) with medium A (200 mM HEPES buffer pH 7.4, containing 120 mM KCl , 2 mM $MgCl_2$, 1 mM Ethylene glutamate tetra acetate (EGTA) and 5 mg/ml bovine serum albumin (BSA) and was homogenized with Potter-Elvehjem tissue homogenizer.

The homogenate was centrifuged at (4000 rpm for 10 min at 4 ºC) and the supernatant was collected (13). The obtained pellet was resuspended in medium A and was centrifuged at 4000 rpm, for 10 min at 4 ºC. These two supernatants were combined, filtrated, and re-centrifuged at 15000 rpm for 10 min at 4 º C.

The pellet obtained after the last centrifugation was resuspended in 10 volumes of medium B (20 mM HEPEs buffer pH 7.4, containing 300 mM sucrose and 1 mM EGTA) and re-centrifuged at 15000 rpm for 10 min at 4º C. The resulting pellet, which contained muscle mitochondria, was suspended in medium B and stored at -70 ºC until analyzed (3, 4). Aliquots of this pellet were used for determination of protein concentration.

D) Determination of the Activities of the Enzyme Complexes of the Respiratory chain in mitochondrial extract

1) Activity of NADH Dehydrogenase (Complex I): was measured according to Birch-Machin *et al.* (13) in which the mitochondria was disrupted in 25 mM potassium phosphate buffer, pH 7.2. The reaction mixture contained phosphate buffer (pH 7.2), 2 mM $MgCl_2$, 2.5 mg BSA, 2 mM KCN (to inhibit complex IV), 2 μg antimycin A (to inhibit complex III), 0.13 mM NADH, 65 μM ubiquinone and 50 μl mitochondrial suspension in a final volume of 1 ml. The activity of NADH dehydrogenase was measured by recording the decrease in absorbance at 340 nm for 3-5 min. 2 μg rotenone (to inhibit complex I) was added, after which the decrease in absorbance was followed at 340 nm for additional 3 minutes. The enzymatic activity of complex I was calculated by a standard curve using $NADHH^+$ as standard. One unit of enzyme activity was defined as nmol $NADHH^+$ hydrolyzed per min at 25ºC under standard assay conditions. Complex I activity is expressed as nmol/min/mg protein.

2) Activity of Succinate Dehydrogenase (Complex II): It was determined in mitochondrial extract according to Birch-Machin *et al.* (13). The mitochondria extract was pre-incubated with in 0.5 ml of 25 mM KH_2PO_4 buffer

pH 7.2, containing, 5 mM $MgCl_2$, 20 mM succinate at 30 ºC for 10 min. 0.5l ml of the solution containing 2 mM KCN and 2 mg antimycin A, 2 μg rotenone, and 50 μM 2,6-dichlorophenolindophenol was added and a base-line rate was recorded for 3 min. The reaction was started with addition of 16 μg ubiquinone. The enzyme catalyzed reduction of 2,6-dichlorophenolindophenol was measured for 3-5 min by recording the decrease in absorbance at 600 nm. An extinction coefficient of 19.1 mmol L^{-1} was used to calculate absolute changes. Complex II activity was expressed as nmol/min/ mg protein.

3) Activity of Ubiquinol-Cytochrome c Reductase, (Complex III): It was determined according to **Carols, et al.** (14) by monitoring the reduction of cytochrome c at 550 nm. 50 μl of mitochondrial extract was equilibrated at room temperature in 1.0 ml KH_2PO_4 buffer (pH 7.2). The containing 2 mM $MgCl_2$, 2 mg BSA, 2 mM KCN, 430 μl distilled water and 60 μM ferricytochrome c for 7 min. 0.13 mM NADH was added, and the increase in absorbance was monitored at 550 nm for 1 min. 4 μg rotenone was added and the increase in absorbance was monitored at 550 nm for additional 1 min. The rotenone specific activity was calculated. A blank was prepared exactly as the sample tested but without mitochondria. One unit of enzyme activity defined as μmole cytochrome c Fe^{3+} hydrolyzed per min at 25ºC under standard assay conditions. Complex III activity is expressed as nmol/min/mg protein.

4) Activity of Cytochrome-c Oxidase (Complex IV): Complex IV activity was determined according to **Rustin et al.** (15) by monitoring the oxidation of cytochrome c at 550 nm. 1.0 ml reaction mixture contained 25 mM KH_2PO_4 buffer (pH 7.2), 2 mg Rotenone, and 60 μM reduced cytochrome c, 0.5 mg dodecylmaltoside and 50 μl mitochondrial extract was added. The changes in absorbance were followed at 550 nm for 2 min. The enzymatic activity of complex IV was calculated from a previously established standard curve using cytochrome c Fe^{3+} as standard. One unit of enzyme activity was defined as μmole cytochrome c Fe^{3+} formed per min at 25 ºC under standard assay conditions. Complex IV activity is expressed as nmol/min/mg protein.

E) Mitochondrial protein concentrations: It was determined by the method of **Lowry et al.** (16) that depends on formation of a protein-copper complex in alkaline medium.

Statistical Analysis:

The mean, standard deviation (S.D.) and *p* values were calculated by using the Statistical Package for Social Science (SPSS) (version 13, USA). Student's *t* test was used to compare mean values among groups. Mean of different groups was tested by one-way analysis of vari-

ance (ANOVA) followed by Tukey_s significant difference test *P-value* considered significant when $p < 0.05$. Pearson's correlation coefficient was also used.

Results

The body weight was significantly decreased in rats kept on hypoenergetic hypoprotein diet (group II), hypoenergetic protein refed (group III) and hypoenergetic protein refed plus L-carnitine (group IV) as compared to the control fed group (group I), as presented in Table 2.

Activities of serum ALT and AST showed a significant increase (in group II) compared to control ($p < 0.001$). There was a significant decrease in ALT and AST activities of group III and group IV compared to hypoenergetic group ($p < 0.001$) (Figure 1). Serum ALP activity was significantly decreased in all hypoenergetic rat groups compared to that of the control group (p<0.001). It was found that there was a significant increase in the activity of ALT of group III (hypoenergy + protein refed) and group IV (hypoenergy + protein refed + carnitine) compared to group II (hypoenergy + hypoprotein), p<0.01, <0.05, respectively. In addition, a significant increase in ALP activity of group III with still significant difference as compared to control rats was also observed (Figure 1).

Muscle glycogen concentration showed a significant decrease in all hypoenergetic groups (group II, III & IV) compared to control group ($p< 0.001$, $p< 0.001$ & $p< 0.01$, respectively). A significant increase was evaluated in the content of muscle glycogen in protein plus L-carnitine group (group IV) compared to hypoenergetic group (group II) (p<0.01) (Figure 2).

The activity of NADH dehyrogenase (Complex I) showed a significant decrease in both group II and group III compared to control group ($p < 0.001$). A significant increase in the activity of Complex I was found in protein refed plus L-carntine group (group IV) compared to hypoenergetic group (group II) (P <0.001), (Table 3).

The activities of Succinate Dehydrogenase (Complex II) and Ubiquinol-Cytochrome c Reductase, (Complex III) decreased significantly in groups II, III & IV compared with those of the control group. However there was a significant increase in the activities of complex II and
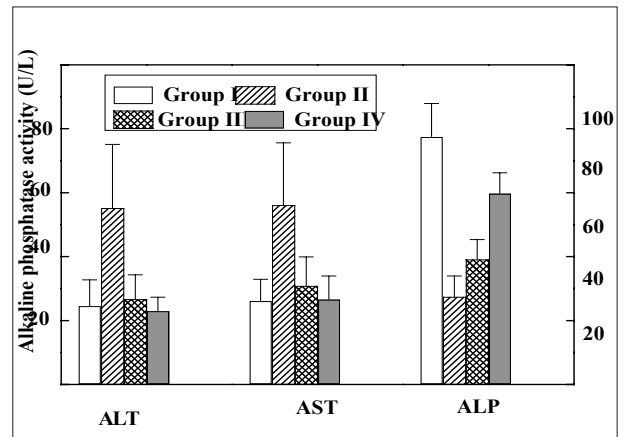

Figure 1. Effect of different diet regimen on the activities of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phoshatase (ALP).
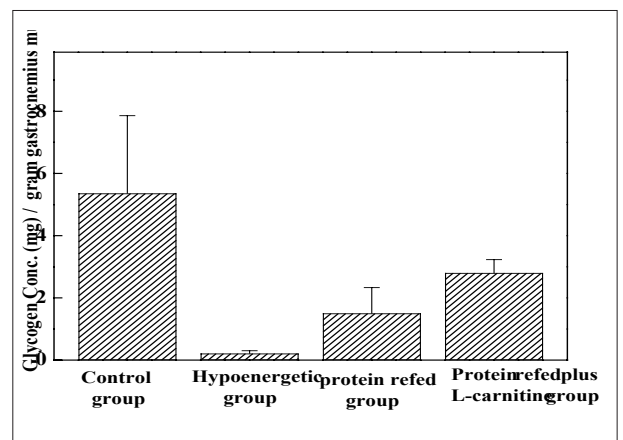

Figure 2. Effect of different diet regimen on the concentration of muscle glycogen.

Table 2. Effect of different diet regimen on body weights of adult male rats before and after feeding

Groups	Body weight(g)		% change of body weight	Paired-t
	At start	At sacrifice		p
Group I (n) Mean ±SD.	(10) 158.80±7.38	(10) 191.00±12.76	21.6% ↑	32.15 0.001*
Group II(n) Mean ±SD	(10) 160.5±14.42	(8) 132.5±10.56	20.6% ↓	8.18 0.001*
Group III(n) Mean ±SD	(10) 170.5±6.7	(8) 139.38±13.13	17.7% ↓	8.06 0.001*
Group IV(n) Mean ±SD	(10) 167.67±8.66	(9) 148.78±19.14	11% ↓	3.51 0.001*

n = number of cases.   P = At start vs at sacrifice * significant <0.05

Table 3. Effect of different regimen on the activities of complexes I-IV in mitochondrial fraction isolated from gastronomies muscles of rats.

Complex IV (nmol/ mg protein)	Complex III (nmol/ mg protein)	Complex II (nmol/mg protein)	Complex I (nmol/mg protein)	Parameters Groups
307-507 411.5±67.5	750-1400 1065±222.4	59-114 81±15.66	54-127 88.6±24.50	Group I Range Mean±S.D.
210-485 306.2±82.3 N.S	160.5-230 174.2±70.2 <0.001	6.1-14.1 11.5±2.4 <0.001	25-57 37±10.5 <0.001	**Group II** **Range** **Mean±S.D.** ***p*[(a)]**
250-360 350.6±36.4 <0.01 N.S.	250-488 368.8±78.7 <0.001 <0.05	34-65.5 44.2±9.1 <0.001 <0.001	53-63 57.4±3.7 <0.001 N.S.	**Group III** **Range** **Mean±S.D.** ***p*[(a)]** ***p*[(b)]**
322-490 370±56.3 N.S. N.S. N.S.	425-800 586.2±112.8 <0.001 <0.001 <0.05	43-78 57.3±13.9 <0.001 <0.001 N.S.	54-98 75.1±13.3 N.S. <0.001 N.S.	**Group IV** **Range** **Mean±S.D.** ***p*[(a)]** ***p*[(b)]** ***p*[(c)]**

p (a): < 0.5 value vs group I, p (b): < 0.5 value vs group II, p (c): < 0.5 value vs group III.

III after addition of protein with or without L-carnitine (groups III & IV) compared to hypoenergetic group (group II), with more elevation in their activities in protein refed plus L-carntine group as compared to control group.

The activity of Cytochrome-c Oxidase (Complex IV) showed a significant decrease in protein refed group (group III) as compared to control   (P< 0.01) (Table 3). Complex IV activity showed insignificant change among all the study groups as compared with group I, except in group II the activity of cytochrome-c oxidase was decreased significantly compared to its activity of group I (p<0.01).

There was a significant positive correlation between not only all the complexes with both body weight and muscle glycogen but, also between all the complexes with each other except I *vs*. IV, as shown in Table 4.

Discussion

Malnutrition is a persistent health problem affecting people from many communities in the world in particular children during first years of life. This study revealed that the control balanced diet significantly increase the body weight of control rats by about 21.6% but the body weight of hypo-energetic group and hypo-energetic pro-tein refed group decreased by 20.6% and 17.7%, respectively. The main causes of weight loss may be due to the reduced energy intake and due to the low protein content of the experimental diet.

The obtained results were in agreement with Briet *et al*. (8) who reported a significant loss in the body weight occur in rats allocated on hypo-energetic diet compared to rats kept on normal diet and compared to rats re-fed with protein and carbohydrate that had been manifested as restoration of total body mass. Malnutrition also caused a significant reduction in hind limb muscles (17) and reduction in diaphragm muscle weight (18). The current study showed a significant increase in body weight of hypo-energetic rats refed with protein plus L-carnitine. Ramsay *et al*. (19) reported that L-carnitine is used as a nutritional supplement due to its importance in β−oxidation of fatty acids and cellular energy production. In contrast, Susanna *et al*. (20) found that final body weight did not differ in either young or old rats after L-carnitine treatment.

As regards liver enzymes, the current study showed a significant increase in serum AST and ALT activities but a significant decrease in ALP in hypo-energetic group as compared to control group. Addition of pro-tein with or without L-carnitine results in a significant

Table 4. Correlation between mitochondrial complexes with body weight and glycogen concentrations in all the studied groups (n=35 rat).

Parameters	complex I (mol/ mg protein)		complex II (nmol/ mg protein)		complex III (nmol/ mg protein)		complex IV (nmol/ mg protein)	
	r	p	r	p	r	p	r	p
Body weight (g)	0.570	<0.01	0.687	<0.001	0.820	<0.001	0.426	<0.01
glycogen concentration (mg)	0.344	<0.05	0.597	<0.01	0.579	<0.01	0.297	N.S.
complex I			0.779	<0.001	0.813	<0.001	0.313	N.S.
complex II	0.779	<0.01			0.859	<0.001	0.451	<0.01
complex III	0.813	<0.01	0.859	<0.001			0.477	<0.01
complex IV	0.313	N.S.	0.451	<0.01	0.477	<0.01		

decrease in serum AST and ALT activities and a significant increase in ALP as compared to hypoenergetic group (group II).

These results are in accordance with Waterlow (21) who observed an increase in the activities of hepatic AST and ALT in the animals fed with protein deficient diet. Moreover, Pond *et al.* (22) reported that there is an increase in ALT activity in protein deficient pigs. This may explain that during hepatic disorder, which may result from hypo-energetic nutrition, amino acids are released from exaggerated tissue breakdown and in order to metabolize these amino acids, the process of transamination enhanced leading to increased activity of the related enzymes AST and ALT (23, 24).

Also Waterlow, (21) reported that ALP decreased when rats or humans exposed to protein caloric malnutrition. Animals fasted for a longer time have had their protein reserves depleted in liver and intestine to the extent that they have lost the ability to synthesize sufficient enzyme and this can be ameliorated by feeding the malnourished rat or human by high protein diet. In contrast, Lee *et al.* (25) stated that since enzymes are proteins, a fall for protein might be reflected in a reduction in the amounts of enzymes in blood or tissues, hence measurement of enzyme activity might provide a useful tool for assessing the state of protein nutrition. Davenport *et al.* (26) postulated that protein restricted diet results in many hepatic and extra hepatic conditions that cause an increase in products of ALP iso-enzymes from bone and hepatobiliary source.

As regards muscle glycogen in the present study, its concentration was significantly decreased in hypo-energetic group as compared to the control group, which is in accordance with many reports (27, 28). The decrease in peripheral skeletal muscle glycogen level after under nutrition and the significant effect of malnutrition on glycogen stores in liver and skeletal muscle is by increasing the utilization of glucose by tissues (27). Addition of protein with or without L-carnitine resulted in significant increase in glycogen level as compared with

hypoenergetic-hypoprotein. This was in agreement with Morifuji *et al.* (29) who reported that protein refeeding causes a decrease in phosphofructokinase activity and may increase glycogen synthase in skeletal muscle, so protein refeeding may increases glycogen content by regulation of these enzymes. Re-feeding with L-carnitine boosts energy by stimulating the body's burning of triglycerides as fuel and sparing the supply of glycogen stored in the liver for heavier exertion (30).

Regarding mitochondrial enzyme complexes there was a significant decrease in the activities of complex I, II & III, with insignificant change in the activity of complex IV in hypo-energetic feeding group as compared with the control group. Because nutritional manipulations alter protein metabolism, it is likely that the effects on activities of complexes I–III may be due to changes in mitochondrial protein synthesis, breakdown, or both (31).

Moreover, María & Carolina, (2) reported that malnutrition not only alters protein metabolism, but also it has an effect on the activities of the complex enzymes of electron transport chain (ETC), which may be due to changes in mitochondrial protein synthesis, breakdown, or both. Addition of protein to the hypoenergetic-hypoprotein diet in this study resulted in a significant increase in complexes activities of ETC, except complex IV as compared to the hypoenergetic-hypoprotein group.

Briet *et al.*(3) reported that protein refeeding, but not glucose refeeding, restored the activities of mitochondrial I-III in the gastronomies muscle. The rapid and specific effect of protein, but not of glucose refeeding, suggested that increased amino acid availability rather than increased insulin concentrations (glucose effect) is important in restoring the activities of mitochondrial complexes (32).

Briet *et al.*(1) also reported that complex I activity was restored within 24 h by protein feeding, suggesting that alteration in mitochondrial enzyme synthesis or catabolism, rather than mitochondrial proliferation, is likely to be the mechanism of nutritional effects. Furthermore, addition of protein and L-carnitine to the hypoenerget-

ic-hypoprotein diet resulted in pronounced elevation in activities of complex I, II and III of ETC. Kumaran *et al*. (5) reported that supplementation of L-carnitine to young and aged rats improved the levels and activities of the complexes of ETC in aged rats in time dependent manner when compared with young rats .

Thus, it is unlikely that limitations in substrate entry such as pyruvate, succinate and glutamate, may explain the observed reduction in the activities of some enzyme complexes of ETC in rats kept on hypoenergetic-hypoprotein diet. Similarly, since these substrates enter the citric acid cycle at different steps, it is also unlikely that the reduced generation of $NADHH^+$ or $FADH_2$ may be responsible for the reduced activities of the enzyme complexes of ETC observed in the hypoenergetic-hypoprotein fed rats.

Taken all together, it can be concluded that the protein-energy restricted diet may be altered the energy yield of the cell. Feeding with protein plus L-carnitine supplementation can normalize the altered liver function, muscle energetic and the mitochondrial oxidative phosphorylation without remarkable effect on the responding weight gain. It is necessary to study a larger number of malnourished rats at various stages of response to refeeding to clarify the role of protein and energy intake on mitochondrial complexes of electron transport chain.

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