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Effects of Exercise and Supplementation of L-Carnitine and Antioxidants on Mitochondrial Function in Rats

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ABSTRACT

This study was investigated the effects of exercise and supplementation of L-carnitine and antioxidants on hepatic mitochondrial function, especially oxidative phosphorylation (OXPHOS). Isolated hepatic mitochondria from 4 rat groups were functionally tested by an analysis of respiration and the coupling of this process to ATP synthesis in the presence of ADP. Four groups were non-trained, non-supplemented group (NTNS), non-trained, supplemented group (NTS), long term-trained, non-supplemented group (LTNS), and long term-trained, supplemented group (LTS). The trained rats run on a treadmill (grade 10°, 20 m/min) for 60min/day for 8 weeks. The supplemented rats were treated with L-carnitine (0.5% diet), vitamin E (0.5mg/g BW), vitamin C (0.5mg/g BW) and melatonin (1 μg/g BW) for 8 weeks. There were exercise effects on improving mitochondrial OXPHOS. Within non-supplemented groups, exercised rats resulted in a significant decrease in state 4 oxygen consumption, which increased the respiratory control (RC) ratio and ADP : O (P/O) ratio. There were supplementation effects on improving mitochondrial OXPHOS, too. Within non-exercised rats, supplemented rats resulted in a significant decrease in state 4 oxygen consumption, which increased the RC ratio and P/O ratio. There were additive effects of exercise and supplementation on OXPHOS. Within supplemented rats, exercise resulted in an increase in RC ratio. Significant effects of exercise-supplement interaction on improving OXPHOS were identified. It suggests that exercise and supplementation of L-carnitine and antioxidants might improve more efficiently the impaired OXPHOS efficiency in mitochondrial dysfunction that recognized as is an important cause of degenerative diseases. (*J Community Nutrition* 4(3) : 187~194, 2002)

KEY WORDS : mitochondrial function · oxidative phosphorylation · exercise · L-carnitine, antioxidants.

Introduction

Mitochondrial dysfunction is increasingly recognized as an important cause of human diseases (Larsson, Luft 1999 ; Wallace 1992). There were large numbers of patients with biochemical and morphological evidence of mitochondrial respiratory chain dysfunction (Holt, Harding 1988). The symptoms of mitochondrial dysfunction are diabetes mellitus, heart failure, renal failure, liver disease, dementia, movement disorder, loss of eyesight, and aging (Brown, Wallace 1994 ; Larsson, Luft 1999 ; Wallace et al. 1995). One of the chief functions of mitochondria is to supply the cell with ATP made via OXPHOS. Mitochondrial ATP production via OXPHOS is essential for normal function and maintenance of human organ systems. Slightly decreased efficiency of OXPHOS might result in a small but significant decrease in the amount of ATP (Matschinsky 1996). If there is a reduction in the amount of ATP available for ATP-dependent cell functions, that cell's function will be impaired. Buttgereit and Brand (1995) have described a hierarchy of ATP-consuming processes that is consistent with this hypothesis of how a small 'error' in ATP synthesis can have large consequences when viewed over a lifetime of small but incremental losses of function.

OXPHOS is composed of five multi-unit enzyme complexes that span the mitochondrial inner membrane. The five complexes contain proteins that are encoded by both mitochondrial and nuclear genome. OXPHOS biogenesis requires at least 100 gene products and is under dual genetic control (Brown, Wallace 1994 ; Wallace et al. 1995). The involve-

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ment of two genomes in the biosynthesis of complexes of OXPHOS means that the inheritance pattern in patients with genetic defects is complicated. Since OXPHOS biogenesis depends on both nuclear- and mitochondrial-encoded gene products, mutations in either of these genomes can result in impaired electron transport and ATP synthesis, thus causing tissue dysfunction and, ultimately, human diseases (Wallace 1992 ; 1994). Although there is a strong genetic element, degenerative diseases have a multifactorial etiology in which environmental factors like diet, supplements and exercise are important modifiers. The control of OXPHOS has attracted increasing attention in recent years.

It is well established that exercise plays an important role in regulation of mitochondrial OXPHOS. Physical exercise provides a topological basis for interaction between the ATP generating reaction and the intramitochondrial ATP requiring OXPHOS process (Chen, Gollnick 1994). Regular exercise increased in mitochondria number, size, and ATP production capacity (Venditti et al. 1999). Namely, OXPHOS efficiency was improved as endurance exercise for mammals (Berthon et al. 1995).

The beneficial effects of L-carnitine supplementation on mitochondrial function have been reported (Nicot et al. 2001 ; Paradies et al. 1992 ; Villa et al. 1988). L-carnitine supplementation resulted in significant increase in cellular respiration. It is known that carnitine transports the activated long-chain fatty acids into the mitochondrial matrix and removes the short chain fatty acids from the mitochondrial membranes space thereby decreasing the acyl-CoA/CoA ratio which stimulates the activity of pyruvate dehydrogenase and increasing the oxidative potential of glucose (Bieber 1988 ; Siliprandi 1985). Several studies (Arenas et al. 1994 ; Huertas et al. 1992) reported carnitine effects on increase in electron transport system components. L-carnitine added to the mitochondrial suspension is sufficient to act as a buffer for the increase in mitochondrial OXPHOS efficiency. Recently, Brass and Hiatt (1994 ; 1998) reported ATP generation during exercise was dependent on adequate carnitine stores.

Several studies have been reported that antioxidants are depleted by the oxidative stress induced by exercise (Baskin et al. 2000 ; Leeuweenburgh, Heinecke 2001 ; Schroder et al. 2000). During endurance exercise, energy production will be enhanced and thus radical oxygen species (ROS) accumulation will be also increased. OXPHOS is the major source of ROS in the cell. Under physiological conditions, these deleterious species are removed effectively by the antioxidant systems. However, prolonged exercise may cause a transient reduction of tissue antioxidants. Supplementation of antioxidants may protect against oxidative defect of OXPHOS in exercise. Therefore, during endurance exercise, supplementation of L-carnitine and antioxidants may improve more increase in mitochondrial function without an increase in oxidative stress.

In turn, it is suggested that exercise and supplementation of L-carnitine and antioxidants may improve more efficiently increase in mitochondrial OXPHOS efficiency. This study investigated the effects of exercise and/or supplementation of L-carnitine and antioxidant on hepatic mitochondrial OXPHOS efficiency.

Materials and Methods

1. Animals and diets

Twenty four male Sprague-Dawley rats were divided into four groups (6/group) : NTNS (non-trained non-supplemented group), NTS (non-trained supplemented group), LTNS (long-term trained non-supplemented group) and LTS (long-term trained supplemented group). The rats were fed AIN-76 diet for 8 weeks. The rats were housed individually in hanging, wire mesh cages in a room in which room temperature (21 ± 1℃), humidity (45 − 50 %), and lighting (lights on 8 : 00 AM to 8 : 00 PM) were controlled. The trained rats ran on a treadmill (grade 10°, 20 m/min) for 60min/day for 8 weeks. Supplements consisted of the addition of L-carnitine (0.5% diet) and the administration of vitamin E (0.5mg/g BW), vitamin C (0.5mg/g BW) and melatonin (1 μg/g BW) into the stomach of rats for 8 weeks. Rats were weighed and food intakes were determined every week.

2. Mitochondrial preparations

The rats were euthanized by decapitation and the livers were quickly excised, chilled in Tris-buffered (pH 7.2) 0.25M sucrose, and weighed. Mitochondria were prepared by the procedure of Johnson and Lardy (1967). The liver was homogenized in cold, Tris-buffered 0.25M sucrose and the mitochondria were isolated from the homogenate by differential centrifugation. The mitochondria were then washed and resuspended three times. After the final wash, the mitochondria were resuspended in the buffer. Mitochondrial protein

Table 1. Effects of exercise and/or supplementation of L- carnitine and antioxidants on gained body weight, liver weight, RLS and food intake

	Non-exercise		Exercise		Analysis of variance[2]		
	NTNS	NTS	LTNS	LTS	E	S	E*S
Gained	202.50[a]	184.85[a]	134.17[b]	129.48[b]	0.05	NS	NS
Body weight(g)	(25.35)	(8.60)	(27.08)	(9.81)			
Liver weight(g)	14.12[a]	9.51[a]	10.58[a,b]	11.11[a,b]	NS	NS	0.05
	(2.71)	(3.02)	(2.95)	(2.72)			
RLS[1]	3.20[a]	2.85[a]	2.73[a,b]	2.91[a,b]	NS	NS	0.05
	(0.65)	(0.49)	(0.40)	(0.44)			
Food intake	6.39[a]	6.14[a]	6.15[a]	6.06[a]	0.05	0.05	NS
(g/100g BW/day)	(0.28)	(0.46)	(0.42)	(0.48)			

All values are Mean ± SD, values with different superscripts are significantly different ($p < 0.05$).
[1] RLS, relative liver size.(liver weight/body weight) × 100
NTNS : Non-trained non-supplemented group, NTS : Non-trained supplemented group, LTNS : Long-term trained, non-supplemented group, LTS : Long-term trained, supplemented group ; n = 6., E : exercise, S : supplementation
[2] Analysis of variance. Significant excercise, supplementation or exercise-supplementation interaction effects are shown $p < 0.05$ or NS, non significant

content was determined by the biuret method using bovine serum albumin as a standard.

3. Determination of oxidative phosphorylation

Oxygen consumption was determined with oxygen meter (Yellow Springs Instrument Co., Yellow Springs, USA). The reaction chamber was fitted with a magnetic stirrer and temperature was controlled at 25℃. Respiration buffer (75mM glycine ; 10mM phosphate buffer, pH 7.4 ; 75mM KCl ; 5mM $MgSO_4$; 10mM Tris-HCl, pH 7.2) was pre-equilibrated with air by shaking in a water bath at 25℃ and introduced into the chamber by syringe (Trounce et al. 1994). All subsequent additions to the chamber were made with Hamilton syringes passed through the capillary on top. The 0.65M succinate (pH 7.2) was stored frozen (−80℃) in small aliquots. In a typical experiment, freshly isolated mitochondria were added to the chamber containing respiration buffer and 5mM succinate. After 2min, ADP was added to stimulate state 3 respiration. State 3 (O_2 consumed by isolated mitochondria upon addition of ADP) and state 4 (O_2 consumed once all the added ADP is phosphorylated to ATP) oxygen consumption rates were calculated according to Chance and Williams (1955) using assumptions from Reynafarje et al. (1985). Respiratory control (RC) ratio was calculated as the ratio of oxygen consumption rate for state 3 to that for state 4. The ADP : O (P/O) ratio was calculated as the amount of added ADP to the amount of oxygen used during the state 3 respiration (Estabrook 1967). Subsequently, ATP contents were calculated as ADP/O × state 3 respiration.

4. Statistical analysis

SAS version 6 (SAS Institute, Cary, NC, USA) was used for statistical analysis. All values are expressed as group means and standard deviation. Significances of differences were determined by 2 way analysis of variance (ANOVA). The differences among groups were tested by Duncan's multiple range test.

Results

The gained body weight, liver weight, relative liver size (RLS) and food intake are presented in Table 1. The rats were similar food intakes. Exercise effects on body weight were observed. The exercised rats (LTS, LTNS) gained less weight than the non-exercised rats (NTS, NTNS). This is a typical response to exercise. An effect of exercise-supplement interaction on liver weight and RLS was significant when these data were subjected to an analysis of variance. Exercise and supplementation would be additive with respect to their effects on liver weight and RLS.

Succinate-supported respiration was measured in absence and presence of ADP (Fig. 1 − 5). In NTNS rats, the state 3 respiration rate (in the presence of ADP), the state 4 rate (after the added ADP had been converted to ATP), the RC ratio and the P/O ratio were similar to those previously reported (Kim, Berdanier 1998) and were an evidence of well prepared mitochondria for this study.

With respect to state 3 and state 4 respiration rates, exercise, supplementation and exercise-supplementation decreased in

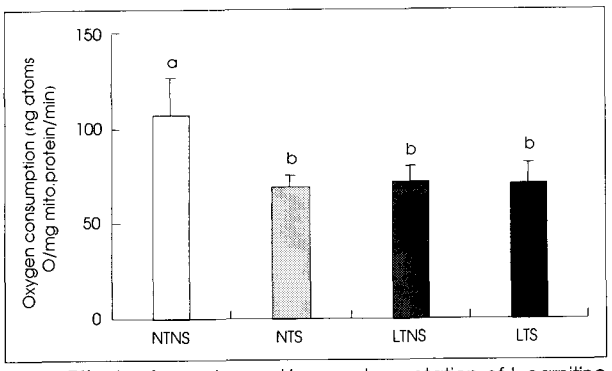

Fig. 1. Effects of exercise and/or supplementation of L-carnitine and antioxidants on state 3 respiration in hepatic mitochondria. Each bar with a different letter is significantly different ($p < 0.05$). NTNS : Non-trained, non-supplemented group, NTS : Non-trained, supplemented group, LTNS : Long-term trained, non-supplemented group, LTS : Long-term trained, supplemented group, n = 6.

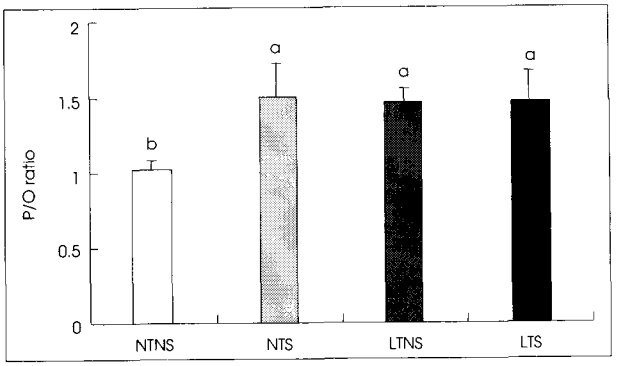

Fig. 4. Effects of exercise and/or supplementation of L-carnitine and antioxidants on P/O ratio in hepatic mitochondria. Each bar with a different letter is significantly different ($p < 0.05$). NTNS : Non-trained, non-supplemented group, NTS : Non-trained, supplemented group, LTNS : Long-term trained, non-supplemented group, LTS : Long-term trained, supplemented group, n = 6.

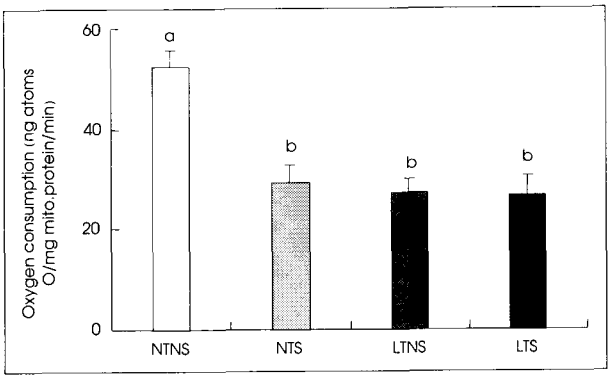

Fig. 2. Effects of exercise and/or supplementation of L-carnitine and antioxidants on state 4 respiration in hepatic mitochondria. Each bar with a different letter is significantly different ($p < 0.05$). NTNS : Non-trained, non-supplemented group, NTS : Non-trained, supplemented group, LTNS : Long-term trained, non-supplemented group, LTS : Long-term trained, supplemented group, n = 6.

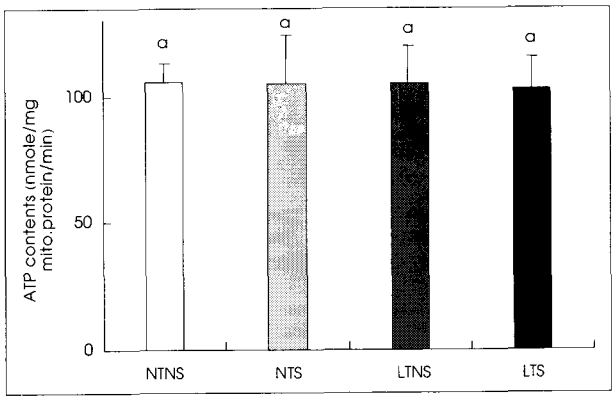

Fig. 5. Effects of exercise and/or supplementation of L-carnitine and antioxidants on ATP contents in hepatic mitochondria. Each bar with a different letter is significantly different ($p < 0.05$). NTNS : Non-trained, non-supplemented group, NTS : Non-trained, supplemented group, LTNS : Long-term trained, non-supplemented group, LTS : Long-term trained, supplemented group, n = 6.

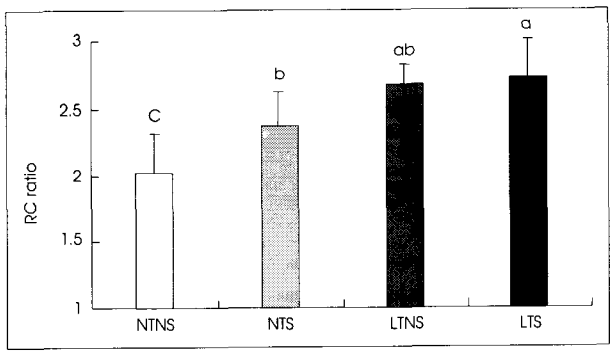

Fig. 3. Effects of exercise and/or supplementation of L-carnitine and antioxidants on RC ratio in hepatic mitochondria. Each bar with a different letter is significantly different ($p < 0.05$). NTNS : Non-trained, non-supplemented group, NTS : Non-trained, supplemented group, LTNS : Long-term trained, non-supplemented group, LTS : Long-term trained, supplemented group, n = 6.

state 3 and state 4 oxygen consumptions (Fig. 1, 2). There were no differences in state 3 and state 4 oxygen consumptions among the 3 groups ; NTS, LTNS, LTS. With respect to RC ratio, exercise, supplementation and exercise-supplementation increased in RC ratio (Fig. 3). Characteristically, there were statistically significant differences between the supplemented rats with or without exercise (LTS vs. NTS). Within exercised groups, supplemented rats resulted in a slight increase in RC ratio (LTNS vs. LTS). But there were no significant differences. With respect to P/O ratio, exercise, supplementation and exercise-supplementation increased in P/O ratio (Fig. 4). There were no differences in P/O ratio among the 3 groups ; NTS, LTNS, LTS. With respect to ATP contents, there were no significant differences in ATP contents among 4 groups ;

Table 2. Effects of exercise and/or supplementation on state 3, state 4, R/C ratio, P/O ratio and ATP content by 2-way ANOVA*

	Exercise	Supplementation	E*S
State 3	NS	0.01	0.01
State 4	0.001	0.001	0.001
RC ratio	0.001	NS	0.01
P/O ratio	0.05	0.01	0.001
ATP content	NS	NS	NS

*Analysis of variance. Significant exercise, supplementation or exercise-supplement interaction effects are shown $p < 0.05$ or NS, non significant.
E : exercise, S : supplementation

NTNS, NTS, LTNS, LTS (Fig. 5).

These data were subjected to an analysis of variance (Table 2). Significant exercise effects on state 4, RC ratio and P/O ratio were identified. With respect to supplementation, there were significant effects on state 3, state 4 and P/O ratio. In exercise-supplementation interaction, significant effects were on state 3, state 4, RC ratio and P/O ratio.

Discussion

One of the chief functions of mitochondria is to supply the cell with ATP made via OXPHOS. Mitochondria ATP production via OXPHOS is essential for normal function and maintenance of human organ systems. Defects of OXPHOS are increasingly being recognized as an important cause of degenerative diseases. Therefore, the metabolic control of OXPHOS has attracted increasing attention. This study investigated the effects of exercise, supplementation of L-carnitine and antioxidants and exercise-supplementation on mitochondrial OXPHOS. An isolated preparation of hepatic mitochondria is functionally tested by an analysis of its respiration and the coupling of this process to ATP synthesis. Mitochondria were assessed by measuring state 3 (O_2 consumed by isolated mitochondria upon addition of ADP), state 4 (O_2 consumed once all the added ADP is phosphorylated to ATP) respiration, the RC ratio, ADP : O (P/O) ratio and ATP contents.

There were exercise effects on improving mitochondrial OXPHOS. The rising O_2 consumption concomitant with exercise was positively correlated with decrease in state 4 oxygen consumption. Within non-supplemented groups, exercise resulted in a decrease in state 4 oxygen consumption, which affected RC ratio. The RC ratio was higher for the mitochondria from exercised rats than was mitochondria from non-exercised rats. Well coupled mitochondria from exercised rats utilized the chemical energy inherent in succinate and produced energy which was trapped in high energy bounds of ATP. And thus P/O ratio was higher in the mitochondria from exercised rats than in the mitochondria from non-exercised rats. The high level of RC ratio, together with P/O ratio in exercised rats, indicated a concomitant increase in the capacity to form ATP. These results presented good efficiency of mitochondrial ATP synthesis in exercised rats. These results are consistent with earlier reports (Starrit et al. 1999 ; Terblanche et al. 2001 ; Venditti et al. 1999 ; Yoon 1999). Based on earlier studies, well coupled mitochondria from exercised rats seem to occur mainly through an increase in mitochondria number, size and mitochondrial content.

There were supplementation effects on improving mitochondrial OXPHOS, too. The high level of RC ratio, together with P/O ratio in supplemented rats, indicated more coupled and more efficient with respect to mitochondrial ATP synthesis. These results are consistent with earlier reports. Treatment of young rats with L-carnitine documented a stimulating effect on ATP production (Mollica 2001). Also, L-carnitine supplementation resulted in significant increase in cellular respiration (Hagen et al. 1997 ; Nicot et al. 2001). Carnitine added to the mitochondrial suspension is sufficient to act as a buffer for the increase in mitochondrial OXPHOS (Sahlin 1990). Huertas et al. (1992) reported carnitine effects on increase in electron transport system components. Arenas et al. (1994) documented a marked increase in activities of complexes I, III and IV of the respiratory chain in endurance athletes receiving carnitine. Based on earlier studies, supplemented rats, indicated more coupled and more efficient with respect to mitochondrial ATP synthesis, may have higher levels of electron transport system components in mitochondria. As is well known, OXPHOS is the major source of radical oxygen species (ROS) in the cell. In this study, addition of antioxidants may protect against oxidative defect in OXPHOS in exercised rats. Supplementation of L-carnitine and antioxidants may prevent damage of mitochondrial function and may increase in electron transport system components. It suggests that the mitochondrial targeted L-carnitine and antioxidants may manipulate mitochondrial function.

The data of this study showed that there were additive effects of exercise and supplementation on improving OXPHOS. Earlier studies have reported that exercise performance is improved by carnitine supplementation (Ahmad et al. 1990 ;

Brass, Hiatt 1998 ; Lancha et al. 1995) or antioxidants supplementation (Bejma et al. 2000 ; Pincemail et al. 1988 ; Robertson et al. 1991). Under the no supplementation of L-carnitine and antioxidants, during endurance exercise, energy production will be enhanced and thus ROS accumulation will be also increased. Therefore, supplementation of L-carnitine and antioxidants may improve more efficiently mitochondrial functions without an increase in oxidative stress in exercise. We have an instance in which supplementation of L-carnitine and antioxidants elicited the expected increase in mitochondrial OXPHOS in exercise. These observations would be useful in developing an understanding of effects of exercise and supplementation on hepatic mitochondrial OXPHOS in rats and may provide an exciting new avenue for exploring exercise-supplement interactions in the control of hepatic OXPHOS.

Lastly, with respect to ATP contents, the results of the present work were of interest. Although there was an increase in efficiency of OXPHOS, ATP contents were of no significant differences among the four groups. This result is consistent with earlier reports. Infante and Huszagh (2000) reported that ATP homeostasis was not significantly altered by mitochondrial function. There might be regulatory mechanism for ATP production (Corral-Debrinski 1991). The regulatory mechanism is still unknown. It is necessary to elucidate the mechanism in further studies.

The present study provides considerable insight into the role of exercise and supplementation of L-carnitine and antioxidants in the control of mitochondrial function, especially oxidative phosphorylation. It suggests that exercise and supplementation of L-carnitine and antioxidants might improve more efficiently impaired OXPHOS in mitochondrial dysfunction that is recognized as an important cause of degenerative diseases. Further studies are needed to investigate the effects of exercise and supplementation of L-carnitine and antioxidants on OXPHOS in animals predestined to develop degenerative diseases.

Summary and Conclusion

This study investigated the effects of exercise and supplementation of L-carnitine and antioxidants on hepatic mitochondrial function, especially oxidative phosphorylation (OXPHOS). Isolated hepatic mitochondria from 4 rat groups were functionally tested by an analysis of its respiration and the coupling of this process to ATP synthesis in the presence of ADP. Four groups were non-trained, non-supplemented group (NTNS), non-trained, supplemented group (NTS), long term-trained, non-supplemented group (LTNS), and long term-trained, supplemented group (LTS). The trained rats ran on a treadmill (grade 10°, 20m/min) for 60min/day for 8 weeks. The supplemented rats were treated with L-carnitine (0.5% diet), vitamin E (0.5mg/g BW), vitamin C (0.5mg/g BW) and melatonin (1 μg/g BW) for 8 weeks. The results are as follows :

1) There were exercise effects on improving mitochondrial OXPHOS. Exercise of non-supplemented rats resulted in a decrease in state 4 and an increase in RC ratio and P/O ratio. Significant exercise effects on state 4, RC ratio and P/O ratio were identified by an analysis of variance.

2) There were supplementation effects on improving mitochondrial OXPHOS. Supplementation of non-trained rats resulted in a decrease in state 4 and an increase in RC ratio and P/O ratio. Significant supplementation effects on state 3, state 4 and P/O ratio were identified by an analysis of variance.

3) There were additive effects of exercise and supplementation on OXPHOS. Within supplemented rats (LTS vs. NTS), exercise resulted in an increase in RC ratio. Significant supplementation effects on state 3, state 4, RC ratio and P/O ratio were identified by an analysis of variance.

It suggests that exercise and supplementation of L-carnitine and antioxidants might improve more efficiently impaired OXPHOS efficiency in mitochondrial dysfunction that recognized as is an important cause of degenerative diseases.

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