Vol. 188, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1992 Pages 102-107

# RESPIRATORY CHAIN ENZYMES IN MUSCLE OF ENDURANCE ATHLETES: EFFECT OF L-CARNITINE

Rosa Huertas*, Yolanda Campos*, Enrique Díaz*, Jesús Esteban*, Leonardo Vechietti+, Giussepe Montanari+, Stefania D'Iddio§, Marco Corsi§ and Joaquín Arenas*[1]

* Centro de Investigación, Hospital 12 de Octubre, Madrid, SPAIN
+ FIDAL, Biomedical Center of Gubbio, ITALY
§Medical Research Dept, Sigma-Tau, Pomezia, ITALY

Received August 4, 1992

SUMMARY. The effects of L-carnitine on respiratory chain enzymes in muscle of long distance runners were studied in 14 athletes. These subjects received placebo or L-carnitine (2 g orally b.i.d.) during a 4-week period of training. Athletes receiving L-carnitine showed a significant increase ($p < 0.01$) in the activities of rotenone-sensitive NADH cytochrome c reductase, succinate cytochrome c reductase and cytochrome oxidase. In contrast, succinate dehydrogenase and citrate synthase were unchanged. No significant changes were observed after placebo administration. The levels of both total and free carnitine from athletes receiving placebo were significantly decreased ($p < 0.01$) after treatment. By contrast, total and free carnitine levels were markedly increased ($p < 0.01$) after supplementation with L-carnitine. Our results suggest that L-carnitine induces an increase of the respiratory chain enzyme activities in muscle, probably by mechanisms involving mitochondrial DNA. 

Endurance training promotes an increase in the volume and mass of mitochondria relative to other cellular constituents within muscle fibers of the exercised limbs (1). These morphological changes are accompanied by parallel increases in the maximum activities of tricarboxylic acid cycle enzymes of fatty acid transport and oxidation and in part of respiratory chain enzymes (2).

Carnitine performs a crucial role in the energy supply of the muscle during exercise, by controlling the influx of fatty acids into mitochondria (3). In addition, carnitine facilities

[1]To whom correspondence should be addressed.

0006-291X/92 $4.00

oxidation of pyruvate and branched amino acids (4), and contributes to the protection of cells from these deleterious agents by preventing the accumulation of acyl CoAs (5). During the exercise of long duration, the increased esterification of muscle carnitine reduces the free carnitine pool in muscle, leading to carnitine insufficiency (6).

L-carnitine has been shown to stimulate pyruvate dehydrogenase complex (PDHC) in human skeletal muscle (4). In the same way, the aim of the present study is to asses whether treatment with L-carnitine increases the activities of respiratory chain enzymes in muscle of endurance athletes.

## MATERIALS AND METHODS

Subjects and experimental protocol. All the subjects were volunteers and expressed their informed consent to participate in the study. Fourteen well-trained athletes (double blind, parallel groups) were studied. All of them were long-distance runners (LDR), a specialty requiring physical endurance. They have a dietary regimen of 3500 to 4000 Kcal/day, of which proteins represent 13 to 15% of the total caloric intake, and lipids 25% to 30%. Muscle needle biopsies (Vastus lateralis, average net weight: 100-150 mg) were taken at rest to determined basal values of the respiratory chain enzymes and carnitine content. The LDR were then divided at random in two groups: the first group (7 males) was treated with 2 g orally b.i.d. of L-carnitine (Sigma-Tau. Pomezia. Italy) for 28 days; the other group (7 males) received placebo during the same period of time. Carnitine supplementation was suspended 12 hours before muscle sampling. At the same time, the athletes of the two groups started a 4 weeks endurance training program. The weekly training program consisted of running at 40% to 50% $VO_2$ max for 90 minutes/day for 5 days, and at 70% to 80% of the $VO_2$ max for 60 minutes for the other 2 days, corresponding to 130-140 Km/wk. At the end of the training period (28 days) a second biopsy was performed (at rest) and carnitine content, as well as enzyme activities, were re-examined.

Carnitine assay. Free carnitine (FC), short-chain acylcarnitine (SCAC), long-chain acylcarnitine (LCAC) and total carnitine were measured in muscle homogenates as described by Di Donato et al (7).

Enzyme assays. Muscle biopsies were homogenized in 15 volumes of 0.15 M KCl, 50 mM Tris-HCl pH 7.4 in all glass homogenizers and mitochondrial enzymes were measured in supernatants after centrifugation at 800xg for 10 minutes. Describe spectrophotometric assays (8) were used to measure succinate cytochrome c reductase (complex II+III), rotenone-sensitive NADH-cytochrome c reductase (complex I+III), succinate dehydrogenase (complex II), citrate synthase and cytochrome c oxidase. The latter was determined by monitoring the decrease in absorbance at 550 nm of reduced cytochrome c. Reduced cytochrome c was freshly prepared before each experiment by adding to a 1% solution in 10 mM K-Phosphate buffer (pH 7.0). Protein concentration was determined by Lowry method (9).

Statistical analysis. Statistical analysis was performed by one way analysis of variance and Student's test for paired and unpaired comparison.

## RESULTS

Respiratory chain enzymes (Table 1): The basal levels of all respiratory enzymes as well as citrate synthase in muscle of LDR were markedly increased compared to normal age and sex matched control values. The pretreatment activities of all the enzymes in athletes receiving L-carnitine or placebo were similar. LDR receiving L-carnitine showed a significantly increase ($p< 0.01$) of the activities of NADH cytochrome c reductase, succinate cytochrome c reductase and cytochrome c oxidase after treatment, compared to both the pretreatment and the posttreatment receiving placebo levels. In contrast, succinate dehydrogenase and citrate synthase activities remained unchanged. No significant changes were observed after placebo administration.

Carnitine (Table 2): In basal conditions, muscle free and total carnitine content in muscle of LDR were both markedly increased compared to normal controls. No significant differences in the pretreatment amounts of TC, FC, SCAC and LCAC were observed between carnitine-treated athletes and placebo-receiving athletes. In LDR receiving placebo the levels of both TC and FC were significantly lower ($p< 0.01$) after treatment than before treatment, whereas SCAC was unchanged and LCAC was significantly increased ($p< 0.01$). By contrast, TC and FC levels were significantly higher ($p< 0.01$) after treatment than before treatment in LDR supplemented with L-carnitine. However, SCAC and LCAC remained unchanged. In addition, the difference between carnitine-treated athletes and placebo-receiving athletes after treatment was markedly significant ($p< 0.01$) for FC and TC.

## DISCUSSION

In agreement with previous reports (1,2), the values of all respiratory chain enzymes as well as the citrate synthase basal

Table 1. Succinate dehydrogenase (SDH, complex II), rotenone-sensitive NADH-cytochrome c reductase (NADH cyt c red, complex I+III), succinate cytochrome c reductase (succ cyt c red, complex II+III), cytochrome c oxidase (Cox, complex IV) and citrate synthase (CS) in muscle of endurance athletes

| | n | SDH | NADH cyt c red | succ cyt c red | Cox | CS |
|---|---|---|---|---|---|---|
| Controls | 25 | 10.3±3.6 | 11.7±4.0 | 7.5±3.1 | 52.8±16.7 | 120±38.5 |
| Long distance runners | 14 | 15.2±4.3* | 24.3±6.95* | 13.1±4.8* | 129.6±26.6* | 309.8±68.1* |
| Placebo | 7 | | | | | |
| Before treatment | | 14.9±3.8 | 25.5±7.7 | 13.5±5.6 | 131.7±27.7 | 307.3±68.2 |
| After treatment | | 14.8±2.2 | 27.2±8.3 | 14.4±6.7 | 130.8±31.5 | 304.9±67.9 |
| L-carnitine | 7 | | | | | |
| Before treatment | | 15.6±4.7 | 23.2±6.2 | 12.7±4.1 | 127.5±25.4 | 312.3±73.2 |
| After treatment | | 15.8±5.7 | 41.1±9.8+ | 22.6±7.2+ | 198.8±22.7+ | 299.8±66.5+ |

Activities expressed as nmol of substrate utilized. $min^{-1}$. mg $protein^{-1}$ (mean±SD).
* Significant differences vs controls ($p< 0.01$; unpaired t test).
+ Significant differences vs both pretreatment levels and receiving placebo posttreatment levels ($p< 0.01$; one way variance analysis).

Table 2. Muscle free carnitine (FC), short chain acylcarnitine (SCAC), long chain acylcarnitine (LCAC) and total carnitine (TC) in endurance athletes

| | n | FC | SCAC | LCAC | TC |
|---|---|---|---|---|---|
| Controls | 30 | 19.3±0.8 | 2.86±1.50 | 0.40±0.05 | 22±1.10 |
| Long distance runners | 14 | 27.5±3.3* | 2.75±0.70 | 0.36±0.075 | 30.7±3.7* |
| Placebo | 7 | | | | |
| Before treatment | | 27.3±3.1 | 2.71±0.71 | 0.34±0.07 | 30.4±3.5 |
| After treatment | | 24.1±3.2+ | 2.76±0.80 | 0.48±0.09+ | 27.3±3.7+ |
| L-carnitine | 7 | | | | |
| Before treatment | | 27.8±3.5 | 2.8±0.7 | 0.38±0.08 | 30.98±3.8 |
| After treatment | | 31.7±2.9§ | 2.7±0.8 | 0.37±0.09 | 34.8±3.4§ |

Values are expressed in $\mu$mol. $g^{-1}$ of noncollagenous protein (mean ± SD).
* significant differences vs controls ($p< 0.01$; unpaired t test).
+ significant differences vs pretreatment levels ($p< 0.01$; paired t test).
§ significant differences vs both pretreatment and posttreatment receiving placebo levels ($p< 0.01$; one way variance analysis).

values (table 1) were markedly increased in muscle of long-distance runners (LDR). The mechanisms leading to these effects are still unclear, but have been extensively reviewed elsewhere (10).

Our results show that treatment with L-carnitine causes a significantly increase of the activities of NADH cytochrome c reductase, succinate cytochrome c reductase and cytochrome c oxidase in muscle of LDR. The mechanism whereby L-carnitine induces this shift in the respiratory chain enzyme pattern is unknown yet, but mtDNA is likely to be involved (i.e. by stimulation of either mtDNA replication or transcription, or both of them), because of mtDNA codes for several polypeptides of the complexes I, III and IV (11). In contrast, the activities of succinate dehydrogenase, a mitochondrial respiratory chain enzyme encoded by nuclear DNA, and citrate synthase, a enzyme of the mitochondrial matrix, remained at pretreatment levels. Some recent findings indicate that acetyl-L-carnitine treatment is able to stimulate mitochondrial transcription under altered metabolic conditions, such as ageing (12) and hypothyroidism (13). In these conditions, the correct structure and function of mitochondrial membranes appear to be altered (14,15), but acetyl-L-carnitine normalizes the induced alterations. In the same way, in endurance exercise, membrane destabilizing agents, such as acyl CoAs can accumulate giving rise to reduced free carnitine pool in muscle (6,16).

Our results indicate that oral carnitine supplementation is able to "stabilize" muscle carnitine pool, thus preventing a loss of carnitine from tissues to plasma-urine compartments (6). A larger carnitine availability might normalize the alteration in the lipid composition of human muscle mitochondria. The latter is particularly relevant for mtDNA transcription influencing factors such as optimal mitochondrial concentration of ATP and cations (17), import into mitochondria of RNA polymerase and other nuclear DNA encoded proteins required for mtRNA synthesis (18) and processing (19).

However, mitochondrial enzymes levels remained unchanged after treatment in athletes receiving placebo. These data indicate that the training program had little influence, if any, on mitochondrial function.

Acknowledgments: Supported by grants of CICYT (91-0126) and FIS (92/0363). R. Huertas and Y. Campos were supported by grants of FIS and Sigma-Tau España respectively.

REFERENCES

1. Saltin B., and Gollmick P.D. (1983) In Handbook of Physiology. Skeletal muscle. pp 555-631. American Physiological Society, Bethesda.
2. Holleszy J.O., and Coyle E.F. (1984) J. Appl. Physiol. 56, 831-838.
3. Bremer J. (1983) Physiol. Rev. 63, 1420-1480.
4. Uziel G., Garavaglia B., Di Donato S. (1988) Muscle Nerve 11, 720-724.
5. Stumpf D.A., Parker W.D., Angelini C. (1985) Neurology 35, 1041-1045.
6. Arenas J., Ricoy J.R., Encinas A.R., Pola P., D´Iddio S., Zeviani M., Di Donato S., and Corsi M. (1991) Muscle Nerve 14, 598-604.
7. Di Donato S., Rimoldi M., Garavaglia B., Uziel G. (1984) Clin. Chim. Acta 139, 13-21.
8. Di Mauro S., Servidei S., Zeviani M., Di Rocco M., De Vivo D., Di Donato S., Uziel G., Berry G., Hoganson K., Johnsen G., and Johnson P.C. (1987) Ann. Neurol. 22, 498-506.
9. Lowry O.H., Rosenbrough N.J., Farr A.L., and Randall R.J. (1951) J. Biol. Chem. 193, 265-275.
10. Sanders Williams R. (1986) In Biochemical aspects of physical exercise (Benzy L et al; eds) Elsevier, pp 171-180. Amsterdam.
11. Attardi G. (1984) Trends Biochem. Sci. 6, 86-103.
12. Gadaleta M.N., Petruzzella V., Renis M., Fracasso F., and Cantatore P. (1990) Eur. J. Biochem. 187, 501-506.
13. Gadaleta M.N., Petruzzella V., Fracasso F., Fernández Silva P., and Cantatore P. (1990) FEBS Lett. 277, 191-193.
14. Hansford R.G., and Castro F. (1982) Mech. Ageing Dev. 19, 191-201.
15. Hafner R.P., Leake M.J., and Brond M.D. (1989) FEBS Lett. 248, 175-178.

16. Angelini C., Vergani L., Costa L., Martinuzzi A., Dunner E., Marescotti C., and Nosadini R. (1986) Adv. Clin. Enzymol. 4, 103-110.
17. Gaines G., Rossi C., and Attardi G. (1987) J. Biol. Chem. 262, 1907-1915.
18. Fisher R.P., Topper J.N., and Clayton D.A. (1987) Cell 50, 247-258.
19. Topper J.N., and Clayton D.A. (1990) Nucl. Acid. Res. 18, 789-793.