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Effects of L-carnitine on the pyruvate dehydrogenase complex and carnitine palmitoyl transferase activities in muscle of endurance athletes

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Abstract

The effects of L-carnitine on the pyruvate dehydrogenase (PDH) complex and carnitine palmitoyl transferase (CPT) were studied in muscle of 16 long-distance runners (LDR). These subjects received placebo or L-carnitine (2 g orally) during a 4-week period of training. Athletes receiving L-carnitine showed a dramatic increase ($P < 0.001$) in the PDH complex activities. By contrast, the levels of CPT, both 1 and 2, were unchanged. No significant changes were observed after placebo administration. We previously reported [Huertas R. et al., Biochem. Biophys. Res. Commun. 188 (1992) 102–107] that L-carnitine induces an increase in the activities of complexes I, III and IV of the respiratory chain in muscle of LDR. Taken together, our data suggest that the improvement in (maximal oxygen consumption) $V_{O_2\,max}$ observed in LDR after L-carnitine administration is based on these biochemical findings.

Key words: Endurance; Carnitine palmitoyl transferase; Pyruvate dehydrogenase complex; Mitochondria

1. Introduction

The energetic demand during endurance exercise relies almost exclusively upon the aerobic metabolism of carbohydrates and fatty acids. The pyruvate oxidation rate is controlled by pyruvate dehydrogenase complex activity. The PDH complex is composed of three catalytic enzymes (PDH, lipoamide transacetylase, and lipoamide dehydrogenase) and two regulatory enzymes (PDH kinase and PDH phosphatase) [1].

The long-chain fatty acid (LCFA) oxidation rate is controlled by CPT activity. CPT has two functional locations within the mitochondrion [2]: CPT 1, is located on the inner surface of the outer mitochondrial membrane and CPT 2 is situated on the inner surface of the inner mitochondrial membrane.

The oxidation of LCFA is almost completely dependent on carnitine [2]. Moreover, carnitine and carnitine acetyl transferase (CAT) are known to control the acetyl CoA/CoA ratio and therefore pyruvate oxidation [3].

Recently, we documented [4] a marked increase in activities of complexes I, III and IV of the respiratory chain in muscle of endurance athletes receiving L-carnitine. The aim of the present work is to assess how endurance exercise modifies PDH and CPT activities in long-distance runners with and without carnitine supplementation.

2. Materials and methods

2.1. Experimental protocol

All the subjects were volunteers and expressed their informed consent to participate in the study. Sixteen well-trained male athletes (double blind, parallel groups) were studied. All of them were LDR, a speciality requiring physical endurance. Age was 28.3 ± 7.1 years (mean ± S.D.), body weight was 67 ± 5.1 kg (mean ± S.D.). Controls ($n = 22$) were all sedentary age-matched males. Body weight was 69.2 ± 6.7 (mean ± S.D.).

Both, controls and athletes, had a dietary regimen of 3,500 to 4,000 kcal/day, of which proteins represented 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 determine basal values of the PDH complex and CPT. The LDR were then divided at random in two groups: the first group was treated with 2 g orally of L-carnitine (Sigma-Tau) for 28 days; the other group received placebo during the same period of time.

Carnitine supplementation was suspended 12 h 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 below the anaerobic threshold, 40% to 50% of the maximal oxygen consumption ($V_{O_2\,max}$, for 90 min/day for 5 days, and at the anaerobic threshold, 70% to 80% of the $V_{O_2\,max}$, for 60 min for the other 2 days, corresponding to 130–140 km per week. At the end of the training period, 28 days, a second biopsy was performed (at rest) and the PDH complex and CPT activities, were re-examined. All biopsies were immediately frozen and stored in liquid nitrogen until analysis, which was done at the end of the protocol.

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2.2. Biochemical assays

Muscle biopsies were homogenized in 15 volumes of 0.15 M KCl, 50 mM Tris-HCl pH 7.4 in all-glass homogenizers. CPT was measured in total muscle homogenates by the forward reaction (CPT 1) [5], and by the backward reaction (CPT 2) [6]. PDH complex activity was determined in total muscle homogenates by a radiochemical method as described [7]. Non-collagen protein (NCP) was measured by the method of Lilienthal et al. [8].

2.3. Statistical analysis

Statistical analysis was performed by one-way analysis of variance and Student's *t*-test for paired and unpaired comparison.

3. Results

3.1. PDH complex activities (Table 1)

Basal levels of the PDH complex in muscle of LDR were significantly increased ($P < 0.05$) compared to age- and sex-matched control values. Pretreatment activities in athletes receiving L-carnitine or placebo were similar. LDR receiving L-carnitine showed a dramatic increase ($P < 0.001$) in the activities of PDH complex after treatment compared to pretreatment levels. By contrast, in LDR receiving placebo, no significant changes were observed after placebo administration. After treatment, LDR receiving L-carnitine had activities markedly higher ($P < 0.001$) than LDR receiving placebo.

3.2. CPT activities (Table 1)

Under basal conditions, CPT activities, both 1 and 2, in muscle of LDR were markedly increased ($P < 0.001$ and $P < 0.01$, respectively) compared to age- and sex-matched control levels. Pretreatment activities in athletes receiving L-carnitine or placebo were similar. Both in athletes receiving placebo and in those receiving carnitine pretreatment levels did not differ significantly from post-treatment levels.

3.3. Carnitine and citrate synthase activities

Data on carnitine content and citrate synthase (CS) activities in muscle of these LDR have been recently reported elsewhere [4], (Table 2).

Under 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 total carnitine (TC), free carnitine (FC), short chain acylcarnitine (SCAC) and long chain acylcarnitine (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.

The basal levels of CS in muscle of LDR were markedly increased compared to normal age- and sex-matched control values. CS of LDR receiving L-carnitine remained unchanged. No significant changes were observed after placebo administration in CS activity.

4. Discussion

We found that the activities of the PDH complex were significantly increased in muscle of long-distance runners (Table 1). The reasons for these exercise-induced biochemical effects are unknown yet. Possible mechanisms involved have been reviewed elsewhere [9].

Our results show that LDR had a marked increase in CPT 1 levels, and a less marked, but very significant, increase in CPT 2 activities. These findings suggest that during prolonged exercise both CPT 1 and 2, could play a central role in controlling the flux of fatty acids into the oxidation pathway on human skeletal muscle. Exercise-induced increase of CPT 2 activity has been previously shown in rat skeletal muscle [10]. However, to our knowledge, this is the first report that demonstrates an increase of CPT 1 and 2 in muscle of LDR during endurance exercise.

Our data indicate that treatment with L-carnitine dramatically increased the activities of PDH complex in muscle of LDR. Moreover, oral carnitine supplementation is able to 'stabilize' muscle carnitine pool. By contrast, the activities of CPT remained at pretreatment levels. The catalytic efficiency of PDH complex is regulated by a phosphorylation–dephosphorylation mechanism of the α-subunit [11]. PDH kinase, the inhibiting and phosphorylating compound is stimulated by a high acetyl-CoA/CoA ratio. During endurance exercise, py-

Table 1
PDH complex, and CPT (CPT1 and CPT2) activities in muscle of endurance athletes

	n	PDH complex	CPT1	CPT2
Controls	22	2.60 ± 0.80	0.28 ± 0.051	13.5 ± 3.5
Long distance runners	16	3.82 ± 0.94*	1.45 ± 0.19^{+}	21.7 ± 4.7§
Placebo	8			
Before treatment		3.90 ± 0.96	1.48 ± 0.20	22.1 ± 4.8
After treatment		3.88 ± 0.98	1.47 ± 0.22	22.0 ± 4.7
L-Carnitine	8			
Before treatment		3.74 ± 0.92	1.42 ± 0.18	21.3 ± 4.6
After treatment		6.96 ± 1.02**	1.43 ± 0.17	21.7 ± 4.8

Activities are expressed in nmol of substrate utilized · min^{-1} · mg NCP^{-1} (mean ± S.D.). *Significant differences vs. controls ($P < 0.05$); unpaired *t*-test. $^{+}$Significant differences vs. controls ($P < 0.001$); unpaired *t*-test. §Significant differences vs. controls ($P < 0.01$); unpaired *t*-test. **Significant differences vs. both pretreatment levels and receiving placebo post-treatment levels ($P < 0.001$; one-way variance analysis).

Table 2
FC, SCAC, LCAC, TC and CS in muscle of endurance athletes

	n	FC	SCAC	LCAC	TC	CS
Controls	30	19.3 ± 0.8	2.86 ± 1.50	0.40 ± 0.05	22 ± 1.10	120 ± 38.5
Long distance runners	14	27.5 ± 3.3*	2.75 ± 0.70	0.36 ± 0.075	30.7 ± 3.7*	309.8 ± 68.1*
Placebo	7					
Before treatment		27.3 ± 3.1	2.71 ± 0.71	0.34 ± 0.07	30.4 ± 3.5	307.3 ± 68.2
Aftertreatment		24.1 ± 3.2^{+}	2.76 ± 0.80	0.48 ± 0.09^{+}	27.3 ± 3.7^{+}	304.9 ± 67.9
L-Carnitine	7					
Before treatment		27.8 ± 3.5	2.8 ± 0.7	0.38 ± 0.08	30.98 ± 3.8	312.3 ± 73.2
After treatment		31.7 ± 2.9§	2.7 ± 0.8	0.37 ± 0.09	34.8 ± 3.4§	299.8 ± 66.5^{+}

Carnitines: Values are expressed in $\mu mol \cdot g^{-1}$ of noncollagenous protein (mean ± S.D.). *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).

Citrate synthase: Activities are expressed in nmol of substrate utilized $\cdot min^{-1} \cdot$ mg NCP^{-1} (mean ± S.D.). *Significant differences vs. controls ($P < 0.01$); unpaired *t*-test. $^{+}$Significant differences vs. both pretreatment levels and receiving placebo posttreatment levels ($P < 0.001$; one-way variance analysis).

ruvate is oxidized at high rates. As the production rate of acetyl-CoA becomes higher than the rate of its utilization through the Krebs cycle, acetyl-CoA accumulates in mitochondria. CAT, together with L-carnitine, provide a clearance mechanism by transforming the excess of acetyl-CoA into acetylcarnitine. A larger carnitine availability in muscle, therefore, can stimulate PDH complex activity by forming larger amounts of acetylcarnitine from pyruvate-derived acetyl-CoA. In agreement with our results, Uziel et al. [7] in an 'in vitro' study on isolated human muscle mitochondria, reported that at pyruvate concentrations above 0.25 mM, only carnitine concentrations greater than 0.1 mM stimulate PDH complex activity.

Endurance athletes upon L-carnitine loading increase the values of $V_{O_2\,max}$, which may be of practical importance in improving tolerance during exercise [12]. Several lines of evidence, at the biochemical level, seem to support such a physiological response; first, L-carnitine induces an increase of the respiratory chain enzyme activities in muscle [4]; second, L-carnitine stimulates PDH complex activity in muscle; and third, L-carnitine loading prevents the accumulation of LCFA [13], which are potentially deleterious inhibitors of adenylate translocase in muscle. However, we can not rule out the possibility that L-carnitine treatment may affect further factors, i.e. β-oxidation enzymes.

We conclude that in muscle from endurance athletes, when pyruvate is oxidized at high rates, L-carnitine and CAT are able to regulate PDH activity by maintaining a favorable acetyl-CoA/CoA ratio. This conclusion is consistent with our data previously reported [13], which showed that in LDR L-carnitine treatment increased the concentration of acetyl-carnitine in plasma and its excretion in the urine.

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