Effects of L-, D-, and DL-Carnitine on Morphometric Parameters of Skeletal Muscle and Exercise Performance of Laboratory Animals Receiving Carnitine-Deficient Diet

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Serum concentration of L-carnitine, the mean thickness of the skeletal muscle fiber, and exercise performance in the forced swimming test decreased in rats receiving a carnitine-deficient diet. Treatment with L-carnitine compensated for carnitine deficiency, while racemate and D-stereoisomer did not increase its level. L-Carnitine, but not racemate and D-stereoisomer, promoted recovery of the skeletal muscle fiber thickness and exercise performance of rats.

Key Words: *L-, D-, and DL-carnitine; stereopharmacology; carnitine deficiency; exercise performance; skeletal muscles*

Many synthetic drugs are mixtures of spatial isomers with different characteristics. L-carnitine (L-β-hydroxy-γ-N,N,N-trimethylaminobutyric acid, vitamin B_T) involved in the transport of long-chain fatty acids into the mitochondrial matrix, regulation of metabolism of medium-chain acyl-CoA and acyl-CoA with bronched carbohydrate chain, and in conjugation reactions with xenobiotics is an example of stereopharmacological efficiency [1,9]. L-Carnitine is effectively used in combined therapy of anorexia, chronic fatigue syndrome, cardiovascular diseases, hypoglycemia, male sterility, renal diseases, and in hemodialysis. D-Carnitine is biologically inert and causes side effects, manifesting in interference between the D- and L-carnitine [10].

We compared the effects of L-, D-, and DL-carnitine on the morphological parameters of skeletal muscles (*m. gastrocnemius*) and exercise performance of animals receiving carnitine-deficient (CD) diet.

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MATERIALS AND METHODS

Experiments were carried out on 75 outbred male rats (90-100 g). Before the experiment the animals were kept under standard vivarium conditions with free access to water and food. Systemic alimentary carnitine deficiency [12] was induced by CD diet with 0.1% mildronate (Grindex).

The rate and depth of CD deficiency was controlled by measuring serum L-carnitine concentration using L-Carnitine and Enzymatic UV test standard kits (Roche Diagnostics GmbH). Serum L-carnitine concentration below 20 μmol/liter indicated the development of medium severe systemic CD. Oral therapy with optical isomers of carnitine (Sigma) in a dose of 200 mg/kg (10% solution) was carried out for 30 days. Some animals received water after the diet.

The compensation of L-carnitine deficiency (X) was calculated by the formula:

$$X = \frac{C_I - C_D}{C_C - C_D} \times 100\%,$$

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where C_C is L-carnitine concentration in animals after treatment with optical isomers of carnitine, C_D L-carnitine concentration in animals receiving CD diet, and C_I that in intact animals.

For evaluation of exercise performance the rats were forced to swim in a glass cylinder (water temperature 28-32°C) with a load of 10% body weight fixed at the root of the tail (stay under water for more than 10 sec was considered as an indicator of physical fatigue).

Skeletal muscle tissues were histologically studied twice: 80 days after CD diet and 30 days after treatment with carnitine optical isomers. The animals were narcotized with sodium ethaminal (40 mg/kg intraperitoneally).

Histological material was fixed for 24 h in 10% neutral buffered formalin (pH 7.2-7.4), after which 2 fragments (0.5×0.5×0.5 cm) were obtained. Some specimens were processed by routine histological methods in ascending alcohols and chloroform and embedded in paraffin. Sections (5-6 μ) were sliced from paraffin blocks on a rotor microtome and routinely stained with hematoxylin and eosin.

Other specimens were used for preparing cryostate sections (10-15 μ) on freezing microtome. These sections were stained with Sudan III.

Morphometrical analysis was carried out using Video-Test-Morpho-4.0 software. Randomly selected visual fields of micropreparations were photographed using a Pixera digital camera (1.4 megapixels) attached to a Micros microscope (×400). Volume percentage of lipid vacuoles and the mean size (μ^2) of fatty vacuoles in the hepatocytes, cardiomyocytes, and skeletal myocytes, mean thickness (μ) of cardiomyocyte sarcoplasma and striated muscles were measured.

The data were statistically processed using unifactorial analysis of dispersions and Scheffe's test.

RESULTS

The content of L-carnitine in CD rats decreased significantly on day 80 (by on average 56.49%: 19.70±4.97 μmol/liter *vs.* 45.27±6.97 μmol/liter in intact animals). After treatment with L-, D-, and DL-carnitine the compensation of L-carnitine was 118.55 (p<0.001), 18.95, and 9.77%, respectively. Carnitine level in animals treated with DL- and D-carnitine was significantly lower (by 72.61 and 66.47%) in comparison with animals treated with L-carnitine. By the compensation of plasma CD in rats, optical carnitine isomers can be placed in the following series: L-carnitine>>D-carnitine>DL-carnitine.

The duration of swimming during the first 80 days was 11.33±2.53 min in intact animals and 4.08±0.28 min in CD group (p<0.05). After treatment with optical carnitine isomers, the duration of swimming for animals treated by L-carnitine was comparable to that in the control group. For rats treated with DL- and D-carnitine the duration of swimming was by 37.16 and 33.08% below the control, respectively, and by 34.12 and 29.85% lower than in animals treated by L-carnitine (Table 1).

Morphological study of cryotome sections of skeletal muscles showed no accumulation of fatty droplets in intact and CD animals. Evaluation of the mean thickness of muscle fibers showed significant differences (p<0.05) in comparison with the control only in rats treated with carnitine L-stereoisomer (Table 1). The data are comparable with the data indicating that decreased fatty acid metabolism under conditions of carnitine deficiency leads to accumulation of lipids in the liver and to destruction of myofibrils and mitochondrial aggregation at the ultrastructural level [5].

Hence, CD is associated with decreased thickness of skeletal muscle fibers. Correction of CD with L-stereoisomer promoted recovery of the skeletal muscle fiber thickness. Correction of CD with racemate and D-stereoisomer did not lead to appreciable morphological and morphometric differences in the severity of skeletal muscle atrophy.

High concentrations of D-carnitine block L-carnitine transport in the small intestine and reverse reabsorption in the kidneys [3,6]. Pharmacological doses of L-carnitine are less effective than its low doses in normal carnitine-balanced diet [4]. High concentration of D-carnitine (as admixture in drugs or bioactive food additives) can reduce *a priori* low bioavailability of synthetic L-carnitine, thus causing a competitive deficiency of L-carnitine at the level of reaction with the same transporting systems in the intestine [11]. This leads to a significant reduction in L-carnitine level in the skeletal muscles and myocardium [7,8,10].

TABLE 1. Effects of L-, D-, and DL-Carnitine on Morphometric Parameters of Skeletal Muscles and Exercise Performance of Rats Fed CD Diet ($M\pm m$; n=10)

Group	Thickness of muscle fibers, μ	Duration of swimming, min
Intact	37.70±3.40	19.97±6.64
CD	29.10±1.40*	14.94±4.74
CD+L-carnitine	34.00±1.11**+	19.05±3.28
CD+D-carnitine	28.44±1.97*	12.55±3.34
CD+DL-carnitine	30.37±1.85	13.36±4.08

Note. p<0.05 compared to *intact, **CD, +D-carnitine-treated animals.

L-Carnitine is prescribed as an anabolic in anorexia caused by nervous and physical exertion, after surgery and diseases. After treatment with optical carnitine isomers the duration of swimming increased in rats treated by L-carnitine in comparison with CD animals, but virtually did not change in animals treated with DL- and D-carnitine. Correction of CD with L-stereoisomer led to recovery of the skeletal muscle fiber thickness and reduced cardio- and myotoxic symptoms (myasthenia and arrhythmia).

REFERENCES

1. H. Czeczot and D. Scibior, *Postepy Hig. Med. Dosw.* (Online), **59**, 9-19 (2005).
2. M. L. Dubelaar, C. M. Lucas, and W. C. Hulsmann, *Am. J. Physiol.*, **260**, No. 2, Pt. 1, E189-E193 (1991).
3. J. M. Duran, M. J. Peral, M. L. Calonge, and A. A. Ilundain, *J. Membr. Biol.*, **185**, No. 1, 65-74 (2002).
4. A. M. Evans and G. Fornasini, *Clin. Pharmacokinet.*, **42**, No. 11, 941-967 (2003).
5. E. F. Gilbert, *Pathology*, **17**, No. 2, 161-171 (1985).
6. C. J. Gross and L. M. Henderson, *Biochim. Biophys. Acta*, **772**, No. 2, 209-219 (1984).
7. R. Mehvar, D. R. Brocks, and M. Vakily, *Clin. Pharmacokinet.*, **41**, No. 8, 533-558 (2002).
8. P. Molstad, *Biochim. Biophys. Acta*, **597**, No. 1, 166-173 (1980).
9. Monoghraph. L-Carnitine, *Altern. Med. Rev.*, **10**, No. 1, 42-50 (2005).
10. D. J. Paulson and A. L. Shug, *Life Sci.*, **28**, No. 26, 2931-2938 (1981).
11. C. J. Rebouche, *J. Nutr.*, **113**, No. 10, 1906-1913 (1983).
12. M. Spaniol, H. Brooks, L. Auer, *et al.*, *Eur. J. Biochem.*, **268**, 1876-1887 (2001).