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Potential for L-carnitine application in sports practice

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Abstract

The article is devoted to investigating of the L-carnitine effects on the structural and functional characteristics of skeletal muscles and of the myocardium during an intense physical activity. The research was done in 2 stages: experiments on animals (white laboratory mice and rats) and clinical (young athletes). The histochemical study of skeletal muscle tissue of rats has shown that after a regular swimming exercise, L-carnitine did not influence the ratio of the phenotypes muscles (m. soleus, m.plantaris) and did not promote hypertrophy in the muscle fibers of various types. L-carnitine slightly stimulates dynamic physical working capacity in experiment and clinic. The cytoprotective features of L-carnitine during an intense exercise revealed with the electron microscopic technique were confirmed by the findings of the biochemical study in clinic.

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1.1 Introduction. Owing to its pharmacological properties L-carnitine is a promising medicinal agent for the sports pharmacology [1]. Russian scientists regard carnitine as an efficient agent for correcting dysfunctional

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disorders of the organs and organ systems in athletes, induced by an intense physical exercise and by an accompanying stress. The scientists emphasize first and foremost the effects of L-carnitine on the state of the cardiovascular system due to its interference into the myocardial metabolism [2-5].

The main component of the mechanism of carnitine action is believed to be maintenance of the transport of long-chain fatty acids via the inner membrane of mitochondria meant for subsequent oxidation and ATP production (these acids are the main source of myocardial energy). In addition, during an intense exercise, carnitine maintains the pool of a free co-enzyme A that provides the normal metabolic processes (protecting the cells from excessive toxic combinations of acyl-co-enzyme A). It also reduces the activity of pyruvate dehydrogenase and maintains the activity of an aerobic glycolysis, diminishing lactate production which is the main reason of muscle fatigue [6].

Despite such a convincing theoretical substantiation, L-carnitine ability to enhance muscular vigor and performance and speed up recovery after exertion has been debatable up till now [7]. The research findings presented in the international database of PubMed both prove or deny carnitine ability to elevate maximal oxygen consumption, to enhance enzymatic activity in the respiratory chain and decrease lactate level [8,9]. As for the effect of exogenic carnitine in the morpho-functional organization of the skeletal muscular tissue, the available study has no systemic character.

In view of this, the goal of our research was to provide a clinic-experimental substantiation of potential use of exogenic L-carnitine for optimization of the structure and function of both different-type skeletal muscles and of the myocardium during an intense physical exertion.

1.2 Materials and methods. The research was done in 2 stages. In the preclinical stage, experiments were performed on white laboratory mice, 20 to 24 g of body mass and on white laboratory rats, 250 to 300 g of body mass. The test animals were kept in standard setting of a vivarium, with an unlimited access to water and food. All the experimental manipulations were carried out in conformity with the Helsinki declaration regulating the work with test animals.

In order to model a static load, the mice were daily placed into a laboratory plant designed as in [10, 11]. Once daily, for 10 days a run, the animals were kept in a forced vertical position on wooden conical rods with the bases mounted into a metallic grid that was under a direct current (20 to 40 V). The duration of the mice stay on a dielectric rod was regarded as a criterium of their physical endurance. A dynamic load was modelled by daily swimming «to capacity» in water at 28 to 32°C temperature, with a 10% load to the body mass [11, 12] for 14 days (mice) or 20 (rats). Thirty six mice (12 in each series) and 18 rats (6 in each series) were tested in the experiments with a dynamic exercise eighteen mice (6 in each series) were involved in a static exercise test.

The animals residing in the settings of standard motor activity made up group 1 (intact), the rodents with a variety of loads made up group 2 (control), the animals with an administered course L-carnitine («Elcar») from 50 to 100 mg/kg made up group 3 (test group). The drug was injected once a day intraperitoneally, 15 to 20 min prior to a workout. Twenty-four hours after the end of the last workout, the rats were withdrawn from the experiment by ether anesthesia; thereafter the musculus soleus were prepared, which are predominantly of an «oxidative» type of energy production, along with the musculus plantaris that belong to a «glycolytic» type.

The muscles were thereafter frozen and placed into a cryostat-microtom OTF5000 (Bright, Great Britain). A series of transverse section, 13 μ m of thickness were made which were then stained to determine the activity of alkali-resisting adenosine triphosphate (ATP-ase) of myosin and to determine succinate dehydrogenase (SDG) activity [13,14]. Photographing was done by eclipse Ni on a microscope, and digital photcamera DS-Fi1 (Nikon, Japan). After that, the number of typed muscle was evaluated, the diameter of the fibers was determined by a direct-morphometry technique.

To perform an electron microscopy, the muscles were additionally fixed in 4% buffered paraformaldehyde and in 1% OsO₄ solution on a phosphate buffer with added saccharose, after which all this was quenched in Epon 812 (Fluka). Fine transverse sections were prepared on ultramicrotom LKB-III (Sweden), contrasted with uranyl-acetate and lead citrate and thereafter viewed on transmission microscope Hitachi HT 7700.

The clinical study was carried out at the Republican Clinical Children's hospital with an approval of the local ethics committee at the Mordovia State University. Forty athletes (36 boys of them), 12 to 16 of age, going in for biathlon and ski race not less than for 3 years with the intensity of trainings not less than 8 to 10 hours per week were included into the study. During the basic training period, the athletes of the test group (n=20) received 20% solution of L-carnitine «Elcar»; (PIK Pharma company) 50 to 75 mg/kg/day (not more than 3g) orally, in two divided doses for 1,5 month. The control group athletes did not receive any metabolic supplements.

Physical performance was evaluated by a PWC₁₇₀ and maximal oxygen consumption (MOC) in exercise test on bicycle ergometer (Kettler, Germany). The electrophysiological characteristics of the myocardium were studied by the findings of Holter monitoring (HM) of the ECG on a computer – assisted complex «Kardioteknika-04» (Inkart, Russia). The structural and functional indices of the myocardium were assessed by the findings of the echocardiography (Echo CG) on Aloka SSD – 5500 apparatus, Toshiba Aplio-400 (Japan), where the systolic and diastolic functions and the heart dimensions were evaluated. The cardio – and cytoprotective effects of Elcar were determined by the levels of creatin-phosphokinase and lactatdehydrogenase (CPK and LDG) and also by brain natriuretic peptide and troponin I levels on an automatic analyser Pathfast (USA).

The obtained data were processed by using the generally accepted methods of variant statistics. Unequal variances in the obtained data were determined by the Student’s t-test for department and independent samples, «χ²» criterium, with assumed probability of an infallible 95% prognosis.

1.3 Results. At stage I, a comparative analysis of L-carnitine effect upon the skeletal muscles was made in the static and dynamic patterns of physical exercise in a mice group. It was determined that L-carnitine does not prolong the time period of «the animals ability to keep a static position», but it was able to intermittently prolong the duration of «swimming to capacity» (fig. 1).

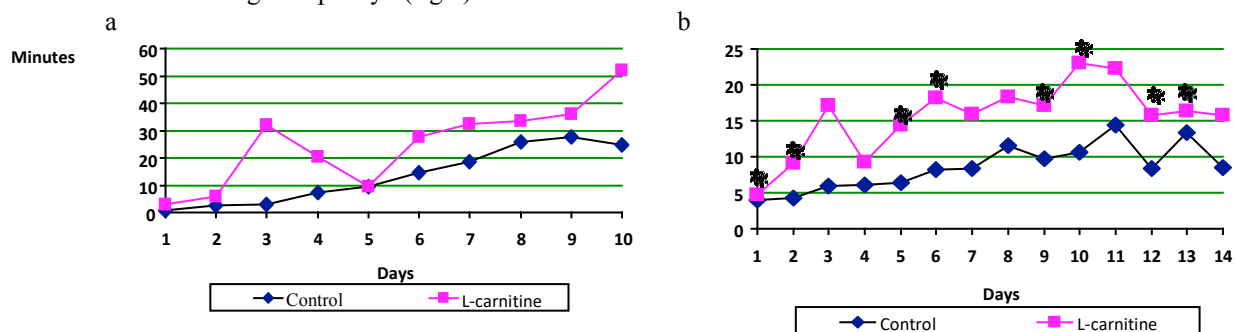


Fig. 1. The effect of L-carnitine on the physical endurance of test mice with applied static (a) and dynamic (b) exercise load. Notes: * statistically significant distinctions in relation to a carries – ponding point in the control group (at p<0,05).

The obtained findings laid the basis for a further study of morphological changes in the skeletal muscles of the rats with predominantly glycolytic (m. plantaris) and oxidative (m. soleus) metabolism in the fibers induced by static and dynamic exercise and mediated by L-carnitine correction effect. L-carnitine in a 100 mg/kg dose did not produce a statistically valid effect either on the type of muscles fibers in both the muscle types, or on the morphometrical indices that evidenced a rise of exertional hypertrophy (Table 1).

Table 1. The diameter of muscle fibers (mcm) in a test for succinate dehydrogenase (SDG) activity and ATP-ase of myosin in the skeletal muscles of the rats.

Muscle	Typing method	Fiber type	№ group		
			1	2	3
Musculus plantaris	ATP-ase of myosin	Type I (slow)	34,3±0,51	34,1±0,48	35,4±0,58
		Type II (fast)	42,4±0,63	47,4±0,68 [#]	46,8±0,84 [#]
	SDG – activity	High	33,0±0,44	35,1±0,35 [#]	36,1±0,42 [#]
		Intermediate	44,8±0,51	47,1±0,56 [#]	48,3±0,60 [#]
Musculus soleus	ATP-ase of myosin	Low	54,6±0,66	58,6±0,60 [#]	59,4±0,69 [#]
		Type I (slow)	37,1±0,92	42,1±0,68 [#]	40,0±1,34
	SDG – activity	Type II (fast)	44,4±0,70	51,9±0,65 [#]	50,0±0,82 [#]
		High	43,6±0,60	44,7±0,52	44,6±0,68
		Intermediate	50,7±0,56	54,7±0,59 [#]	52,5±0,56 [#]

Note: [#] - the distinctions and valid when compared to group 1 (p<0,05).

Further on, electrone microscopy allowed to reveal that an excessive dynamic exercise is followed by a standard set of intracellular note an impairment of the nucleus membrane, sites with loose fibres and ruptured

myofibrils, consolidation or, on the contrary, facilitation of the mitochondrial matrix, with damaged cristae. Edematic areas were perinuclear, or between the myofibrils, around mitochondrial clusters. In the latter case, the edematic areas not infrequently contained fragments of damaged myofibrils. All the mentioned changes in their totality brought disorder into a regular alternation of isotropic and anisotropic discs. In the rats receiving L-carnitine during the dynamic exercise test, the destructive changes mentioned above occurred relatively seldom (Fig.2,3). They had a character intermediate between the changes that occurred in the animals on a standard motion regimen and those subjected to intense exercise.

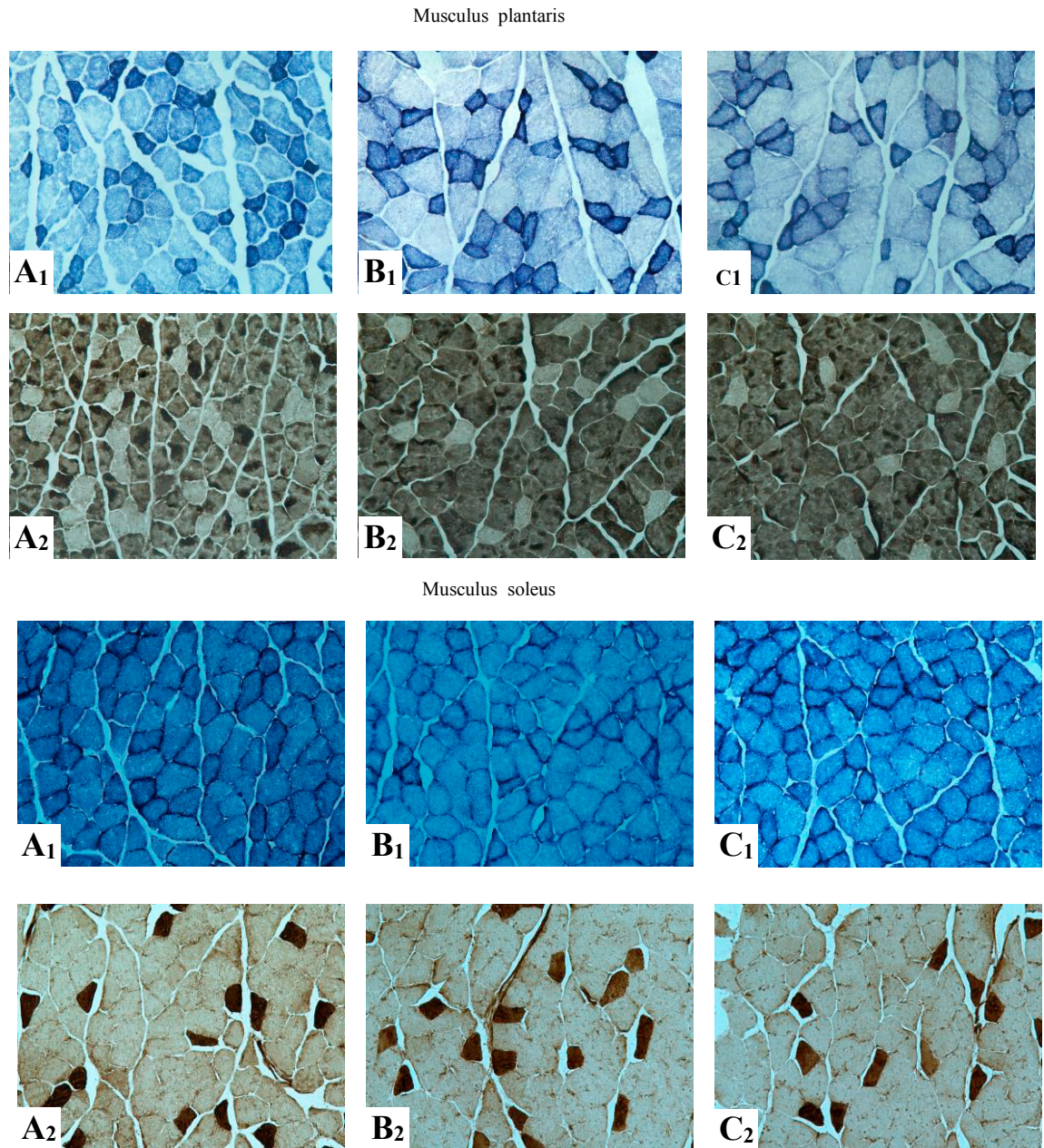


Fig. 2. The effect of dynamic physical activity and L-carnitine (100 mg / kg, ip) on succinate dehydrogenase (A1-C1) and ATPase (A2-C2) activity of plantar and soleus muscles in rats. Magnification x 200:
A - intact animals, B - swimming for 20 days, C - swimming + L-carnitine.

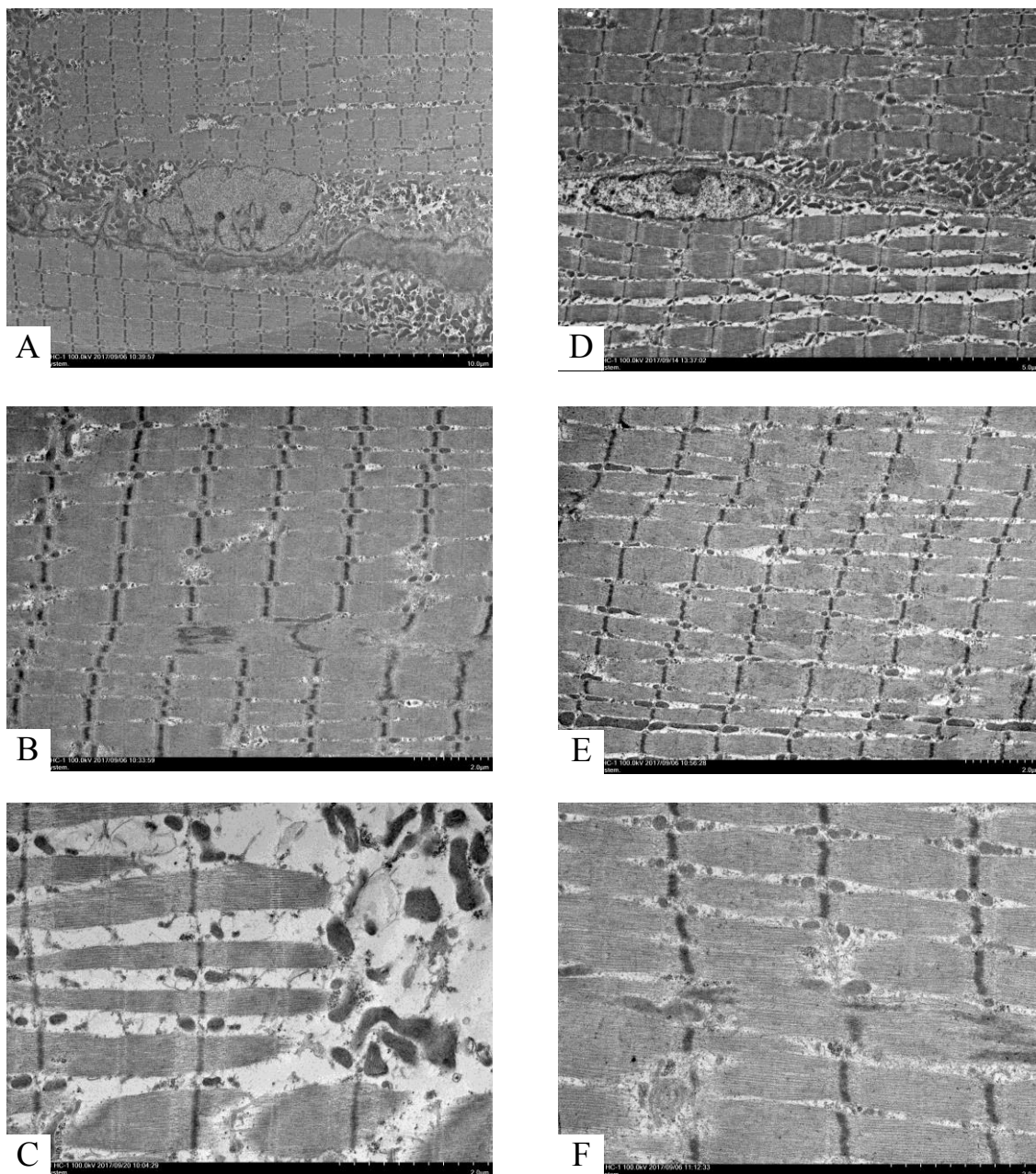


Fig. 3. Ultrastructural myosin myofibrils in *m. soleus* (A, B, E, F) and *m. plantaris* (C, D) of rats subjected to dynamic physical activity (left column) and the action of L-carnitine at a dose of 100 mg / kg (right column). Magnification: upper micrographs x 10000, middle 15000, lower 25000.

In the clinical study L-carnitine course administered to young athletes enhanced their capacity for work along with subjective improvement (elevated muscles tonicity, diminished tiredness after training, a stronger desire for sport activity and offers). The athletes heart rate reached a submaximal level within a more prolonged time interval (11,8% to the original) and they were able to do a more considerable amount of work.

A more prolonged exercise time period and a slower elevation of heart rate (HR) and of arterial pressure at this stage contributed to an increase in capacity for work (PWC₁₇₀ increased by $4,3 \pm 2,1\%$, $p < 0,05$); MOC was seen

to grow (by $5,2 \pm 1,7\%$, $p < 0,05$) (Fig.4). Noteworthy, that the dynamic exercise test was never accompanied by any arising disturbances of cardiac rhythm and conduction or any changes in repolarization (ST-T) on the ECG, while initially 20% of the athletes had such disorders. In the control group, physiology – related myocardial disorders do not undergo changes in response to an intense exercise.

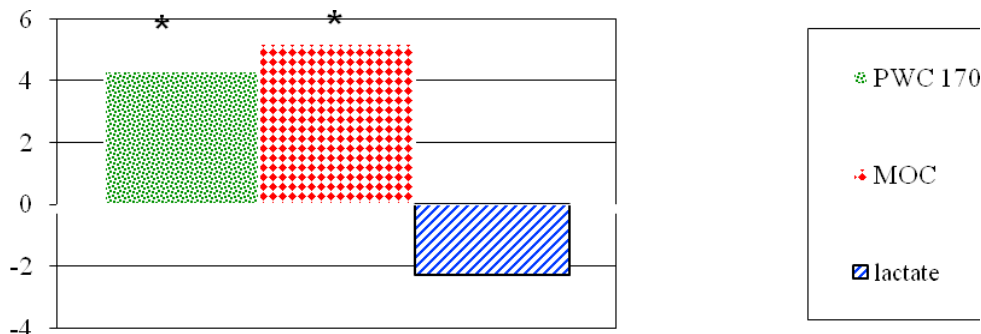


Fig. 4. The dynamics of PWC₁₇₀, MOC and of lactate levels in athletes after the administered course of L-carnitine (in % to the original level).
Notes: * the distinctions from the original values are valid at $p < 0,05$.

Further on, we processed to the study of the dynamics of the structural and functional indices of the myocardium in young athletes who received L-carnitine. We took into consideration the data obtained in the exercise tests as well as the findings of the preliminary examination which revealed signs of dysadaptation of the cardiovascular system of a cardiomyopathy type caused by stress and physical overexertion in 40% of young athletes (in half of the athletes a full variant was identified, in the other half – some signs).

According to the findings on standard ECG and HM, the administration of L-carnitine was seen to reduce electric instability of the myocardium (QTc interval from $385,9 \pm 11,3$ ms to $360,4 \pm 10,2$ ms, $p < 0,05$). It also contributed to complete disappearance of single systoles and ST-T disorders (T-wave inversions and ST-segment depressions), which initially were noted in 17,5% of the children. Besides, the sinus node functioning was seen to improve, as minimal HR within a 24-hour period increased (by 4,7% to the original); the duration in the heart rhythm pauses was seen to shorten (from $1725,3 \pm 108,6$ to $1430,2 \pm 98,7$ ms, $p < 0,05$). In the control group, the signs of myocardial electric instability were seen to reduce (from 15% to 5%, $p > 0,05$), while the sinus node dysfunction retained at the same level. The findings of repeated Echocardiography gave evidence that L-carnitine improved the myocardial contract ability by increasing the ejection fraction (EF) of left ventricle (LV) by 5,7 to 6,4% to the original level within the normal values in the athletes with initially reduced systolic function ($p < 0,05$) (Fig.5). In addition, there was a tendency toward diminished myocardial hypertrophy and diminished myocardial body mass index in the left ventricle (from $93 \pm 16,8$ g/m²) due to improved diastolic function of the LV myocardium. This tendency may also be due to the changes in the main index of the diastolic function, i.e. in the ratio of the rates of early and late diastolic filling a shorter rate from $1,92 \pm 0,13$ to $1,67 \pm 0,18$ ($p < 0,05$) was recorded.

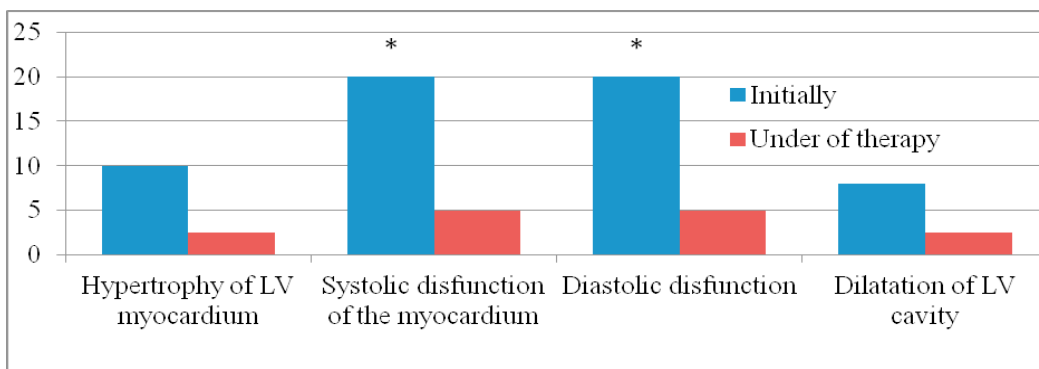


Fig. 5. Dynamics of morpho-functional myocardial disturbances in the athletes, received L-carnitine (in %).
Note: *- the distinctions from the original values are valid at $p < 0,05$.

A statistically significant reduction of creatinphosphokinase level of cardiospecific enzymes (troponin, brain Na-uretic peptide) was recorded in L-carnitine group of athletes (Table 2).

Table 2. Dynamics of some biochemical indices in adolescent athletes who received L-carnitine

	Comparison group		Group of L-carnitine	
	Baseline	After 1,5 month	Baseline	After 1,5 month
Creatinphosphokinase, U/l	157,6±23,25	129,4±14,18	161,5±12,17	101,5±20,72*
Lactatdehydrogenase, U/l	459,0±27,41	439,3±36,73	457,4±43,6	421,3±49,86
Troponine I, ng/ml	0,12±0,014	0,09±0,006	0,11±0,013	0,06±0,008*
Na-uretic peptide, pg/ml	129,4±22,6	104,8±17,5*	128,5±32,7	92,6±24,1*

Note: *The distinctions from the corresponding original values are valid at $p < 0,05$.

Discussion. The bindings obtained in the clinical study have given evidence that L-carnitine (at a 50 mg/kg dose) did not produce any effect on duration of the skeletal muscle tissue work in the presence of static exercise, while in dynamic exercise tests, this drug (100 mg/kg) proved to be more efficient, but its effect was not stable. The histochemical study of skeletal muscle tissue has shown that after a regular swimming exercise, L-carnitine did not alter the ratio of the myosymplastal phenotypes both in oxidative and glycolytic muscles and did not promote hypertrophy in the muscle fibers of various types.

However, the electron microscopic study has revealed L-carnitine ability to correct negative changes in the ultrastructure of the myosymplast in both skeletal muscle types that were caused by excessive dynamic exertion and accompanying stress.

In the clinical study we have confirmed the ability of carnitine to enhance capacity for sport activity. We assume, that this effect may be associated not only with its direct “ergogenic” impact on the skeletal musculature, but also with the ability of this agent to produce a favourable effect on the cardiovascular system. L-carnitine treatment course was noted to improve the morphofunctional characteristics of the myocardium that underwent changes due to an excessive physical exertion, this reducing the number of individuals with signs of remodeled myocardium (from 40 to 10%, $p < 0.05$). Noteworthy is the fact that was not a single full variant of cardiomyopathy induced by overexertion and stress.

In comparison group the number of children with the signs of a remodeled myocardium did not change.

The cytoprotective features of carnitine during an intense exercise revealed by the electron microscopic technique were confirmed by the findings of the biochemical study. In our opinion, the obtained data have proved that the use of L-carnitine in sports medicine is promising, as it prevents myocardial remodeling (induced by an excessively intense exertion and sport stress) and speeds up recovery of the athletes after exercise due to stimulation of intercellular regeneration of the muscle tissue.

Conclusion:

- L-carnitine slightly stimulates dynamic physical working capacity in experiment and clinic.
- L-carnitine 20-day course with a 100 mg/kg dose did not promote exertional hypertrophy of the skeletal muscles, but demonstrated an efficient correction of dysadaptation related changes in the myocardium caused by stress and overexertion.
- L-carnitine course in young athletes (50-75 mg/kg/day for 1,5 month) effectively corrects dysadaptive changes in the myocardium caused by stress and overtraining.

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