

# Immunological aspects of the use of L-carnitine in sports nutrition

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**Abstract.** *The purpose of the work is to study the immunomodulating activity of L-carnitine in junior athletes during the training period. Materials and methods. 20 junior athletes (masters of sports and candidates for masters of sports in swimming) aged 14-18 years participated in the study. Athletes were divided into 2 groups of 10 people each. Athletes of the main group received L-carnitine (600 mg per day) for 4 weeks in addition to the basic diet. Athletes of the control group received only basic diet without sports nutrition. Examination of athletes was performed at the beginning and after 4 weeks of the observation period. Results. As a result of a comprehensive survey of junior athletes, the positive effect of L-carnitine intake on erythrocyte hemoglobin content ( $30.2 \pm 0.4$  pg vs  $28.3 \pm 0.3$  pg at the beginning) was observed. The relative content of basophilic leukocytes in athletes of the main group statistically significantly decreased by the end of the observation period - from  $0.64 \pm 0.05\%$  to  $0.45 \pm 0.04\%$ , which indicated an increase in the body's resistance to allergic reactions. The biomarkers of the immunotropic effect of L-carnitine are a decrease in the expression of the apoptotic marker CD95/Fas on peripheral blood lymphocytes and suppression of the production of pro-inflammatory cytokines synthesized by Th1 lymphocytes with switching the response to humoral immunity. An evidence base for the effectiveness of the use of L-carnitine in sports nutrition for restoring immune dysfunction and adaptive potential of junior athletes has been provided.*

**Keywords:** *sports nutrition, junior athletes, L-carnitine, immunity.*

## I. INTRODUCTION

The indicators of the immunity system in athletes due to physical and psycho-emotional overstrain can go beyond reference values, causing an increase in morbidity and a decrease in sports performance. Currently, much attention is paid to the study of violations of immunoregulation and methods of effective immunocorrection in highly qualified athletes. A wide range of immunomodulating pharmacological drugs for the prevention of transient immunodeficiency in adult athletes, is unacceptable for junior athletes. In this regard, the issue of using nutrients with immunomodulatory properties in youth sports is of particular relevance. One of the most commonly used components of specialized foods for athletes is L-carnitine. Given the evidence-based effectiveness of the use of L-carnitine in sports practice, there are ambiguously interpreted results of its immunomodulating effect. The purpose of the

work is to study the immunomodulatory activity of L-carnitine in junior athletes during the training period.

## II. MATERIALS AND METHODS

The study was conducted with the participation of 20 junior athletes (masters of sports and candidates for master of sports in swimming) aged 14-18 years. Athletes were divided into 2 groups of 10 people each. Athletes of the main group for 4 weeks in addition to the main diet received L-carnitine at 600 mg / day, which amounted to 200% of the adequate daily intake and did not exceed the reference limits of consumption. Athletes in the control group received the main diet without the inclusion of L-carnitine. A survey of athletes of both groups was carried out at the beginning and after 4 weeks of the observation period.

The quantitative composition of the lymphocyte subpopulations in the peripheral blood of the subjects was studied using an FC-500 flow cytometer (BeckmanCoulter, USA) and the Cytomics CXP Software program using double combinations of monoclonal antibodies (Beckman Coulter, USA). At the same time, the percentages of the T-cell population were evaluated: the total number of T-lymphocytes (CD3 +), the number of T-helpers (CD3 + CD4 +), cytotoxic T-lymphocytes (CD3 + CD8 +), natural killer cells (NK cells-CD3- CD16 + CD56 +), natural killer cells with the properties of T-lymphocytes (NKT cells -CD3 + CD16 + CD56 +), B-cell population (CD19 +) lymphocytes, as well as the relative content of lymphocytes carrying activation markers (CD3 + HLA- DR +, CD3 + CD25 +), and apoptosis marker antigen CD45 + CD95 +. As isotypic controls, we used: CD45 / CD14 (for identification of the leukocyte population and isolation of the lymphocyte gate by small-angle and lateral light scattering) and IgG1 / IgG2 (for control of non-specific binding of lymphocytes to antibodies and isolation of lymphocyte-negative gate fluorescence). The immunoregulatory index (IRI) was expressed as the ratio of T-helper cells to T-cytotoxic lymphocytes. Erythrocyte hemolysis was performed automatically at the TQ-PREP sample preparation station (Beckman Coulter, USA).

Determination of the cytokine level: GM-CSF (granulocyte-macrophage colony stimulating factor), INF-Interferon- $\gamma$ , IL-12p70, IL-13, IL-18, IL-1 $\beta$ , IL-2, IL-4, IL -5, IL-6, TNF- $\alpha$  in the blood serum of athletes was performed by the method of multiplex immunoassay using the commercial kit "eBioscience HumanTh1 / Th2 Extended 11-Plex" (Bender Med Systems GmbH, Austria). Measurements

were performed on a Luminex 200 multiplex analyzer (Luminex Corporation, USA).

Hematological parameters were determined on a CoulterACTTM5 diff OV hematology analyzer (Beckman Coulter Int. S.A, USA). Defined parameters: the content of red blood cells, white blood cells, platelets, the concentration of hemoglobin, hematocrit, the average volume of the red blood cell, the average concentration of hemoglobin in the red blood cell, the average concentration of hemoglobin in the red blood cell, the leukocyte formula, the average platelet volume, the relative volume of platelets in the whole blood sample.

Statistical processing of the results was performed using the IBM SPSS Statistics 20.0 software package (IBM, USA). The calculation included the determination of the sample mean, standard error, median, the probability of accepting the null hypothesis about the coincidence of the distributions of the compared samples according to the Student, Mann-Whitney and ANOVA criteria. Differences were considered significant at a significance level of  $p < 0.05$ .

### III. RESULTS AND DISCUSSION

Hematological indicators of athletes. The vast majority of the studied hematological parameters of athletes of the main and control groups at the beginning and at the end of the observation period were within the reference values [1]. In the group of athletes who consumed L-carnitine for 4 weeks, a statistically significant increase in the hemoglobin content in the erythrocyte was  $30.2 \pm 0.4$  pg vs  $28.3 \pm 0.3$  pg at the beginning. This is an important hematological indicator for athletes, especially with aerobic exercise. The results are consistent with data [2] on the stimulation of erythropoiesis factors by L-carnitine.

The content of basophilic leukocytes in athletes of the main group significantly decreased by the end of the observation period (from  $0.64 \pm 0.05\%$  to  $0.45 \pm 0.04\%$ ), which indicates an increase in the body's resistance to allergic reactions. In the control group, this indicator did not change significantly:  $0.60 \pm 0.04\%$  at the beginning of the examination and  $0.70 \pm 0.15\%$  after 4 weeks. It was found that basophilic granulocytes contain histamine granules. The main function of these cells is their participation in the immune response of immediate and delayed type [3].

Indicators of cellular immunity of athletes. The determination of the subpopulation composition or phenotype of lymphocytes is currently an important diagnostic criterion that allows us to assess the state of the immune system and its disorders in various pathological conditions, including during sports loads. All studied indicators of cellular immunity in athletes of the main and control groups at the beginning and at the end of the study were within the reference values [4]. In the group of athletes consuming L-carnitine, a statistically significant decrease in the expression of the apoptotic marker CD95 / Fas on peripheral blood lymphocytes was detected ( $4.08 \pm 0.17\%$  at the beginning of the observation period and  $2.87 \pm 0.01\%$  after 4 weeks), which is a reflection of the processes contributing to the regression of apoptosis of lymphocytes. In the control group, this indicator tended to increase ( $p < 0.01$ ):  $3.99 \pm 0.76\%$  at the beginning of the observation period and  $4.28 \pm 1.51\%$  after 4 weeks.

Cytokine profile of athletes' blood serum. The humoral component of intercellular interactions in the immune system is represented by cytokines, which are protein or polypeptide products of activated cells. Cytokines are mediators in the immune response, hematopoiesis and the development of inflammation. Cytokines are divided into several groups: interleukins, interferons, tumor necrosis factors, colony stimulating factors, and others. The functional specialization of CD4 + T-lymphocytes during the development of the immune response is ensured by their differentiation into T-helper cells of the 1st (Th1) and 2nd (Th2) type, which are characterized by a different spectrum of cytokines [5, 6]. It was established that Th1 lymphocytes produce: IL-2, INF- $\gamma$ , TNF- $\alpha$  and  $\beta$ , carrying out the development of a predominantly cellular immune response. The Th2 subpopulation produces: IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 and is responsible for the development of a humoral immune response [7]. Both types of cells produce IL-3 and GM-CSF. The proliferative activity of Th1 and Th2 lymphocytes is maintained autocrine: growth factor for Th1 is IL-2, for Th2 - IL-4. The regulation of differentiation and the ratios of these subpopulations is also influenced by the products of non-lymphoid cells, in particular macrophages.

The spectrum of cytokines studied in the work can be divided into the following groups: regulators of differentiation of Th1 / Th2 - cell populations: IL-4, INF- $\gamma$ , IL-12p70, IL-13, IL-18; hematopoiesis regulators; GM-CSF, IL-2, IL-5, IL-6; inflammatory factors and inducers of apoptosis: TNF- $\alpha$ , IL-1b.

As a result of studying the serum cytokine profile in athletes who consumed L-carnitine for 4 weeks, a statistically significant decrease in levels (pg / ml, median, minimum-maximum) was revealed from 11 cytokines studied: INF- $\gamma$  - from 18.1 (11.08-64.75) to 8.7 (4.76-50.00), IL-18 - from 102.4 (56.16-192.27) to 48.3 (22.93 -121.82) and TNF- $\alpha$  - from 2.1 (0.00-3.01) to 1.6 (0.00-2.96). In addition, one can note a tendency ( $p < 0.10$ ) to a decrease in the content of IL-13 (pg / ml, median, minimum-maximum) - from 3.4 (0.48-5.13) to 1.3 (0.00-6.15). In the control group, the values of these cytokines did not have statistically significant differences and amounted to: (pg / ml, median, minimum-maximum): INF- $\gamma$  - from 19.7 (12.36-35.62) to 20.3 (10.22-33.17), IL-18 - from 72.2 (37.01-247.39) to 71.5 (34.05-242.57), TNF- $\alpha$  - from 2.9 (0.90-5.74) to 2.7 (0.82-5.20), IL-13 - from 1.3 (0.81-4.46) to 1.5 (0.98-5.13). The results obtained indicate the presence of anti-inflammatory activity of L-carnitine in conditions of intense physical activity.

As is known, L-carnitine as a cofactor is involved in the transfer of long-chain fatty acids through cell membranes from the cytosol to the mitochondrial matrix, in which they are  $\beta$ -oxidized to acetyl-CoA, the substrate for the formation of ATP in the Krebs cycle [2, 8]. Carnitine contributes to a more economical expenditure of glycogen and glucose reserves during prolonged intensive training, participates in the exchange of ketone bodies and choline, and inhibits the formation of lactate and apoptosis [8]. It has now been established that L-carnitine stimulates the physical performance of athletes by switching metabolism under conditions of intense exercise from anaerobic to a more energetically beneficial aerobic route [9].

#### IV. CONCLUSION

Thus, as a result of a comprehensive survey of junior athletes who consumed L-carnitine for 4 weeks in addition to the main diet, it was found:

- L-carnitine increases the content of hemoglobin in the red blood cells;
- L-carnitine increases the body's resistance to allergic reactions;
- Biomarkers of the immunotropic effect of L-carnitine are a decrease in the expression of the apoptotic marker CD95 / Fas on peripheral blood lymphocytes and suppression of the production of pro-inflammatory cytokines synthesized by Th1 lymphocytes, with a response switching to humoral immunity.

The results of this study provide an evidence base for the effectiveness of the use of L-carnitine in sports nutrition to restore immune dysfunction and adaptive potential of junior athletes.

#### REFERENCES

- [1] Zborovsky A.B., Zborovskaya I.A. Internal diseases in tables and diagrams. Directory. M.: Medical Information Agency LLC. 2011. 668 p.
- [2] Balykova L.A., Ivyansky S.A., Pixikina O.A., Efremova Yu.A. Justification of the use of L-carnitine in sports medicine. Sports medicine: science and practice, 2011. Vol.1. Pp. 22-29.
- [3] Kozinets G.I. Laboratory diagnosis. Blood and urine tests. Clinical significance. M.: "Practical medicine." 2011. 160 p.
- [4] Zemskov A.M., Zemskov V.M., Karaulov A.V. Clinical immunology. Moscow. "GEOTAR Media", 2005. 319p.
- [5] Zurochka A.V., Khaydukov S.V. Cytometric analysis of T-helper subpopulations (Th1, Th2, Treg, Th17, T-activated helper cells). Medical Immunology, 2011. Vol.13 (1), Pp. 7-16.
- [6] Kepshinsky C.A. The role of T-helper types 1 and 2 in the regulation of cellular and humoral immunity. Immunology, 2002. Vol.2. Pp. 77-79.
- [7] Yarilin A.A. Symbiotic relationships of cells of the immune system. Immunology, 2001. Vol. 4. Pp. 16-20.
- [8] Aartsma-Rus A., Van Ommen G. J. and Kaplan J.C. Innovating therapies for muscle diseases. Handb.Clin. Neuro, 2013. Vol. 113. Pp. 1497–1501.
- [9] Kraemer W.J., Volek J.S., Dunn-Lewis C. L-Carnitine Supplementation: Influence upon Physiological Function. Curr. Sports Med. Rep., 2008. Vol. 7. Pp. 218-223.