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Imbalance of n6/n3 fatty acids in the training period and after competition in cross-country Skiers

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Abstract: The aim of the study was to investigate the level of essential polyunsaturated fatty acids (PUFAs) in athletes into the training period and after endurance race (n=15 high-trained male cross-country skiers, real members of national skiing team). The PUFAs in plasma was determined by the gas-liquid chromatography method. Results demonstrate a deficiency of plasma level of the n-3 PUFA of the almost all skiers during the training period. Competition activity modified the essential PUFA profile in blood across more twice increase of the n-3 docosahexaenoic acids (DHA) (p=0.112) and n-3 alpha-linolenic (ALA) (p=0.048) levels and decrease n-6 PUFA relative to baselines, which expressed in a decrease in n6/n3 ratio (p=0.017). Relationships between n-3/n-6 ratio and base heart rate, diastolic pressure and Maximal oxygen uptake, show on the significant role essential PUFAs in regulation of the cardiovascular system in cross-country skiers.

Key Words: Essential fatty acids, Heart rate, Blood pressure, Cross-country skiers, Competition.

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1. Introduction

Research of essential substrates, for example n-3 polyunsaturated fatty acids (PUFAs), meet with great interest in the world sport because of their key role in enhancing physical performance [1, 2]. Linoleic acid (LA) and alpha-linolenic acid (ALA) are the major n-6 and n-3 fatty acids.

In the body, LA is metabolized to arachidonic acid (AA), and ALA is metabolized insignificantly to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [3]. Studies on the effects of n-3 PUFA supplementation have predominantly focused on their potential ability to reduce cardiovascular risk factors and peripheral neuromuscular function [4],

improves endothelial function, maximal oxygen uptake [1, 5] in endurance-trained athletes. n-3 PUFAs have been shown to decrease the production of inflammatory eicosanoids, cytokines, and reactive oxygen species; have immunomodulatory effects; and attenuate inflammatory diseases [6].

Some studies [7, 8] have reported that medium intensity exercise and exercise till exhaustion changes the percentage of individual saturated FAs. Little is known about the effect of exercise on percent distribution of the essential fatty acids in blood highly trained athletes, especially after competitions. Determination of these metabolites pre- and post-exercise could be good markers in order to evaluate the diet and training strategy. The aim of this work was the estimate of the essential PUFA levels of skiers for the training period and after competition.

2. Materials and Methods

Fifteen healthy male high-trained athletes (age: 20.7±5.4 years; body mass: 68.9±5.2 kg; body mass index: 22.2±1.3 kg/cm2, body fat: 9.4±3.1%) participated in the study. We studied real members of the Komi Republic and national skiing team during the general training season as well as during competitions. Heart rate (HR) and work load were continuously recorded, Breath-by-breath measurements (VO2 and VCO2) were taken throughout exercise by using an automated gas analysis system (Jaeger Oxycon Pro, Wuerzberg, Germany). Maximal oxygen uptake (VO2max) was 4.3 ± 0.4 l min-1, and maximal oxygen uptake by RER (VO2max RER) was 3.5 ± 0.6 l min-1.

The subjects provided written informed consent to participate in the present study. The experimental protocol was in accordance with the Declaration of Helsinki. The study was designed and performed according to the guidelines of the Local Research Bioethics Committee of the Institute of Physiology of the Komi Scientific Centre of the Ural Branch of the RAS.

The athletes performed a 15-km race (medium distance, medium intensity exercise) on National Cup competitions in classical cross-country style in Russia. Blood sampling was taken fasting in the pre-competition period at rest and after 5 min medium distance - race 15 km which was performed during the day. The plasma profile of total fatty acids was determined using gas-liquid chromatography («Crystal 2000M» Chromatek, Russia, with a flame ionization detector attached to a SupelcoWAX 25 m x 0.23 mm), as described by Lyudinina et al. (2018) [8]. The FA concentrations were expressed as a weight percentage of the total level of FAs. The reported fasting means («reference») of plasma total FA (mol%) in healthy adults were taken from a literature source [9].

Statistical analyses were performed using Statistica software (version 6.0, StatSoft Inc., 2001, USA). All results were presented as Average ± Standard Error. The correlation coefficients between two variables were determined by Spearman rank analysis. The value $p<0.05$ was accepted as statistically significant.

3. Results

The profile of essential fatty acids of skiers at rest into the training period is characterized by deficiency of n-3 linolenic (ALA), n-3 eicosapentaenoic acids (EPA) and n-3 docosahexaenoic acids (DHA) in blood plasma compared with the reference dates of the almost all participants. At the same, percentage of n-6 PUFAs was higher than the reference means [9] (Figure1).

The n-6 PUFAs consisted of 36.7 ± 1.5% LA (C18:2), 9.2 ± 0.7% AA (C20:4). The average value of the n-3 ALA (C18:3) was 0.3 ± 0.04%, EPA (C20:5) was 0.7 ± 0.2% and level of DHA (C22:6) was 1.3 ± 0.3%. The n6/n3 balance by skiers at rest was within 8.8-40.3 in average 29.8± 6.4%.

A positive correlation was found between n-3/n-6 ratio with HR at rest (rs = 0.42; p=0.001) and negative correlation n-3/n-6 ratio with Diastolic pressure (rs = -0.50; p=0.023) and VO2max (rs= -0.52; p=0.017) in the high-trained skiers at rest (Table 1). Moreover, the relationships between EPA and HR (rs= - 0,69, p=0.018) and links ALA with systolic pressure (rs= - 0,71, p=0.009) show on the significant role essential PUFAs in regulation of the cardiovascular system in cross-country skiers.

Table 1. Correlation analysis between levels of n-3 PUFA in total lipids with blood pressure and heart rate at rest from high-trained skiers at rest (Rs/p)

	n-3 C18:3	n-3 C20:5	n-3 C22:6	n6/n3
Heart rate	0.16 / p=0.458	**-0.69 / p=0.018**	-0.04 / p=0.837	**0.42 / p=0.001**
Systolic pressure	**-0.71 / p=0.009**	0.16 / p=0.495	-0.31 / p=0.177	0.133 / p=0.564
Diastolic pressure	0.10 / p=0.656	0.26 / p=0.236	0.08 / p=0.709	**-0.50 / p=0.023**
VO2max	-0.23 / p=0.318	0.29 / p=0.179	0.36 / p=0.124	**-0.52 / p=0.017**

Note: Rs = Spearmen correlation coefficient

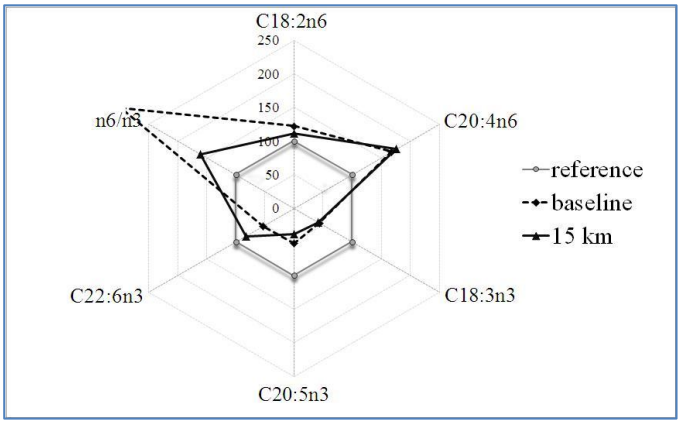

Figure 1. Plasma essential polyunsaturated fatty acids at rest and after competition in cross-country skiers to compare baseline (100%)

Competitive activities (races 15 km) have modified the profile of the essential PUFA in the blood. We marked an increase in the level of n-3 ALA (C18:3) (p=0.048), DHA (C22:6) (p=0.112) and decrease of the n-6 LA (p=0.078) and n-6 AA (p=0.232) relative baseline. We found a decrease of the n6/n3 ratio from 29.8±6.5 to 16.1±0.9 after endurance race (p=0.017).

A negative correlation was found between plasma levels of n-6 linoleic acid (C18:2) (Rs=-0.69; p=0.006) and docosahexaenoic acid (C22:6) (Rs=-0.66; p=0.014) with the total score in the proceeding of the competition (data from source www.flgr-results.ru).

4. Discussion

The PUFA composition of plasma and cell membranes is, to a great extent, dependent on dietary intake [9, 10]. The average percentage of ALA in plasma in athletes in the training period consisted 0.3%, the levels of EPA and of DHA – 0.7% and 1.3%, respectively. Findings data were twice less relative to the recomended [9]. The low proportion of ALA was observed in all the skiers, DHA - in 92% of subjects, which is probably due not only to inadequate intake of essential acids. Previous studies have shown that n-3 PUFAs are important cell-signaling molecules which rapidly and directly alter the transcription of specific genes [1]. Moreover, ALA may function as a fuel substrate during long-duration exercise, especially in the later stages when carbohydrate stores are depleted [3], that can also be the cause of its low levels in blood.

The higher values of n-6 linoleic, arachidonic acid and more high n6/n3 ratio (29.8/1) in skiers comparison with recomended means (5-10/1) (Figure 1) may be related to inflammation and coagulation markers whereas excessive radical formation and trauma during high-intensity exercise

leads to an inflammatory state. Moreover, the adequate balance of omega-6/omega-3 fatty acids is an important determinant in decreasing the risk for coronary heart disease [1].

We observed that the level of n-3 linolenic acid (C18:3) rose considerably (50%) after competition distances. We also noted a significant, twofold increase in DHA (C22:6) concentrations upon completion of 15-km races. Nieman (2015) found that the ALA is strongly mobilized during exercise, with plasma levels increasing nearly 6-fold following prolonged, intensive exercise [11]. The increase levels of the n-3 DHA (C22:6) and ALA (C18:3) in some of the subjects after the competition might be attributed to the released from membrane phospholipids by cellular phospholipases or made available to the cell from the diet or other aspects of the extracellular environment [1].

PUFAs are known to be the substrates most vulnerable to the active oxygen species impact. Therefore, oxidative destruction of PUFA n3 might act as indirect anti- rather than pro-oxidant in vascular endothelial cells and may be followed both by cell membrane parameters alteration and by the change in functional activity of membrane-bound protein compounds, i.e. enzymes, receptors, transporters [12]. Omega-3 fatty acids supplementation improves endothelial function and maximal oxygen uptake in endurance-trained athletes [5]. Levels of n-6 AA and LA decreasing during the recovery after competition that it was accounted for by the lipid peroxidation processes variation. It might be supposed that the decrease observed in AA and LA levels was due to its being involved as substrate into free-radical oxidation and acute hypoxia-induced metabolic changes in glycerol-containing lipids. Chronic hypoxia decreased the proportion of n-6 PUFA, particularly linoleic acid, which was accompanied by a proportional increase in n-3 PUFA, particularly DHA in young rat hearts [6].

5. Conclusions

The obtained results demonstrate deficiency of plasma level of the n-3 PUFAs at rest of the almost all skiers requiring adjustments during the training period. Competition activity modified the essential PUFA profile in blood across increase of the DHA and ALA levels and decrease n-6 PUFA relative to the basal data. Relationships between n3/n6 ratio and base heart rate, diastolic pressure and Maximal oxygen uptake show on the significant role essential PUFAs in regulation of the cardiovascular system in cross-country skiers.

Results may be application by optimization of the training process, correction nutrition programs becouse adequate supply of the body with n3 PUFA could play role in physical resistance and for prevent the inflammation in muscles and joints and for overall health of the athletes.

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Author Contributions

A.Yu. Lyudinina carried out the biochemical studies, performed the statistical analysis and drafted the manuscript; **E.R. Boyko** conceived of the study, and participated in its design and project administration. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

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Conflict of interest

The authors declare no conflict of interests regarding the publication of this manuscript. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the

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