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Essential Fatty Acid Associated with Heart Rate Variability in Highly Trained Male Cross-Country Skiers: A Pilot Study

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Abstract: Polyunsaturated fatty acid (PUFA) metabolites play important roles in the modulation of vascular tone, heart rate variability (HRV), and cardiovascular diseases. This study was undertaken to examine the relationship between HRV and the plasma levels of essential acids. Methods: Highly trained cross-country skiers participated in the study (n = 19). Time-domain and frequency-domain HRV analyses were performed. The plasma levels of fatty acids were determined using gas–liquid chromatography. Results: Plasma eicosapentaenoic acid and docosahexaenoic acid were found to be negatively correlated with resting heart rate (HR) (p = 0.026). The plasma levels of alpha-linolenic acid (ALA) were positively associated with the relative value of high-frequency power (rs = 0.465, p = 0.045) and negatively correlated with the sympathovagal balance ratio (rs = −0.493, p = 0.032) and the absolute and relative values of low-frequency power (rs = −0.490, p = 0.028). The plasma levels of arachidonic acid (ARA) were positively associated with the relative value of high-frequency power (rs = 0.59, p = 0.006) and negatively correlated with the sympathovagal balance ratio (rs = −0.54, p = 0.017) and the relative values of low-frequency power (rs = −0.52, p = 0.022). No correlation was found between n6/n3 and HRV parameters except for HR and pNN50. Conclusions: n-3 PUFAs and ARA play an important role in the autonomic regulation of heart rate in highly trained skiers. Athletes with substantial deficiencies in plasma ALA and excess levels of ARA had increased sympathetic and decreased parasympathetic activity.

Keywords: autonomic nervous system; heart rate variability; alpha-linolenic acid; arachidonic acid; essential fatty acids; cross-country skiers

Citation: Lyudinina, A.Y.; Markov, A.L.; Bojko, E.R. Essential Fatty Acid Associated with Heart Rate Variability in Highly Trained Male Cross-Country Skiers: A Pilot Study. *Physiologia* **2024**, *4*, 54–63. https://doi.org/10.3390/physiologia4010003

Academic Editors: Anastassios Philippou and Michael Koutsilieris

Received: 1 December 2023
Revised: 10 January 2024
Accepted: 16 January 2024
Published: 19 January 2024

1. Introduction

Heart rate variability (HRV) is a method of assessing the regulatory mechanisms of physiological functions in humans and is extensively used in sports medicine [1–3]. High HRV and low heart rate (HR) have been identified as hallmarks of health [4,5] and are associated with the risk of cardiac events in the general population [6].

Currently, the classical assessment of HRV is carried out using time-domain and spectral parameter HRV. The standard deviation of normal RR intervals (SDNN) is an index of the overall HRV and reflects both sympathetic and parasympathetic influences. The pNN50 reflects the parasympathetic neural regulation of the heart. The total power (TP) is regarded as an index of comprehensive autonomic activity. The power spectrum of the HF band is affected by the activity of the vagal nerve, while the power spectrum of the LF band mainly relates to the activity of the sympathetic nerve. The ratio of absolute power in the LF and HF bands (LF/HF) is considered to be an indicator of sympathetic–parasympathetic balance [1].

The Olympic endurance sports are associated with a high risk of cardiovascular disease. There are few works in the literature on the study of HRV in cross-country skiers [7,8], in which a higher sympathetic tone is considered a risk factor for exercise-related sudden

cardiac deaths in men. At the same time, of particular interest is the study of HRV in cross-country skiers since they have the highest rates of maximal oxygen consumption among all sports [3,8,9].

The functional state of highly trained athletes includes complex endocrine and/or neural regulatory mechanisms, in which essential fatty acids play an important role. Fatty acids metabolized by cardiomyocytes are used not only as energy substrates (contribution to ATP synthesis reaches 40–60%) but also for the synthesis of the triglycerides and phospholipids of cell membranes, affecting the structure and functional activity of the heart [5]. The mechanisms of their action on the cardiovascular system are different and still remain a source of discussion. To date, works devoted to the connection between blood metabolites and the parameters of heart rate under maximal physical activity in highly trained athletes are single cases.

A meta-analysis study demonstrated a reduction in the risk of cardiovascular events by increasing essential polyunsaturated fatty acid (PUFA) intake [10]. Previous work has shown that n-3 polyunsaturated fatty acids (n-3 PUFAs) are required for normal health, especially brain development and cardiovascular function [11,12]. Some studies suggest that n-3 PUFAs protect against coronary artery disease [12], ventricular extrasystoles, thrombosis, arrhythmia, and inflammation [11,13,14], but data have been inconsistent. Particular importance is attached to essential n-3 PUFAs in optimizing physical and aerobic performance [11]. n-3 PUFAs are components of membrane phospholipids throughout the body, but docosahexaenoic acid (DHA) is highly concentrated in the central nervous system and also abundant in cardiac tissue, which might modulate the autonomic control of the heart [12].

A number of experimental and clinical studies demonstrate that n-3 PUFAs both lower resting HR and increase resting HRV [4,12,15–18]. Long-term supplementation with n-3 PUFAs might exert effects on vagal tone and, hence, HRV; the incorporation of them into synaptic membranes could potentially influence the autonomic control of the heart [12]. ALA is not equivalent in its physiological effects to the long-chain n-3 PUFAs found in marine fish oils [5] and is also of interest for cardiovascular disease prevention. Alpha-linolenic acid (ALA, 18:3n-3), a plant-derived n-3 fatty acid, can be partially metabolically converted into long-chain n-3 PUFAs, including eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) [11,19]. The enzymatic conversion of ALA into EPA and DHA is relatively inefficient in humans [13,20]. There are a few epidemiological and interventional studies on the effect of n-3 PUFAs on HR of healthy humans [12,15–18]. At the same time, there are no data regarding the relationship between n-3 PUFA levels in the plasma and HRV of highly trained cross-country skiers as a model of high aerobic performance. The purpose of this study was to examine the relationship between heart rate variability and the plasma levels of n-6 and n-3 essential fatty acids in cross-country skiers. Since n-3 PUFAs are completely essential acids, we hypothesized that a low concentration of them in the blood may contribute to the tension of regulatory systems and decreased HRV in athletes.

2. Results

The data for the HRV of cross-country skiers are presented in Table 1.

The HRV data analysis indicated a shift in the autonomic nervous system balance to an increase in the activity of the parasympathetic nervous system. Observations included increases in the pNN50 level and the relative value of HF.

The median percentage of ALA (18:3n-3) in the plasma of athletes was 0.21% (with a range of 0.01–0.53%), while that of EPA (20:5n-3) was 0.7% (with a range of 0.08–1.97%), and that of DHA (22:6n-3) was 1.3% (with a range of 0.07–2.04%); these median values are lower than the recommended percentages [19]. Low levels of 18:3n-3 and 22:6n-3 were observed in all studied men. The median percentage of ARA (20:4n-6) in the plasma of skiers was in the reference range and equaled 6.7% (with a range of 0.3–10.3%). The average value of linoleic acid (LA) (18:2n6) was 35.4% (limits, 20.7–41.7%), which was detected at the upper

limit of reference. The percentage of essential PUFA levels in the relative recommended norms [21] is presented in Figure 1.

Table 1. Heart rate variability parameters in cross-country skiers.

Parameters	Median (25–75th Percentiles)
HR (bpm)	57.00 (51.50–64.00)
pNN50 (%)	45.40 (28.60–54.50)
SDNN (ms)	65.44 (60.11–87.37)
TP (ms^2)	3978.56 (3057.90–5972.84)
HF (ms^2)	1562.33 (988.92–1742.63)
LF (ms^2)	1177.46 (899.86–2166.45)
HF, %	49.00 (25.75–57.55)
LF, %	38.10 (31.15–53.40)
LF/HF	0.79 (0.55–1.86)

Note: HR—resting heart rate; pNN50—the percentage rate of times a successive RR interval was greater than the previous interval by >50 ms; SDNN—the standard deviation of normal RR intervals; TP (ms^2)—total power; LF/HF ratio—the ratio of absolute power in low-frequency power (LF = 0.04–0.15 Hz, in ms^2 and %) and high-frequency power (HF = 0.15–0.4 Hz in ms^2 and %).

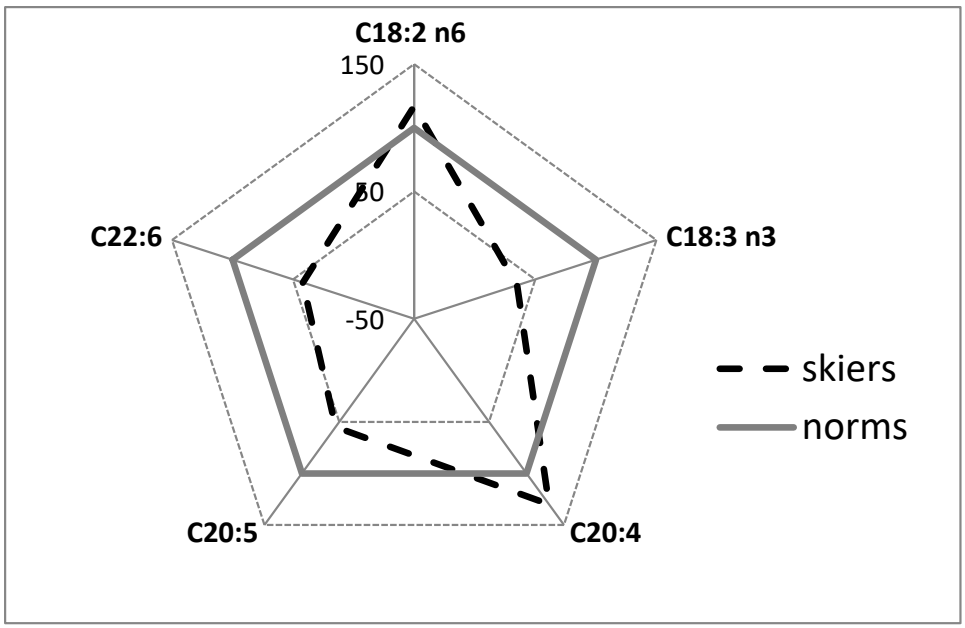


Figure 1. The percentage of essential PUFA levels in cross-country skiers in the relative recommended norms [21]. Note: Data are presented as variations in PUFA norms, taken as 100%. ALA—alpha−linolenic acid (C18:3n-3); EPA—eicosapentaenoic acid (C20:5n-3); DHA—docosapentaenoic acid (C22:6n-3); LA—linoleic acid (C18:2n-6); ARA—arachidonic acid (C20:4n-6).

Spearman correlation analysis showed that ALA was negatively correlated with LF/HF (rs = −0.493, p = 0.032), the absolute value of LF (rs = −0.490, p = 0.028), and the relative value of LF (rs = −0.478, p = 0.038) (Table 2). The plasma levels of ALA (18:3n-3) were positively associated with the relative value of HF (rs = 0.465, p = 0.045). No significant correlations were observed between ALA and HR (rs = 0.111, p = 0.652), pNN50 (rs = 0.207, p = 0.395), SDNN (rs = −0.279, p = 0.247), TP (rs = −0.405, p = 0.077), or the absolute value of HF (rs = 0.161, p = 0.509). The plasma levels of ARA were positively associated with the relative value of high-frequency power (rs = 0.59, p = 0.006) and negatively correlated with the sympathovagal balance ratio (rs = −0.54, p = 0.017) and the relative values of low-frequency power (rs = −0.52, p = 0.022).

Table 2. Spearman correlation coefficients between heart rate variability parameters and essential fatty acids (mol%).

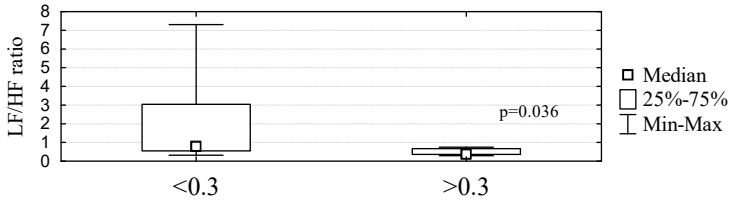
Parameters	Alpha-Linolenic Acid (ALA)		Eicosapentaenoic Acid (EPA)		Docosahexaenoic Acid (DHA)		Linoleic Acid (LA)		Arachidonic Acid (ARA)		n6/n3	
	Spearman, r	*p*-Level	Spearman, r	*p*-Level	Spearman, r	*p*-Level	Spearman, r	*p*-Level	Spearman, r	*p*-Level	Spearman, r	*p*-Level
HR (bpm)	0.11	0.652	**−0.47**	**0.037**	**−0.49**	**0.026**	0.23	0.335	0.29	0.215	**0.575**	**0.009**
pNN50 (%)	0.21	0.395	0.16	0.498	0.20	0.391	−0.17	0.478	−0.14	0.569	−0.44	0.060
SDNN (ms)	−0.28	0.247	0.14	0.569	−0.02	0.919	−0.18	0.454	−0.28	0.222	−0.18	0.454
TP (ms^2)	−0.41	0.077	0.12	0.613	−0.08	0.743	−0.27	0.237	−0.36	0.113	−0.13	0.586
HF (ms^2)	0.16	0.509	0.05	0.835	0.10	0.663	0.10	0.673	0.27	0.256	−0.26	0.283
LF (ms^2)	**−0.49**	**0.028**	0.13	0.537	−0.10	0.672	−0.27	0.256	−0.38	0.111	−0.07	0.775
HF, %	**0.46**	**0.045**	−0.14	0.552	0.20	0.392	0.29	0.204	**0.59**	**0.006**	−0.10	0.678
LF, %	**−0.48**	**0.038**	0.04	0.865	−0.18	0.442	−0.24	0.308	**−0.52**	**0.022**	0.11	0.639
LF/HF	**−0.49**	**0.032**	0.08	0.724	−0.19	0.423	−0.25	0.291	**−0.54**	**0.017**	0.11	0.639

Data are correlation coefficients and *p*-values. Spearman's rank correlation was used. Note: HR—resting heart rate; pNN50—the percentage rate of times a successive RR interval was greater than the previous interval by >50 ms; SDNN—the standard deviation of normal RR intervals; TP (ms^2)—total power; LF/HF ratio—the ratio of absolute power in low-frequency power (LF = 0.04–0.15 Hz, in ms^2 and %) and high-frequency power (HF = 0.15–0.4 Hz, in ms^2 and %); ALA—alpha-linolenic acid (C18:3n-3); EPA—eicosapentaenoic acid (C20:5n-3); DHA—docosapentaenoic acid (C22:6n-3); LA—linoleic acid (C18:2n-6); ARA—arachidonic acid (C20:4n-6). *p*-values<0.05 are shown in boldface type.

No correlation was found between EPA, DHA, and HRV except for heart rate (rs = −0.47, p = 0.037 for EPA and rs = −0.49, p = 0.026 for DHA). No correlation was found between LA and heart rate variability parameters. No correlation was found between n6/n3 and heart rate variability parameters except for heart rate (rs = 0.58, p = 0.009) (Table 2).

The links between n-3 PUFA levels and HRV are presented in Figure 2.

The LF/HF ratio was significantly higher in subjects with ALA levels < 0.3% than in subjects with ALA levels > 0.3% (p = 0.036), and a similar trend was noted for DHA (p = 0.107). No significant differences in the LF/HF ratio were observed for different concentrations of EPA (p = 0.939).



Plasma level a-Linolenic acid, mol%

Plasma level Eicosapentaenoic acid, mol%

Figure 2. *Cont.*

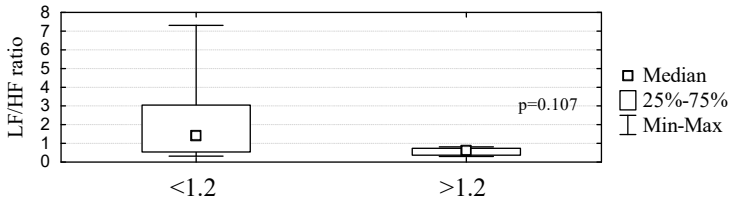


Figure 2. Comparison of the LF/HF ratio in subjects with different degrees of ALA, EPA, and DHA deficiency. Note: LF/HF ratio—the ratio of absolute power in low-frequency power (LF = 0.04–0.15 Hz, in ms^2 and %) and high-frequency power (HF = 0.15–0.4 Hz, in ms^2 and %); ALA—alpha-linolenic acid (C18:3n-3); EPA—eicosapentaenoic acid (C20:5n-3); DHA—docosapentaenoic acid (C22:6n-3).

3. Discussion

We believe that this is the first study to investigate a possible relationship between essential PUFAs and HRV as an indicator of cardiac autonomic function in highly trained skiers. We demonstrated lower n-3 PUFA levels in skiers relative to the recommended value [21], and FA levels presented a correlation with one or more HRV parameters or with mean heart rate.

Arachidonic acid was also negatively correlated with LF, indicating increased vagal activity. We assume that the connection between ARA and HRV indicators occurs through its metabolites, for example, the autonomic modulation of circulating cytokines and catecholamines [5]. ARA has several physiological functions as a constituent in the phospholipid bilayer of cell membranes, as a precursor for a crucial group of biologically active compounds known as eicosanoids (C20 unsaturated lipids), as a regulator of gene expression, as an inflammatory intermediator, and as a vasodilator/vasoconstrictor [10], and it is also related to many chronic diseases, especially cardiovascular diseases (CVDs) [22].

A number of experimental and clinical studies demonstrate that n-3 PUFAs both lower resting HR and increase resting HRV [12,14–18]. It should be noted that not all studies report a positive effect from n-3 PUFAs on HR and HRV [23,24].

According to our data, the n-3 PUFA values in the plasma of skiers during the pre-competition period were lower than the recommended levels (Figure 1). According to this source, an n6/n3 ratio over 8.0 is a marker of cardiovascular risk (hazard ratios) [21]. A negative correlation was observed between EPA and DHA concentrations (mol%) in total plasma lipids with resting HR (EPA—rs = −0.47, $p = 0.037$; DHA—rs = −0.49, $p = 0.026$). No correlation was observed between ALA and HR (rs = 0.111, $p = 0.652$). Unfortunately, there are few studies on the effect of ALA on HR. Humans with high ALA consumption tend to have decreased HR [25]. Some data, however, do not reveal any association between ALA and HR [16]. Similarly, our study detected no significant effect from ALA on HR. Our study shows the relationship between the levels of n-3 PUFAs in plasma, not dietary n-3 PUFAs themselves, and HR.

The mechanism behind this association remains a source of controversy. Common hypotheses are that n-3 long-chain PUFA intake affects HR and HRV via (a) autonomic modulation, (b) changes in cardiac electrophysiology, and/or c) the autonomic modulation of circulating cytokines and catecholamines [5]. The prolonged consumption of alpha-linolenic acid is associated with the long-term enhancement of acetylcholine receptors and an increase in brain acetylcholine concentrations [26], resulting in increases in parasympathetic tone, with corresponding decreases and increases in HR and HRV, respectively [5,27].

Another mechanism by which n-3 PUFAs potentially alter HR is by directly influencing myocardial voltage-gated ion channels. n-3 PUFAs affect a myriad of molecular pathways; for example, they can alter the physical and chemical properties of cellular membranes, and they can also directly interact with and modulate membrane channels and proteins [28]. By modulating the conductance of the myocardial Na and Ca channels, n-3 PUFAs increase the

depolarizing current required to elicit action potential and prolong the refractory period, resulting in an overall reduction in HR [5,29]. Together, these effects suggest that the antiarrhythmic potential of diets enriched with plant-derived n-3 PUFAs results, in part, from direct effects on cardiac ion channels.

Various PUFA metabolites include prostaglandins, prostacyclin, thromboxanes, hydroxyeicosatetraenoic acids, leukotrienes, lipoxins, and epoxyeicosatrienoic acids. n-3 PUFAs directly affect inflammatory prostaglandin and cytokine (IL-1, 2, and 6; TNF-) production and are released through various methods, including competition with n-6 fatty acids for shared enzymatic pathways and the altered expression of inflammatory genes via transcription factors (which play a role in regulating and reducing 12/15-lipoxygenases and may act as coactivators of PPAR-) [11]. The upregulation and/or downregulation of the three different ARA metabolic pathways (involving cyclooxygenase (COX), cytochrome P450 (CYP) enzymes, and lipoxygenase (LOX)) lead to or protect against CVDs [10]. This suggests that n-3 PUFAs can also affect circulating inflammatory factors independent of autonomic modulation, which, in turn, may affect HR and HRV [5].

We measured ALA, EPA, and DHA as components of total plasma lipids because they are significant biomarkers of the short-term dietary intake of essential PUFAs [21]. The levels of ALA and DHA in all athletes were below the recommended values [21]. A study by Wilson and Madrigal (2016) also shows relatively low intakes of omega-3 PUFAs in athletes [30]. That study indicates the insufficient intake of PUFAs by athletes and may also be associated with their intensive expenditure. Dietary ALA, similar to other dietary fatty acids, undergoes significant utilization by tissues, e.g., the heart and muscle, for energy, or it is recycled by tissues to be used as a carbon source for the production of other fatty acids, amino acids, and sterols in various organs, e.g., the brain and liver [19]. This may also be the cause of the low blood levels of n-3 PUFAs.

ALA is a common constituent of green leaves and a number of different seeds (flaxseeds, chia seeds) and their oils, and some nuts also contain considerable amounts of alpha-linolenic acid [19]. The enzymatic conversion of ALA into EPA and DHA is relatively inefficient in humans, with less than 1% converted into DHA and 0.3% to 8% into EPA in men, while up to 21% is converted into each product in women [18]. Although the efficacy of individual n-3 PUFAs on cardiac rhythm has not been extensively studied, the relative potency of n-3 PUFAs may be as follows: DHA $\geq$ EPA >> ALA [4].

The results of our study indicate links between ALA and HRV. The ratio of absolute power in the LF and HF bands (LF/HF) is considered an indicator of sympathetic–parasympathetic balance [1]. We assumed that athletes with deficiencies in ALA and DHA in the plasma had increased sympathetic and decreased parasympathetic activity. A decrease in parasympathetic activity and the appearance of higher-order slow waves indicate the nonoptimal regulation of heart rhythm. Thus, it is possible that with a severe deficit of ALA in the plasma of athletes, the tension of the regulatory mechanisms of the circulatory system is likely to increase, and functional reserves are likely to decrease.

In a large cohort of over 5000 men and women aged >65 years, habitual fish consumption, providing omega-3 long-chain PUFA intake in the normal dietary range (300–500 mg/day) and higher, was associated with improved HRV, particularly in parameters indicating augmented vagal activity and reduced erratic sinus-node firing [28]. Christensen and co-workers (2005) found low SDNN values in women with ALA deficiency, and linear multiple regression analysis revealed that ALA was positively associated with HRV [13]. The results of a meta-analysis of 15 studies showed that high-frequency power increases significantly with the consumption of fish oil, but the LF/HF ratio trends downward, indicating enhanced vagal tone [17]. La Rovere et al. (2013) showed that, with the use of 1 g/day of n-3 PUFAs for 3 months, the mean RR interval, SDNN, and very low-frequency power increased [31].

Limitations

This study is not without limitations. First, the study sampling is relatively small, so further studies with larger athlete populations are needed to define the precise effect of essential fatty acids on cardiac autonomic function in skiers. Second, a control group was not included in the study design. Third, from a statistical standpoint, this study lacks the power to assess causality, and there is a risk of spurious correlations between variables due to multiple statistical tests. In addition, when working with athletes, there is always the possibility of individual differences and deviations.

4. Materials and Methods

4.1. Study Participants

A pilot, prospective study of athletes was conducted. Nineteen highly trained male cross-country skiers (median age: 18.0 years, height: 180.0 cm, body mass: 70.3 kg, body mass index: 22.5 kg/m^2, body fat: 9.5%, fat-free mass: 65.7kg) from national and regional skiing teams in Russia were recruited to participate in the study during the general training season. The current members of the regional ski team were repeatedly examined in the training (June–September) period. Data on the anthropometric characteristics of the cross-country skiers are presented in Table 3.

Table 3. Anthropometric characteristics of the participants.

Characteristics	Median (25–75th Percentiles)
Age, years	18.0 (18.0–29)
Body height, cm	180.0 (167.0–190.1)
Body mass, kg	70.3 (57.0–77.0)
Body mass index, kg/m^2	22.5 (18.5–24.3)
Fat mass, %	9.5 (4.8–13.9)
Fat-free mass, kg	65.7 (48.5–68.8)

The criteria for inclusion in the study were active sports experience and age (16–33 years). All athletes were under the direction of strength coaches and had undertaken a minimum of 5 years of cross-country skiing practice as part of their main training and competition schedule. The exclusion criteria were availability of acute, chronic diseases (for example, bronchial asthma) and taking drugs and alcohol.

Body mass and height parameters were determined with standard methods. All participants provided written informed consent after being informed of the study design. This study was approved by the Local Research Bioethics Committee of the Institute of Physiology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences (approval date: 1 November 2013 and 28 December 2022) in accordance with the Declaration of Helsinki.

4.2. Procedures

4.2.1. Body Composition Assessment

Body composition was assessed using a tactile bioelectrical impedance device, AC-CUNIQ BC380 (Republic of Korea). Prior to body composition measurement, participants received instructions on how to be adequately hydrated to enable the precise measurement of FFM, which was used in further analysis: the day before the study, participants were recommended to restrict intense physical activity, exclude a late dinner, and limit caffeine and high salt in foods. Participants were advised to empty their bladder before the procedure.

4.2.2. Heart Rate Variability

Prior to the HRV measurement, subjects rested for 10 min. An electrocardiogram (ECG) was recorded for 5 min in the supine position. The ECG recordings were analyzed using the Ecosan-2007 complex. These complexes were released in a special series for experiments

in the "Mars 500" project [1]. The following parameters of heart rate variability were analyzed: resting heart rate (HR), the standard deviation of normal RR intervals (SDNN), the percentage rate of times a successive RR interval was greater than the previous interval by >50 ms (pNN50), total power (TP = 0–0.4 Hz), high-frequency power (HF = 0.15–0.4 Hz, in ms^2 and %), low-frequency power (LF = 0.04–0.15 Hz, in ms^2 and %), and the ratio of absolute power in the LF and HF bands (LF/HF).

4.2.3. Measurement of Fatty Acids

Venous blood samples were collected from athletes after at least 12 h of fasting. The samples were collected into vacutainer tubes (Becton Dickinson BP, UK) containing heparin as an anticoagulant. The plasma levels of essential fatty acid (FA) were determined using gas–liquid chromatography. Sample preparation included extracting lipids from plasma and obtaining fatty acid (FA) methyl ethers using methanol and acetyl chloride, as described by Lyudinina et al. (2018) [9]. Gas–liquid chromatography analysis of the FA methyl ethers was performed with a gas chromatograph ("Crystal 2000M", Chromatek, Russia) with a flame ionization detector attached to a SUPELCOWAX (25 m × 0.23 mm) capillary column (Supelco, Bellefonte, USA) at a temperature range of 170 °C to 250 °C (retention time, 2 min). The temperature increase rate was 4 °C/min (overall time, 25 min). Helium was used as the carrier gas, the volume rate was 0.6 mL/min, and the flow separation rate was 1/65. The evaporator temperature was 260 °C, and the detector temperature was 200 °C. FA identification was performed using Sigma standards. The quantitative analysis of the FA concentrations was performed using the internal standard of margarine acid solution (C17:0). The FA concentrations are expressed as a weight percentage of the total weight of FAs. The reported fasting recommended means of plasma total FA (mol%) in healthy adults were taken from a pre-existing source [21]. We measured essential n-3 ALA; EPA and DHA; and n-6 LA and ARA as components of total plasma lipids because they are significant biomarkers of the short-term dietary intake of essential PUFAs, and an n6/n3 ratio over 8.0 is a risk marker of cardiovascular risk (hazard ratios) [21].

To examine the influence of ω3 PUFA levels on HRV, we divided the athletes into two groups relative to the reference values. Since almost all of the subjects had a deficiency in n-3 PUFAs, we classified their deficiency as more or less than 50%. For ALA, the 50% cutoff value was 0.3% (13 skiers out of 19); for EPA and DHA, the cutoff values were 0.7 and 1.2%, respectively (11 and 12 skiers out of 19). Since the plasma level of n-6 PUFAs in most athletes was in the normal range, we did not divide them into groups based on this factor.

4.2.4. Questionnaire of Fats (QFat)

As we described earlier [32], all the athletes were on a standardized balanced diet (in the SAF RK of the Center for Sports Training of National Teams) and lived in the same city. The athletes used QFat, an online screening questionnaire developed by us independently; it includes an assessment of the consumption frequency of fat-containing products and the actual amount. Consumption of n-3 PUFAs in all surveyed young men averaged 2.1 g/day and was generally within the reference values. However, dietary deficiency in EPA + DHA was noted in more than 70% of all volunteers, as well as α-LNA deficiency in 35% of athletes.

4.3. Statistical Analysis

Statistical analyses were performed using the Statistica software (version 6.0, StatSoft Inc., 2001, Tulsa, OK, USA). All results are presented as the median and 25th and 75th percentiles. Normality was analyzed with the Shapiro–Wilk test to determine the homogeneity of the variances. Differences between the groups were analyzed using the Mann–Whitney U test. A Spearman correlation test was used to analyze the correlation of HRV with ALA, EPA, and DHA. A value of $p < 0.05$ was accepted as statistically significant.

5. Conclusions

We demonstrated nonoptimal heart rhythm regulation in athletes with essential PUFA deficiency in blood plasma. With ALA and ARA deficiency, athletes experience an increase in the activity of the sympathetic nervous system. The links shown between ALA and ARA with HRV parameters (a positive association with the relative value of high-frequency power and a negative correlation with the sympathovagal balance ratio and the relative values of low-frequency power) indicate the significant role of essential PUFAs in the high performance of cross-country skiers.

A more than twofold decrease in the levels of ALA and DHA in the plasma relative to the recommended values is associated with a decrease in heart rate variability. Although the exact mechanism of this impairment is still unknown, deficiency in n-3 and n-6 PUFAs contributes to autonomic dysfunction in these skiers. These results may be useful for optimizing sympathetic–parasympathetic balance, which can be a preventive measure to improve the physical function of athletes. Further studies with larger athlete populations are needed to define the precise effect of fatty acids on cardiac autonomic function.

Author Contributions: A.Y.L. carried out the biochemical studies, performed the statistical analysis, and drafted the original manuscript; A.L.M. obtained data on HRV and helped to draft the manuscript and methodology; E.R.B. conceived of the study and was responsible for supervision and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Program for the Fundamental Research of RAS 2022-2026 (project № 1021051201877-3).

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Local Research Bioethics Committee of the Institute of Physiology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences (approval dates: 1 November 2013, 28 December 2022 without no.).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. We obtained written informed consent from all participants before the study began. We conducted all procedures in accordance with the ethical standards of the 1964 Helsinki Declaration.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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