Dynamics of homocysteine precursors and methylation index in high-class biathletes at the stages of the preparatory period of the annual cycle

UDC 612.829.34

PhD **M.A. Dikunets**[1]
G.A. Dudko[1]
Dr. Sc.Chem. **E.D. Viryus**[1]
Dr. Hab. **E.V. Fedotova**[1]
[1]Federal Science Center of Physical Culture and Sport (VNIIFK), Moscow

Corresponding author: dikunets.m.a@vniifk.ru

Received by the editorial office on 05.06.2024

Abstract

Objective of the study was to identify the dynamics of S-adenosylmethionine, S-adenosylhomocysteine and methylation index in high-class biathletes at the stages of the preparatory period of the annual cycle.

Methods and structure of the study. Participants in the experiment are athletes of the Russian national biathlon team (n=23). Quantitative analysis of homocysteine precursors in blood samples of study participants was performed on an ultrafast liquid chromatography-mass spectrometer with triple quadrupole LCMS-8060 (Shimadzu, Japan).

Results and conclusions. The features of the dynamics of the concentrations of homocysteine precursors and the methylation index were studied within the preparatory period of the annual cycle of high-class biathletes. At the stages of the preparatory period of different orientation, intensity and specificity of the preparation means used, the frequency of occurrence of cases of a reduced level of the methylation index was revealed: at the general preparatory stage – 14,52%, at the special preparatory stage – 32,26%, at the pre-competition stage – 5,00%. It was established that the decrease in the methylation index during the training period under consideration is due to a shift in the direction of training loads towards high-intensity motor modes.

Keywords: *methylation index, S-adenosylmethionine, S-adenosylhomocysteine, biathlon, preparatory period.*

Introduction. Various physical exercises induce changes in circulating inflammatory RNAs and microRNAs previously proposed as biomarkers of heart disease [2]. Several studies have reported elevated levels of homocysteine (Hcy) in the blood of athletes in endurance sports [5, 7], which were negatively correlated with work time [3]. At the same time, there is no consensus regarding the effect of regular physical exercise on Hcy content due to the high variability of loads used by athletes in the annual training cycle, which differ in intensity, duration and modes of muscle work.

Hcy is a non-protein sulfur-containing amino acid, the increased concentration of which in plasma is a risk factor for cardiovascular diseases (CVD) caused by endothelial dysfunction, oxidative stress mechanisms and inflammatory vascular processes [5]. Hcy is also associated with one-carbon metabolism and epigenetics through S-adenosylmethionine-dependent (SAM) DNA methylation [6], the product of which is S-adenosylhomocysteine (SAH), which can be transformed to Hcy. The latter is converted into methionine through the process of remethylation, followed by potential conversion to SAM, thus completing the methyl donor cycle. High bioavailability of SAM is necessary for the synthesis of methyl compounds spent during training for recovery (DNA, adrenaline, acetylcholine, carnitine, creatine) [4] and the synthesis of creatine phosphate, an energy source for muscle contraction [10]. SAH and its hydrolysis product, Hcy, are biological inhibitors of transmethylation. Because of this tightly coupled metabolism, biochemistry and medicine have considered SAM, SAH, and their ratio as an indicator of cellular methylation potential or ca-

pacity index [8] and have proposed their use as sensitive markers for CVD prognosis [10]. At the same time, studies aimed at studying the influence of high-intensity physical activity performed by athletes as part of the training process on the dynamics of the levels of Hcy precursors and the methylation index have not been previously conducted.

Objective of the study was to identify the dynamics of SAM, SAH and the methylation index in high-class athletes specializing in CVS (using the example of biathlon) at the stages of the preparatory period.

Methods and structure of the study. The experiment involved 23 athletes undergoing centralized training as part of the Russian men's national biathlon team (age – 25,29±3,20 years; body weight – 76,02±7,60 kg; body length – 179,5±6,7 cm; relative content of muscle and fat mass, respectively, 51,58±1,59% and 9,45±1,45%; MIC per kg of body weight – 69,61±6,65 ml/min/kg.). All participants were healthy; at least 24 hours before the examination, they were prohibited from performing developmental loads of a cyclic and strength nature. The study was approved by the ethics committee of the Federal Scientific Center VNIIFK (protocol No. 2 of April 1, 2021) and was conducted in accordance with the Declaration of Helsinki. The work was carried out within the framework of the state assignment of the Federal Scientific Center VNIIFK No. 777-00001-24 (subject code No. 001-22/3).

Biochemical control was carried out at the stages of the preparatory period of the annual training cycles of athletes 2021–2023. Quantitative analysis of SAM and SAH in plasma was performed on an ultrafast liquid chromatography mass spectrometer with triple quadrupole and external heated flow electrospray ionization source LCMS-8060 (Shimadzu, Japan).

Results of the study and discussion. The average SAM concentration calculated at the stages of the preparatory period was in the range of 38,54±5,84 ng/ml, which corresponds to the physiological norm for healthy untrained individuals [1]. Statistical analysis did not reveal significant differences between the levels of SAM concentration in the plasma of highly qualified biathletes within the stages of the preparatory period.

In the dynamics of SAH concentration on average for the group of biathletes during the training period under consideration, a more pronounced wave-like character was noted. The average group concentration of SAH at the end of the general preparatory stage (GPE) had a slight tendency to decrease, but did not reach a significant value. At the beginning of the special preparatory stage (SPE) (in July), its value was the highest for the entire period and significantly higher than at the previous stage of preparation (April–June) and the pre-competition stage (PSE) ($p<0,05$). There was also a significant ($p<0,05$) increase in SAH during preparation for the SPE. When moving from SPE to PSE, the concentration of SAH decreased, but only at the trend level ($p>0,05$). On average for the group, the methylation index at the beginning of the SPE (July) was significantly lower than the same parameter at the SPE and PSE ($p<0,05$), while no significant differences were found within the SPE itself ($p>0,05$). The methylation index in the middle of the EPE (August) was significantly lower relative to the values measured at the end of the EPE (June) and at the ESE (October) ($p<0,05$).

As part of the OPE of the annual cycles under consideration, athletes performed physical exercises mainly in a low-speed motor mode under normoxic conditions. Starting from the second half of the stage (from June), the specificity of the exercises gradually increased, determined by the degree of their similarity to the competitive movement in the mode of muscle work, and the proportion of exercises simulating individual phases of the competitive movement and performed with increased intensity of muscle effort increased. During the SPE, the specificity of the muscle work mode in exercises biomechanically similar to competitive exercises increased even more: from low-speed, high-intensity to high-speed, high-intensity modes, simulating competitive and super-competitive motor modes of muscle work. In September, the final month of SPE, the intensity of the muscle work regime reached its maximum, with high-intensity exercises performed under hypoxic conditions.

The identified features of the dynamics of SAH concentration and methylation index in biathletes in terms of the training loads performed, differing in the modes of muscle work, allow us to conclude that the use of variants of low-speed modes of work – low-intensity and high-intensity – in the OPE does not entail a decrease in the functional capabilities of the cardiovascular system. A shift in the direction of training loads towards high-intensity motor modes, characteristic of SPE, leads to an increase in SAH concentration and a decrease in the methylation index.

Previous studies have found that a decrease in the methylation index to 4,4 and below is associated with

oxidative stress and impaired endothelial nitric oxide synthesis [9]. Using this criterion as a grouping variable when conducting frequency analysis in our pilot study, it was possible to identify differences between the stages of the preparatory period in the frequency of occurrence of cases of decreased methylation index in this group of biathletes: in the OPE – 14,52%, in the SPE – 32,26% , on PSE – 5,00%. Taken together, the features of the dynamics of the methylation index identified in the study with changes in the direction and intensity of the load at the stages of preparation allow us to consider this indicator as one of the markers of the functional state of the CVS of athletes of the central nervous system.

A decrease in the cardiorespiratory performance of athletes with a shift in the direction of training loads could theoretically be associated with the accumulation of SAH. By causing feedback inhibition of SAM-dependent methyltransferases, an increase in SAH concentration can affect the pattern of DNA methylation and gene expression, as well as induce apoptosis in endothelial cells [11], contributing to a decrease in the functionality of the cardiovascular system under the influence of high-intensity cyclic loads localized in this area. preparation stage.

Conclusions. For the first time, a quantitative determination of the concentrations of SAM and SAH in the plasma of high-class biathletes was carried out, the methylation index was calculated, and differences in the nature of the dynamics of indicators of this complex were identified within the preparatory period of the annual cycle. It has been established that a decrease in the methylation index at the stages of the preparatory period is associated with a shift in the direction of training loads towards high-intensity motor modes.

References

1. Bravo A.C., Aguilera M.N.L., Marziali N.R., et al. Analysis of S-adenosylmethionine and S-sdenosylhomocysteine: method optimisation and profiling in healthy adults upon short-term dietary intervention. Metabolites. 2022;12(5):373.
2. De Gonzalo-Calvo D., Dávalos A., Fernández-Sanjurjo M., et al. Circulating microRNAs as emerging cardiac biomarkers responsive to acute exercise. Int. J. Cardiol. 2018;264:130–136.
3. Deminice R., Ribeiro D.F., Frajacomo F.T.T. The effects of acute exercise and exercise training on plasma homocysteine: a meta-analysis. PLoS One. 2016;11(3):e0151653.
4. Herrmann M., Schorr H., Obeid R., et al. Homocysteine increases during endurance exercise. Clin. Chem. Lab. Med. 2003;41(11):1518–1524.
5. Iglesias-Gutiérrez E., García-González Á., Montero-Bravo A., et al. Exercise-induced hyperhomocysteinemia is not related to oxidative damage or impaired vascular function in amateur middle-aged runners under controlled nutritional intake. Nutrients. 2021;13(9):3033.
6. Lind M.V., Lauritzen L., Vestergaard H., et al. One-carbon metabolism markers are associated with cardiometabolic risk factors. Nutr. Metab. Cardiovasc. Dis. 2018;28(4):402–410.
7. Mrakic-Sposta S., Gussoni M., Vezzoli A., et al. Acute effects of triathlon race on oxidative stress biomarkers. Oxid. Med. Cell. Longev. 2020;2020:3062807.
8. Schalinske K.L., Smazal A.L. Homocysteine imbalance: a pathological metabolic marker. Adv. Nutr. 2012;3(6):755–762.
9. Stabler S.P., Allen R.H. Quantification of serum and urinary S-adenosylmethionine and S-adenosylhomocysteine by stable-isotope-dilution liquid chromatography-mass spectrometry. Clin. Chem. 2004;50(2):365–372.
10. Xiao Y., Su X., Huang W., et al. Role of S-adenosylhomocysteine in cardiovascular disease and its potential epigenetic mechanism. Int. J. Biochem. Cell Biol. 2015;67:158–166.
11. Zhang Z., Wang L., Zhan Y., et al. Clinical value and expression of Homer 1, homocysteine, S-adenosyl-l-homocysteine, fibroblast growth factors 23 in coronary heart disease. BMC Cardiovasc. Disord. 2022;22(1):215.