Prolonged adaptability of highly qualified athletes to training stresses, studied by longitudinal metabolomic analysis of biofluids

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Abstract

Objective of the study is to determine the feasibility of longitudinal metabolome analysis of biological samples from elite athletes to identify previously unknown and highly sensitive metabolic processes associated with long-term adaptation to intense training.

Methods and structure of the study. To identify metabolic pathways associated with long-term adaptation to training in elite athletes, a computerized search and analysis of relevant scientific publications describing the capabilities of longitudinal metabolomic analysis of biological samples was performed. The search was conducted in the electronic scientific citation databases PubMed, Scopus and Web of Science to identify relevant studies.

Results and conclusions. Careful planning of the pre-analytical phase (including selection of study participants, definition of the analyzed sample, choice of methodology and approach to profiling) is the key to successful longitudinal metabolomic analysis of biological fluids in professional athletes involved in a long-term training cycle. Using such opportunities allows identifying significant shifts in the functioning of the physiological systems of the body of the trainee (endocrine, cardiovascular, oxygen transport system, energy metabolism at the cellular level), which are reflected in the metabolomic profile of the studied biological fluid.

Systematic multi-month training forms a specific phenotype of a highly qualified athlete, determined by his individual ability to adapt and having a unique metabolomic expression. Longitudinal metabolomic analysis of biological fluids provides information necessary for an in-depth study of the mechanisms of long-term adaptive changes occurring in the body of professional athletes.

Keywords: *training loads, long-term adaptation, metabolic profiling, functional state, qualified athletes, mechanisms of adaptation processes.*

Introduction. Physical activity, as an external stimulus, leads to an immediate response of the body to its impact. In turn, metabolites, being substrates and end products of cellular metabolism, directly reflect the cellular activity of the body, and their concentrations react to minor disturbances in cellular homeostasis caused by physical activity [4, 1]. Sports metabolomics, as an "omics" direction of sports biochemistry, evaluates and studies changes in the content of low-molecular compounds (up to 1.5 kDa) in biological fluids of athletes under the influence of physical activity in order to identify metabolic pathways and biomarkers associated with changes in the functional state of the athlete's body [2]. Today, the following tasks are successfully solved based on the metabolomic approach [10,14,5]:

– identification of biomarkers characterizing the level of general fitness of an athlete and allowing to evaluate the effectiveness of training programs and the quality of recovery processes;

– identification of biomarkers that allow reliable identification of the discrepancy between training loads and the functional capabilities of an athlete and to detect the risk of overtraining at early stages.

However, despite the large number of works aimed at solving the above problems, only a few of them are devoted to the use of longitudinal metabolomic pro-

filing of biological fluids (hereinafter referred to as LMPB) of qualified athletes to identify new metabolic pathways that are highly sensitive to functional shifts and associated with long-term adaptation to training loads.

Objective of the study is to identify the potential of longitudinal metabolomic profiling of biological fluids of qualified athletes to identify new metabolic pathways that are highly sensitive to functional shifts and associated with long-term adaptation to training loads.

Methods and structure of the study. A computerized search for relevant articles reflecting the results of studies on the LMPB of qualified athletes was conducted using the electronic scientific citation databases PubMed, Scopus and Web of Science. The search time frame was five years (from January 2020 to January 2025). Only works with an experiment duration of at least 12 weeks were considered.

Results and conclusions. As a result of a computerized search for the keywords "metabolomics and sports", 858 articles were found. Works with an experiment duration of less than 12 weeks were excluded for further consideration. Only scientific materials in which qualified athletes participated (at least five years of experience) were analyzed. Thus, nine articles met the criteria we established. Table 1 presents data on the analyzed objects, the methods used and the strategies of metabolomic profiling in these works.

Most studies used single stress testing or short periods of training exposure. Table 1 shows the lack of uniformity in the choice of objects to be analyzed when conducting the LMPBZh of qualified athletes. In the studies [8, 6, 15, 9], despite the invasiveness of the biomaterial selection procedure, the object of metabolomic profiling was blood, since it contains all the molecules secreted or excreted by the body's tissues. In the studies [13, 12, 11, 3], urine was chosen as the object of study due to the non-invasive method of collection and low requirements for sample storage, however, the high bacterial content in combination with the elevated transportation temperature can lead to a noticeable distortion of its composition.

Only in two studies [7, 9] saliva was used as the LMPBZh object of study. Despite the non-invasiveness of the selection procedure and the wide spectrum of low-molecular metabolites present in saliva, the main disadvantage of this research object is the ultra-low content of metabolites (several picograms per milliliter). In this context, the choice of the object under study is of fundamental importance when conducting LMPBS. When choosing a profiling method, there is a virtually uniform view on its implementation.

Eight studies [8, 13, 12, 11, 6, 3, 15, 9] used liquid chromatography-mass spectrometry with electrospray ionization (HPLC-MS), which allows identifying thermolabile and polar molecules of metabolites present in ultra-small quantities in complex biological matrices. The use of gas chromatography-mass spectrometry (GC-MS) for metabolomic profiling [7, 9] is advisable due to the possibility of identifying

Table 1. Research objects, methods and strategies of metabolomic profiling in the selected scientific publications

Sport / Group	Object	Period	Method	Strategy	Authors
Weightlifting (12 men), middle, long and marathon distance running (10 men), control group (12 men)	blood	1 year	HPLC-MS/MS	target	[8]
Football (41 men)	urine	10 months	HPLC-MS/MS	non-targeted	[13]
Basketball (10 men, 10 women), control groups (10 men, 10 women)	saliva	12 weeks	GC-MS	target	[7]
Football (23 men, 28 women)	urine	11 months	HPLC-MS/MS	target	[12]
Football (134 men)	urine	11 months	HPLC-MS/MS	non-targeted	[11]
Football (42 men)	blood	6 months	HPLC-MS/MS	target	[6]
Football (24 women)	urine	2 years	HPLC-MS/MS	target	[3]
American Football (23 men)	blood	9 months	HPLC-MS/MS	non-targeted	[15]
Basketball (10 men, 10 women), control group (10 men, 10 women)	blood, saliva	12 weeks	GC-MS, HPLC-MS/MS	target	[9]

metabolites using libraries containing mass spectra of several hundred thousand compounds and the absence of matrix effects. However, the need for an additional derivatization stage at the sample preparation stage complicates large-scale studies using this method. In studies [8, 13, 11, 15], scientists used a non-targeted strategy of metabolomic profiling. It is important to note the transition from the non-targeted [13, 11] to the targeted strategy [12, 3] of the Rodas group, confirming the need for verification and validation of the identified markers after using the non-targeted strategy.

The principle of the latter is to conduct a "panoramic" metabolomic study aimed at detecting, identifying and semi-quantitatively determining the maximum number of metabolites in biological samples without a pre-formulated biological hypothesis. The target strategy is used to identify and quantify metabolites associated with the biological processes/pathways under study that characterize the biological function of interest. Multivariate statistics methods were used to process the obtained data in all studies.

Regular performance of specific and high-intensity, high-volume loads over several years leads to the regulation of mitochondrial energy metabolism, amino acid metabolism, fatty acid oxidation, and cellular signaling [8]. A “metabolic trace” associated with the metabolism of tryptophan, purine, phenylalanine, tyrosine, a number of steroid hormones, histidine, methionine, and cysteine has been identified [13, 12, 11, 3]. Increased regulation of glutathione metabolism in basketball players has been revealed for the first time, indicating a significant role of antioxidant protection in the long-term adaptation of athletes in team sports to training loads [7, 9].

Attention to preanalytical aspects (formation of a group of subjects, the analyzed object, the choice of the method and profiling strategy) determines the capabilities of the LMPBZh of qualified athletes participating in a multi-month training process. The implementation of these capabilities affects the potential for detecting significant changes in the functioning of the body systems (endocrine, cardiovascular, oxygen transport, energy supply of intracellular metabolism) of a qualified athlete, reflected in the metabolomic profile of the analyzed biological fluid.

Conclusions. Regular training for several months leads to the development of a characteristic phenotype of a qualified athlete in accordance with his adaptation potential, which has an individual "metabolomic trace". LMPBZh provides an opportunity to obtain data necessary for a deep understanding of the mechanisms of long-term adaptation processes occurring in the athlete's body. Funding

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