

## Crystalloscopic characteristics of saliva in student-athletes with different levels of physical training

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### Abstract:

The issue of using non-invasive methods for diagnosing human fitness remains an important topic in the fields of physical education and sports. **Purpose:** To investigate the features of crystallization and initiating properties of saliva among student-athletes and students who do not participate in sports. **Materials and Methods:** The study involved 86 participants from a university in the Volga Federal District. The participants were divided into two groups: Group 1 (n=42) consisted of students who did not engage in sports, and Group 2 (n=44) consisted of student-athletes who regularly trained in various university sports sections. Saliva samples were collected once. The activity of crystal formation and their excitatory potential in the saliva were assessed. Three categories of salivary indicators were distinguished in the picture of crystal formation: single crystals, polygonal forms, and amorphous structures. Quantitative coefficients Q and P were calculated. The degree of cellular density, element distribution uniformity, degree of pattern destruction signs, and the formation process of the micropreparation marginal zone were analyzed. The crystallogenic bioenvironment was assessed using static methods. **Results:** The study of saliva crystallization revealed statistically significant differences in the crystallogenic and stimulating properties between students with different attitudes towards regular sports. The test showed activation of the biological fluid stimulating ability and optimization of texture characteristics in combination with a decrease in pattern element destruction degree, as well as a moderate increase in the margin of the micropreparations' border in student-athletes compared to those who did not participate in sports. **Conclusions:** Regular sports activities contribute to the normalization of metabolic processes in the body, increasing the adaptive capacity and tolerance of the athlete to training loads. The method of crystalloscopic examination of saliva can be applied in physical education and sports to monitor human fitness and manage education and training processes.

**Key Words:** physical activity, metabolism, saliva, physical training, student-athletes

### Introduction

Physical activity performed by a person during physical education and sports activities causes significant changes in the body. It is important that, along with morphological changes in tissues and organs, physical activity increases adaptive reserves and can act as a factor in leveling the body's maladaptation states (Nuutila et al., 2017). An important area of medical control is the development of simple, informative methods for assessing the state of the reserve potential of athletes and athletes (Tummala et al., 2018; Goodman et al., 2018; Vynohradov et al., 2021). Numerous scientific publications mainly present information on the possibilities of optimizing the training regimen and the specifics of the effect of various types of physical activity on the body (Mendes et al., 2018; Kiselev et al., 2019). The issues of monitoring the functional state of the human body in the dynamics of its use to correct physical activity remain insufficiently studied (Bykov, & Kolomiets, 2016).

The determination of systemic hemodynamic indicators, as well as the study of the vegetative support of the body's activity using the heart rate variability analysis method (Catai et al., 2020; Bocharin et al., 2021) can serve as an informative criterion for assessing adaptive reserves. The literature contains materials on the study of the variability of hemodynamic parameters at different intensities of physical activity (Martusevich et al., 2022a), and the results of changes in the hormonal status of the body of a person involved in sports are described (Saad et al., 2020; Foretic et al., 2020). In the scientific literature, relatively little attention is paid to the study of the metabolic characteristics of people engaged in physical activity (Mednes et al., 2018; Kokornaczyk et al., 2021). Existing scientific research in the field of metabolism in individuals involved in sports and physical education is aimed primarily at studying the biochemical processes during muscle contraction of skeletal muscles (Neufer, 2018; Sims et al., 2023; Santangelo et al., 2024). In this regard, it is of interest to assess the adaptive reserves using non-invasive laboratory diagnostics, which allows for effective monitoring of metabolic changes during physical activity (Neufer, 2018; Schulze, Busse, 2024). Also, it is necessary to adhere to simplifying the testing and collection procedure of biological material to reduce the risk of possible complications. In connection with the above, attention is drawn to the possibility of using a non-invasive method for diagnosing the salivary glands, which allows the researcher to obtain informative indicators of mixed human saliva (Jordanishvili, 2019; Martusevich et al., 2022b). The technology for collecting diagnostic material makes it possible to obtain it outside of any medical institution, including on the territory where the training process takes place. The use of non-invasive methods for monitoring the reserve capabilities of the athlete's and athlete's body for dynamic monitoring allows to improve their physical fitness (Reneker, 2018; Lucas de Albuquerque Freire et al., 2020). Monitoring the state of biological fluids in an athlete allows you to make adjustments to the process of his physical training (Budzyn et al., 2018), choose modern restorative procedures (Chiriac Paul Bogdan et al., 2021). The use of biological fluid monitoring for the prevention of injuries in sports activities is of great importance (Mardiana et al., 2021). The scientific literature presents the results of using the method of studying the crystal-forming ability and initiating potential of saliva to study the component composition of the human body (Jordanishvili, 2019), as well as in students who had different levels of physical health (Martusevich et al., 2022a). However, the results of using the method of studying oral fluid require further study of the issue to obtain comprehensive information about the features of the crystallogenic properties of saliva in individuals who perform different levels of regular physical activity.

**Purpose.** To study the features of the crystal-forming and initiating properties of saliva in student-athletes and students who do not play sports.

### **Material & methods**

The study involved 86 students (aged  $18.2 \pm 1.4$  years) studying at a university in the Volga Federal District (Nizhny Novgorod, Russia). Using a random method, the young men were grouped into two teams: the first ( $n=42$ ) included students who ignore sports, and the second group ( $n=44$ ) included students who regularly attend sports sections at the university. The exclusion criteria from the study were the presence of various pathologies in the subjects, including diseases of the oral cavity and nasopharynx. At the beginning of the research project, all participants in the experiment voluntarily signed an informed consent to participate in it. The experiment was carried out in accordance with the Helsinki Declaration of 2008, which provides for compliance with moral and ethical norms and rules when conducting medical and biological research involving humans. Before collecting saliva samples, the mouth was rinsed twice with distilled water. The obtained saliva samples were evaluated for crystal formation activity and their excitatory potential (Martusevich et al., 2022). The crystal-forming properties of saliva were studied using the traditional method of classical crystalloscopy. The comparative tesigraphy method was used to study the initiating properties of students' oral fluid. The use of this method makes it possible to evaluate the process of crystal formation in saliva and obtain diagnostic crystallized samples, as well as describe individual structural components in the analyzed preparation. The main substance in the tesigraphic study was physiological saline. Morphologically, the structural elements of saliva were grouped into three categories: single crystals, polycrystalline forms, and amorphous bodies. Using the generally accepted technique, quantitative indicators of the tesigraphic image of saliva were calculated, in particular, the main tesigraphic coefficient  $Q$ , which shows the average number of crystallization centers in the test sample compared to the control. In addition, the characteristics of semi-quantitative parameters were given: the degree of expression of cellular density (coefficient  $P$ ), the uniformity of distribution of elements, the degree of signs of pattern destruction, which provide an integral description of the indicator of the correctness of the crystallogenesis process. A description was given of the process of formation of the border of the marginal zone of the micropreparation, which indicates the relative content of protein in the studied medium. The crystallogenesis of the bioenvironment was assessed in points using static assessment methods.

For statistical processing of the obtained digital material, the Statistica 10.0 and Microsoft Excel 2016 software packages were used. To identify the statistical significance of the differences obtained in the absence of normal distribution of the obtained digital materials according to the Shapiro-Wilk criterion, the Mann-Whitney U-test was used. When assessing the differences in criteria, a statistically significant level was accepted at  $p < 0.05$ .

## Results

As a result of the crystalloscopic and tesigraphic comparative analysis of saliva, statistically significant differences in the values of the indicators were established between student-athletes and students who ignore sports. In the group of young men who do not play sports, the number of such elements as "rectangle" was 43.9% less, "prism" was 68.2% less compared to the number of these elements in the group of student-athletes. In young men who do not perform regular physical activity, the form of structural elements "pyramid" was revealed, which was absent in student-athletes, Table 1.

**Table 1. Qualitative and quantitative indicators of crystalloscopic properties of saliva in the observed groups of students, (n=86), M±m**

Structural element/ Parameter	Untrained students (n=42)	Trained students (n=44)
<b>Single crystals</b>		
Rectangular shape	4.1±0.1	2.3±0.1*
Prismatic shape	2.2±0.1	0.7±0.1*
Pyramidal shape	0.8±0.1	–
Octagonal shape	–	1.3±0.1
<b>Dendritic (polycrystalline) structures</b>		
Linear shape	0.9±0.2	1.8±0.1*
Rectangular shape Прямоугольная форма	0.6±0.1	1.3±0.1*
"Moss" and onion"	2.8±0.2	–
Cruciform shape	0.4±0.1	–
"Horsetail"	–	6.2±0.2
"Rose"	–	–
<b>Amorphous bodies</b>		
Size	average	average
Number	moderate	numerous

Note: \* - statistically significant differences between groups,  $p < 0.05$

Analysis of the results of studying the semicrystalline structures of micropreparations showed an increase in the density of linear and rectangular polycrystalline elements in the group of trained individuals. At the same time, only among the subjects who do not regularly engage in physical activity were figures of the type "moss", "onion" and "cross" found, and among trained students - elements of the type "horsetail".

To determine the shifts in the results of structural changes in biological fluid in the study groups, the results of the crystalloscopic test were compared with the tesigraphic result, Table 2.

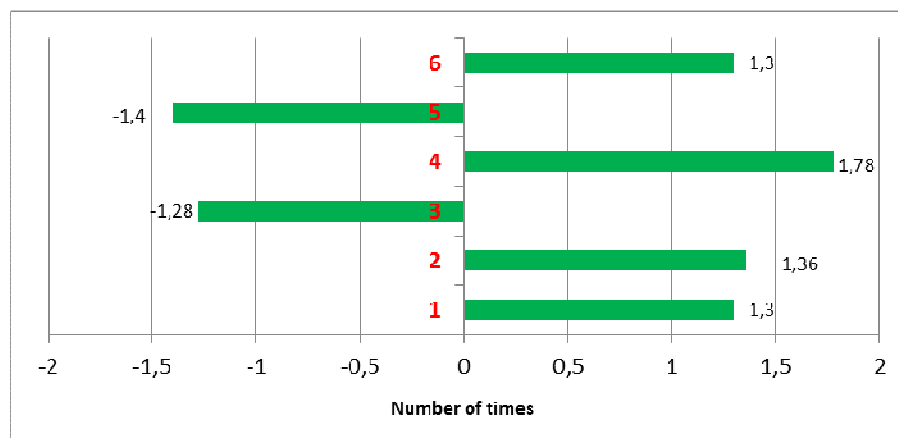
**Table 2. Ratio of semi-quantitative coefficients of saliva in the observed groups of students, (n=86), M±m**

No	Parameter	Untrained students (n=42)	Trained students (n=44)
1.	Coefficient Q	2.1±0.1	2.8±0.1*
2.	Coefficient P	1.4±0.1	1.9±0.1*
3.	Coefficient I	1.8±0.1	1.4±0.1*
4.	Coefficient R	1.4±0.1	2.5±0.1*
5.	Degree of facies destruction	2.1±0.1	1.5±0.1*
6.	Expression of marginal zone	1.6±0.1	2.1±0.1*

Note: \* - statistically significant differences between groups,  $p < 0.05$

According to the value of the initiating potential of saliva (coefficient Q), 1.3 times greater activation of the structuring of the main substance in the presence of biological fluid was registered,  $p < 0.05$ .

In addition, an expansion of the zone of tezigraphic drawings and a 1.36-fold increase in the coefficient among student athletes was established compared to the value of this indicator among young men who ignore sports,  $p < 0.05$ , Figure 1.



Note: 1, 2, 3, 4, 5, 6 – parameter numbers from Table 2

**Fig. 1. Difference in the values of semi-quantitative coefficients in student-athletes compared to young men who do not play sports, number of times**

Analysis of the results of the study of semi-quantitative parameters of excited crystallogenes of oral fluid shows a moderate decrease in the number and size of crystals in the biological preparation, which is apparently associated with the influence of regular physical activity in student-athletes. In these students, the changes correlate with an increase in the homogeneity of the texture and spatial distribution of crystals and amorphous bodies in the sample. This is evidenced by an increase in the value of the coefficient R by 1.78 times,  $p < 0.05$ , Figure 1.

The integral indicator of crystallogenes is the degree of destruction of the facies and the severity of the border of the marginal zone of the micropreparation. It was found that the manifestation of the degree of destruction of the facies was statistically significantly 1.3 times less in student-athletes compared to the group of students who ignore sports,  $p < 0.05$ . Moreover, the expression of the marginal zone was 1.36 times greater in student athletes compared to young men who do not play sports,  $p < 0.05$ .

Thus, the discovered features of saliva in the group of trained individuals indicate the optimization of their metabolic processes, protein composition and organ-mineral balance of biological fluid caused by regular dosed physical activity, which increases their tolerance to physical exertion and stress.

## Dicussion

Sports and regular physical education activities cause profound morphofunctional changes in the body of the participant. Diagnostics of such changes in the human body, assessment of the physical health of the participant, his adaptive reserves and tolerance to physical activity is an important area of sports medicine (Catai et al., 2020; Bocharin et al., 2021; Martusevich et al., 2022a). Along with traditional testing of the level of physical fitness using motor tests and studying the biochemical parameters of the body (Neufer, 2018; Sims et al., 2023; Santangelo et al., 2024), methods for studying biological materials, including oral fluid, have begun to be used in physical education and sports activities. Such a study refers to a non-invasive method for diagnosing the salivary glands and allows the researcher to assess the adaptive capabilities of a person (Jordanishvili, 2019; Martusevich et al., 2022b). Therefore, the scientific topic of this study is relevant and important in physical education and sports practice.

The study of the functional state of the body of individuals engaged in regular physical activity allows us to assess both the individual physiological cost of the impact of physical activity and to study the body's tolerance from the standpoint of the metabolic component. In aggregate, this can significantly improve athletic performance and neutralize the states of tension in regulatory systems and maladaptation (Nuutila et al., 2017). For this purpose, the crystallographic method of studying biological environments can be successfully used, which was shown in the scientific works of A.K. Jordanishvili (2019) and A. Martusevich et al. (2022b).

According to our data, according to the crystalloscopic test, among untrained students there was a significant decrease in the number of elements "rectangle", "prism", and the appearance of the "pyramid" shape, which are absent in student-athletes. When observing the dendritic component, an increase in the density of linear and rectangular elements was found among trained students. A characteristic feature of the group of trained individuals was the activation of the structuring of the main substance, as well as the expansion of the zone of tesigraphic patterns. In addition, the results of the analysis of the study of semi-quantitative characteristics indicate a moderate decrease in the size and number of "crystalline islands". According to our data, the identified features of the severity of the marginal zone and the degree of destruction of the facies indicate the optimization of the protein composition of the biological fluid in students engaged in regular dosed physical activity. Such properties of saliva indicate that regular dosed physical activity helps to normalize

metabolic processes in the body, which increases the reserve capacity of the human body. These data are confirmed by the previously obtained materials of the study of saliva in students who played football at different stages of the annual macrocycle (Bocharin et al., 2022). The results of this study necessitate a more in-depth biochemical examination of biological fluids in students who have different levels of athletic training. The scientific data obtained during the study can be used to optimize the training regimen and individualize the volume and intensity of physical activity, taking into account the body's adaptation to it.

### Conclusions

Crystallographic and tesigraphic comparative analysis of saliva revealed statistically significant differences in the values of the indicators between student athletes and students who ignore sports. These differences are manifested in the qualitative and quantitative transformation of the process of crystal formation in the salivary fluid among single-crystal, dendritic structures and amorphous bodies.

The tesigraphic test showed activation of the initiating ability of biological fluid and optimization of textural characteristics in combination with a decrease in the degree of destruction of pattern elements, moderate expansion of the border of the marginal zone of micropreparations in students who play sports. The crystal-forming and initiating picture of saliva may indicate that regular dosed physical activity contributes to the normalization of metabolic processes in the body, increases human adaptive abilities and tolerance to physical activity.

**Conflicts of interest.** The authors declare no conflict of interest.

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