

Saliva diagnostics of skiers during competitions

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Abstract:

A necessary condition for achieving high athletic performance is the application of effective diagnostic methods to assess athletes' functional state. **Purpose:** To predict the recovery of skiers' bodies in running disciplines by studying the characteristics of saliva crystalloscopic properties during the competitive phase of sports activity. **Materials and methods:** The study involved 36 skiers, who comprised the main observation group (MG), and 165 healthy, untrained students (comparison group, CG). The age of participants in both groups ranged from 18 to 21 years (19.4 ± 0.9 years). Crystallogenic properties of saliva were assessed using classical crystalloscopy, while the initiating potential of the biological fluid was evaluated through comparative tesigraphy. **Results.** The study revealed distinctive features of saliva crystallization in athletes compared to healthy individuals. These included a predominance of branched (dendritic) structures, an increased proportion of amorphous bodies, and an expansion of the marginal zone, with the latter in athletes specifically characterized by the presence of fractures. In the post-competition period, saliva micropreparations showed a further increase in dendritic elements, accompanied by narrowing of the marginal belt and a reduction in the initiating potential of the biological environment. After a short rest (30 min), partial recovery of the crystallogenic and initiating properties of saliva was observed. **Conclusions.** These findings highlight the potential of this approach to non-invasively assess the rate of recovery of athletes' functional state after physical exertion and to promptly detect the onset of overtraining and maladaptation.

Key Words: skiers, physical testing, training, physical training, saliva diagnostics.

Introduction

Despite considerable progress in the development of medical and biological support for sports activities, the range of informative and accessible technologies capable of reliably tracking the dynamics of athletes' functional state and metabolic processes remains limited (Baker, & Wolfe, 2020; Carrard et al., 2022). A commonly used approach to address this issue is heart rate variability analysis (Bocharin et al., 2022). However, this method offers only an indirect assessment of metabolic status and is constrained by several methodological limitations (Esteves et al., 2019; Carrard et al., 2022; Martusevich et al., 2022; Kolokoltsev et al., 2021). Moreover, it requires the involvement of a dedicated specialist. In recent years, the analysis of hormonal status has also been introduced as an additional method for evaluating athletes' condition (Foretic et al., 2020). Conventional invasive methods, such as blood sampling or muscle biopsy, can be applied as diagnostic tools to monitor adaptation processes, assess performance, and detect overtraining or overexertion (Dudko et al., 2021). However, these approaches are painful, time-consuming, and impose strict requirements for sample storage and transportation. Such limitations restrict their applicability for routine and stage-by-stage monitoring of the functional state of athletes in skiing disciplines, for whom no comprehensive non-invasive diagnostic methods of functional reserves are currently available (Jones et al., 2021; Claus Henn et al., 2022; Hintikka et al., 2022; Bolotin, et al., 2022). This underscores the relevance of identifying alternative research objects that avoid these disadvantages.

Saliva represents an informative and promising material for monitoring the condition of athletes (Liu et al., 2025). It is known to contain molecular components closely resembling those of blood serum, which makes it a valuable source of diagnostic information about the body's state. Literature reports describe a home-based method that allows daily monitoring of pregnant women and reasonably accurate prediction of delivery dates by analyzing crystallization patterns in saliva (Li et al., 2022). Furthermore, the determination of glutathione and malondialdehyde in saliva is increasingly being applied to assess oxidative stress, offering a practical alternative to similar analyses traditionally performed on blood serum (Kanioura et al., 2023).

Saliva analysis in athletes and physically active individuals provides valuable insights into training stress, adaptation, and the effectiveness of exercise (Ntovas et al., 2022). Observations indicate that changes in saliva composition during and after training reflect both the role of physical activity and its potential risks to the body. Factors such as training intensity, physical condition, and overall health influence the levels of hormones, immunoglobulins, and salivary enzymes, which can serve as important health indicators. This highlights the need to explore and validate innovative laboratory technologies, including methodologies for studying the crystallogenic activity of biological substrates and environments in humans and animals, which have been actively developed in recent decades (Martusevich, & Kamakin, 2007; Jordanishvili, 2019; Kokornaczyk et al., 2021). These studies have provided data on the physiological patterns of crystalloscopic and tesigraphic profiles of various biological fluids, including blood serum and plasma, mixed saliva, and urine (Russo Krauss et al., 2013; Pearlmutter et al., 2020; Mischenko, et al., 2021). On this basis, an innovative methodology—biocrystallogics—has been developed. This interdisciplinary field investigates crystallization and structuring processes in biosystems (Martusevich et al., 2022, 2023). However, these technologies have not yet been fully applied in sports medicine, and no studies have examined the crystalloscopic state of saliva in skiers during competitive activity. We suggest that monitoring the crystalloscopic properties of saliva in skiers during the competitive stage of training will make it possible to predict recovery processes and help prevent the negative consequences of intense physical exertion.

Research aim. This study aims to predict the recovery of skiers in running disciplines and to examine the crystalloscopic properties of saliva during the competitive stage of sports activity.

Material & methods

The study was performed at the National Research Lobachevsky State University of Nizhny Novgorod (Nizhny Novgorod, Russia). The main observation group (MG) consisted of 36 cross-country skiers of the 1st sports category, with an average skiing experience of 8.9 ± 1.1 years. The comparison group (CG) was randomly selected from 165 healthy students not engaged in any type of sport. Participants in both groups were young men aged 18–21 years, with a mean age of 19.4 ± 0.9 years. The study was approved in advance by the Local Ethics Committee of the National Research Lobachevsky State University of Nizhny Novgorod and complied with the ethical principles outlined in the 2008 Declaration of Helsinki regarding human participation in research. All participants provided voluntary written informed consent prior to inclusion in the study. The project was performed in collaboration with researchers and specialists from universities in Russia, Kyrgyzstan, Kazakhstan, and Bulgaria. Oral fluid samples were collected from all participants in dry, sterile test tubes. For athletes, sampling was performed at three control points: before the start, immediately after the finish, and 30 min after the race. In the CG, saliva was collected and analyzed once, following two rinses of the mouth with distilled water. The same procedure was performed for athletes before the start of the race. The physicochemical properties of mixed saliva in both groups were assessed using two complementary methods. Classical crystallography involved the description of biosubstrate structuring in individual preparations, while comparative tesigraphy was based on the co-dehydration of the studied bioenvironment with a reference crystal-forming substance (Martusevich et al., 2022, 2022a; 2023). A sterile 0.9% sodium chloride solution was used as the crystal former. The primary method for analyzing saliva crystallograms was visuometria, which involved assessing the density of individual crystalline and amorphous structures in the field of view, averaged over three fields. These measurements were complemented by a criterial assessment (Martusevich et al., 2022a), calculating semi-quantitative parameters such as the degree of facies destruction (DFD), severity of the marginal zone (Kz), cellularity (I), and the uniformity of distribution of crystalline and amorphous elements across the sample texture (R). The second component of the biocrystallogomic analysis involved describing tesigrams using a set of qualitative and quantitative indicators. Based on our methodology (Martusevich et al., 2022, 2022a, 2023), the most significant tesigraphic criteria are:

- 1) the leading tesigraphic coefficient Q , calculated as the ratio of the density in the co-crystallized saliva sample with the crystal former to that of the facies of the pure base substance, in points;
- 2) the zonation coefficient P , defined as the ratio of the radii of the maximum and minimum crystallization belts in the saliva tesigram, in points;
- 3) the quantitative parameters described above for crystallograms were also applied.

Digital processing of the collected data was performed using Statistica 6.0 and IBM SPSS 24 software, employing standard methods of variation statistics. The normality of feature distributions was assessed using the Shapiro–Wilk test. Statistical significance of differences between groups was evaluated using nonparametric

methods, including the Mann–Whitney U test, Wald–Wolfowitz runs test, and Pearson's χ^2 test. A critical significance level of $p \leq 0.05$ was applied throughout.

Results

The study showed that the crystallogenic activity of oral fluid in skiers outside of competition load (at the first control point) differs significantly from that of untrained individuals (Table 1).

Table 1. Visuometric analysis of oral fluid crystallograms in athletes and untrained students

Structures	Untrained students*, n = 165	Skiers, n=36		
		At the start **	At the finish* (+)	After 30 min *
Single-crystal figures				
Rectangles	4	2	1	2
Prisms	2	0-1	0	0-1
Pyramids	1	0	1	0-1
Octahedrons	0	1	0	2
Dendritic (polycrystalline) structures				
Ruled	1	2	0-1	2
Rectangles	0-1	1	0-1	0-1
Crystals of the type "Moss", "Onion", "Comet"	2-3	0	0	0
Cross-type crystals	0-1	0	0	0
Horsetail-type crystals	0	6	5-7	5
Rosette-type crystals	0	0	0	0
Amorphous bodies				
Size	Average	Average	Small	Average
Quantity	Average	Many	Average	Many

Note. The nature of the interaction of crystals and amorphous bodies: "" – adhesion, "***" – displacement; (+) – presence of cellular facies*

In these comparisons, we identified the most specific morphological features of mixed saliva crystallograms in professional skiers. These included the formation of large crystalline and amorphous elements with a pronounced predominance of dendritic structures, as well as characteristic transformations of the marginal zone of micropreparations, marked by deep "cracks" and an expansion of the facies belt.

At the end of the competition, the crystallogenic properties of saliva underwent significant changes. These changes were driven both by systemic dehydration and by shifts in the component composition of the biological fluid. Such modifications were reflected in the dynamics of saliva crystallization, assessed via classical crystalloscopy, as well as in changes in the initiating potential measured by tesigraphy. Visual-metric analysis of crystalloscopic facies revealed that immediately after the finish, the crystalloscopic pattern was depleted, with a reduction in the density of single-crystalline and dendritic elements. This trend was also observed in the amorphous components of the facies (Table 1).

At the third control point, corresponding to the recovery period 30 min after the finish, a partial normalization of the saliva crystalloscopic pattern was observed. However, not all components of the facies returned to their original configuration. It is noteworthy that the rate of recovery was individual-specific, reflecting differences in the functional state of athletes in response to the same competitive load.

Analysis revealed that the crystallogenic activity of skiers' saliva shifted toward a moderate increase in cellularity, increasing from 2.62 ± 0.03 to 2.85 ± 0.04 points ($p < 0.05$) compared to the pre-start state. At the same time, the uniformity of structural density in the micropreparations showed a tendency toward a statistically significant decrease of 28.5%, decreasing from 2.07 ± 0.04 to 1.48 ± 0.02 points ($p < 0.05$) (Fig. 1).

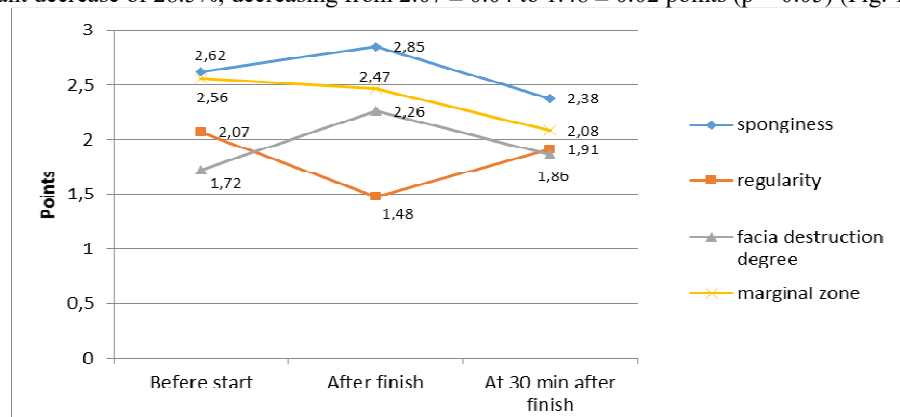


Fig. 1. Dynamics of saliva crystalloscopic parameters during competition

Additionally, a 31.4% increase in the degree of destructive processes in the crystalline elements of the facies and a 3.5% narrowing of the marginal protein zone in micropreparations were observed compared to the start of the competition ($p < 0.05$). These changes indicate a reduction in the amount of conformationally preserved proteins in the biological environment. Furthermore, the initiating potential of skiers' saliva also underwent significant changes upon reaching the finish line (Fig. 2).

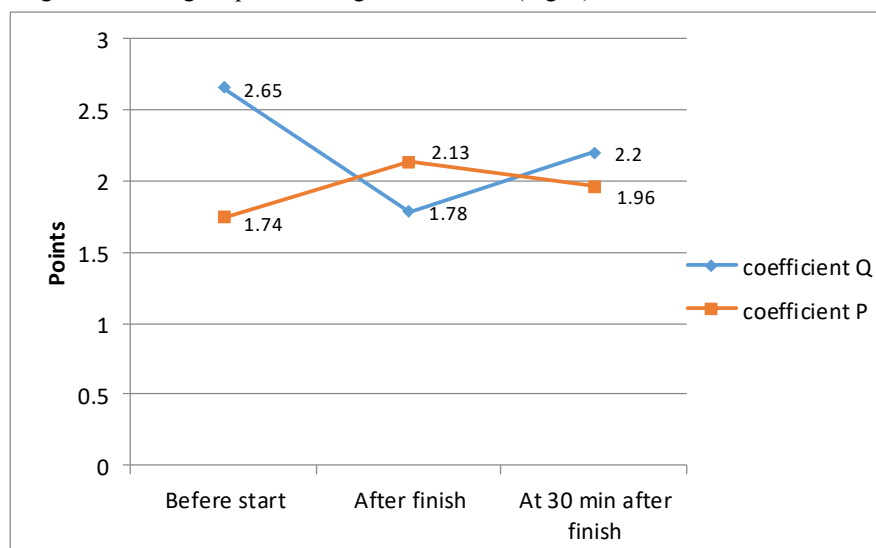


Fig. 2. Dynamics of tesigraphic indicator values in skiers (Q – leading tesigraphic coefficient, P – zonation coefficient)

The initiating ability of saliva relative to the physiological base substance (0.9% sodium chloride solution) decreased significantly by 32.8% compared to the first control point, decreasing from 2.65 ± 0.04 to 1.78 ± 0.03 points ($p < 0.05$). At the same time, the gradient of molecular weights of the biological fluid components increased relative to pre-start levels, as reflected in a 22.4% increase in the zonality coefficient, from 1.74 ± 0.04 to 2.13 ± 0.06 points ($p < 0.05$).

Analysis of the tesigraphic facies of biological fluid using additional assessment indicators confirmed the presence of pronounced metabolic stress at the second control point, as evidenced by the most substantial parameter shifts relative to baseline. Among the evaluated indicators, the most sensitive markers of the body's functional state were the degree of facies destruction and the radius of the marginal protein belt in micropreparations. Consistent with the dynamics observed in crystallograms, 30 min after competition (third observation point), the tesigraphic facies showed partial normalization of saliva's crystallographic properties in athletes, Figure 2.

Dicussion

The study of issues related to the search for new and modernization of existing methods for assessing the functional state of athletes continues to be a relevant area of scientific research for scientists and specialists in the field of sports (Romanova et al., 2023).

Currently, saliva diagnostics is actively developing as a promising direction of non-invasive diagnostics for typical functional and pathological conditions (Gatti, & De Palo, 2011; Esteves et al., 2019; Li et al., 2022; Kanioura et al., 2023). This is due to the presence of a clear correlation between the metabolomes of saliva and blood plasma (Nonaka, & Wong, 2022), as well as the conjugacy of the component composition and physicochemical properties of oral fluid with the metabolic characteristics of the body. These patterns are fully realized in athletes (Gatti, & De Palo, 2011; Ntovas et al., 2022; Nonaka, & Wong, 2023). However, to fully utilize the capabilities of saliva diagnostics, informative and accessible methods for extracting metabolic information are needed. These include biocrystallography technologies based on the ability of any biological fluids to undergo specific structuring with the formation of a crystalloscopic pattern (facies) depending on the composition of the dried biomedium (Jordanishvili, 2019; Kokornaczyk et al., 2021; Martusevich et al., 2022, 2023).

Previously, we made successful attempts to use crystalloscopic studies of blood plasma and mixed saliva of athletes, which made it possible to identify the features of dehydration structuring of these biological fluids in individuals professionally involved in sports (Bocharin et al., 2022a; Martusevich et al., 2022). At the same time, to compile a comprehensive description of variations in the crystallogenic activity of biomedium in various functional states associated with sports (individual phases of the training cycle, taking into account the

level of training and the presence of overtraining, the course of recovery processes, etc.), extensive research is required. Taking into account the above, we conducted a comprehensive analysis of the athletes' own and initiated crystallization of mixed saliva in the dynamics of the competition (using cross-country skiers as an example). The performed studies confirmed previously observed features of crystalloscopic and tesigraphic facies in professional athletes, such as the presence of "fractures" in the marginal zone of micropreparations, which are related to specific characteristics of the saliva proteome (Russo Krauss et al., 2013; Esteves et al., 2019; Martusevich et al., 2023; Romanova, et al., 2023; Ferrari et al., 2025). In addition, the observed dynamics of the crystallogenic properties of saliva during competitive activity indicate a rapid response of its physicochemical characteristics to metabolic shifts associated with exercise and subsequent recovery processes. Thus, the study highlights the potential of biocrystallomic analysis of mixed saliva as an indicator for rapid monitoring of athletes' functional state. However, further extensive data are required to develop a standardized diagnostic algorithm.

Conclusion

The study revealed distinct features of saliva crystallization in skiers compared to healthy individuals. These included a predominance of branched (dendritic) structures, an increased proportion of amorphous bodies, and expansion of the marginal zone of the facies. A specific characteristic of this marginal zone in athletes was the presence of faults. In the post-competition period, saliva micropreparations exhibited a further increase in dendritic elements, accompanied by narrowing of the marginal belt and a reduction in the initiating potential of the biological environment.

After a short rest (30 min), partial restoration of the crystallogenic and initiating properties of saliva was observed. These patterns enable non-invasive assessment of athletes' functional recovery rates and facilitate timely detection of overtraining and early signs of severe maladaptation.

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

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