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Hormonal parameters of saliva and blood of participants of the 21-day Arctic marine expedition

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Introduction. It is relevant to study the adaptive changes among the participants of the Arctic marine expedition, which allow for a certain period of time to maintain a stable working capacity necessary to perform various professional tasks.
The study aims to identify the dynamics of hormonal parameters in saliva and blood serum samples of the expedition group members who temporarily come to work in the extreme conditions of the Arctic.
Materials and methods. Scientists examined 40 participants of the expedition of the Arctic Floating University: 24 women and 16 men aged from 19 to 56 years (the average age is 26 years). They collected saliva in the morning and evening at the beginning, middle and end of the 21-day route. Specialists performed blood sampling in the morning on an empty stomach at the beginning and at the end of the expedition. The levels of cortisol, dehydroepiandrosterone and testosterone were determined by enzyme immunoassay.
Results. The concentration of morning salivary cortisol is maximum at the beginning of the route for both men (32.2 nmol/l) and women (31.8 nmol/l), then it decreases significantly by the end of the expedition (8.2 and 8.7 nmol/l, respectively). The reverse dynamics is shown for evening cortisol levels: the maximum values at the end of the route compared with the beginning in men (6.6 versus 1.4 nmol/l) and women (5.5 versus 1.7 nmol/l). The content of morning and evening dehydroepiandrosterone is maximal in women at the beginning of the expedition, then decreases by the end of the route (1.52 and 0.91 nmol/l for morning concentrations, $p<0.0001$; 0.35 and 0.21 nmol/l for evening concentrations, $p=0.013$), while it does not significantly change in men. Testosterone levels increase in the middle of the route, more significantly in women (0.26 versus 0.05 nmol/l, $p<0.0001$). The content of serum hormone concentrations does not significantly change except for the level of dehydroepiandrosterone in men, whose level decreases by the end of the route (47.6 versus 65.4 nmol/l, $p=0.038$). Limitations. Most of the participants did not have the opportunity to measure the background values of the indicators before the route, which, of course, would have been of great value.
Conclusion. *The hormone content in saliva, rather than in serum, is an informative indicator reflecting hormonal changes in the body of participants in the Arctic expedition associated with the presence of acute stress caused by natural factors and specific working conditions. An increase in evening cortisol and testosterone levels indicates a violation of their circadian dynamics and the possible development of desynchronosis in the expedition members by the end of the route. An increase in dehydroepiandrosterone at the beginning of the expedition and testosterone from the middle of the route may indicate their adaptive and neuroprotective role under the influence of a complex of stressful conditions of the expedition and increased mental stress.*
Ethics. The study was conducted in compliance with Ethical standards (Minutes No. 2 of the meeting of the Ethics Committee of the N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences dated 06.06.2024).

Keywords: *cortisol; dehydroepiandrosterone; testosterone; saliva; serum; marine expedition; Arctic*
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Introduction. According to Decree of the President of the Russian Federation No. 164 dated March 5, 2020 "On the Fundamentals of the State Policy of the Russian Federation in the Arctic for the period up to 2035", one of the main tasks in the field of science and technology development in the interests of Arctic exploration is to increase activities for the implementation of integrated expeditionary research in the Arctic.

Arctic scientific marine expeditions can be viewed from the perspective of a three-week watch at high latitudes. At the same time, participants in such expeditions are exposed to a whole range of adverse factors: in addition to high noise levels, pitching, unfavorable work and rest conditions, and social isolation, they are additionally affected by extreme climatic conditions, especially the polar day and low temperatures, which can lead to increased neuromotional and other functional stress. It is necessary to monitor the adaptation process, establish criteria for sustainable adaptation, and develop measures to stimulate adaptation processes and maintain appropriate adaptability of researchers to working conditions.

One of the criteria for assessing the adaptive reserves of the body is the monitoring of cortisol levels. Glucocorticoids are known to mediate the escape or fight response to stress, but at the same time suppress numerous immune functions. On the contrary, the androgenic steroid hormone of the adrenal glands dehydroepiandrosterone (DHEA) and its sulfated metabolite dehydroepiandrosterone sulfate (DHEA-S) have anti-stress, antidepressant, immunomodulatory effects and have an antiglucocorticoid effect [1–3]. The anti-stress mechanisms of DHEA-S include changes in the metabolism of cortisol to its inactive metabolite cortisone, the resulting ratio of which redistributes energy and restores homeostasis [4]. In a meta-analysis, F. Dutheil with co-authors the use of DHEA as a biomarker of acute stress has been demonstrated, regardless of the type of fluid (blood or saliva) and the measurement method [5]. Testosterone levels are also able to flexibly change in response to stressors, which may be an important mechanism by which the hormone promotes adaptive behavior by reducing social anxiety [6]. Experts noted an increase in testosterone levels after a long route among employees of the Yakutsk River Transport Company [7]. In a study by Eremenko V.V. and co-authors, a direct relationship was established between the level of stress tolerance in men and the level of testosterone in the blood [8].

In conditions of marine expeditions, it is not always possible to collect blood and conduct its sample preparation, so saliva research is becoming relevant. One of the important characteristics of saliva is that it contains free concentrations of steroid hormones and, thus, their biologically active part. It has been shown that when exposed to acute stress, the level of salivary cortisol increases [9]. In addition, cortisol changes in response to physical activity are more pronounced in saliva compared to blood [10]. The results of the effect of acute psychosocial stress on hormonal parameters also demonstrate an increase in DHEA levels in saliva immediately after a stress test and a direct relationship with cortisol levels [11, 12]. Crewther et al. showed a more significant relative increase in testosterone in saliva during high-intensity sprinting compared with a relative increase in total and free testosterone in plasma, which suggests that saliva testing to assess the hormonal response to exercise is more sensitive [13]. Thus, the content of hormone levels in saliva is an adequate indicator reflecting hormonal changes in the human body associated with the presence of acute and chronic stress [14].

The study aims to identify the dynamics of hormonal changes in saliva and blood serum samples of the expedition group members who temporarily come to work in the extreme conditions of the Arctic.

Materials and methods. Scientists conducted a study aboard the scientific expedition vessel Professor Molchanov as part of the Arctic Floating University project. They examined 40 participants of the expedition "Arctic Floating University — 2024". Among them, 24 women and 16 men aged 19 to 56 years (*Me*=26 years) signed an information consent to participate in the study and filled out detailed questionnaires, including questions about the presence or absence of diseases, current condition, and bad habits. The study was approved by the Ethics Committee (Minutes No. 2 of the meeting of the Ethics Committee dated 06.06.2024). The researchers collected saliva three times during the route: on the 2–3 day of the expedition, in the middle of the route, and 2–3 days before the end of the 21-day route. Given that the synthesis of cortisol and testosterone has a clear circadian rhythm, the scientists performed saliva sampling twice on the days of the study. Morning saliva samples were obtained from 7 a.m. to 9 a.m. immediately after waking up on a strict empty stomach before brushing teeth, evening saliva samples were obtained from 21 to 23 hours 2 hours after the last meal and pre-rinsing the mouth with water. Further, the samples were frozen until the analysis was carried out. After defrosting, the samples were centrifuged at 3000 r/m for 15 minutes, after which the filler fraction was collected. The researchers conducted blood sampling on an empty stomach with from 7 a.m. to 10 a.m. twice — at the beginning and at the end of the route then serum and plasma were obtained by centrifugation for 10–15 minutes at a speed of 1500–2000 r/m, followed by the freezing. Using in vitro enzyme immunoassay on an ELISYS Uno tablet autoanalyzer (Human GmbH, Germany), levels of cortisol, DHEA-S and testosterone were determined in saliva and serum samples using kits from HEMA (Russia), and DHEA levels using kits from DRG (Germany).

The scientists carried out statistical data processing using the STATISTICA 10.0 and *p*-level GraphPad Prism 8.4.3 application software package. The Shapiro-Wilk test revealed a deviation of most of the studied parameters from the law of normal distribution, and therefore nonparametric analysis criteria were applied. The results are presented as *Me [min; max]*, where Me is the median, min is the minimum value, and max is the maximum value. The comparison of indicators between dependent samples the authors have carried out using the Friedman criterion, followed by a posteriori comparison using the Dunn criterion, while the values at $p<0.05$ were considered significant.

Results. The analysis of morning and evening saliva samples revealed significant changes in the levels of all the studied indicators in the route dynamics ***(Table 1)***. Moreover, the nature of the changes in men and women is similar in most cases.

The morning cortisol concentration was highest at the beginning of the route, with 56% of men (9/16) and 38% of women (9/24) having higher than normal levels. By the middle of the route, only 17% of women (4/24) had exceeded the norm, and 12.5% of men (2/16) and 8% of women (2/24) had exceeded the norm by the end of the route. At the same time, the opposite dynamics of evening cortisol levels was revealed: at the beginning of the expedition, 19% of men (3/16) and 25% of women (6/24) exceeded the reference values, then in the middle of the route, the frequency of elevated values reached 44% (7/16) and 33% (8/24), and by the end — 69% (11/16) and 75% (18/24), respectively.

The morning concentration of DHEA in both men and women is also maximal at the beginning of the route, with values exceeding the norm observed in 54% (13/24) of women, while only 19% (3/16) of men. A significant decrease in DHEA levels by the end of the route was registered only in women. We would also like to note an increase in the proportion of people with elevated evening concentrations of DHEA by the end of the route for men: from 19% (3/16) at the beginning and middle of the route to 37.5% (6/16), while for women the proportion of people with high DHEA values is 17–25% (4–6/24) at all stages of the route.

The morning and evening concentrations of free testosterone in saliva significantly increase in the middle of the route, compared with the beginning, in both men and women, and in the female sample, the morning samples exceeded the standard values in 37.5% (9/24) of cases. Further, by the end of the route, morning testosterone levels remain high in men and significantly decrease in women. Evening levels remain elevated in both groups.

At the same time, analysis of the serum hormone levels in the expedition participants did not reveal significant changes in cortisol levels in either men or women, although the limits of its fluctuations in men widen by the end of the route ***(Table 2)***.

It is possible to note high median values of DHEA levels in both men and women at the beginning of the expedition and a significant decrease in its concentration in men by the end of the expedition. At the same time, the content

Table 1

Hormone levels in saliva of Arctic expedition participants in route dynamics

Indicator, nmol/l	Sex	Route stages			*p*-level
		1 the beginning	2 the middle	3 the end	
Cortisol morning 2.5–31.9	male	32.2 0.2; 59.7	9.1 1.5; 30.8	8.2 1.2; 61.0	0.001 (1–2) 0.011 (1–3)
	female	31.8 2.1; 100.7	13.2 0.9; 63.4	8.7 0.0; 39.9	0.008 (1–2) 0.001 (1–3)
Cortizol evening <2.8	male	1.4 0.0; 7.0	2.4 0.4; 12.6	6.6 0.0; 30.4	0.0002 (1–3)
	female	1.7 0.2; 83.0	2.2 0.6; 22.9	5.5 1.1; 18.5	0.034 (1–3)
DHEA morning m: 0.36–2.01 f: 0.28–1.72	male	1.38 0.20; 4.11	1.12 0.22; 2.49	1.13 0.08; 3.69	>0.05
	female	1.52 0.33; 3.36	1.08 0.19; 3.72	0.91 0.12; 3.03	0.031 (1–2) <0.0001 (1–3)
DHEA evening	male	0.58 0.13; 1.65	0.27 0.04; 1.88	0.27 0.02; 1.53	>0.05
	female	0.35 0.11; 2.91	0.38 0.03; 1.56	0.21 0.03; 1.94	0.013 (1–3) 0.013 (2–3)
Testosterone morning m: 0.2–1.16 f: 0.03–0.47	male	0.39 0.04; 0.66	0.74 0.15; 2.16	0.54 0.21; 1.78	>0.05
	female	0.05 0.02; 0.56	0.26 0.05; 2.96	0.16 0.01; 1.33	<0.0001 (1–2) 0.020 (2_3)
Testosterone evening	male	0.08 0.04; 0.74	0.54 0.16; 2.64	0.41 0.20; 2.61	0.012 (1–2) 0.001 (1–3)
	female	0.02 0.00; 0.29	0.22 0.02; 2.38	0.15 0.00; 0.55	0.0002 (1–2) 0.022 (1–3)

Table 2

Changes in the serum hormone levels of the Arctic expedition participants during the route

Indicator, units of measurement	Sex	Этапы рейса		*p*-уровень
		начало	конец	
Cortisol, 140–600 nmol/l	m	548.8 486.8;849.3	520.5 108.5; 740.0	>0.05
	f	539.2 404.8; 709.3	559.0 132.7; 1020	>0.05
DHEA, nmol/l m: 2.88–62.72 f: 0.42–40.04	m	65.64 33.20; 102.6	47.34 15.87; 82.89	0.038
	f	73.57 11.24; 139.6	61.24 15.64; 140.3	>0.05
DHEA-S., mmol/l m.: 8.45–41.83 (18–30 liters) 3.98–30.55 (31–50 liters) f: 1.95–30.55 (18–30 liters) 0.81–25.61 (31–60 liters)	m	13.47 3.08; 27.80	12.38 2.090; 29.21	>0.05
	f	8.685 1.76; 21.64	9.790 1.86; 24.26	>0.05
Testosterone, nmol/l m: 1.36–38 (18–39 L) 6.9–21 (40–55 L) f: <4.6	m	13.68 7.70; 24.58	13.85 5.97; 25.24	>0.05
	f	1.44 0.45; 4.39	1.62 0.39; 6.22	0.077

of DHEA-S in all members of the expedition is within the normal range and does not significantly change. Women tend to have a slight increase in testosterone levels by the end of the route.

Discussion. It is known that the effect of cortisol causes an increase in the sensitivity of sensory systems and the excitability of the nervous system, which causes cognitive and behavioral changes that are crucial for successful adaptation [15]. Hyperproduction of cortisol is considered a normal reaction to acute stress, necessary to maintain survival functions, leading to an increase in glucose levels, while simultaneously conserving energy by suppressing reproductive, immune, and digestive functions [16], compensating for the effect of the cold factor on the human body. This explains its elevated levels at the beginning of the expedition, indicating the presence of tension from the new environment. Further, cortisol levels gradually decrease towards the end of the route, which could indicate a decrease in stress levels during the expedition. At the same time, it was found that evening cortisol concentrations increased by the end of the route. Such a violation of the dynamics of cortisol production may be accompanied by a strain on the regulatory mechanisms of the body's adaptation in response to environmental influences, as well as indicate the possible development of desynchronosis in the expedition members. We believe that the disruption of circadian rhythms of cortisol secretion may be due to a complex of factors affecting participants during the route. The expedition took place from June 25 to July 15, during the polar day. Due to the specifics of the work on oceanographic sections and ornithological observations, most of the participants conducted research around the clock during part of the route, changing each other in shifts. In addition, due to weather conditions, night landings sometimes occurred to carry out work and collect material on the islands of the archipelagos of Franz Josef Land and Novaya Zemlya. As is known, exposure to light at night reduces the production of melatonin secretion [17, 18], which has a number of adverse metabolic consequences, and may also be the cause of an increase in evening cortisol levels, and is consistent with data from a survey of employees of the Ministry of Emergency Situations working in the Arctic zone of Russian Federation [19].

As already noted, in addition to cortisol, the physiological response to stress includes the release of anabolic hormones that play a protective and regenerative role — testosterone, as well as DHEA and DHEA-S with pronounced neuroprotective, antioxidant, anti-inflammatory and anti-glucocorticoid effects. Increased concentrations of DHEA in both men and women at the beginning of the route may be explained by its protective effect aimed at leveling the effects of cortisol [20], which was also noted for DHEA in saliva [11], and, in addition, its effect on cognitive functions for the successful implementation of scientific work on the route. In the study of Korneeva Yu., Simonova N., an increase in the level of cortisol in saliva was also noted along with an increase in stress tolerance, operator efficiency and activity among shift workers in the oil and gas industry in the Arctic [21], which, in our opinion, can be explained by an increase in DHEA levels. The more pronounced increase in DHEA levels in women in the present study is consistent with data indicating a higher increase in DHEA levels in women after acute stress [5].

From the data in the table, it can be seen that morning concentrations of free testosterone in saliva increase in the middle of the route, compared with the beginning, for both men and women, and then they tend to decrease by the end of the route. It is known that cortisol and testosterone are antagonists: as the concentration of one increases, the level of the other decreases. In this case, there is a significant decrease in cortisol levels already in the middle of the route, which may lead to an increase in testosterone concentration. Also, an increase in testosterone levels during physical exertion has been repeatedly demonstrated in athletes, confirming its significant anabolic role in the short-term adaptation of the body [10, 22]. Perhaps the increase in testosterone, starting from the middle of the expedition, may indicate its adaptive role under the influence of a complex of stressful conditions of the expedition.

However, attention is drawn to the increased evening testosterone levels in the middle and end of the route, which practically corresponds to the morning, which is a violation of the physiological daily fluctuations and may also indicate the development of desynchronosis in the expedition members.

Regarding serum concentrations of the studied hormones, high values of cortisol and DHEA can be noted at the beginning of the expedition, however, a significant decrease by the end of the route is shown only for DHEA in men, which confirms the informative value of studying free fractions of hormones, as well as their salivary levels in marine expeditions.

It is also necessary to analyze the individual dynamics of salivary and serum hormone levels, as well as hormonal indices, in order to identify typological reactions of adaptation and criteria for the stress of adaptive reserves, which will be a continuation of this study.

Conclusion. *The hormone content in saliva, rather than in serum, is an informative indicator reflecting hormonal changes in the body of participants in the Arctic expedition associated with the presence of acute stress caused by natural factors and specific working conditions. An increase in evening cortisol and testosterone levels indicates a violation of their circadian dynamics and the possible development of desynchronosis in the expedition members by the end of the expedition. An increase in dehydroepiandrosterone at the beginning of the route and testosterone from the middle of the route may indicate their adaptive and neuroprotective role under the influence of a complex of stressful conditions of the expedition and increased mental stress.*

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