SPORTS MEDICINE

Short Highly Intense Exercise Causes Changes in Salivary Concentrations of Hydrocortisone and Secretory IgA

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The dynamics of salivary hydrocortisone during exercise depends on the professional status of the athlete. Hydrocortisone concentrations increase and those of secretory IgA decrease significantly during short-term highly intense exercise. Presumably, basal serum hydrocortisone level is the key factor in restoration of the secretory IgA concentration after exercise by inhibition of lymphocyte, macrophage, and monocyte functions through an increase in glucocorticoid level under the effect of physiological stressors.

Key Words: *saliva; hydrocortisone; secretory immunoglobulin A; exercise*

Exercise triggers many biochemical, molecular, and genetic mechanisms underlying the adaptation reactions to physiological stress [9]. The saliva now attracts the attention of scientists in athletic medicine due to its good prospects as a diagnostic fluid. Many substances detected in the saliva reliably reflect the effects of exercise of different intensity on human body (α-amylase [15], secretory IgA [15], hydrocortisone [7], testosterone [5]).

The effects of exercise on the immune system are determined by the intensity and duration of exercise [15]. Secretory IgA is the main immunoglobulin of the mucosal system. It provides resistance to respiratory infections. This immunoglobulin located in the mucosae and secretion differs by its structure and characteristics from circulating IgA produced in the lymph tissue. Numerous lymphocytes and plasma cells responsible for the production of secretory IgA are present in the stroma of the salivary glands. Passing through the epithelial cells, immunoglobulin binds the secretory component and is released into the ductal lumen as secretory IgA.

The data on changes in the secretory IgA level in the saliva in response to exercise are contradictory [15]. Moderate exercise (50-80% of maximum oxygen consumption (MOC) for 15-45 min) leads to an increase in the concentration of secretory IgA [4], while long-term intense exercise [14] and short-term highly intense exercise [11] can reduce the level of secretory IgA. According to some authors, the level of secretory IgA does not change after exercise [12].

No relationship between the physical training status of examinees and the basal status of secretory IgA or changes in secretory IgA concentration in response to a stress exposure was detected [15].

Hydrocortisone, released into the saliva by passive transport from the plasma through salivary glands (SG), is one more salivary substance significantly changing its concentration in response to stress. The capillaries adjacent to the SG are patent for many substances released from the blood system directly through ductal or acinus cell membranes. Steroid hormones (estriol, hydrocortisone, testosterone) are relatively small (<400 Da) notpolar molecules, and hence,

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easily pass through the blood–saliva barrier. Salivary levels of these hormones correspond to the levels of their free forms in the serum [6].

Hydrocortisone (glucocorticoid hormone) is a final product of the parasympathetic autonomic nervous system activation in humans. Hydrocortisone secretion increases in response to stress of any kind, physiological (disease, injury, fatigue, fever) and psychological [8]. Increasing the volume of exercise and chronic stress leads to a drop of the basal level of hydrocortisone [13].

Hydrocortisone acts through specific cell-cell receptors and influences many physiological systems, including the immune function, glucose regulation, heart rhythm, bone metabolism, muscle contractility [3], plays an important role in adaptation changes in athletes [7].

The effects of short highly intense exercise on the salivary biochemical profiles, specifically, on changes in the concentrations of secretory IgA and hydrocortisone were not described. The use of various tolerance tests and different methods for collection and storage of salivary specimens used by different research teams preclude the comparison of their results and correctly determining the effect of exercise on human organism. Hence, a universal methodologically standardized protocol is needed for evaluation of the training status of athletes and exercise tolerance by analysis of salivary biochemical profiles.

We analyzed changes in secretory IgA (local immunity component) in response to short highly intense exercise and evaluated the relationship between functional training status of athletes and the dynamics of hydrocortisone levels.

MATERIALS AND METHODS

The study was carried out in 48 athletes with qualification of master of sports candidates and higher (age 22.1±6.0 years, body length 181.7±7.3 cm, body weight 79.1±10.4 kg). All volunteers gave informed written consent to participation in the experiment. Experiments were approved by the Ethic Committee of the Institute.

Exercise tolerance tests were described previously [1]. The material for studies (venous blood and saliva) was collected at rest before exercise and directly and 30 min after it. The serum was separated by the standard method. Salivary specimens were collected using Salivette kit (Sarstedt) consisting of a neutral cotton tampon and a tube. The saliva was removed from the tampon by centrifugation. The specimens were stored at -80°C. One hour before collection of the saliva, the volunteers did not smoke, eat, or drink anything but water, chewed no gum, used no steroid creams or lotions, and rinsed the mouth with mineral water directly before collection.

The concentrations of total protein, secretory IgA, and hydrocortisone were measured by spectrometric analysis of the saliva using Microlab IK-Fourier spectrometer (ML, A2 technologies) as described previously [2].

The concentrations of hydrocortisone (DRG), creatine phosphokinase (CPK), AST, ALT (Human), and other biochemical markers were measured in the sera collected from all athletes before and after exercise for standardization of the results and evaluation of the athletes' physical status.

The significance of differences between the studied parameters was evaluated using Mann–Whitney's *U* test. Spearman's rank correlation coefficient was used for statistical evaluation of the relationships between the parameters.

RESULTS

Short-term highly intense physiological stress observed in athletes during competition was modeled in this study. The volunteers were subjected to graded treadmill exercise tests. The duration of tolerance test was 820±133 sec. By the results, the volunteers were distributed into 2 groups in accordance with their physiological parameters. Anthropometric parameters and the main integral parameters of the working capacity of volunteers in each group are summed up in Tables 1 and 2.

The groups differed significantly by body weight, MOC, duration of test exercise, duration of work before attaining the anaerobic threshold (ANT) and work after it ($p<0.05$). The performance of the test exercise (954±65 sec) and the work before ANT (717±104 sec) were longer and MOC was higher (65.9±9.1 ml/min/kg) in group 1 athletes in comparison with group 2 individuals (725±70 sec, 541±84 sec, and 53.8±7.4 ml/min/kg, respectively).

TABLE 1. Physiological Characteristics of Volunteers

Parameter	Group	
	1 (*n*=20)	2 (*n*=28)
Age, years	21.9±5.1	22.2±6.6
Body weight, kg	73.7±5.1	82.6±11.6*
Body length, cm	179.1±5.4	183.4±7.9
MOC, ml/kg/min	65.9±9.1	53.8±7.4*

Note. Here and in Table 2: *$p<0.05$ compared to group 1.

It is known that CPK, AST, and ALT are detected in the serum in cell membrane injury, for example, in the skeletal muscle myocyte membrane injury. Activities of CPK, ALT, and AST before and after test exercise were within the range of mean physiological values, which indicates the absence of myocyte and hepatocyte membrane damage. Enzyme activities increased just slightly, presumably indicating the minimum myocyte injury. No appreciable differences in these parameters were detected in the athletes.

All volunteers exhibited an increase of total protein level after the test with a trend to normalization during rest. During the exercise, salivary protein level could increase, because salivary secretion was mainly stimulated by adrenergic mediators. As we know, exercise stimulates the sympathetic activity. In addition, a high level of proteins after exercise could be caused by the SG β-sympathetic hyperactivity. No appreciable differences in the changes of total protein content under the effect of exercise were detected in the two groups of volunteers. Protein content increased 3.3±1.9 times after exercise and after rest differed 1.9±0.9 times from the basal level.

Salivary specimens were collected by the standardized method using Salivette kits as described previously [10], because the method of collection of salivary specimens is essential for the resultant hydrocortisone concentration [7]. Testing of athletes was carried out during the early hours of the day, so that the effects of circadian rhythms of hydrocortisone on the results were minimized.

Tolerance test of increasing intensity led to a significant increase in salivary hydrocortisone concentration, which was in line with the previous data [8] obtained on volunteers performing other exercise tests.

Salivary hydrocortisone concentrations in athletes of two groups differing by their training status were virtually the same before testing (12-20 mmol/liter). Salivary hydrocortisone increased greater after exercise in better trained athletes than in less trained athletes (Fig. 1). A trend to an increase of hydrocortisone concentration persisted after 30-min rest, the pattern of increase being identical in the two groups of athletes.

A correlation between salivary hydrocortisone concentrations after 30-min rest and directly after exercise was detected in athletes of higher qualification (r=0.78, p<0.05). Correlations between basal hydrocortisone level and the hormone concentration after exercise (r=0.94, p<0.05), directly and 30 min after exercise (r=0.89, p<0.05), and between basal level and level after rest (r=0.81, p<0.05) were observed in the group of less trained athletes.

Salivary hydrocortisone concentration correlates with free hydrocortisone concentration in the serum [6,10], due to which it is possible to monitor the dynamics of active hormone form in response to stress by noninvasive methods. About 80% of total serum hydrocortisone is bound to hydrocortisone-binding globulin (HBG), 10% to albumin, and 10% is the free form of the hormone. Hydrocortisone-binding globulin is a passive regulator of hydrocortisone bioactivity, determining its content needed for the organism. The saliva contains only the free hormone form, its share being just 3-4% of total serum hydrocortisone.

TABLE 2. Mean Values of Physiological Characteristics after Exercise Tolerance Test

Parameter	Group	
	1 (n=20)	2 (n=28)
Work duration, sec	954±65	725±70*
Work duration before ANT, sec	717±104	541±84*
Work duration after ANT, sec	238±76	184±66*
Respiratory coefficient	1.04±0.09	1.07±0.09
ANT, ml/kg/min	56.5±8.3	45.2±6.6
Heart rate, bpm	194±9	189±11
Lactate, mmol/liter	9.4±2.3	10.1±2.3

Analysis of hydrocortisone synthesis, transport, and regulation system suggested that athletes with a higher training status develop a high hydrocortisone response after short exercise either at the expense of greater stimulation of the hypothalamic—pituitary—pancreas system and higher production of the hormone or at the expense of lesser binding of hydrocortisone to HBG. Inhibition of HBG is also possible (endoge-

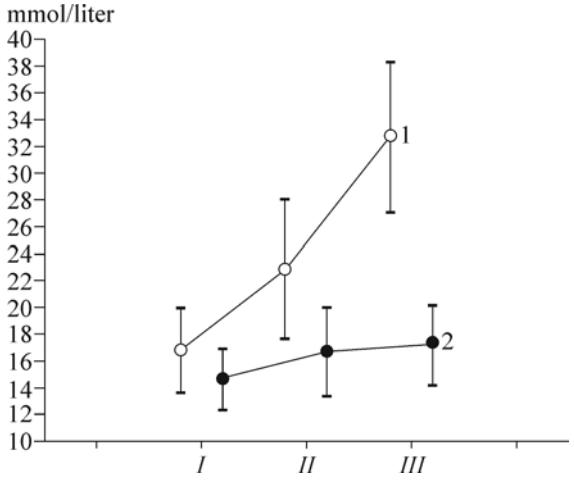

Fig. 1. Dynamics of hydrocortisone concentrations in the groups of athletes with different training status. *1*) group 1; *2*) group 2. *I*) before exercise; *II*) after exercise; *III*) 30 min after exercise.

nous hormones, IL-6), as well as the release of greater amounts of neutrophil elastase, leading to destruction of the hydrocortisone–HBG complex and release of greater amounts of the free hormone into circulation.

Water evaporation during test exercise can have a concentrating effect on the saliva. In addition, changes in salivary secretion, caused by exercise, should be taken into consideration. In order to rule out this problem when evaluating the changes in secretory IgA levels, the concentration of secretory IgA was standardized for total salivary protein level. Fluctuations in the secretory IgA concentrations are characterized by circadian rhythms with a significant decrease in the morning hours and stable level to 20.00 [14]. Experiments were carried out from 10.00 to 12.00, due to which the impact of circadian rhythms of secretory IgA concentrations for the results was minimized.

Basal level of secretory IgA was 217.3±121.4 mg/liter (normal) in all examined subjects.

Short highly intensive exercise stimulated a significant reduction of secretory IgA level in all volunteers ($p<0.05$). All athletes could be divided into two groups significantly differing by changes in the secretory IgA after 30-min rest. In one group ($n=21$), the "immunological gap" was replaced by a jump-wise increase in the level of secretory IgA (Fig. 2, group B), in the other ($n=27$) the reduction of this component of local immunity persisted (Fig. 2, group A).

Analysis of changes in the secretory IgA level in point 3 (after 30-min rest) in comparison with point 1 (before exercise) and basal levels of serum hydrocortisone concentration in athletes showed a significant correlation between the secretory IgA3/secretory IgA1 and basal serum hydrocortisone concentration in group A athletes ($p<0.05$, $r=0.73$; Fig. 3). The higher was basal serum hydrocortisone level, the slower was recovery of local immunity.

We can explain these regularities and the reduction of secretory IgA level, if we suggest that physiological stressors stimulate the glucocorticoid level (the observed increase of hydrocortisone concentration after a short highly intense stress is described above). Glucocorticoids inhibit lymphocyte, macrophage, and monocyte functions; this can lead to a reduction of the secretory IgA level [14].

The diagnosis by analysis of salivary specimens is an important sphere of physiology, endocrinology, immunology, psychology, dentistry, and athletic medicine, which is gradually extending.

The composition and rate of salivary secretion depend on coordinated effects of several transmitters and biological messengers on the secretory cells. Changes in the biochemical profile of the saliva (protein, electrolyte, hormone composition) in response to exercise can manifest as a result of complex integral interactions between different regulatory systems. We used a model short-term highly intense stress, leading to significant shifts in the salivary biochemistry. A short highly intense exercise leads to an increase of hydrocortisone concentration. This can indicate an increase of the parasympathetic activity. The qualification of athletes correlated with the intensity of response to model exercise. In addition, exercise testing described in this paper leads to a short-term immunological gap, recovery after which was different in the athletes. The degree of secretory IgA recovery after 30-min rest following highly intense exercise correlated with the basal serum hydrocortisone level. Hence, a short

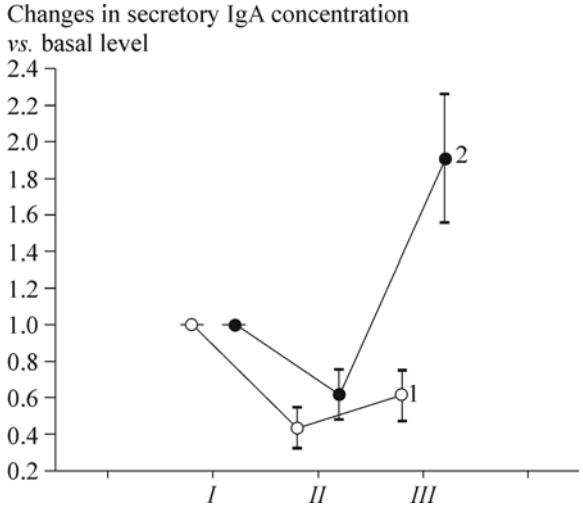

Fig. 2. Changes in secretory IgA concentration *vs*. its basal level in the saliva. *1*) group A; *2*) group B. *I*) basal level; *II*) after exercise; *III*) 30 min after exercise.

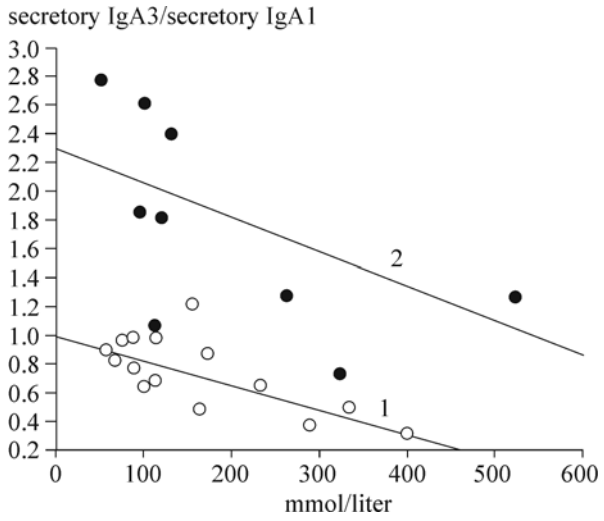

Fig. 3. Relationship between changes in secretory IgA after 30-min rest and basal serum hydrocortisone level ($r=0.73$, $p<0.05$ group A; $r=0.62$, $p>0.05$ group B). *1*) group A; *2*) group B.

highly intense exercise can be used as a model test for evaluation of the athlete's training status and exercise tolerance by analysis of changes in the salivary biochemical profile, specifically, dynamics of hydrocortisone and secretory IgA.

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REFERENCES

1. D. A. Sakharov, A. V. Stepanov, M. U. Shkurnikov, and A. G. Tonevitsky, *Byull. Eksp. Biol. Med.*, **147**, No. 3, 335-340 (2009).
2. S. A. Khaustova, M. U. Shkurnikov, E. S. Grebenyuk, *et al.*, *Ibid.*, **148**, No. 11, 597-600.
3. J. P. Ahtiainen, A. Pakarinen, M. Alen, *et al.*, *Eur. J. Appl. Physiol.*, **89**, No. 6, 555-563 (2003).
4. T. Akimoto, Y. Kumai, T. Akama, *et al.*, *Br. Sports Med.*, **37**, No. 1, 76-79 (2003).
5. M. Bouget, M. Rouveix, O. Michaux, *et al.*, *J. Sports Sci.*, **24**, No. 12, 1297-1302 (2006).
6. J. L. Chicharro, A. Lucia, M. Pérez, *et al.*, *Sports Med.*, **26**, No. 1, 17-27 (1998).
7. B. T. Crewther, T. Lowe, R. P. Weatherby, *et al.*, *J. Strength Cond. Res.*, **23**, No. 7, 2046-2053 (2009).
8. M. Hasegawa, M. Toda, and K. Morimoto, *Biomed. Res.*, **29**, No. 1, 43-46 (2008).
9. J. A. Hawley, M. J. Gibala, S. Bermon, *et al.*, *J. Sports Sci.*, **25**, Suppl. 1, S115-S124 (2007).
10. J. G. Lewis, *Clin. Biochem. Rev.*, **27**, No. 3, 139-146 (2006).
11. L. T. Mackinnon, E. Ginn, and G. J. Seymour, *Eur. J. Appl. Physiol. Occup. Physiol.*, **67**, No. 2, 180-184 (1993).
12. S. L. McDowell, K. Chaloa, T. J. Housh, *et al.*, *Ibid.*, **63**, No. 2, 108-111 (1991).
13. A. Moreira, F. Arsati, Y. B. de Oliveira Lima Arsati, *et al.*, *Eur. J. Appl. Physiol.*, **106**, No. 1, 25-30 (2009).
14. N. P. Walsh, N. C. Bishop, J. Blackwell, *et al.*, *Med. Sci. Sports Exerc.*, **34**, No. 10, 1632-1637 (2002).
15. N. P. Walsh, A. K. Blannin, A. M. Clark, *et al.*, *J. Sports Sci.*, **17**, No. 2, 129-134 (1999).