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Consideration of the Issue of Non-Invasive Diagnostics of Hormone Level in Athletes

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ANNOTATION

The article presents overview information on the current state of the issue on non-invasive methods for determining the level of a number of hormones in athletes in both sexes in saliva, possible in the process of training and competition, as well as at different phases of exercise. It is shown that local and foreign authors are keen to actively introduce modern methods and means of salivodiagnostics of the hormonal profile in athletes. Questions of correlation of hormone levels in blood and saliva are discussed, advantages and disadvantages of both methods of diagnostics are described.

Keywords: Athletes; Hormones; Saliva Diagnostics; Non-Invasive Methods; Saliva; Features of the Definition; Exercise

Abbreviations: MCS: Microcrystallization; OMC: Ovarian-Menstrual Cycle; NFP: Natural Family Planning; ABB: Acid-Base Balance; SDI: Sexual Dimorphism

Aim of Study

The purpose of this article is to review and analyze the available modern data on the use of modern methods for determining the level of hormones in the body of athletes and. in particular, with the use of taking saliva from athletes for research.

Material and Methods

When writing this article, its author used the method of critical literary analysis, available, at the time of this research and the writing of the article itself, domestic and foreign sources of information.

Introduction

Saliva is one of the biological fluids produced by the human body [1-15]. A number of domestic and foreign studies have reliably established that the determination of markers of hormones and a number of other substances in saliva is an alternative method for determining the same substances in the blood serum of the subject [1,4,6-15,16,17]. A significant advantage of determining the level of hormones in the blood is its simplicity, accessibility, painlessness, and non-invasiveness. Also, the advantages of modern methods for determining the level of hormones in saliva is that, unlike blood sampling, saliva biomaterial can be taken at any time of the day, both during physical activity and during the athlete's rest period, but with strict adherence to the conditions of the obtained biomaterial [1,4,12,16,17]. Naturally, an important point in the more active implementation of this method in practical clinical and sports medicine is the determination of standards for the content of a number of hormones (cortisol, cortisone, male and female steroid hormones, insulin, etc.) in saliva and the determination of correlation values for the level of these and other hormones in saliva, blood serum and urine. The technique of collecting material from the oral cavity does not require a private room, which is necessary when collecting urine and blood, i.e. this method is an alternative where collecting blood and urine is not possible [1,16,17,18].

According to research and practical experience cited by a number of authors, saliva samples are stable for 7 days at room temperature, 4 weeks at 2–8°C, and for a long period at temperatures below –20°C. Such high stability makes it possible to widely use saliva samples for analysis [1-3,6-9,17]. The problem that researchers continue to work on is the choice of reagents, preservatives, packaging material and its composition for collecting, storing and transporting saliva samples, as well as expanding the scope of application of this research method into practice, in particular in sports medicine, endocrinology and gy-

necology [1,4,7-20,19,20,21]. Domestic authors such as T.P. Vavilova, I.G. have been working quite long and fruitfully on the practical application of methods for determining hormones in saliva and all related aspects of this problem. Ostrovskaya, A.E. Medvedev, O.O. Yanushevich (2011, 2014) [2,3]. Under the leadership of T.P. Vavilova et al. A number of research papers have been published on the biochemistry of saliva and its research [2,3]. Of the domestic researchers involved in saliva diagnostics, I would also like to note the basic, classical works of such scientists as L.P. Gotovtseva, 2005; S.S. Mikhailov, E.V. Rosengart, 2008-2012; S.I. Piskov, 2008; S.A. Khaustova, 2010; L.V. Belskaya, O.A. Golovanova, V.G. Turmanidze, E.S. Shukaylo, 2011; V.A. Kurashvili, 2012; S.N. Didenko, G.D. Aleksayants, 2014; I.V. Evstigneev, 2014.

Among the foreign authors dealing with this problem, I would like to note the interesting studies of Vining R.F., McGinley R.A. (1986), reflected in the work "Hormones in saliva", as well as in the works of Higashi T. (2012) "Salivary hormone measurement using LC/MS/MS: specific and patient-friendly tool for assessment of endocrine function" and Zolotukhin S. (2013) "Metabolic hormones in saliva: origins and functions" [15]. One of the latest works that deserves close attention, in our opinion, is the work of L.D. Hayes, N. Sculthorpe, B. Cunniffe, F. Grace "Salivary Testosterone and Cortisol Measurement in Sports Medicine: a Narrative Review and User's Guide for Researchers and Practitioners" - "Salivary Testosterone and Cortisol Measurement in Sports Medicine: a Narrative Review and User's Guide for researchers and practitioners" [15]. Classic works, especially regarding the introduction into practice of such an important index value as the "anabolism index" (AI), were introduced by S.K. Chang, H.F. Tseng, N.F. Tan, Y.D. Hsuuw, J. Lee-Hsieh, 2005 [12]. Also interesting are the works of such foreign authors as T.J. Cieslak, G. Frost, P. Klentrou, 2003 [12]; D.A. Edwards, K. Wetzel, D.R. Wyner, 2006 [14]; W.J. Kremer, A.D. Rogol, 2008 [19]. Here it is worth noting the earliest practical developments on this issue by S.K. Chang, H.F. Tseng, N.F. Tan, Y.D. Hsuuw, J. Lee-Hsieh, 2005, the authors of which laid the foundation for the use of saliva diagnostics in conducting research on testosterone, cortisol, their ratios, as well as describing the methodology of the saliva sampling process and conducting research procedures, which were taken into account and used in their works by other researchers this issue [12].

According to the authoritative opinion of such researchers as T.P. Vavilova et al. (2011, 2014), and V. Evstigneeva "In clinical and laboratory diagnostics, an important issue is the ratio of the concentration of steroid hormones in the blood and saliva" [2,3,17]. This is really of decisive importance, since thanks to the research of a number of domestic and foreign authors (S.S. Mikhailov, V. Rosengart, 2008, 2012; L.V. Belskaya, O.A. Golovanova, V.G. Turmanidze, E. S. Shukaylo, 2011), it was found that in saliva, unlike blood serum, a number of hormones, such as cortisol and testosterone, are in a free, unbound form, and their quantitative determination in the saliva of athletes is more reliable and informative [1,3,7,8,10-15,18]. This, taking into account the simplicity of collecting material and the ability to carry out express collection of material in any conditions and at any time of the day, makes saliva diagnostics an alternative and competitive one [1,3,6,10-12,14].

Results and Discussion

What hormones can be determined in a saliva sample? Today, many diagnostic laboratory centers determine the level of 17-OH-progesterone, androstenedione, DHEA, cortisol, progesterone, testosterone, free estriol, E2 estradiol [1,3,6-8,10-15,17,18]. From a physiological point of view, measurements of progesterone concentration in saliva can be used to monitor the menstrual cycle in women to determine the time of ovulation and to assess the function of the corpus luteum, which is very important in the early stages of pregnancy. Due to the constant fluctuations in progesterone levels, which also depend on the individual condition, it is very convenient to carry out several successive measurements of the hormone in saliva [4,9-21,16,18,19]. Control of estradiol and progesterone in the saliva of female athletes can not only help in controlling the menstrual cycle and its phases, both for building rational training and competitive micro, meso and macrocycles, but also conduct adequate express monitoring of the individual state of reproductive health of female athletes [1,6,7,12,15,16,17]. Human saliva and oral fluid contain the same hormones as blood serum (total and free thyroxine, total and free triiodothyronine, thyroid-stimulating hormone, cortisol, progesterone, prolactin, testosterone, follicle-stimulating and luteinizing hormones), but in significantly lower concentrations, in different at least for different hormones than in blood serum [1,3,6-8,10-15,18,17].

The concentration of progesterone and estrogen varies both in the blood and in saliva, depending on the phase of the menstrual cycle and the woman's age [1,3,6-8,10-15,18,17]. Estradiol has a significantly higher concentration in oral fluid than in blood serum. There is a direct relationship between the concentration of hormones in blood serum and oral fluid or saliva [1,3,6,15-19,18,17]. Oral fluid hormones adequately reflect sex differences in serum hormones. In the blood serum and oral fluid, the concentration of estradiol, prolactin, FSH and LH in women is higher than in men, and in the luteal phase - progesterone [1,5,10,20]. In men, the concentration of testosterone in blood serum and oral fluid is many times higher than in women. In postmenopausal women, the concentration of estradiol and progesterone in the blood serum and oral fluid is reduced, and the latter was not detected in the oral fluid [3]. When determining dehydroepiandrosterone (DHEA) in the saliva of young female athletes (prepubertal, pubertal and adolescence), it allows us to determine such phenomena as delayed puberty or its premature manifestation [1,3].

Interest in determining the level of hormones at different age periods in athletes of both sexes, with their different levels of sports qualifications, as well as at different stages of physical activity, with different psychological components (in the pre-competitive, competitive and post-competitive periods), as well as during training and rest and rehabilitation - this is not a complete list of questions to which, in our

opinion, coaches, sports doctors and psychologists, physiologists, and the athletes themselves would like to receive answers [1,16,17,20]. In this regard, L.V. Belskaya et al. (2011), rightly note that "The main reason for the negative impact of physical activity is the insufficient use of modern quantitative methods that allow for careful medical monitoring during the training process" [1]. It is important to monitor the dynamics of hormone formation during physical activity using strength, speed, complex coordination and other exercises. And here it is difficult to imagine an attempt by a researcher to collect blood and urine from athletes at all these stages. Taking a saliva sample appears to be a comprehensive alternative here [1,3,4,12,17].

Unfortunately, there is not yet a comprehensive practice of a widespread, fully algorithmized (standardized) procedure for this type of research, although there is a sufficient number of works on this issue [17]. When considering the methods used, the use of equipment and reagents, there is also no unity, since the authors carried out their studies using different methods, equipment and reagents [6-8,17]. What did the authors most often use in their research? So, for example, I.V. Evstigneev, when studying steroid hormones in saliva (testosterone, androstenedione, dehydroepiandrosterone sulfate, progesterone, estradiol, cortisol), recommends using an immunoassay based on enhanced chemiluminescence [17]. Arguing for the practical application of the luminescent immunoassay (LIA) method, I.V. Evstigneev points out that "LIA allows one to determine with high accuracy and specificity the level of fT (free testosterone) in saliva, which is an adequate marker for assessing androgen status in men" [17]. The author, in his studies on determining the level of free testosterone in saliva, used special containers (SaliCaps, IBL - Hamburg, Germany) and a special tube connected to the container, which was made of a material that does not absorb steroids, to obtain and store saliva samples [17]. Therefore, having extensive practical experience in conducting saliva diagnostics, I.V. Evstegneev (2014) makes the following practical conclusion: "The level of free biologically active testosterone in saliva strongly correlates with the biologically active form of the hormone in the blood (cT plus albumin-bound testosterone).

The level of testosterone in saliva is a more adequate indicator of biological activity than serum testosterone, especially when the binding ability of specific transport globulin changes" [17]. When determining the level of cortisol in saliva, the author points out that "Determination of cortisol in saliva is used as an alternative method when conducting functional tests with dexamethasone, adrenocorticotropic hormone (ACTH), insulin, while the stressful effect of repeated blood sampling on the hypothalamic-pituitary-adrenal system is reduced (GGAS). Studying the concentration of free cortisol in saliva is more suitable for assessing physiological fluctuations in HPA axis activity over time compared to determining the level of cortisol in the blood" [17]. And it's hard to disagree with this, especially having practical experience in conducting this kind of research both in clinical practice and among athletes. The author, when studying the determination of Free cortisol, used special saliva collection systems Salivette (Sarstedt) [7]. The resulting saliva sample was examined using the ECLA method on an automatic analyzer. After analysis, saliva samples were frozen at a temperature of – 70°C for ELISA using Salivary Cortisol ELISA Kit SLV-2930 (DRG) diagnostic kits [3,4,17].

The method proposed by LV. Belskoy et al., 2011б is quite well-reasoned and has proven itself positive over many years [1]. According to their opinion, based on many years of practical experience, "The most promising new diagnostic technology is the morphological study of biological fluids. The crystallographic (tesigraphic) method has significant sensitivity, and therefore has found wide application, first in the practice of forensic chemical analysis, and then in sports medicine. Data on microcrystallization of oral fluid can be used as a method for assessing the general condition of the human body and, in particular, to determine the state of the body before and after physical activity" [1]. To determine the type of saliva microcrystallization (MCS), the authors propose to use the methods of Leus P.A. and Puzikova O.Yu. [1]. It should be noted that this method has been used in practical medicine (gynecology, reproductive and forensic medicine) for quite a long time. Thus, the ability of saliva to crystallize with the "creation" of a pattern of varying degrees of severity, in different phases of the ovarian-menstrual cycle (OMC), is used in the practical application of natural family planning (NFP) methods, in particular in the work of the Arbor® minimicroscope and the Vesta device®", as well as the "Maybe Mom®" test microscope for determining ovulation using a drop of saliva [4,21,22]. Crystallization patterns of saliva in different phases of OMC are presented in the Figure 1.

Note: No ovulation beginning of ovulation Ovulation.

Figure 1: The structure of saliva in different phases of OMC under a microscope.

It should be noted that with a meager content of female sex steroids in the blood serum and saliva during the postmenstrual period of OMC (estrogens and progesterone), the degree of crystallization of saliva will be unexpressed, in the form of "sand", as it approaches ovulation (preovulatory period of OMC) and, accordingly, the level of sex steroids in the blood and saliva increases, clusters of crystals appear in the saliva sample under a microscope. The peak of crystallization, with a pronounced manifestation of the process of their loss, in women occurs in the middle of their individual OMC, which is characterized by the maximum concentration of microcrystals both in the blood serum and in saliva samples [4,21,22]. It should be noted that similar changes occur in the cervical mucus of women, depending on the phases of its OMC, which was the basis for such a practical EPS method as the Ogino-Knaus method [4,21,22]. Informative and relevant, from a practical point of view, is to determine the level of cortisol and adrenal hormones in saliva. This will be very important and practically in demand among female athletes, with clinical manifestations of hyperandrogenism of varying degrees, especially in female athletes, with an identified shift in the values of the index of sexual dimorphism (SDI) in sexual somatotypes - from gynecomorphic to mesomorphic and, especially, andromorphic [3,4-17,6,16,20].

Since this dynamic phenomenon, with changes in the values of the Index of Sexual Dimorphism (SDI), is inherent in many female athletes in most types of modern sports, express diagnostics of the level of cortisol and a number of other hormones (hypothalamic-pituitary, steroid) in saliva will help in clarifying the course of the adaptive process changes and correction of reproductive health disorders in many athletes, both in reproductive age, and in young athletes in prepuberty and puberty, and former athletes in premenopause and menopause [3,4-17,6,16,20]. Also relevant for the phenomena of hyperandrogenism and the phenomena of hirsutism will be the determination in saliva of such an indicator as the level of androstenedione, which is formed in the adrenal glands and gonads and is an intermediate product in the synthesis of both testosterone and estrone [3,6,4-17,20]. According to T.P. Vavilova et al., in the saliva of women, the concentration of androstenedione is normally low, but with hirsutism it increases 2-6 times. In men, the level of this hormone in saliva does not differ from that in women [3].

The practical application of saliva diagnostics in sports is given attention by such authors as S.S. Mikhailov and E.V. Rosengart. Their joint research, the data of which was published in 2008-2012, has significant theoretical and practical interest in matters of sports physiology. Thus, in their joint review article "Saliva as an object of biochemical control in sports", 2008 [7], provides a lot of valuable information about changes in the indicators of a number of hormones in saliva during physical activity of varying intensity in athletes [3]. The authors show the possibilities of saliva diagnostics in determining the level of acid-base balance (ABB) and immune status (in particular IgA values), amylase, peroxidase, etc. in athletes, as well as cyclic changes in the spectrum of steroid hormones in athletes in a number of sports (rugby, volleyball, handball, judo) [7,8,10]. An important point in carrying out saliva diagnostics of various directions, incl. and when determining the level of hormones in saliva, there is a unity of methods associated with collecting this biological material from patients, which, in our opinion, requires strict algorithmization. In this regard, in his many years of fundamental research concerning the determination of the level of a number of hormones in human saliva, T.P. Vavilova, I.G. Ostrovskaya, A.E. Medvedev (2014), give the following valuable practical recommendations: "Mixed saliva can be collected both in standard measuring glass containers and in special polypropylene containers, as well as using a moisture-absorbing cotton or synthetic swab placed at the bottom of the mouth. The reduced content of hormones in stimulated saliva samples may be due to their adsorption on paraffin and cotton swabs. Replacing a cotton swab with a synthetic one or using special systems such as Salivette ("Sarstedt", Germany), Quantisal ("Immynalysis", Canada), Saliva Collection System ("Greiner BioOne", Austria) improves the quality of analysis and affects the level of hormones in studied saliva samples" [3].

This is a very valuable recommendation, which allows us to unify the algorithm and methodology for collecting saliva samples from a contingent of people (patients, athletes), in whose saliva it is planned to determine the level of the hormones being studied. Further, the authors of the study provide valuable practical advice: "The level of hormones in saliva is also affected by the conditions of its storage. A number of hormones, primarily steroid hormones (androgens and glucocorticoids), retain their properties when saliva samples are stored at room temperature for several days. The addition of preservatives significantly prolongs the stability of salivary steroid molecules, which is essential, for example, for sending saliva samples by mail" [3]. The authors focus special attention on the comparison of methods for determining hormones in saliva, their informativeness, advantages and disadvantages of different methods of saliva diagnostics [3]. In addition, the authors point to another, fundamentally important, in our opinion, problem. The point is that "the determination of various hormones is affected by the growing number of reagent kits on the world market from different manufacturing companies. In order to speak with complete confidence about the similarities or differences in the determination of hormone concentrations by one or another test system, it is necessary to eliminate possible variations that arise when conducting analyzes in different laboratories, in different cities, which is inevitably associated with freezing/thawing of samples, their transportation. Therefore, it is advisable to determine the concentrations of hormones in saliva within the same laboratory using test systems from the same manufacturer" [3].

Conclusion

1. Saliva diagnostics is a promising modern method for diagnosing the level of hormones and a number of other biologically active substances in the saliva of athletes, both at different stages of the training and competition cycle, and at rest.

2. As a simpler, non-invasive, accessible and accurate method, in most cases, saliva diagnostics is an alternative to studies with blood serum.

3. The use of saliva diagnostics in determining the level of hormones of the hypothalamic-pituitary, adrenal and ovarian zones in female athletes - diagnostics of their hormones in women, taking into account their individual characteristics and phases of OMC - a relevant and modern way of taking into account the adaptation of female athletes to physical activity.

4. It is necessary to standardize and algorithmize the saliva diagnostic procedure, with mandatory correlation of hormone indicators in blood serum and saliva.

5. The saliva diagnostic method in sports physiology and medicine should become an accessible and mandatory diagnostic method in any sports team when preparing athletes during training and competition.

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