ΙΑΤΡΙΚΗ ΣΧΟΛΗ

Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών

Διπλωματική εργασία στο Μεταπτυχιακό Πρόγραμμα

“ Μοριακή κι Εφαρμοσμένη Φυσιολογία"

Τίτλος:

“ ΜΕΛΕΤΗ ΤΟΥ STRESS ΣΕ ΚΡΙΤΕΣ ΡΥΘΜΙΚΗΣ ΓΥΜΝΑΣΤΙΚΗΣ ΕΠΙΣΗΜΟΥ ΠΡΩΤΑΘΛΗΜΑΤΟΣ ΚΑΤΑ ΤΗΝ ΕΝΑΡΞΗ ΚΑΙ ΤΗ ΛΗΞΗ ΤΩΝ ΑΓΩΝΩΝ”

Δήμητρα Σκούρα

Ιούνιος 2015

Copyright

MEMBERS OF COMMITTEE

1. Koutsilieris Michalis,

Professor of the graduate program of "Molecular and Applied Physiology" , Medical School of Athens

2. Tsopani Despina,

Assistant Professor in Coaching of Rhythmic Gymnastics, Faculty of Physical Education and Sports Science of Athens

3. Maridaki Maria,

Associate Professor in Biology of Exercise, Faculty of Physical Education and Sports Science of Athens

ACKNOWLEDGES

Special thanks to the professor of the graduate program of " Molecular and Applied Physiology" Mr Philippou Tasos, without the help of which the investigation could not be completed. His knowledge and his advice in laboratory tests and statistics was really important and necessary.
Also, Mrs Tsopani Despina, for the confidence she showed to me once again, the help and the support during the study.
I would like to thank The Greek Federation of Gymnastics for giving us the permission to take samples from the judges and of course the judges- subjects for their participation in the research, the good mood and the cooperation.
Furthermore, my fellow student in the graduate program Mrs Thodou Sissy for her help during the experiments and the measurements in the laboratory.
Last but not least, special thanks to my family, to which I owe all my strength.

TABLE OF CONTENTS

SUMMARY

Rhythmic Gymnastics is an aesthetic event balancing between art and sport. It has a perfomance rating system (Code of points) and it is one of the sports which competition results greatly depend on th judges' evaluation. The main cause for unintensional mistakes of the judges in the rating is the fatigue of the judges.

Stress is a fundamental process. The human stress response involves complex signaling pathway among neurons, somatic cells and chemical interactions to keep the homeostasis. Cortisol, is an important indicator of stress, and its secretion presents secretory spikes, especially in the morning hours and weaker in the evening and as a result at the first eight hours of the morning, it is secreted 70% of the total amount of cortisol produced the whole 24 hours of the day. Oxytocin, is a neurohypophysial peptide which is produced in the paraventricular nuclei of the hypothalamus and is called th " love" hormone responsible for the feelings of joy and euphoria (for example at women eho breastfeed a.o.), but also is released by the pituitary gland in response to various stressful stimuli, including pain, fear and exposure to an unfamiliar environment. The purpose of this study is to find the stress responses of the judges of Rhythmic Gymnastics at the beginning of the competition and then in the end after fourteen hours under stressful conditions, full of work and judging, estimating how the fatigue and the pressure of the workload can affect the stress hormones. We measured 17 judges, by taken samples of saliva, and applied the competitive immunoassay (ELISA) using ELISA kits for cortisol and oxytocin. Also, they answered the scientific questionnaire for assessing depression and stress, the Anxiety Inventory (Laux 1981) and the Self- Rating Depression Scale(Zung 1965).

The results show decline of the cortisol levels after the competition from 4,68+- 1,29 ng/ml to 3,37+- 0,64ng/ml (t-test: 0,0004), because of the biorhyth and the secretion of the hormone, but the decline was not so great as others studies found for people under normal conditions. Oxytocin levels were, also, lower at the end of the competition, 0,74+- 0,47 ng/ml to 0,51+- 0,36 ng/ml (t-test:0,012), as the contents of the judges' work is not based on positive procedures and euphoria, but instead it provokes stress. According to Pearson's method, the flunctuation between the two hormones for each judge seperately shows no statistical significant negative correlation as it was expected. The average score for the judges at the Anxiety Inventory was normal, < 70 (100th percentile).

PART 1
BIBLIOGRAPHIC REVIEW

JUDGING IN RHYTHMIC GYMNASTICS

Rhythmic Gymnastics (RG) is an aesthetic event balancing between art and sport that also has a performance rating system (Code of Points) given by the International Gymnastics Federation (FIG), the official governing body for gymnastics in the world (Flessas et al., 2015). RG is one of the sports (e.g. artistic gymnastics, diving, figure skating and synchronized swimming) in which competition results (scoring and ranking of gymnast's performance) greatly depend on the judges' evaluation. (Leskosek B. et al., 2012)

In those kinds of sports where results are calculated in kilograms, seconds, meters etc, the accuracy of the results is constantly growing thanks to the perfection of the technology and the precise technical equipment. Where, however, refers to the subjective rating of the judges, progress can be achieved by the refinement of the scoring system by experts. Strongly there is a need for study and evaluation (the completion) of the data currently available in order to create a single system best scoring methods that take into account both the modernization of the scoring method, and the structure of the human factor in the jury-scoring system the games (Karpenko L.A. et al. , 2007).

Rhythmic gymnastics judging demands great mental capacity because it combines emotional perception with objective technical evaluation; therefore, the judge must simultaneously appreciate expressive, artistic and technical parameters.

In RG at the world class level extremely difficult bodily movements are performed in combination with skillful handling of the apparatus resulting in a fascinating spectacle. During a typical rhythmic gymnastics competition, a judge must evaluate approximately 150 performances that last approximately 1 minute and 30 seconds each (in the case of an individual competition). These exercises are executed by gymnasts of different levels. Also a particular task that the judge of RG is faced with when officiating a five gymnast ensemble is to attend simultaneously to all gymnasts following their performance in real time and detecting errors in performance based on specific scoring rules (a basic mechanism for focusing attention on a particular object of interest is to direct foveal vision to it). In ensemble competitions judges of RG have to track the performance of five gymnasts simultaneously for 2 min 15 s to 2 min 30 s. These gymnasts move within a large competitive field of size 13 13 m, coming close to each other and then spreading out running or jumping at high speed. Each of them also holds an apparatus (rope, ribbon, and hoop) that at certain time points becomes separated from the gymnast after a complex moving trajectory of its own. The judge must detect several possible errors in body shape, position and coordination, apparatus trajectory and contact with the gymnast, as well as combinatorial errors in the coordination of all

gymnasts and apparatuses (Flessas et al., 2015)

DIFFICULTIES IN JUDGING, FATIGUE AND STRESS

The main cause for unintentional mistakes of the judges in the rating is the fatigue of the judges. Fatigue causes not only the weakening of memory and attention. The weakening of attention can result to very important mistakes when grading an exercise. The judge at work should isolate the attention of all the rest, irrelevant subjects, so that the image of the athlete becomes more clear and unambiguous, representations and thoughts held in consciousness by the time the whole activity is completed, process until the goal is achieved.

When fatigue occurs, there is most often the need for volitional efforts to organize the attention of judges, which causes errors in the assessment - rating the level of athletes.

Special studies have been studying the concentration of attention of the judges, the variation in the degree of concentration of the judges according to the shift of competitions, the components that determine the initial mood of the judges.

The comparative analysis of the concentration of attention of the judges in the first and second shift showed that there are remarkable differences. Statistically, significantly different and the indicators of attention between the first and the third party. This means strongly expressed fatigue for judges and vigorous swing of attention after the first part. Indeed, the indicators of attention is not fully restored even after the break (when the shift of attention happens) and before the third party.

The efficiency of the work of the judge and the concentration of the attention are in close relationship. In second part (shift) they are weakened. It's weakened the degree of self-reactive concentration. The judges go fully in ordinary scoring stereotypes (based on their experience). We can assume that the accuracy and precision grades begin to depend on the experience of the judge, and the abilities of the least experienced of the judges - pundits.

Observations showed that the presentation of the degree of an experienced judge and a new in the first part of the games simultaneously. Starting from the second part, the fatigue occurs, and judges with less experience show their score with little delay, oriented by the judge authority. According to the data of psychologists, in this case, operates the equalising or stabilizing psychological mechanism.

The effectiveness of the work of judges is also dependent on the original mental state and their psychological moods. Greater than the work of the judges have such indicators of mental status such as energy, anxiety, attention. Psychologists note that the energy of the person is important to maintain a constant concentration of attention. (Karpenko et al., 2007)

Judges' work efficiency depends on their psychological status, activity, anxiety, attention. Mental activity plays important role, which is connected with solution of different perceptual and mental tasks. These factors facilitate “loosening” of mark from its increasing to reduction that underlines dependence of judges on the state of their central nervous system. Such problems can also be connected with peculiarities of memory. By the data of Yu.Ye. Titov, L.I. Turischeva the capacity of human memory is lower than requirements of modern rules of competitions, according to which in one composition female gymnast carry out more than 20 elements by body, not considering the load of equipment, which is more than 1000 bits of memory. A judge must see and evaluate all this. Thus, formation of integral idea about factors of influence on judges’ work as one of main problems not objective and unqualified refereeing, determination of total result and progressive development of calisthenics in Olympic sports system, conditions the urgency of the research.

Subjective change of mark, connected with executive skill of female gymnasts is additional activity, i.e. solution of other tasks in the process of main information memorizing. For example, evaluating an exercise, judges have not only to memorized the made by sportswomen mistakes, but also to solve tasks, connected with determination of additional markups. The next factor, which influences on judging quality, is judges’ tiredness. Tiredness causes both memory and attention weakening. Attention weakening is very important factor, which can bring to substantial mistakes in refereeing. Judges’s activity is characterized mostly by visual memory. Often visual memory requires volitional efforts. Special scientific works were devoted to: concentration of attention, judge’s work efficiency, components, determining final psychic status of referees. Each of these factors influences on judge’s work efficiency (Perederij V. V., 2013).

Besides capacity limitations of the attentional system, other factors are thought to affect judgment of RG judges. One such factor is a priori bias. Bias in sports officiating is a known phenomenon in competitive sports, examples being social biases, crowd noise, etc. The effects of biased officiating are potentially more dramatic in sports, like RG, in which the officials actually determine the outcome of the competition with a score of points resulting from the evaluation of the performances of gymnasts (Leskosek B. et al., 2012). Experience and training of judges certainly play an important role in their ability to perform this task (Fernandez- Villarino Maria A. et al., 2013). Nevertheless, they are required to perform a formidable cognitive task that lies beyond the normal limits of human attentional capacity, even taking into consideration the fact that attention can be maintained at objects outside the focus of visual fixation.

STRESS

Stress is a fundamental process. It affects everybody, from the simplest bacteria protozoans, to the complex eukaryotes such as mammals. In unicellular organisms, but also in individual cells in our bodies, molecules have been developed triggering a series of emergency systems, which protect basic cellular functions of unexpected external challenges and their internal consequences. For example, specific molecules called heat shock proteins (heat-shock) guide damaged proteins where they can repair or break up quietly, thus protecting cells from toxicity or malfunction. In complex organizations such as human, the stress systems have evolved a complicated process to help in the confrontation of any abnormal activity may afflict them. These systems use the cellular protection mechanisms as building blocks to a broader network protection from stress (http://www.brainsource.com/stress_&_health.htm)

The main concepts of stress derived from the ancient Greek spirit. The first evidence that mental function is based on the brain goes back to Pythagoras and the first evidence of homeostasis made by Alkmeona. Hippocrates supported throughout viotheoria and introduced the ancient medicine to the idea that the body is constantly under the influence of threatening forces, which juxtaposes contrasting adaptive forces, achieving health and therefore balance or harmony. Nowadays, the threatening forces against homeostasis are named stressful stimuli and the counter adjustment forces, the balance between the opposing forces homeostasis and the condition of the affected balance is named stress and our knowledge of how this is done result from Neuroendocrinology.

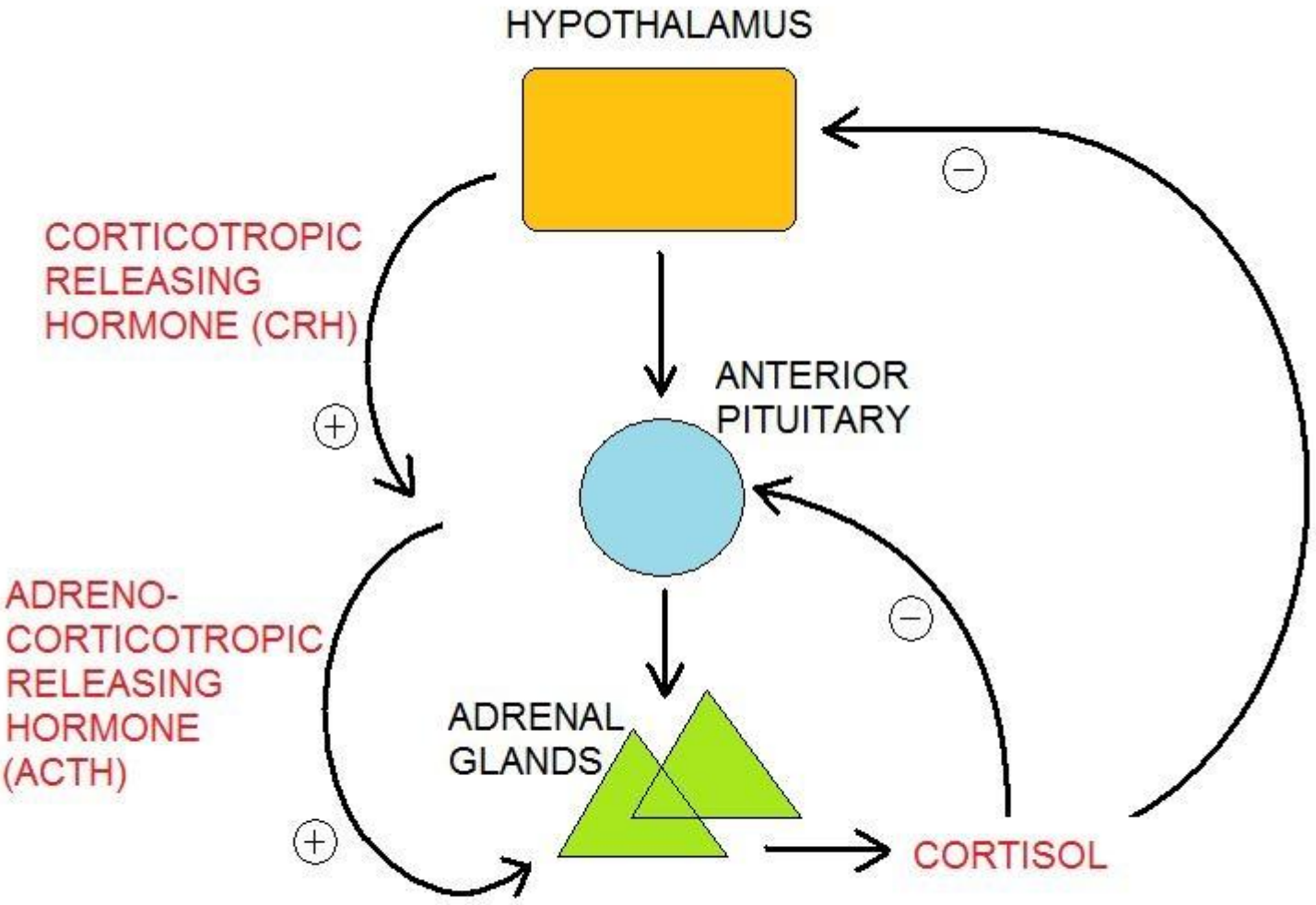
Stress is perceived by the brain and our reaction is determined by him. The cognitive assessment of any situation in the brain interacts with organic signals into the blood stream, such as hormones, nutrients, inflammatory molecules and information peripheral nerves supervising organs and senses vital. The brain combines them all to produce a series of specific and graded answers.
Hormones circulating in the blood are controlled by the brain to help the body to cope with stress. The most easily recognizable answer is the direct activating a system called sympathetic nervous system. After you accept one stressor challenge and calculate the correct response, the brain rapidly activates nerves starting at centers control of the brainstem. This causes noradrenaline release from a variety of structures and adrenaline from the adrenal glands (situated just above by the kidneys). This release establishes the fight or flight response - the classical, immediate reaction which should happen in response to danger. All recognize the initial tingling sensation, the sweating, increased

alertness, fast pulses, the increase in blood pressure and general the feeling of fear we feel, immediately after stressful stimulus. These changes occur because activation of receptors that are a)blood vessels, causing them to shrink, with so that the blood pressure to rise and b) heart, where they cause acceleration of its operation and swipe sense production chest, known as palpitations. Also, receptor activation the skin causing 'lift hair-chill "while activation of receptors in the gut, causing the alarming gastric feelings all perceive as stress. These changes occur to prepare us to give battle or Trap to flee - and to concentrate blood to organs vital that the muscles and brain.

Neurochemistry of stress- How stress affects the body

The human stress response involves a complex signaling pathway among neurons and somatic cells. While our understanding of the chemical interactions underlying the stress response has increased vastly in recent years, much remains poorly understood. The roles of two peptide hormones, corticotropin-releasing hormone (CRH) and arginine-vassopressin (AVP), have been widely studied. Stimulated by an environmental stressor, neurons in the hypothalamus secrete CRH and AVP. CRH, a short polypeptide, is transported to the anterior pituitary, where it stimulates the secretion of corticotropin. Consequently, corticotropin stimulates increased production of corticosteroids including cortisol, the primary actor directly impacting the stress response. Vasopressin, a small hormone molecule, increases reabsorption of water by the kidneys and induces vasoconstriction, the contraction of blood vessels, thereby raising blood pressure. Together, CRH and vasopressin activate the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis comprises the system of feedback interactions among the hypothalamus, pituitary gland, and adrenal glands.
In sum, the hypothalamus releases CRH and vasopressin, which activate the HPA axis. CRH stimulates the anterior pituitary to release corticotropin, which travels through the bloodstream to the adrenal cortex, where corticoptropin then upregulates cortisol production. Vasopressin, the other hormone secreted by the hypothalamus, stimulates the cortical collecting ducts of the kidneys to increase reuptake of water, resulting in smaller volumes of urine formed. As the next section will illuminate, corticosteroids such as cortisol act across the entire body to promulgate the stress response (Michael Randall et al., 2011)

Diagram shows the secretion of cortisol and its feedback loop



CORTISOL

Cortisol (hydrocortisone, compound F) is a steroid hormone synthesized from cholesterol. It is the primary glucocorticoid produced and secreted by the adrenal cortex. The stimulus to secretion given by ACTH, the secreted after induction of adenoupo-nature of the hypothalamic CRF.

Cortisol is found in the blood either as free Cortisol, or bound to corticosteroid-binding globulin (CBG) (Product Manual of Cortisol Elisa kit, 2014).

The secretion of cortisol presents secretory spikes, especially in the morning hours, and weaker in the evening and as a result at the first 8 hours of the morningit it's secreted 70% of the total amount of cortisol produced the whole 24 hours of the day (Kontoaggelos et al., 2013)

Cortisol shows interactions with other chemicals. Primarily, in the metabolic aspect, it influences insulin production by increasing gluconeogenesis, increased levels of free amino acids in plasma, stimulates the production of gastric acid by acting on the hydrogen pump, inhibits sodium loss from the intestine, it acts as an antidiuretic hormone.Also, increases the availability of copper impairs immune function through negative feedback to interleukin-1, promotes the development of osteoporosis, cooperates with epinephrine (adrenaline) in the operation of the short memory, increases blood pressure, inhibits the secretion of corticotropin releasing factor (CRH), affects renal function by promoting the production of weak urine.

Factors that influence the cortisol levels is caffeine, which can increase cortisol levels and sleep deprivation. A study of Leproult et al. At the Dartmouth students found that their complaints of considerable anxiety and pressure and our common responses to stress, lack of sleep, caffeine intake and alcohol consumption raise the amount of cortisol in our bodies, augmenting the very stress we seek to combat. Vigorous exercise (high volume VO2) stimulates the production of cortisol, too.

It has also been shown that plasma cortisol levels elevate in response to stress and variations val / val of BDNF gene in men and variation val / met in women are associated with increased secretion of cortisol in saliva under stressful conditions (Michael Randall et al., 2011).

SALIVARY CORTISOL

Throughout the last decades the use of saliva as an alternative specimen for the determination of hormones has gained increasing attention. Researchers and clinicians in endocrinology, psycobiology, behavioral medicine and psychiatry successfully applied the measurement of steroids in saliva and pointed out the potential advantages of this method over blood analyses.

Although it is well documented that other steroid hormones are also affected by stress, cortisol is still considered a major indicator of altered physiological states in response to stressful stimulation. In the past, most of the studies conducted with humans on this issue faced numerous problems associated with venipuncture for blood sampling. Besides the reactivity of the method, ethical and pravtical constrains as well as economical factors have hampered research on the psychoendocrinology of the human cortisol system. However, recent developments in biochemical assays and progress in methodology now offer the tools to circumvent these difficulties by employing reliable and convenient measurement of cortislo in saliva.

Although the hormone concetration parallels that of the free fraction in plasma, absolute salivary cortisol levels are up to 50% lower compared to the active molecule in blood (Vining et al., 1983a; Brooks and Brooks, 1984; Meulenberg et al., 1987) . This is caused by 11β-hydroxysteroid dehydrogenase, an enzyme converting cortisol to cortisone, which is present in large amounts in saliva.

While no differences in salivary cortisol levels between sexes were found by several research groups, Laudat et al. (1988) reported on a two-fold higher concetration in unstimulated early morning samples of 23 healthy males compared to 35 women. Possibly the high interassay coefficient of variance (14%) of the assy used by this group has caused this discrepant result. In a recent large-scale study at the University of Trier, FRG, with 720 healthy adults who delivered morning, afternoon and evening saliva samples were found evidence for systemically altered unstimulated cortisol levels between sexes or different age- groups (Brandtstadter et al., unpublished data). While in men the mean salivary cortisol levels were not altered at different ages, women showed a steady decline of cortisol concetration with increasing age. Treating the group as a whole, however, the values obtained are comparable to hormone concetration in children (>0,5 years). In agreemnent with other research groups (Riad- Fahmy et al., 1982, who verified their RIA data by comparing these results with obtained by the gas chromatography-mass spectrometry analysis we found the following salivary cortisol concetration for the total group of adults.

7-9 a.m. - 14,32 (+- 9,1) nmol/l

3-5 p.m. - 4,50 (+- 3,5) nmol/l

8-10 p.m. - 1,96 (+- 1,7) nmol/l (Kirschbaum Clemens, et al., 1989)

SALIVARY CORTISOL AND STRESS

It has been shown for a long time that the HPA axis can respond sensitively to external stimulation. A variety of agents and treatments (called stressors) are able to override the feedback systems, leading to enhanced frequency and amplitude of cortisol pulses. Multiple approaches have been employed to elucidate the nature of provoked spotaneous secretory episodes and to relate the hypersecretion of cortisol to cognition, mood and behavior shown by the individual. However, researchers often encounter ethical and methodological problems when blood sampling is required for cortisol assesment. It is suggested that many of these problems can be circumvented by measuring cortisol in saliva.

One indispensable prerequisite of any investigation of stress effects on the cortisol system is the nonreactivity of the sampling method, i.e., that the sampling procedure does not affect the cortisol values. Venipuncture can significally enhance cortisol concetrations in some of the patients investigated, most probably reflecting a psychological stress response. Thos response can either lead to a state of low reactivity of the whole HPA axis (Follenius and Brandenberger, 1986) or even mask a reaction to subsequent stimuli. Another shortcoming of plasma cortisol in stress research is the inconvenience of sampling. Besides the emotive bias of many individuals against blood drawings, medically trained personnel is always required. While in the laboratory the personnel is alwayes available, studies in a natural environment are costly. In contrast, saliva can easily be obtained by the volunteers or patients themselves. Either in the presence of the investigator or independently under various circumstances samples are provided within less that 1 min and there is no evidence for altered cortisol levels following the procedure.

Besides practical considerations, validity problems may be associated with serum/plasma cortisol measures. Since the determination of free cortisol in serum or plasma is time-consuming and demands considerable expertise in biochemical analyses, most researchers and clinicians have to be satisfied with total cortisol assessments when merely blood samples were obtained from volunteer subjects or patients. These values may be misleading under several physiological and/or pharmacological conditions. As outlined, cortisol in saliva is unaffevted in most of these cases known to systemically increase or reduse the concentration of the total hormone in plasma. Moreover, only the the unbound fraction is able to penetrate the membranes of potential target cells on which the hormone exerts its physiobiological actions. Unless the free cortisol is measured, the assessment of salivary cortisol appears to be 'the better measure of adrenal cortical function than serum cortisol' (Vining et al. 1983A, p.329). However, despite obvious advantages of the method rather few papers have been published on salivary cortisol measures under stress so far.

The acceptance of any new methodological approach to the assessmnt of hormones in a given clinical or research environment depends on the ease of sampling, the storage conditions of speciments and the reliability of available assays for analysis. Given that these criteria are satisfactorily fulfilled, the economy of the new technique has to be advantageous to the the conventional methods in use. The measurement of cortisol in saliva does meet all the criteria mentioned above compared to plasma or serum cortisol determination. Thus, in most instances adrenal activity is easier and cheaper to assess in the human if the method of salivary cortisol is employed.

Furthermore, salivary cortisol can be a sensitive measure in stress research. Primarily in short-term laboratory and field experiments cortisol in saliva may be assessed as a convenient and reliable parameter of endocrine stress responses. In studies using psychological stressors it appears to be crucial for any cortisol response that the subjects get involved and anticipate potential negative consequences of the situation. Unlike short-term stress, there are no convincing data available on chronic stress effects on cortisol in saliva (Kirschbaum Clemens et al., 1989).

OXYTOCIN

Oxytocin is a neurohypophysial peptide which is produced in the paraventricular nuclei of the hypothalamus and stored in the posterior pituitary. The molecule consists of nine amino acids linked with a disulfide bond and a semi-flexible carboxyamidated tail. A hormone once thought to be limited to female smooth muscle reproductive physiology, more current findings have determined that oxytocin also functions as a neurotransmitter may be involved in neuropsychiatric disorders, social/ sexual behavior and is important in male reproductive physiology. Oxytocin and the related neurohypophysial peptide, Arg- Vssopressin, maintain renal water and sodium balance.

Oxytocin: H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

Highly conserved across species boundaries, oxytocin- like neurohypophysial peptides are substituted primarily at residues 4 and/ or 8. In the oxytocin- like peptide, mesotocin, a common peptide found in some fishes, reptiles, amphibians, marsupials and nonmammalian tetrapods, the leucine at residue 8 is substituted for isoleucine. Acting in classical endocrine fashion, oxytocin elicits regulatory effects by binding specific cell surface receptors which in turn initiate a secondary intracellurar response cascade via a phosphoinositide signaling pathway.

(Product Manual of Oxytocin Elisa kit, 2014)

OXYTOCIN AND STRESS

The excitement over the hormone began in the 1990s when researchers discovered that breastfeeding women are calmer in the face of exercise and psychosocial stress than bottle-feeding mothers. Recently oxytocin has received increasing attention, both scientifically for its role in stress regulation, social bonding and mental health, as well as in the more popular media. It has been advertised as a universal ‘‘love hormone’’, as the remedy against loneliness, fears, partner relationship and sexual problems (Heinrichs 2003).

Oxytocin is released by the pituitary gland in response to various stressful stimuli, including pain, fear and exposure to an unfamiliar environment. Noradrenergic neurons containing peptides responsible for prolactin release seems to be related, at least in part, by releasing oxytocin in circulation during stressful situations. Experiments on mice have shown that the intense exposure to stressful conditions causes increased levels of oxytocin. This facilitates increased activation Y-Y-E by glucocorticoid release. The release of oxytocin to stressful conditions can cause disorders related to stress, such as depression and anxiety disorders.

Studies show that oxytocin administration decreased fatigue, anger and anxiety and vigour. Additionally oxytocin attenuated learning processes. The wide variety of observed effects has come

to the suggestion that oxytocin has a general effect on cortical arousal rather than a specific effect limited to a certain stage of information processing. The specific effects of central oxytocin as an underlying biological mechanism for the reduction of stress and anxiety and for positive social interactions in humans are yet to be determined (Kontoaggelos et al., 2013).

During pregnancy, oxytocin levels are elevated appear to protect pregnant from an anxiety disorder, e.g. panic attack. In lactating women, a suppression of endocrine stress responses has been observed if breast-feeding starts 30–60 min before stress exposure, depending on the kind of stressor (Altemus et al 1995, 2001; Heinrichs et al 2001, 2002). From the hypothalamus oxytocin is transported to the posterior pituitary gland, the main source of oxytocin detected in the periphery during lactation and parturition.
Stimulates for the secretion of oxytocin with the same mechanism also reach from sensory nerve endings of the myometrium and vagina. The time of life is about 3 min. Oxytocin also causes contraction of the uterus. The uterus is more sensitive to oxytocin in the last weeks of pregnancy. In puerperium, secretion of large amounts of oxytocin during breastfeeding helps the uterus with contractures caused to return to its original megethos. Oxytocin is involved in the regulation of adenohypophysial hormone releasing various particular prolactin of adenokortikotropinis (ACTH) and gonadotropins. Basic and irreplaceable role of oxytocin in human physiology considered milk production during lactation. Milk production occurs by the contraction of myoepithelial cells as a reflex to the irritation of the nipple, but other stimuli such as the baby's image, smell and sounds of prospers production. The properties of the cells also vary oxytocin during childbirth. Electrophysiological studies demonstrated the presence of a positive feedback mechanism (feedback) in the baby's exit, similar to breastfeeding.
The evidence that oxytocin impairs some memory-related tasks also, has led to suggestions that it has a role in the forgetting of delivery pain in mothers.

Recently, down regulation of oxytocin receptors has been associated with the pathophysiology of social phobia and thus explain the cognitive dysfunction accompanying these patients. Finally, the few data from studies indicate low levels of oxytocin in depressed patients (Kontoaggelos et al.,2013)

SALIVARY OXYTOCIN

Research on oxytocin and behavior has been generated mainly from animal models, as there are many challenges in the measurement of oxytocin in humans. These issues include the lack of a documented relationship between central and peripheral effects of oxytocin. Oxytocin does not easily cross the blood brain barrier; although there may be peripheral pathways whereby oxytocin indirectly affects behavior. Early literature reports inconsistent data on the metabolism of oxytocin. Studies may not have controlled for the increased metabolic clearance rate of oxytocin due to the placental increase in enzymes responsible for the degradation of oxytocin. Because of oxytocin's pulsatile release, it is difficult for plasma and urine sampling in humans to capture rapidly changing patterns of oxytocin concentration. Also, due to the interaction between oxytocin and stress response hormones, blood sampling via a single stick venipuncture can induce a stress response, which may then suppress the release or action of oxytocin. Urinary assays restrict the study population to one that is developmentally and psychologically capable of providing accurate sampling, thus excluding infants, fragile individuals, and certain pathological populations. Researchers have attempted to develop a salivary oxytocin assay; however a widely accepted and reliable method has not yet previously been validated due to the fact that the amount of oxytocin is below the detection of most assays (Carter et al., 2007, Horvat- Gordon et al., 2005). Due to the potential influence of oxytocin on behavior, mental health, memory, childbearing, child development and metabolism (Blanks & Thornton, 2003, Carter et al. 2006, Francis et al., 20012, Uvnas-Moberg et al., 2005), a noninvasive measure is critical to advancing our understanding of factors regulating oxytocin levels under naturalistic conditions.

Although oxytocin is believed to affect cortisol via ACTH, an adrenal site of oxytocin action has also been detected. Thus oxytocin is involved at not just one site in the integrated modulation of cortisol concentrations. The physiological modulation of stress is thus a complex network of endocrine control mechanisms, involving several hormones with oxytocin, one of the regulating hormones, being active at at least two sites. It is possible that this very complexity is a safeguard against an inappropriate adrenal response to a rise in oxytocin which was unrelated to a stress stimulus.

The study of Heinrichs et al. (2003) reveals effects of social support and oxytocin on endocrine, mood, and anxiety responses to psychosocial stress in humans. Participants who received both protective factors of social support and oxytocin exhibited the lowest cortisol concentrations during stress exposure, whereas subjects who received no social support
and placebo demonstrated the highest cortisol response. Most notably, we found corresponding results in psychological measures, indicating that subjects without social support and with placebo showed the expected decrease in calmness and an increase in anxiety during
stress. In contrast, participants who received either social support or oxytocin or both protective factors showed increasing calmness and decreasing anxiety scores during the stress procedure. Moreover, pre- and poststress comparisons of anxiety showed an anxiolytic effect of oxytocin administration.

PART 2:
RESEARCH-
MATERIALS AND METHODS

COLLECTION AND PRESERVATION OF SAMPLES

Collection of saliva samples was during the Rhythmic Gymnastics Greek Championship held in the Olympic Village in April 2014. The study was conducted under the auspices of the Greek Federation of Gymnastics (ΕΓΟ) and the participation of the judges was voluntary. The saliva was collected in two stages: in the morning before the competition at 09:00 am The night samples were collected at the end of the competition at 10:00 pm, when the judges are under stressful conditions after full day of work and judging. In total, 17 judges were measured and answered the scientific questionnaire for assessing depression and stress, the Anxiety Inventory (Laux et al. 1981) and the Self-Rating Depression Scale (Zung 1965). Twenty eight items are rated on a 5-point scale, ranking from 1(" not at all") to 5 ("very strongly"). Factor analysis revealed three scales, termed elevated versus depressed mood, wakefulness versus sleepiness, and calmness versus restlessness.

PROCEDURE OF SALIVA COLLECTION

The judges were hungry, without eating or drinking during the last two hours. The saliva was collected in a Salivette test tube (Sarstedt, Nürnbrecht, Germany) equipped with an insert containing a steril cotton-wool swab through which the saliva passes during centrifugation, yielding a saliva free from particles. The swab was drained with saliva during 2 minutes, as recommended by the manufacturer, and was then put back into the insert and maintained in the freezer (Garde A.H.) . None of the test subjects complained of any discomfort in connection with the saliva collection.

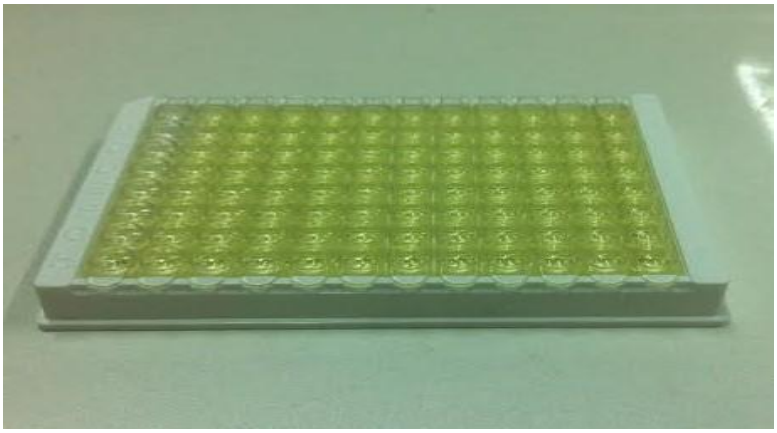
PREPARATION

After collection, samples were centrifuged at 4000rpm for 20 minutes at 4^{o} C. Each sample was collected in eppendorfs, which were kept at -200C until the day of measurement.

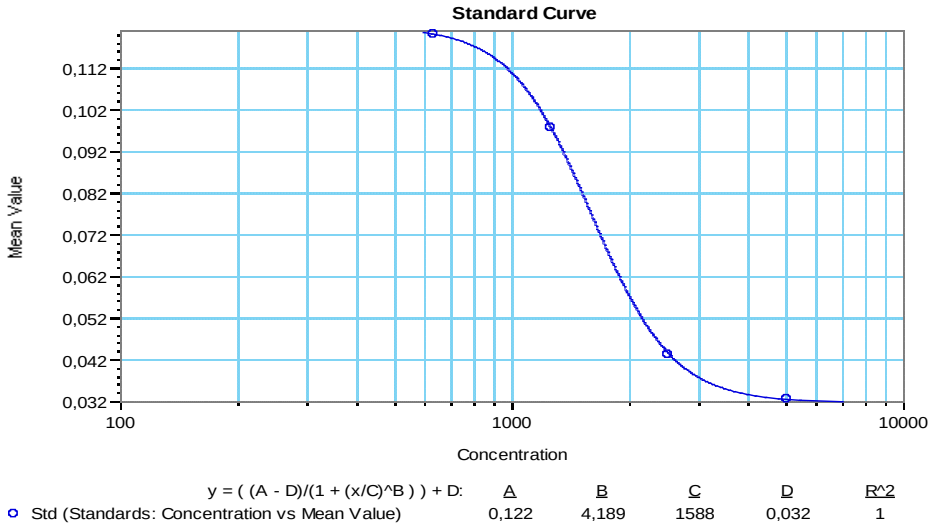
METHOD

Cortisol and oxytocin levels were measured via a competitive immunoassay (ELISA) using the ELISA kit from Enzo Life Sciences. The chemical validity of the EIA was assessed using tests of parallelism, accuracy, and precision. To assess parallelism of a dilution series of saliva to the standard curve, a homogenous pool of saliva samples was made, concentrated and diluted. Precision of the assay was assessed by the variability between high and low controls (inter-assay CV) and within unknown standards (intra-assay CV). Prior to assay, saliva samples were concentrated fourfold (based on the parallelism measurements).

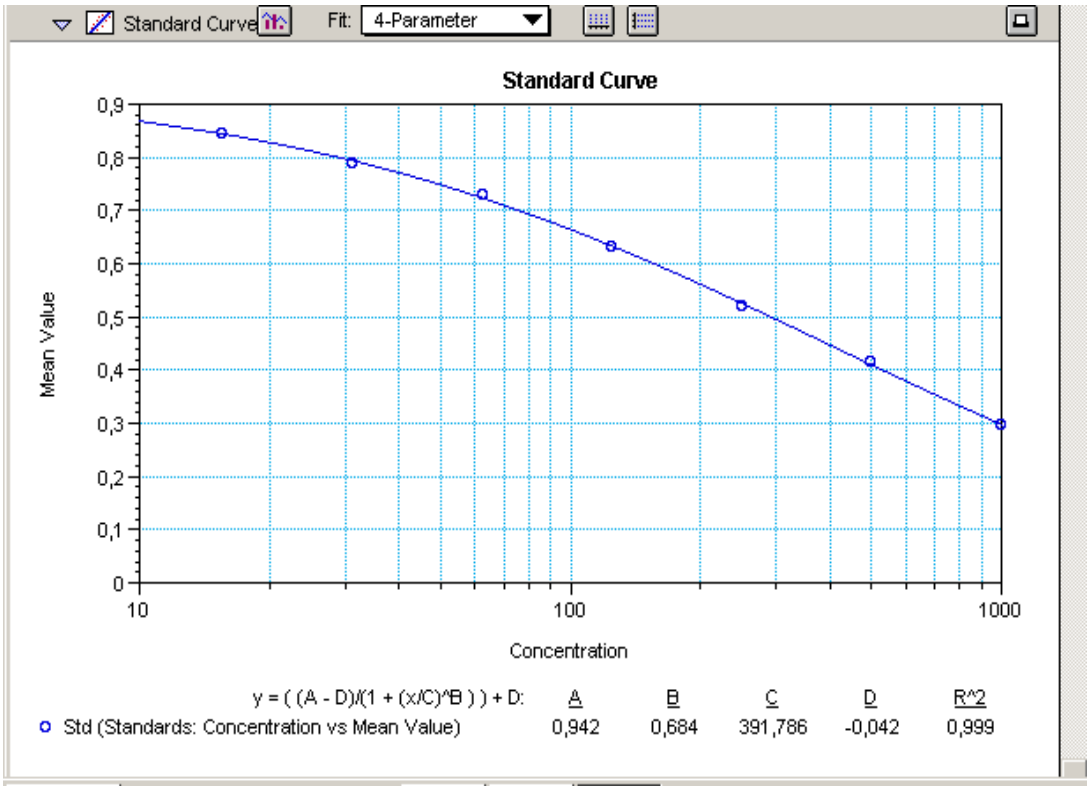
The Cortisol EIA kit is a colorimetric competitive enzyme immunoassay kit with results in 3 hours. It uses a monoclonal antibody to cortisol to bind, in a competitive manner, cortisol in a sample or an alkaline phosphatase molecule which has cortisol covalently attached to it. Absorbance is read at 405 nm. This kit is highly cited in peer-reviewed publications. The ready-to-use liquid color-coded reagents provided are safe, non-radioactive, and reduce error in the lab. The broad dynamic range lets you accurately measure Cortisol levels in a variety of sample matrices.



For the linearity of the typical standard curve, during the sample recoveries, the samples were diluted 4 times, as the manual of the kit recommends for human saliva, and then assayed in the kit. The cross reactivity of cortisol, by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concetrations from 100,000 to 10 pg/ml, was 100%. The precision of numbers is calculated by a 4 parameter logistic curve fitting program.



The Oxytocin EIA kit is a colorimetric competitive enzyme immunoassay kit with results overnight + 1 hour. The kit uses a polyclonal antibody to oxytocin to bind, in a competitive manner, the oxytocin in the standard or sample or alkaline phosphatase molecule which has covalently attached to it. Absorbance is read at 405 nm. Very low reactivity with vasopressin provides confidence in assay results. Sensitive quantification of 15pg/mL oxytocin. Ready-to-use liquid color-coded reagents reduce errors in the lab. The samples were diluted 32 times, as it is recommended for human saliva. Neuropeptide cross-reactivity was reported as < 0.02%.



STATISTICS

Descriptive statistics were conducted on the data of the judges characteristics. A paired t-test measure, parametric test for dependent samples, was conducted to examine if there was a pattern of change in cortisol and oxytocin levels during the competition and for estimating the individual profile relevant to stress for each judge seperately.

Pearson correlation coefficient (r) was used to reveal potential associations between the hormonal responses.

RESULTS

The paired t-test (for dependent samples) of the two measurements revealed differences at the ranges of the stress hormones. Figure 1 shows the decline of the cortisol levels at the end of the competition from 4,68 +- 1,29 ng/ml to 3,37+- 0,64 ng/ml (t-test: 0,0004).

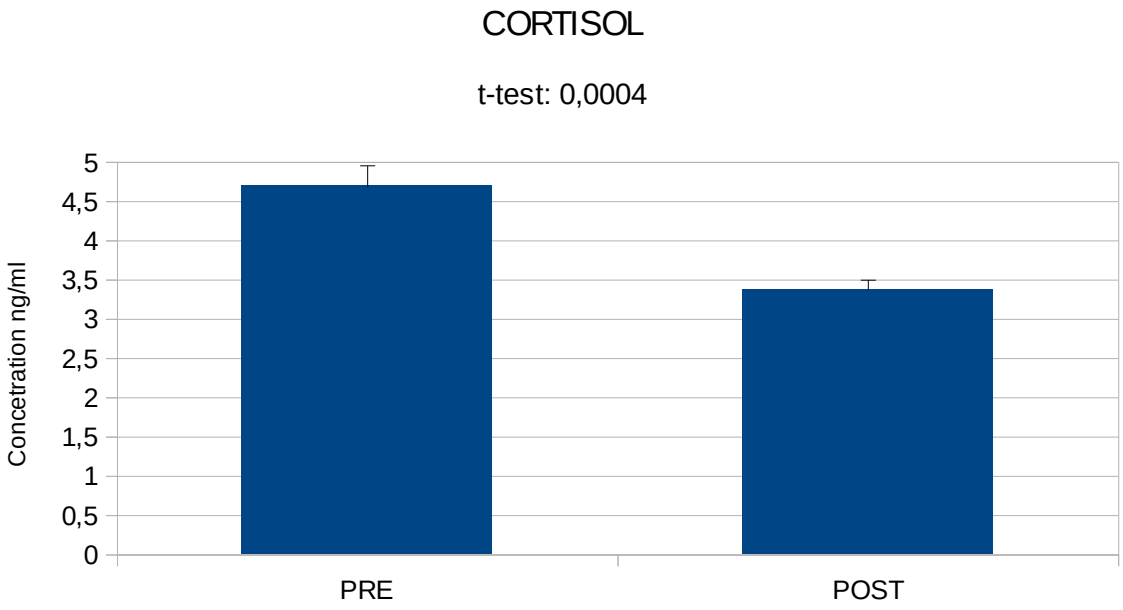
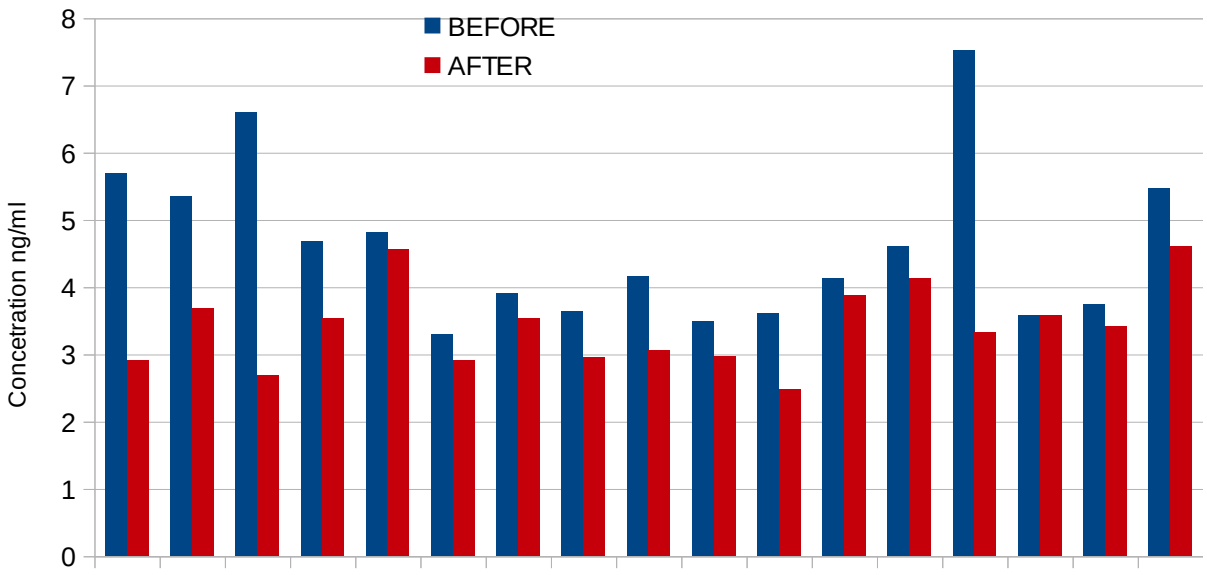


FIGURE 1: CORTISOL

Figure 2 shows seperately for each judge the difference of cortisol levels for two measurements. We can notice that the total amount of the samples had lower cortisol at the second measurement. As we analysed above, the levels of salivary cortisol during the night are signifficantly lower at night, as 70% of the total daily cortisol is externed during the day. It is interesting, however, to see how lower they were expected to be than the findings revealed due to the stressful conditions under which the judges work (analysed in the part of discussion).

FIGURE 2:
CORTISOL



The statistics for the oxytocin reveals that there is a reduction in the levels of the hormone at the second measurement, at the end of the competition. In the morning and at the beginning of the competition, the average of oxytocin was 0,74 +- 0,47 ng/ml and in the night, at the end of the competition, it was 0,51 +- 0,36 ng/ml (t-test: 0,012), as it is also shown at figure 3.

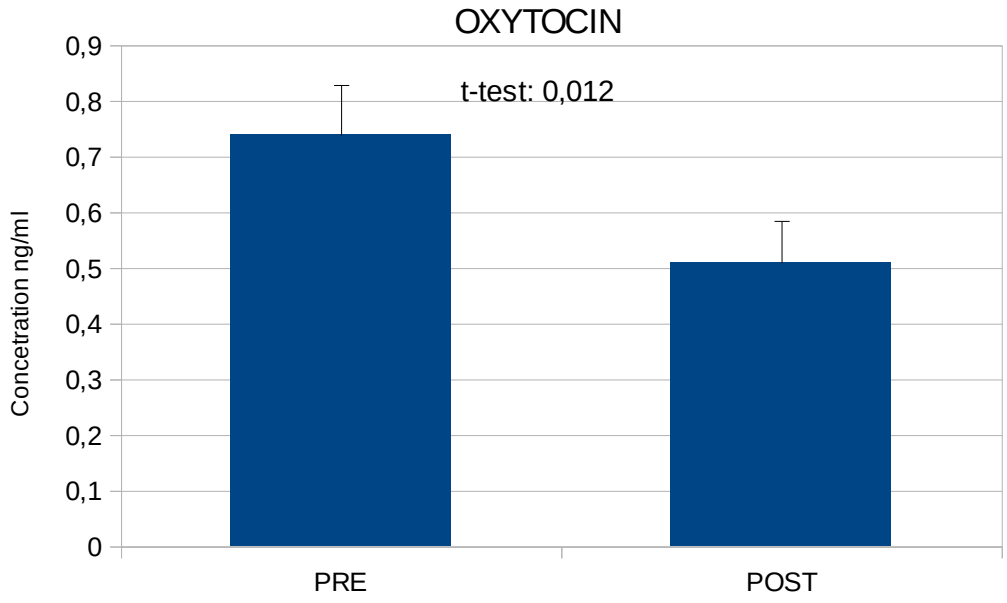
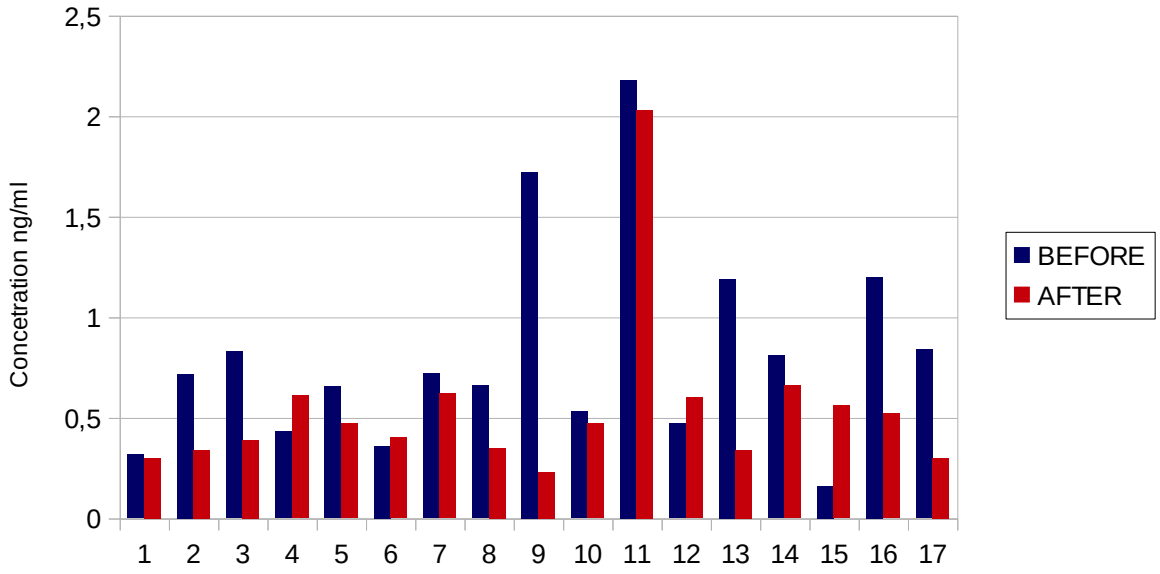


FIGURE 3: OXYTOCIN

Figure 4 shows the range of the salivary oxytocin per judge.

FIGURE 4:
OXYTOCIN



By looking at the profile of each judge seperately we can notice no signifficant statistical correlation between the fluctuation of the two hormones, according to Pearson's method. However, the percentage of declination in cortisol and oxytocin, the lines of tendency at the Scatter plot intersect, so there is a negative correlation between them.

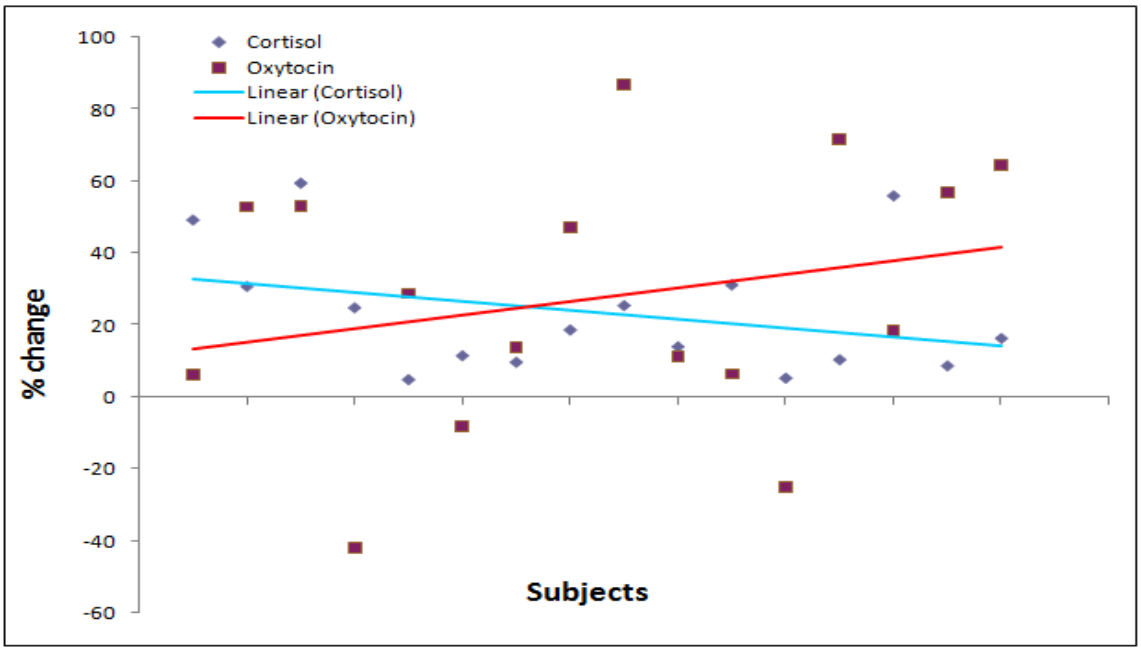
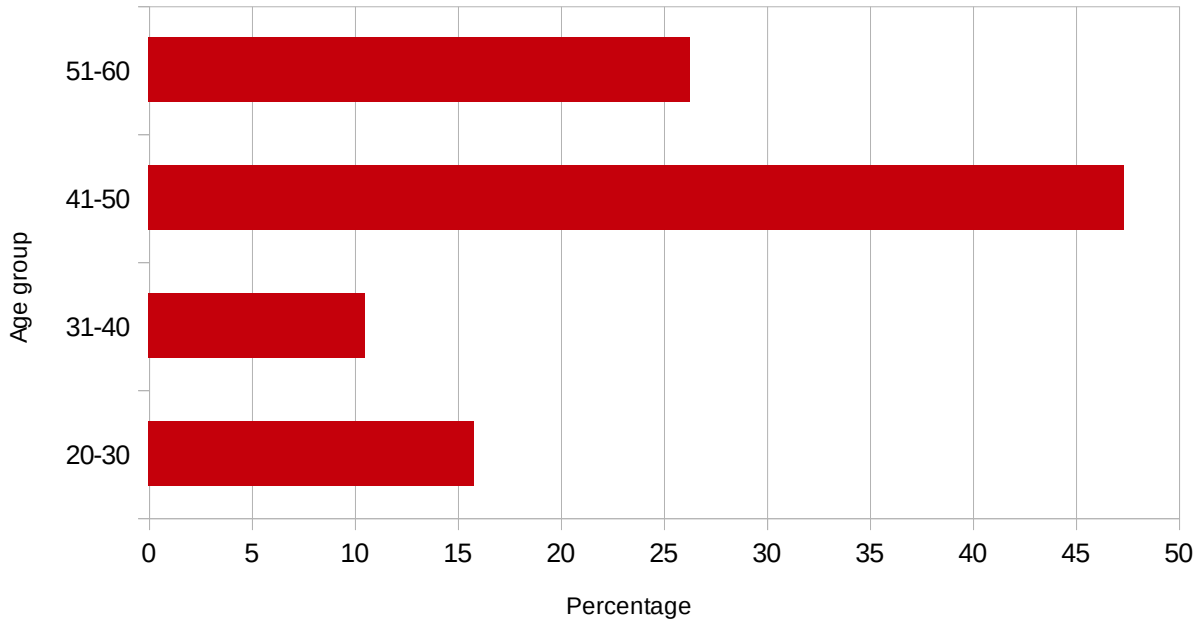


FIGURE 5: CORRELATION OF CORTISOL AND OXYTOCIN (PEARSON)

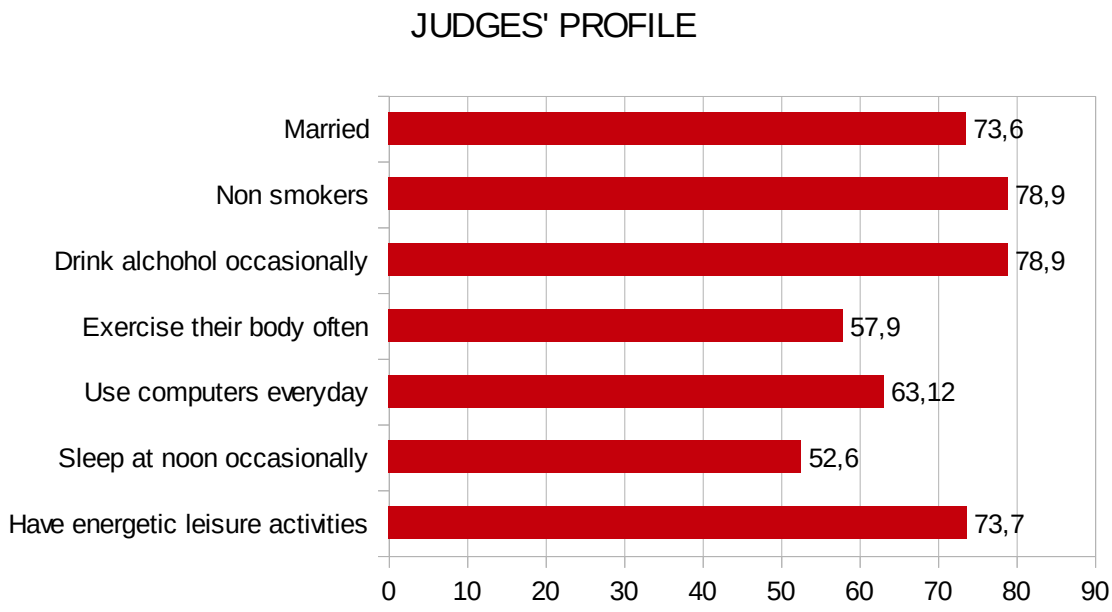
JUDGES' PROFILE

Figure 5 shows the average age group of the judges. 47,5% , almost the half of them, were 41-50 years old, 26,3% were 51-60, 15,8% were 20-30 and only 10,5 % were 31-40 years old.



Judges had no signifficant health problems that could block the conduct of the research or that could have negative effect on the results.

Figure shows the characteristics of the judges' profile that are in normal average ranges.



The counting of the judges' options in the questionnaires, that they filled in, about their personal wellness shows that the average jury has physiological responses under stressful conditions without showing pathological anxiety, fear or nerve reactions. In fact, the average score for the judges at the Anxiety Inventory (Laux et al. 1981) was > 70(the 100th percentile).

In relation to personality typing questionnaire, the highest percentage of responses shows personalities consistent, with patience, industriousness and ambitions.

DISCUSSION

The mean salivary cortisol concetrations determined with the Elisa kit, 4,7 ng/ml at 09:00 a.m. and 3,37 ng/ml at 23:00 p.m. , may be compared with values of other researchs measuring the cortisol in morning and in night hours.

Because the results are lower than expected we can say that we have a mixed result in the response of cortisol from the influence of the biorhythm (time during day) and because of the hormone response. That means that the second measurement shows decline due to the time of the measurement but the percentage of decreasion is lower than the normal rate for the cortisol in nigth under normal conditions. For this reason, we did an approach to results based on proven research on cortisol without giving scientific interpretation. As Kontoaggelos study found, at the first 8 hours of the morning it it's secreted 70% of the total amount of cortisol produced the whole 24 hours of the day. Therefore, it is important to mention that the concetration of the second measurement it is higher that the expected. Actually, if 70% of the 4,7 ng/ml of cortisol is externed during the day, the expected concentration would be at about 1,41 ng/ml. This means that 3,37 ng/ml that our results reveal, is higher than the expected, probably because of the intensive stress and the workload that the scoring system of rhythmic gymnastics requires for the judges. Of course, it is a point that requires investigation in other competitions, too, and in a bigger number of judges. Worth noting, that the original design of the survey included another measurement the same hours (like those two measurements) under normal circumstances and without the stress of the race. This measurement is eventually not reached due to technical difficulties. And, also, we could not had taken the measurements different hours, as the procedures in every competition in rhythmic gymnastics are stable. That means,that all the competitions start early in the morning and finish late in the night, when the whole number of athletes has been presented.

The largest percentage of the judges shows normal fluctuation in the cortisol concetration in both measurements, something that gives the result that the samples are normal with no serious health problems, resulted also by the psychological questionnaires that the judges filled in.

The decline of the mean oxytocin concetrations from 0,74 ng/ ml to 0,51ng/ml reveal that the tasks that the judges deal with, full them temporarily with anxiety and stress, preventing them to express positive emotions that oxytocin ideally provokes.

However, it is sure that they don' t have fears and anxiety about something really vital, because in this case, also, the concetration of oxytocin would be elevated. According to the bibliography, oxytocin has dual roles and as a result it can be analysed by two different meanings. Except for the feelings of love and the positive role of oxytocin which is secreted during pregnancy and when

women breastfead, it can also be secreted in cases with extreme danger, that provokes fear, trying to sign pathways to deal with the stress that shocked the human organism.
Finally, we analysed the correlation between the two hormones for each judge seperately by the Pearson's method and the responses of the participants in terms of correlation of both hormones indicates a mild nonsignificant negative correlation, as we expected. As one hormone is affected by the other, when the concentration of one rises, the concentration of the other decreases. That can be based on the role that each hormone works and the effect that has in human. Under stressful conditions, cortisol is expected to be elevated, whereas oxytocin to be decreased and exactly the oposite under norman conditions without any stressful stimuli.

REFERENCES

Aardal Elizabeth and Aan- Charlotte Holm. Cortisol in Saliva- Reference ranges and relation to cortisol in serum, Eur J Clin Chem Clin Biochem, 1995; 33:927-932

Boen Filip et al., Open feedback in gymnastic judging causes conformity bias based on informational influencing, Journal of Sports Sciences, volume 26, issue 6, 2008, pages 621-628

Evans J John, Oxytocin in the human- regulation of derivations, European Journal of Endocrinology (1997) , 137 559-571

Fernandez- Villarino Maria A. et al., Practical Skills of Rhythmic Gymnastics judges, Journal of Human Kinetics, volume 39/2013, 243-249, Section IV- Behavioural Sciences in Sport

Flessas Konstantinos et al. , Judging the Judges' Performance in Rhythmic Gymnastics, American College of Sports Medicine 2015, unpublished data

Garde A.H. & A°, M. Hansen, Long-term stability of cortisol, Scand J Clin Lab Invest 2005; 65: 433–436

Heinen Thomas, Pia M. Vinken & Konstantinos Velentzas, Judging performance in gymnastics: A matter of motor or vusual experience? , Science of gymnastics journal, Vol. 4 Issue 1: 63 – 72

Heinrichs Markus, Thomas Baumgartner, Clemens Kirschbaum and Ulrike Ehlert: Social support and oxytocin interact to supress cortisol and subjective responses to psychosocial stress, Society of Biological Psychiatry,2003

Karpenko L.A. Et al., Rhythmi Gymnastics, Federation of Rhythmic Gymnastics of Russia, edited greek version by Tsopani D., Publications " Telethrion", 2007

Kirschbaum Clemens, Dirk H. Heillhammer: Salivary Cortisol in Psychobiological Research: An Overview in Neuropsycobiology 1989;22:150-169

Kontoaggelos K. , Papageorgiou X. et al., The role of oxytocin , cortisol , homocysteine, cytokines

in diabetes mellitus and their association with psychological factors, Athens Medical Society (2013)

Leskošek B., Čuk I., Pajek J., Forbes W., Bučar-Pajek M. , Bias of Judging in men's artistic gymnastics at the european championship 2011, Biology of Sport, Vol. 29 No2, 2012 , 107-113

Nevill A.M. et al., The influence of crowd noise and experience upon refereeing decisions in football, Psychology of Sport and Exercise 3 (2002) 261-272

Perederij V. V. The problem of the quality of judging in rhythmic gymnastics. Pedagogics, psycology, medical- biological problems of physical training and sports, 2013, vol 3, pp. 43-46

Polykarpou G., Assays parameters of stress (hormones and cytocines) in saliva of athletes in high level, University of Patra, Study in Clinical Pharmacology, 2007

Product Manual of Oxytocin Elisa kit, Enzo Life Sciences, Catalog#: ADI-900-153A (rev. 2014)

Product Manual of Cortisol Elisa kit, Enzo Life Sciences, Catalog#: ADI-900-071 (rev. 2014)

Randall Michael et al., The Physiology of Stress: Cortisol and the Hypothalamic- Pituitary-Adrenal Axis, Dartmouth Undergraduate Journal of Science, 2011

Ste-marie Diane M., Expertise in women's gymnastic judging: an observational approach, Perceptual and Motor Skills: Volume 90, Issue , pp. 543-546

Ste- Marie Diane M. et al.,Prior processing effects on gymnastic judging, Journal of Experimental Psychology: Learning, Memory, and Cognition, vol 17 (1), Jan 1991, 126-136

White-Traut R., Detection of salivary oxytocin levels in lactating women, Dev Psychobiol., May 2009, 51(4) : 367-373