Corvillo, M.; Timón, R.; Maynar, M.; Brazo-Sayavera, J. y Maynar, J.I. (2013). Excreción urinaria de hormonas esteroideas tras un partido de balonmano femenino / Urinary excretion of steroid hormones after a female handball match. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 13 (52) pp. 737-747. Http://cdeporte.rediris.es/revista/revista52/artexcreccion412.htm

ORIGINAL

URINARY EXCRETION OF STEROID HORMONES AFTER A FEMALE HANDBALL MATCH

EXCRECIÓN URINARIA DE HORMONAS ESTEROIDEAS TRAS UN PARTIDO DE BALONMANO FEMENINO

Corvillo, M.[1]; Timón, R.[2]; Maynar, M.[1]; Brazo-Sayavera, J.[2] & Maynar, J.I.[3]

FIQASAC (Physiology, Analytical Chemistry and Community Health) Research Group. University of Extremadura. Spain.
[(1)]Departament of Physiology. manucorvillo9@hotmail.com; mmaynar@unex.es
[(2)]Department of Musical and Artistic Expression and Body Language. rtimon@unex.es; jbsayavera@unex.es
[(3)]Department of Analytical Chemistry. jimaynar@unex.es

Spanish-English translators: Nina Terry, scrimster@hotmail.com, English School from Villafranca de los Barros (Badajoz – Spain).

Código UNESCO / UNESCO Code: 2411.06. Fisiología del ejercicio / Exercise Physiology.
Clasificación del Consejo de Europa / Council of Europe Classification: 6. Fisiología del ejercicio / Exercise Physiology

Recibido 25 de julio de 2011 **Received** July 25, 2011
Aceptado 18 de enero de 2013 **Accepted** January 18, 2013

ABSTRACT

Performing high-intensity physical exercise constitutes an important stressor which produces important alterations in hormonal metabolism. The aim of this study was to assess the acute effect of a competitive handball match on the urinary excretion of steroid hormones in young female players. To that end, the urinary profiles of the free and glucuroconjugated steroid hormone fractions in a group of 19 players (18.47 ± 2.26 years old), belonging to several teams in the Regional Junior-Senior Extremaduran League, were determined both before and after the same match. In order to determine and quantify the steroids, a gas chromatography-mass spectrometry technique (GC/MS) was used. On the one

hand, the results obtained after the match showed a significant increase in cortisone, tetrahydrocortisol and total glucocorticoids concentrations. On the other hand, when the ratio of the total amount of anabolic hormones to the total amount of catabolic hormones was analysed, an important decrease was also observed. We can therefore conclude that both acute physical exertion and physcological and emotional stress induced by the competitive handball match, were objectively reflected in an immediate alteration of the urinary steroid profile, a decrease in their anabolic state and an increase in their catabolic state.

KEY WORDS: Androgens, Glucocorticoids, Stress, Women, Handball

RESUMEN

La realización de ejercicio físico de alta intensidad es un importante agente estresante y va a provocar importantes variaciones del metabolismo hormonal. El siguiente estudio trató de evaluar el estrés agudo que un partido de competición de balonmano tuvo sobre la excreción urinaria de hormonas esteroideas de jóvenes jugadoras de balonmano. Para ello, se determinó el perfil urinario de la fracción libre y glucuroconjugada de las hormonas esteroideas en un grupo de 19 jugadoras (18,47 ± 2,26 años de edad), de diferentes equipos de la Liga Regional Juvenil-Senior de Extremadura, antes de un partido y después del mismo. Para la determinación y cuantificación de los esteroides se utilizó la técnica de cromatografía de gases acoplada a un espectrómetro de masas (GC/MS). En los resultados obtenidos tras el partido se observó un incremento significativo de las concentraciones de cortisona, tetrahidrocortisol y de los glucocorticoides totales. Por otro lado, al analizar el cociente entre el total de hormonas anabólicas y el total de hormonas catabólicas, también se detectó un descenso importante de dicho cociente. Por tanto, se puede concluir que el esfuerzo físico agudo y el estrés psicológico y emocional que supuso un partido de balonmano de competición, quedó reflejado objetivamente a través de una alteración inmediata del perfil esteroideo urinario, disminuyendo el estatus anabólico y aumentando el estatus catabólico.

PALABRAS CLAVE: Andrógenos, Glucocorticoides, Estrés, Mujer, Balonmano

INTRODUCTION

A vast number of scientific studies have demonstrated that physical exercise constitutes a form of stress, not only physiological and biochemical stress, but also physcological stress (Guezennec et al., 1995; Furuya et al. , 1998). Therefore, physical activity produces a change in the regulation and activation of the hypothalamic-pituitary-testicular axis in men (Goldstein and Kopin, 2007; Timon et al. , 2007) and in the hypothalamic-pituitary-ovarian axis in women

(Warren and Shantha, 2000; Van Eenoo et al. , 2001), which in turn alters hormone production. (Zhou et al. , 2000; Bosco et al. , 2000; Hakkinen et al. , 2000). This alteration of hormone production is especially relevant in the group of steroid hormones, since stress caused by physical exercise can alter the pituitary synthesis of gonadotropins (FSH and LH) and adrencorticotrophic hormone (ACTH), leading to an hormonal imbalance (Warren and Shantha 2000; Traustadottir et al. , 2004).

On the one hand, glucocorticoid levels increase acutely in response to any form of stress which poses a threat to corporal homeostasis. It is widely accepted that the secretion of glucorticoids is a classic endrocrine response to stress situations (Sapolsky et al. , 2000), and many authors consider tetrahydrocortisol and tetrahydrocortisone, the main urinary metabolites of cortisol and cortisone respectively, as indicators of cellular catabolism, the rate of 'wear and tear' caused by stress and the activation of the pituitary-adrenal axis (Nishikaze and Furuya, 1998; Kano et al., 2001; Timon et al., 2007). On the other hand, androgens (testosterone, DHEA and androstenedione) clearly promote anabolism , and these hormones are considered as indicators of 'repair and recovery' from 'wear and tear' caused by stress situations (Nishikaze, 1993; Nishikaze and Furuya, 2000; Kano et al. , 2001). Likewise, acute physical stress has been associated with decreases in urinary levels of androsterone and etiocholanolone, the main testosterone metabolites (Timon et al. , 2008).

Handball is predominantly an aerobic sport which involves explosive-type movements which rely on anaerobic metabolism. At the same time, practising this sport also requires great agility and many conditional factors, such as explosive strength and speed of movement and quick reactions. All of these physical requirements, along with other specific factors in sports competitions, such as the attitude of the spectators, the intervention of the referees, the players' image in front of their friends and relatives, may create physical, psychological and emotional tension in the players, which has a decisive influence on physiological and biochemical parameters.

Hence, the aim of this study is to assess the acute effect of a handball match on the urinary steroid hormone profile of young female handball players.

MATERIAL AND METHODS

Subjects

The participants of this study were 19 female players (18.47 ± 2.26 Age yr; 1.64 ± 0.01 Height cm; 58.96 ± 2.75 Weight kg) belonging to 3 of the 4 teams which played the semifinals of the 'Extremaduran Cup' female handball competition in the Junior-Senior category. Prior to the study, we collected information about the players' menstrual cycles and their general health, and we asked them if they were taking any medicines. Those players who did not meet the eligibility

requirements were excluded from the study. The requirements were: to have regular periods, to have played more than 30 minutes of the match, not to have any illnesses or diseases and not to have taken any hormonal medicines (oral, vaginal or dermal contraceptives) in the 6 months prior to the competition. 12 of the subjects were in the follicular phase of their menstrual cycles and 7 of them were in the luteal phase. This information was obtained from a personal interview based on a direct and simple survey carried out by the members of the research group and designed by medical staff for this very purpose. The nature of the study was explained to them and they were informed of the procedures to be followed.They voluntarily agreed to be the subjects after reading and signing an informed consent form (in the case of minors it was signed by their parents or legal tutors). Each participant was assigned a code in order to protect the anonymity and confidentiality of the research subjects. Once the study had been terminated, the samples were destroyed in order to guarantee that there would be no further use of them.

The analysis of the urine samples

Urine samples were taken from all the subjects before the warm-up prior to the match (initial sample) and 5 minutes after the match had finished (final sample). The handball matches were played between 5pm and 8pm. The samples were then taken to the laboratory where they were frozen at – 20° C in order to prevent deterioration before treating and analysing them. Interpretation of the analytical data of the urine assays requires the sample density to be in the range of 1,005-1,025 g/ml, and the pH to be in the normal range of 4.7-7.8. All the study samples fulfilled these conditions. Likewise, the level of creatinine in all the samples was determined to assign values to steroid concentration in relation to this fundamental urinary parameter, using the Haeckel technique (1981). This parameter is used to determine the degree of urinary concentration, eliminating the possible variations caused by the dilution of the sample (Maskarinec et al., 2005).

The analysis of urinary androgens and estrogens was performed by gas chromatography-mass spectrometry in accordance with Galán et al. (2001) and the analysis of glucorticosteroids in accordance with Rivero et al. (2001). The devices used for androgens were an HP-5890 Series II gas chromatograph equipped with a simple quadruple HP-5972 mass spectrometer (GC/MS system, SIM mode), and a Varian 3800 gas chromatograph coupled to a Saturn 2000 mass–mass (ion-trap) spectrometer (GC/MS/MS system) for glucorticosteroids. The conditions for the analysis of androgens were: carrier gas He N-50, flow-rate 1 ml/min, split 40, temperature 280°C at the detector and 280°C at the injector. The column was a HP-1 (Crosslinked Methyl Silicone Gum) (25m X 0.2 mm X 0.33 µm). The oven temperature program was: initial, 120°C for 2 min; 1st ramp, 20°C/min–200°C; 0 min; 2nd ramp, 5°C/min–240°C, 5 min; 3rd ramp, 30°C/min–300°C, 5min. The detector temperatures were: trap, 200°C; manifold, 50°C; and transfer line 280°C. The detector emission current was 70, and the mass range was from 100 to 700. The conditions for the

analysis of glucorticosteroids were: carrier gas He N-50, flow-rate 1 ml/min, split 40, temperature 280°C at the detector and 280°C at the injector. The column was a Tacer (TRB-1 stationary phase) (25m X 0.25mm X 0.33 µm). The oven temperature program was: initial, 120°C for 2 min; 1st ramp, 20°C/min– 240°C, 7 min; 2nd ramp, 20°C/min–300°C, 5 min. The detector temperatures were: trap, 200°C; manifold, 50°C; and transfer line 280°C. The mass range was from 150 to 650.

The steroid hormones examined were: testosterone, androstenedione, DHEA, androsterone and etiocholanolone (the main metabolites of testosterone) cortisol, cortisone, tetrahydrocortisol and tetrahydrocortisone (the main metabolites of cortisol and cortisone respectively). Two ratios were also examined to assess the anabolic/catabolic state of the female players after the match: testosterone/cortisol and androgens total sum /glucocorticoids total sum.

Table 1 shows the retention times and characteristics of each of the hormones studied.

Table 1. Hormones with their retention times and characteristic ions.

Hormone	***Retention time (min)***	***Ions***
Testosterone	21.949	432,417
Androstenodione	21.743	415,430
DHEA	21.090	417,432,327
Androsterone	20.398	419,434,329,239
Etiocholanolone	20.543	434,419,329
Cortisol	29.897	632,637
Cortisone	28.401	630,616
Tetrahydrocortisol	27.092	637
Tetrahydrocortisone	26.419	635,530,442

The quantative analysis of the samples was carried out using calibration curves of each hormone and the internal standard method. First, standard solutions that had a concentration of 20mg/l were made up. The necessary quantity was taken from these standard solutions so that in 2 ml of synthetic urine there would be a desired steroid concentration of 10, 20, 40, 100, 200, 400 ng/ml. The procedure consisted in adding a known quantity of internal standard to the test sample and calculating the analyte/internal standard area ratio. The resulting ratio was plotted on each of the calibration curves (Table 2).

Table 2. Calibration curves of the steroid hormones studied with their corresponding coefficient of determination.

Hormone	*Equation*	R^2
Testosterone	y=1.2127x-0.0001	0.99
Androstenodione	y=0.8864x-0.0024	0.99
DHEA	y=0.8875x-0.0078	0.99
Androsterone	y= 0.0312x+0.0014	0.99
Etiocholanolone	y=1.9642x+0.0248	0.99
Cortisol	y=0.2648x-0.0647	0.99
Cortisone	y=0.1894x-0.0364	0.99
Tetrahydrocortisol	y=0.1716x-0.0245	0.99
Tetrahydrocortisone	y=0.0090x-0.0012	0.99

In order to carry out the validation of the method, the limits of detection and limits of quantifications (Table 3) were established according to the following calculations. The limit of detection can be defined according to the "3s" criterion, which states that the limit of detection (LOD) is the analyte concentration which provides the net signal which equals 3 times the standard deviation of the target signal (S_b).

$$LOD = (y_c - y_b) / b = (3s_b) / b$$

Where: S_b is the standard deviation of blank, b the calibration curve slope, y_c the critical value of the brute signal and y_b the average of the target signals.

On the other hand, the limits of quantification (LOQ) were obtained by the following formula:

$$LOQ = (10s_b) / b$$

Table 3. Limit of detection and limit of quantification for each of the hormones studied.

Hormone	*LOD* (ng/ml)	*LOQ* (ng/ml)
Testosterone	0.40	1.33
Androstenodione	0.54	1.81
DHEA	0.54	1.81
Androsterone	15.46	51.54
Etiocholanolone	0.25	0.82
Cortisol	1.82	6.07
Cortisone	2.55	8.49
Tetrahydrocortisol	2.81	9.37
Tetrahydrocortisone	53.60	178.68

Statistical analysis

The SPSS 17.0 program for Windows was used for the statistical analysis of the variables. We analysed the normality of distribution of the variables using the

Shapiro-Wilk test and the homogeneity of variance with the Levene test. Once these requisites had been checked, we applied the generalized linear model for repeated measures to check the differences between the results obtained from before and after the handball match. A 95% significance level was required in all cases. The results are expressed as mean ± standard deviation.

RESULTS

Table 4 shows the urinary concentrations of androgenic hormones. No significant differences between the results obtained from before and after the match were observed.

Table 4. Urinary androgen concentrations (ng steroid per mg creatinine). Results are expressed as mean ± standard deviation.

Hormone	*Initial*	*Final*	*Significance level*
Testosterone	24.11 ± 8.57	25.5 ± 7.85	NS
Androstendione	1.66 ± 0.52	1.48 ± 0.7	NS
DHEA	24.99 ± 8.24	19.93 ± 11.63	NS
Androsterone	1006.88 ± 681.04	994.41 ± 777.41	NS
Etiocholanolone	975.41 ± 295.78	933.23 ± 574.02	NS
Total Androgens	2085.31 ± 875.14	1982.33 ± 1038.13	NS

NS Differences not statistically significant.

** Statistically significant (p<0,05).*

Table 5 shows the glucocorticoid values. There are very significant increases (p<0,01) in urinary cortisone, tetrahydrocortisol and total glucocorticoids concentrations. The changes in cortisol and tetrahydrocortisone concentrations were not significant.

Table 5. Urinary glucocorticoids concentrations (ng of steroid/mg of creatinine). Results are expressed as mean ± standard deviation.

Hormone	*Initial*	*Final*	*Significance level*
Cortisol	62.59 ± 33.55	100.01 ± 48.15	NS
Cortisone	51.27 ± 33.69	119.37 ± 60.98	**
Tetrahydrocortisol	953.58 ± 600.38	1841.93 ± 915.11	**
Tetrahydrocortisone	813.03 ± 1025.9	1457.41± 1176.28	NS
Total glucocorticoids	1922.65 ± 737.88	3394.91 ± 1366.78	**

NS Differences not statistically significant.

** Statistically significant (p<0,05).*

Table 6 shows the variations in the anabolic/catabolic ratios after the match. There is a significant decrease (p<0, 05) in the total androgens/total glucocorticoids ratio.

Table 6. Variations in the anabolic/catabolic hormones ratio (steroid ng per creatinine mg). Results are expressed as mean ± standard deviation.

Hormone	*Initial*	*Final*	*Significance level*
Testosterone/Cortisol	0.44 ± 0.32	0.33 ± 0.25	NS
Total Androgens/ Total Glucocorticoids	1.07 ± 0.83	0.58 ± 0.42	*

NS Differences not statistically significant.

** Statistically significant (p<0,05).*

DISCUSSION

With respect to androgens, no significant differences in their urinary concentrations between before and after the match were observed, and for this very reason, not much research is done on androgenic response in women who do physical exercise. Bricout et al. (2003) conclude that doing regular physical exercise could play an important role in androgenic metabolism in women, nevertheless, there is no consensus with respect to androgenic changes caused by acute effort. On the one hand, increases in testosterone and plasmatic DHEA have been observed in women after doing intense exercise (Cumming and Rebar, 1985; Keizer et al., 1987). On the other hand, there are studies which do not find any significant changes in androgenic levels in woman after finishing a basketball match (Filaire and Lac, 2000). Hence, some research studies say that androgenic response to effort in women depends on exercise type and intensity (Tsai et al. , 2001), in such a way that exercise which is predominantly aerobic, such as handball, will not lead to significant changes (Zhou et al. , 2000; Filaire and Lac, 2000). It is important to state that the variations which occur in urinary androgen concentrations in women as a consequence of physical exercise are independent of the phase of the menstrual cycle in which they are, as it has been shown that these urinary levels do not present significant changes throughout the whole menstrual cycle (Burger, 2002; Bricout et al. , 2003).

With respect to glucocorticoids, a general increase in all of them was observed, above all in cortisone, tetrahydrocortisol and total glucocorticoids. Cortisol and cortisone are steroid hormones used to assess stress situations (Loucks and Horvath, 1984; Daly et al., 2005), as are their metabolites (tetrahydrocortisol and tetrahydrocortisone) (Nishikaze and Furuya, 1998; Timon et al., 2008). Therefore, this increase in glucocorticoids shows an increase in catabolic metabolism. As a result, it is widely accepted that the secretion of glucocorticoids is a classic endocrine response to fatigue and competiton (Sapolsky et al., 2000; Daly et al., 2005), and there are many studies which have been carried out on women which show increases in cortisol and cortisone after doing intense exercise, such as marathons (Hale et al., 1983), cycling (Bouget et al., 2006) and even handball (Filaire et al., 1996).

Finally, in order to assess the sportswomen's fatigue level two different ratios

have been used. The androgen/glucocorticoid ratio may indicate the subject's anabolic/catabolic state. (Fischer et al., 1992). The testosterone/cortisol (T/C) ratio is one of the most common ratios used for fatigue assessment. Nevertheless, we must bear in mind that this ratio is used above all in studies on men (Madelenat et al., 1997; Shammin et al., 2001), since levels of plasmatic testosterone in women are lower than in men, and besides, a large amount of female androgens are produced in the suprarenal gland (DHEA and androstendione), so using this ratio in studies on women could lead to biased results. This fact is clear when we observe that the total androgens/total glucocorticoids ratio was sensitive to fatigue induced by the handball match, and in this case, a significant decrease did occur.

CONCLUSIONS

To sum up, acute physical exercise and, possibly, physcological and emotional effort, induced by the match, was reflected objectively in an immediate alteration in the steroid profile. An increase in the levels of glucocorticoids and a decrease in the total androgens/total glucocorticoids ratio occurred. These variations in the urinary steroid profile as a consequence of competiton should be taken into account by national anti-doping organisations and by sports federations which monitor sportspeople's urinary steroid profiles, in order to prevent errors being made in anti-doping analysis.

REFERENCES

Bosco C, Lacovelli M, Tsarpela O, Cardinale M, Bonifazi M, Tihanyi J, Viru M, De Lorenzo A, & Viru A. (2000). Hormonal responses to whole-body vibration in men. Eur J Appl Physiol 81 (6):449-454.

Bouget M, Rouveix M, Michaux O, Pequignot JM, Filaire E (2006). Relationships among training stress, mood and dehydroepiandrosterone sulphate/cortisol ratio in female cyclists. J Sports Sci. 24(12):1297-1302

Bricout VA, Wright F, Lagoguey M. (2003). Urinary profile of androgen metabolites in a population of sportswomen during the menstrual cycle. Int J Sports Med 24:197-202.

Burger HG. (2002). Androgen production in women. Fertil Steril 77(4):3-5.

Cumming D & Rebar RW (1985). Hormonal changes with acute exercise and with training in women. Semin Reprod Endocrinol. 3: 55-64

Daly W, Seegers CA, Rubin DA, Dobridge JD, Hackney AC. (2005). Relationship between stress hormones and testosterone with prolonged endurance exercise. Eur J Appl Physiol. 93(4):375-380.

Filaire E &Lac G (2000). Dehydroepiandrosterone (DHEA) rather than testosterone shows saliva androgen responses to exercise in elite female handball players. Int J Sports Med.21(1): 17-20

Filaire E, Duché P, Lac G, Robert A (1996). Saliva cortisol, physical exercise and training: influences of swimming and handball on cortisol concentrations in women. Eur J Appl Physiol Occup Physiol. 74(3):274-

278.

Fischer HG, Hartmann U, Becker R, Kommans B, Mader A, Hollmann W. (1992). The excretion of 17-ketosteroids and 17-hydroxycorticosteroids in night urine of elite rowers during altitude training. Int J Sports Med 13(1):15-20

Furuya E, Maezawa M, Nishikaze O. (1998). 17-KS sulfate as a biomarker in psychosocial stress. Rinsho Byori 46(6):529-537.

Galán AM, Mariño JI, García de Tiedra MP, Marabé JJ, Caballero Loscos MJ, & Mariño MM. (2001). Determination of nandrolone and metabolites in urine samples from sedentary persons and sportmen. J Chromatogr B Biomed Sci 761(2):229-236.

Goldstein DS & Kopin IJ. (2007). Evolution of concepts of stress. Stress 10(2):109-120.

Guezennec CY, Lafarge JP, Bricout VA, Merino D, & Serrurier B. (1995).Effect of competition stress on tests used to assess testosterone administration in athletes. Int J Sports Med 16(6):368-372.

Häkkinen K, Pakarinen A, Kraemer WJ, Newton RU, Alen M. (2000). Basal concentrations and acute responses of serum hormones and strength development during heavy resistance training in middle-aged and elderly men and women. J Gerontol A Biol Sci Med Sci 55(2):B95-105.

Haeckel R. (1981). Assay of creatinine in serum with use of fuller´s earth to remove interferents. Clin Chem 27: 179-183.

Hale RW, Kosasa T, Krieger J Pepper S. (1983). A marathon: the inmediate effect on female runners´ luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, and cortisol levels. Am J Obstet Gynecol 146(5):550-556.

Kano K, Yamada Y, Arisaka O. (2001). Urinary 17-hydroxycorticosteroids and 17-ketosteroid sulfates in normal children and in children with atopic dermatitis or renal disease. Rinsho Byori 49(8):807-812.

Keizer HA, Kuipers H, de Haan J, Janssen GME, Beckers E, Habets L (1987) Multiple hormonal responses to physical exercise in eumenorrheic trained and untrained women. Int J Sports Med. 8: 139-150.

Loucks AB, & Horvath SM. (1984). Exercise-induced stress responses of amenorrheic and eumenorrheic runners. J Clin Endocrinol Metab 59(6):1109-1120.

Madelenat P, Chuoung T, Driguez P, Belaisch J. (1997). The exercising woman: too much or not enough androgens?. Science & Sports 12(1):46-50.

Maskarinec G, Morimoto Y, Novotny R, Nordt FJ, Stanczyk FZ, Franke AA. (2005). Urinary sex steroid excretion levels during a soy intervention among young girls: a pilot study. Nutr Cancer 52(1):22-28.

Nishikaze O. (1993). Distortion of adaptation--wear & tear and repair & recovery--17-KS-sulfates and stress in humans. J UOEH 15(3):183-208.

Nishikaze O & Furuya E. (1998). Stress and anticortisols--17-ketosteroid sulfate conjugate as a biomarker in tissue repair and recovery. J UOEH. 20(4): 273-295.

Nishikaze O & Furuya E. (2000). Coping with stress in the elderly. Nippon

Ronen Igakkai Zasshi 37(1):68-73.

Rivero-Marabé JJ, Maynar-Mariño JI., García de Tiedra MP, Galán-Martín AM, Caballero-Loscos MJ, Maynar-Mariño M. (2001). Determination of natural corticosteroids in urine samples from sportsmen. J Chromatogr B Biomed Sci Appl 761(1): 77-84.

Sapolsky RM, Romero LM, Munck AU. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. Endocr Rev. 21(1):55-89.

Shamim W, Yousufuddin M, Bakhai A, Coats AJ, Honour JW. (2001). Gender differences in the urinary rates of cortisol and androgen metabolites. Ann Clin Biochem. 38(4):412.

Traustadottir T, Bosch, Cantu T, Matt K. (2004). Hypothalamic-pituitary-adrenal axis response and recovery from high-intensity exercise in women: effects or aging and fitness. J Clin Endocrinol Metab 89 (7):3248-3254.

Timon R, Maynar M, Munoz D, Olcina GJ, Caballero MJ, Maynar, JI. (2007). Variations in urine excretion of steroid hormones after an acute session and after a 4-week programme of strength training. Eur J Appl Physiol 99:65-71.

Timon R, Olcina G, Muñoz D, Maynar JI, Caballero MJ, Maynar M. (2008): Determination of urine steroid profile in untrained men to evaluate recovery after a strength training session. J Strength Cond Res. 22(4):1087-1093.

Tsai L, Johansson C, Pousette A, Tegelman R, Carlström K, Hemmingsson P. (1991). Cortisol and androgen concentrations in female and male elite endurance athletes in relation to physical activity. Eur J Appl Physiol Occup Physiol 63: 308-311.

Van Eenoo P, Delbeke FT, De Jong FH, De Backer P. (2001). Endogenous origin of norandrosterone in female urine: indirect evidence for the production of 19-norsteroids as by products in the conversion from androgen to estrogen. J of Steroid Biochemistry and Molecular Biology 78(4):351-357.

Warren MP & Shantha S. (2000). The female athlete. Best Practice & Resecarch Clinical Endocrinology & Metabolism 14(1):37-53.

Zhou ZH, Liu JH, Jin YL, He P, Zhao PL, Wang X. (2000). Effects of different loading exercises on endogenous sex hormones and their metabolites. Hunan Yi Ke Da Xue Xue Bao 25(1):23-26.

Número de citas totales / Total references: 34 (100%)

Número de citas propias de la revista / Journal's own references: 0