

Urine Steroid Profile of Judo Competitors Affected by Acute Physical Exercises

J.M. Pucsok¹, I. Györe², I. Hollósi², E. Soós², N.R. Ali Ghasemi³, and R. Frenk³

¹National Institute for Sport Talent Care; ²National Institute for Sports Medicine, and ³Semmelweis University, Physical Education Department, Budapest, Hungary

Abstract

A heterogeneous group of 10 male and 15 female judo players are utilized in this study. The subjects complete a standardized maximal treadmill exercise test. Urine samples are collected at the pre- and postexercise stages. The urine steroids are measured using a gas chromatography–mass spectrometry instrument. In rest and after exercise, significantly higher testosterone and epitestosterone concentrations in males ($p < 0.01$) are found. The etiocholanolone–dehydroepiandrosterone (DHEA) ratio is significantly lower in males than females ($p < 0.05$). In both males and females, etiocholanolone concentration significantly decreases with the effect of exercise ($p < 0.05$). 11-OH etiocholanolone concentration also significantly decreases, but only in females ($p < 0.05$). Positive correlation is found between the changes of the etiocholanolone and epitestosterone concentration caused by exercise.

Introduction

Improvements and recent advances (in particular) in laboratory techniques have allowed researchers to gain a better understanding of exercise endocrinology. These investigations were often made using serum, saliva, or urine samples to determine the concentration of steroids and their metabolites. In order to analyze hormonal concentrations from different samples, gas chromatography (GC) and GC–mass spectrometry (MS) are widely used (1).

Basiewicz et al. studied the quantities of testosterone (T), DHEA, androsterone, and etiocholanolone in 24-h urine samples using GC–MS (2). Serum samples were assayed for estradiol, total and free T levels and sex-hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating hormone, and prolactin levels using immunoradiometric assay. Free androgen index, the serum T–estradiol ratio in serum and the androsterone–etiocholanolone ratio in urine were calculated. Accordingly, the volume of information available is expanding rapidly.

Tremblay et al. suggested that chronic exercise training may modulate the pattern of basal hormonal secretion, but may also modify the normal acute hormonal responses to exercise (3).

Standardized environmental and testing conditions help to minimize the influence of variables not directly related to these investigations. Temperature; relative humidity; alcohol, caffeine, nicotine, and food intake; sleep deprivation, and previous exercise may alter the hormonal responses to exercise (3). Most of the investigations indicated a decrease in the concentration of T. For example, after 40 min of continuous exercise, Galbo et al. found lower values (4). Dobrzanski et al. investigated the effect of exercise on steroid response (5). The investigators measured a significant decrease in T concentration after 45 min of a postexercise session.

On the other hand, few studies demonstrated different results. Vogel et al. found significantly higher free and total T values in healthy adults after exercise (6). Two hormones (T and cortisol) with different physiological characteristics provide a good description of the quality of training and condition of the athlete (7). The proportion of these hormones indicates the anabolic or catabolic direction of metabolic processes (7). Researchers such as Perry et al. (8) determined whether the use of the urinary ratio of T to LH (T/LH) is superior as an indicator of exogenous anabolic steroid as compared with the urinary ratio of T to epitestosterone. Yoon and Lee carried out studies measuring the concentration of anabolic steroids in urine in subjects who were orally administered with drugs (9). The researchers used GC–MS microanalysis for the conjugated steroids. A study by Görög (10) gave an overview of the advances during the last 15 years in the analysis of steroid hormone drugs and related materials. The paper discussed the development in steroid hormone drug analysis, which is characterized by the rapid spreading of new techniques, mainly high-performance liquid chromatography and immunoassay methods.

Several studies demonstrated the effect of physical exercise on the level of plasma T and androstendione as well as on the ratio of T–cortisol and T–SHBG. Many investigations found evidence that exercise not only effects the concentration of plasma T and androstendione, but also the ratio of T–cortisol and T–SHBG as well. Hakkinen et al. (11) measured increased values for the T–cortisol and T–SHBG ratio caused by the effect of aerobic exercise. Yap et al. (12) studied the possibility of using absolute concentrations of urinary total T and LH and the T/LH concentration ratios to profile short-term exercise-stress responses in healthy, drug-free male athletes. T and LH concentrations were measured

using GC–MS and microparticle enzyme immunoassay techniques. The stress profiles derived from this study facilitated an assessment of the relationship between the endogenous T, LH concentration, T/LH ratio and the stress responses over a short period of applied exercise stress. There are also several other factors, such as age, gender, training status, etc., which influence the hormonal response to various types of activities.

In the related literature, no data was found regarding exercise-induced steroid profile analysis in combat sports, such as judo. In conclusion, the neuroendocrine response to a specific activity is primarily influenced by the duration and intensity of the given exercise test.

Experimental

Ergometric analysis

Ten male and 15 female judoists, members of the Hungarian national team, participated in this study. The subjects completed a standardized maximal treadmill (LE 580 C, Jaeger, Würzburg, Germany) exercise test. The speed was originally set at 6 km/h, and increased every minute by 1 km/h, with a constant 1.5% incline.

Urine steroid profile analysis

Urine samples were collected at the pre- and postexercise stages. The urine steroids were measured using a GC–MS instrument.

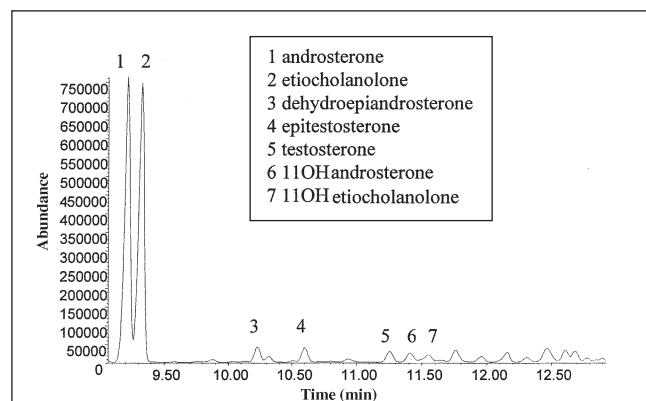


Figure 1. Total ion chromatogram of the seven analyzed steroid hormones.

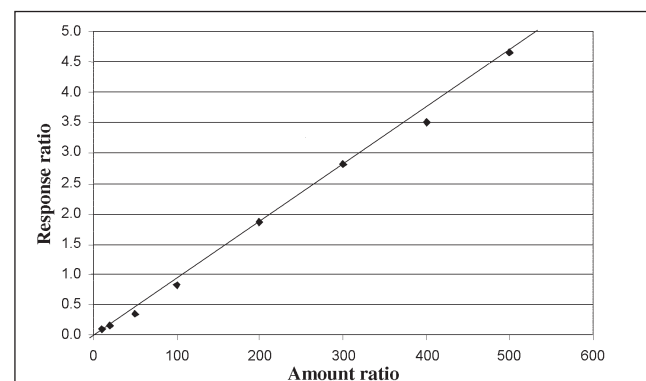


Figure 2. Calibration curve for DHEA hormone. Response ratio = 0.0091x amounts; correlation coefficient (r^2) = 0.998.

The study was approved by the local Ethics Committee (Budapest, Hungary). All subjects were volunteers, who agreed to participate in the study.

Sample preparation

Urine samples were submitted to the routine doping extraction procedure used in the laboratory for the analysis of conjugated steroids shown in Figures 1 and 2 (13). Urine samples (5 mL) were extracted in Sordolit columns (Serva, Heidelberg, Germany) (previously washed with acetone, methanol, and water) and eluted with methanol. The dry residue was dissolved in acetate buffer (pH 5.2), and the free steroids were extracted by diethyl-ether. The glucuronides were hydrolyzed for 2 h with β -glucuronidase from *Escheria coli*, pH was adjusted to 9–10, and conjugated steroids were extracted with ethyl-acetate. The organic phase was evaporated under a nitrogen stream, and the final residue was redissolved in 50 μ L *n*-methyl-trimethyl-silyl-trifluoroacetamide (MSTFA), using trimethyliodosilane (TMSI) as a catalisator (MSTFA–TMSI mixture), to form the trimethyl-silylenol-ether derivatives.

GC–MS analysis

GC–MS analysis in the selected ion monitoring mode were carried out with Agilent 6890 GC with direct coupling to 5973 HP-MSD quadrupol filters (Agilent Technologies, Palo Alto, CA). The system was equipped with automatic sampler model 7673A (Jaeger).

The sample injections (2 μ L) were carried out in the split mode (1:10), and the separation was achieved on J&W Scientific 1-MS capillary column (15-m \times 0.25-mm i.d., 0.25- μ m film thickness).

Chromatographic parameters

The carrier gas used was helium. The oven temperature was set at an initial temperature of 160°C for 0.2 min, then programmed at 10°C/min to 190°C, 3.5°C/min to 255°C, and 15°C/min to 310°C. The injection port was 270°C and transfer line was 310°C.

Quantitative analysis

Quantitative analysis of androsterone, etiocholanolone, dehydroepiandrosterone, T, epitestosterone, 11OH-androsterone, and 11OH-etiocholanolone was performed using a calibration curve prepared by adding known amounts of the compounds. The quantitation of the steroid molecules was based on the response factor of an extracted sample from distilled water, containing 5 ng/mL of methyltestosterone internal standard (0.51-ng/mL limit of detection and 1.12-ng/mL limit of quantitation).

Results and Discussion

In rest and after exercise, a significantly higher T and epitestosterone concentration was found in males ($p < 0.01$). The etiocholanolone–DHEA ratio was significantly lower in males than in females ($p < 0.05$). In both males and females the etiocholanolone concentration significantly decreased with the effect

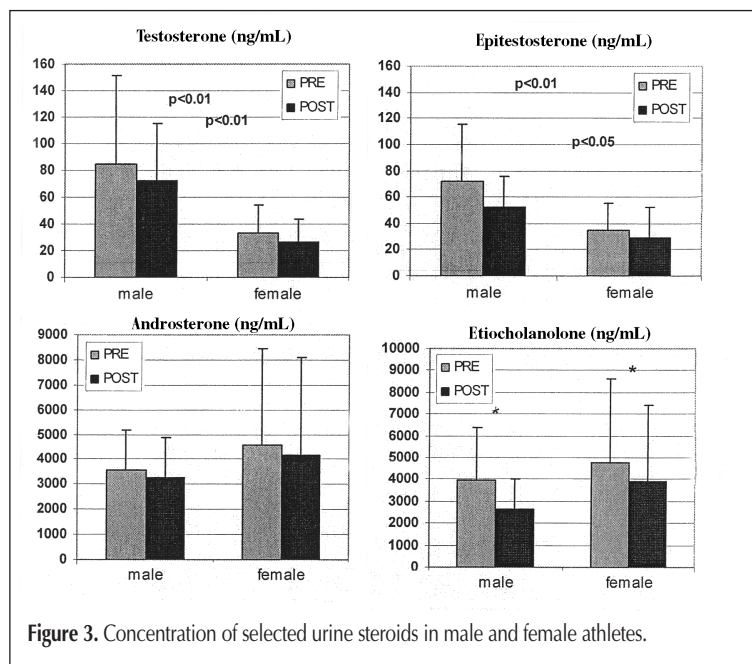


Figure 3. Concentration of selected urine steroids in male and female athletes.

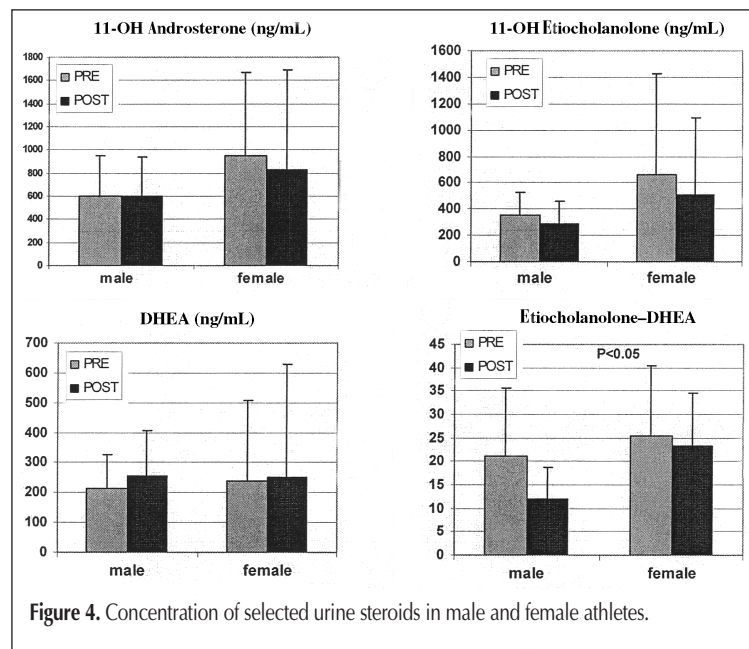


Figure 4. Concentration of selected urine steroids in male and female athletes.

of exercise ($p < 0.05$); the 11-OH etiocholanolone concentration also significantly decreased, but only in females ($p < 0.05$) (Figures 3 and 4).

Conclusion

The judo competitors responded to exercise according to their own individual hormonal mechanism and fitness status (3,14,15). According to the corresponding literature, the intensity and type of (aerobic and anaerobic) exercise have a determinant effect on the hormonal response to exercise (3,14,15). In both males and females, the changes in the concentration of etiocholanolone and

epitestosterone were attributable to the response to acute exercise. The correlation and direction of change of these two hormones may suggest a new pathway of steroid metabolism. These results may provide a new approach for evaluating sports (judo) performance.

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