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RESEARCH LETTER

Urinary endogenous steroids and their relationships with BMD and body composition in healthy young males

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Introduction

Some studies have stated that steroid hormones have associations with the values of strength, bone mass density (BMD), and body composition shown by people [1]. In this way, steroid hormonal changes have been directly or indirectly associated with changes in body composition, fat distribution, lean mass, muscle weakness, osteoporosis, as well as depression [2].

Regarding to this topic, most studies have been carried out using blood samples. However, some research has tried to analyze the correlation among urinary steroid hormones and body mass index, fat mass, or BMD [3, 4]. Urinary samples are easy to obtain in sufficient quantities without the need for invasive techniques. Therefore, in this study, we tested the hypothesis that the values of bone mineral density and body composition are related to the values of urinary endogenous steroid excretion (androgens, estrogens, and corticosteroids).

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Materials and methods

Subjects

Thirty healthy male university students, who did not usually do strength training, were recruited to participate in this study. The mean (SD) physical characteristics of the subjects were: age: 24.2 (2.4) years; height: 1.76 (0.06); weight: 73.3 (8.2) kg. Participants could not ingest any substance that could interfere with the synthesis, metabolism, or excretion of steroids and they could not perform any intense exercise for 48 h before the study.

Steroid hormones analysis

The first morning urine was collected from all subjects, and then frozen at −20°C until processing and analysis. The following hormones—in their free, glucuroconjugated, and sulfoconjugated fractions—were analyzed: testosterone, estrone (E1), B-estradiol (E2), cortisol (F), and cortisone (E). Gas chromatography–mass spectrometry technique (GC/MS) was employed to detect and quantify these hormones, in accordance with Galan et al. [5]. The analysis of the samples was conducted on an Agilent Technologies 6890N chromatograph with MS 5973 Network quadrupole spectrometer. Separation was performed with a factor four capillary column VF-1 ms 25 m × 0.25 mm ID DF = 0.25. The conditions of the analysis started at 100°C for 1.50 min, it rose 10°C/min until 280°C, maintaining the temperature for 5 min, it increased 10°C/min until 300°C, maintaining the temperature for 2 min. The analysis was

performed in splitless mode, with He gas carrier at a flow rate of 0.6 ml/min. The injected volume was 3 µl and the temperature of the injector was 250°C.

Bone mineral density and body composition measurement

Bone mineral content (BMC) and bone mineral density (BMD) were measured using dual-energy X-ray absorptiometry (DXA). In addition, body composition (total fat mass and total lean mass) was also measured with the scanner in total body mode. Quality assurance for DXA, including calibration, was performed every morning, using the standard provided by the manufacturer.

Statistical analysis

Means and standard deviations (SD) were used as descriptive statistics. Correlation between concentrations of steroid hormones and parameters studied was calculated using Pearson's correlation coefficient (r). Moreover, relations between variables were assessed using linear regression for continuous variables.

Results

Table 1 illustrates the significant relations among urinary steroid concentration and bone mineral density and body composition parameters. Urinary estrone and B-estradiol concentrations were positively and independently related with BMI and total fat mass, respectively. Urinary cortisol concentrations were inversely related to BMD and BMC. Also, urinary cortisol concentration was inversely related to total lean mass.

Discussion

The level of urinary testosterone obtained has shown no connection with any of the studied variables. Therefore, it could not be concluded that the urinary excretion of testosterone is a faithful reflection of the total lean mass or body composition parameters. In this way, an important percentage of blood testosterone could be linked to the sex hormone binding globulin (SHBG) and it would not be reduced by the liver [6].

Regarding to estrogens, the results are similar to those of other studies in which it was shown that the levels of estrogens were directly associated with total body fat mass or subcutaneous abdominal fat [7]. In fact, it was proved that obese men with a BMI greater than 30 kg/m^2 had very high levels of estrone [4]. Given that the majority of estrogen levels in men are the result of a transformation process (aromatization) from androgens to estrogens, it could be supposed that subjects with a greater fat mass would show a greater activation of these processes of aromatization [8]. From a clinical point of view, high values of urinary estrogens in men could be used like an indicative of obesity and therefore, they could indicate a greater cardiovascular risk.

Finally, cortisol is an eminently catabolic hormone, so it seems logical that the excess of corticosteroids has a negative impact on bone and muscle formation; reducing the absorption of calcium, decreasing the protein synthesis and affecting the secretion of other hormones (in particular gonadotropins and GH) [9, 10]. These results may have some significant clinical implications. In fact, people with higher urinary cortisol concentrations demonstrate lower bone densities and BMC, so these people would have more possibilities of undergoing osteoporosis or bone fractures.

Table 1 Correlation coefficients and relationships found between urinary steroid concentrations and bone mineral density and body composition

Urinary steroid concentrations	BMD 1.26 ± 0.1 g/cm^2	BMI 23.69 ± 2.1 kg/m^2	Total fat mass 12.97 ± 4.1 kg	Total lean mass 52.25 ± 4.6 kg	BMC 2.88 ± 0.4 kg
Testosterone	$R = 0.161$	$R = 0.249$	$R = 0.118$	$R = 0.244$	$R = 0.209$
29.67 ± 13.4	$P = 0.292$	$P = 0.195$	$P = 0.332$	$P = 0.200$	$P = 0.237$
Estrone	$R = 0.154$	$R = \mathbf{0.527}$	$R = 0.417$	$R = 0.479$	$R = 0.278$
23.18 ± 5.9	$P = 0.300$	$^*P = \mathbf{0.026}$	$P = 0.061$	$P = 0.082$	$P = 0.168$
B-Estradiol	$R = 0.293$	$R = 0.320$	$R = \mathbf{0.680}$	$R = 0.367$	$R = 0.025$
11.53 ± 1.7	$P = 0.155$	$P = 0.132$	$^{**}P = \mathbf{0.003}$	$P = 0.098$	$P = 0.467$
Cortisol	$R = \mathbf{-0.560}$	$R = -0.388$	$R = -0.045$	$R = \mathbf{-0.539}$	$R = \mathbf{-0.622}$
502.46 ± 263.6	$^*P = \mathbf{0.019}$	$P = 0.095$	$P = 0.434$	$^*P = \mathbf{0.023}$	$^{**}P = \mathbf{0.009}$
Cortisone	$R = \mathbf{-0.515}$	$R = -0.231$	$R = -0.038$	$R = -0.400$	$R = \mathbf{-0.476}$
426.73 ± 226.8	$^*P = \mathbf{0.030}$	$P = 0.213$	$P = 0.444$	$P = 0.078$	$^*P = \mathbf{0.043}$

Urinary steroids are expressed as ng steroid/mg creatinine (mean ± SD). R Pearson's correlation coefficient

*, ** Statistically significant

Significant relationships are shown in bold

In conclusion, the urinary excretion of certain steroidal hormones can help us to predict BMD and body composition parameters, as well as to detect or prevent different health problems. The urinary steroid profile could be easily used by general practitioners, specifically to check patient's health. These medical tests would be particularly useful for sedentary and obese young men, and for adults, who are over 60-years old, because important hormonal changes are produced in this time of the life. Anyway, these findings have to be confirmed by longitudinal studies.

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The experiment was developed in agreement with the policy statement of the Declaration of Helsinki and performed with the approval of the Committee on Biomedical Ethics of the University of Extremadura (Spain).

References

1. A.W. Van den Beld, F.H. De Jong, D.E. Grobbee, H.A. Pols, S.W. Lamberts, Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J. Clin. Endocrinol. Metab. **85**(9), 3276–3282 (2000)
2. B. Lunenfeld, Endocrinology of the aging male. Minerva Ginecol. **58**(2), 153–170 (2006)
3. V. Morelli, B. Masserini, A.S. Salcuni, C. Eller-Vainicher, C. Savoca, R. Viti, F. Coletti, G. Guglielmi, C. Battista, L. Iorio, P. Beck-Peccoz, B. Ambrosi, M. Arosio, A. Scillitani, I. Chiodini, Subclinical hypercortisolism: correlation between biochemical diagnostic criteria and clinical aspects. Clin. Endocrinol. **73**(2), 161–166 (2010)
4. J. Teas, J.E. Cunningham, J.H. Fowke, D. Nitcheva, C.P. Kanwat, R.J. Boulware, D.W. Sepkovic, T.G. Hurley, J.R. Hebert, Urinary estrogen metabolites, prostate specific antigen, and body mass index among African-American men in South Carolina. Cancer Detect. Prev. **29**(6), 494–500 (2005)
5. A.M. Galan, J.I. Maynar, M.P. García de Tiedra, J.J. Rivero, M.J. Caballero, M. Maynar, Determination of nandrolone and metabolites in urine samples from sedentary persons and sportsmen. J. Chromatogr. B **761**, 229–236 (2001)
6. M. Maynar, R. Timon, A. González, G. Olcina, F. Toribio, J.I. Maynar, M.J. Caballero, SHBG, plasma and urinary androgens in weight lifters after a strength training. J. Physiol. Biochem. **66**(2), 137–142 (2010)
7. M. Muller, D.E. Grobbee, J.H. Thijssen, A.W. Van den Beld, Y.T. Van der Schouw, Sex hormones and male health: effects on components of the frailty syndrome. Trends Endocrinol. Metab. **14**(6), 289–296 (2003)
8. A. Vermeulen, J.M. Kaufman, S. Goemaere, Van Pottelberg, Estradiol in elderly men. Aging Male **5**, 98–102 (2002)
9. D. Paddon-Jones, M. Sheffield-Moore, M.G. Cree, S.J. Hewlings, A. Aarsland, R.R. Wolfe, A.A. Ferrando, Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress. J. Clin. Endocrinol. Metab. **91**(12), 4836–4841 (2006)
10. I. Chiodini, M. Torlontano, V. Carnevale, V. Trischitta, A. Scillitani, Skeletal involvement in adult patients with endogenous hypercortisolism. J. Endocrinol. Invest. **31**(3), 267–276 (2008)