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Effects of toremifene and tamoxifene on the urinary androgenic steroid profile in males and females

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Introduction

The use of selective estrogen receptor modulators (SERMs) is banned in sports by the WADA since 2005 [1]. The athletes could illicitly use SERMs to increase endogenous testosterone, with the aim to by-pass the specific testing regimens for known synthetic androgens including exogenous testosterone. Previous papers reported that, following the administration of SERMs, the plasmatic levels of testosterone and luteinizing hormone increase significantly only in male subjects [2]; while no data were recorded about the urinary steroids variations. Here we reports the results of a preliminary investigation on the effects of SERMs oral administration on the endogenous anabolic androgenic steroids urinary excretion in both males and females. Urinary variability of testosterone (T), epitestosterone (E), and T/E ratio was measured by GC-MS; whereas the luteinizing hormone (LH), the follicle-stimulating hormone (FSH) and the human chorionic gonadotropin (hCG) urinary levels were measured using immunometric assay with chemiluminescence detection.

Materials and Methods

Chemicals and reagents

Standards of T, E, deuterated testosterone (Td3) and deuterated epitetosterone (Ed3) were purchased from NMI (National Measurement Institute, Pymble, Australia). hCG, LH and FSH were purchased from NIBSC (National Institute for Biological Standards and Control, UK). The enzyme β-glucuronidase from *E. coli* was purchased from Roche (Monza, Italy). N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) was supplied by Alfathech (Genova, Italy). Ammonium iodide (NH_4I) and dithierythritol (DTE) were supplied from Sigma-Aldrich (Milano, Italy). The urine samples were collected to 4 subjects (2 males and 2 females; 35-43 years; 50-78 Kg) before and after oral administration of a single dose of toremifene (60 mg; Fareston® from Orion Pharma, Milano, Italy) or tamoxifen (20 mg; Nolvadex® from AstraZeneca Milano, Italy). The individual baseline variability of

endogenous steroids was set up by collecting urine samples, every three hours, for three days prior to the administration of the drug(s); whereas the study of the effects of a single dose of each drug on the endogenous steroid urinary concentrations was assessed by collecting urine samples every three hours for at least five days. Data obtained after drug(s) administration were then evaluated taking into account the individual baseline variability.

Analytical procedure

Quantitative analysis by GC-MS was performed on an Agilent Technologies 6890 series gas chromatograph coupled to a 5973 MSD quadrupole mass spectrometer in EI ionisation (70 eV), using a 17 m fused silica capillary column DB1 (J&W Scientific), ID 0.20 mm, film thickness 0.11 μm. The carrier gas was helium (split ratio 1:10), and the temperature program was as follows: 180 °C (hold 4.5 min), 3 °C/min to 230 °C, 20 °C/min to 290 °C, 30 °C/min to 320 °C; the transfer line temperature was set at 280 °C. Acquisition was carried out in SIM. The diagnostic ion monitored for T and E was m/z 432. The values of the urinary concentration of T and E were calculated by the peak areas of the detected signals relative to the internal standards Td3 and Ed3 respectively (m/z 435).

Quantitative analysis by immunometric assay with chemiluminescence detection was performed on an Immulite 2000 (DPC instrument), using Siemens FSH, LH and hCG chemiluminescent immunoassay systems (Siemens Medical Solutions Diagnostic, Los Angeles, USA). The detection was made by means of LUMIGEN® PPD [4-metoxy-(3-phosphatephenyl)-spiro-(1,2-dioxethane-3,2'-adamantane)] substrate.

Urine samples were pre-treated according to a specific procedure presently followed by our laboratory for the steroid profile evaluation [3]. Briefly, 3 mL of urine were hydrolyzed using β-glucuronidase from *E. coli*, then a liquid/liquid extraction was carried out with *tert*-butylmethylether. The organic layer was evaporated and the residue was derivatized, at 70 °C for 20 min, using 50 μL of a mixture of MSTFA/NH_4I/DTE (1000:2:4:v/w/w).

Results and Discussion

The results obtained from the SERMs excretion studies indicated that the urinary levels of T and E were altered in male subjects. No variation from the baseline individual profile was recorded for LH, FSH, hGC and T/E ratio. Figures 1A-D show the circadian urinary profiles of T, E, T/E ratio before and after tamoxifen or toremifene administration in male (Figures 1A-B) and in female (Figures 1C-D) subjects. We can notice that significant variations from the baseline values were recorded only in male subject for T and E; whereas no variations

from the baseline levels were measured for the T/E ratio in both male and female subjects. In summary, after SERMs administration: (i) the T and E urinary levels show a transient increase only in males; whereas no variations were recorded for the urinary T/E ratio values (see Figures 1A-D). The blockage of the feedback mechanism on the hypothalamus, seems not modify the qualitative production of T and E from 4-androstenedione; (ii) the toremifene and tamoxifen active metabolites (hydroxyl- metabolites) urinary levels were still high in correspondence to the maximum T excretion values (data not shown); (iii) the increase in urinary testosterone concentrations is higher than the acceptable physiological variations (CV% < 30 for males and CV% < 60 for females) currently employed by WADA to give an atypical or a positive result in case of longitudinal studies evaluation of the steroid profile (see Table 1). Nonetheless the maximum testosterone level reached after SERMs administration is modest and much less than testosterone injections or testosterone transdermal application.

Table 1: Percentage of variation from the baseline T mean value before and after drug administration

Male (tamoxifen)					
Before					
	-24h	-21h	-18h	-15h	-12h
% from baseline	*12*	*1*	*4*	*10*	*14*
First day					
	0h	3h	6h	9h	12h
% from baseline	*1*	*17*	*4*	*10*	*14*
Second day					
	24h	27h	30h	33h	36h
% from baseline	*-2*	*7*	*1*	*13*	*25*
Third day					
	48h	51h	54h	57h	60h
% from baseline	*35*	*46*	*40*	*35*	*20*
Fourth day					
	72h	75h	78h	81h	84h
% from baseline	*13*	*5*	*-9*	*-12*	*15*
Fifth day					
	96h	99h	102 h	105 h	108 h
% from baseline	*5*	*1*	*-7*	*-10*	*-15*

Male (toremifene)					
Before					
	-24h	-21h	-18h	-15h	-12h
% from baseline	*10*	*-3*	*5*	*10*	*9*
First day					
	0h	3h	6h	9h	12h
% from baseline	*4*	*15*	*7*	*10*	*14*
Second day					
	24h	27h	30h	33h	36h
% from baseline	*2*	*5*	*3*	*15*	*21*
Third day					
	48h	51h	54h	57h	60h
% from baseline	*44*	*53*	*48*	*36*	*15*
Fourth day					
	72h	75h	78h	81h	84h
% from baseline	*15*	*4*	*-11*	*-9*	*11*
Fifth day					
	96h	99h	102 h	105 h	108 h
% from baseline	*5*	*31*	*-7*	*-10*	*-10*

References

1. World Anti-Doping Agency. The 2010 Prohibited List. International Standard, Montreal (2010) http://www.wada-ama.org/rtecontent/document/2010_List_En.pdf.
2. Handelsman DJ. Indirect androgen doping by estrogen blockade in sports. (2008) *British Journal of Pharmacology*. **154**, 598-605.
3. Mazzarino M, Braganò MC, Donati F, de la Torre X, Botrè F. Effects of propyphenazone and other non steroidal anti-inflammatory agents on the synthetic and endogenous androgenic anabolic steroids urinary excretion and/or instrumental detection (2010) *Anal. Chim. Acta* **657**, 60-68.

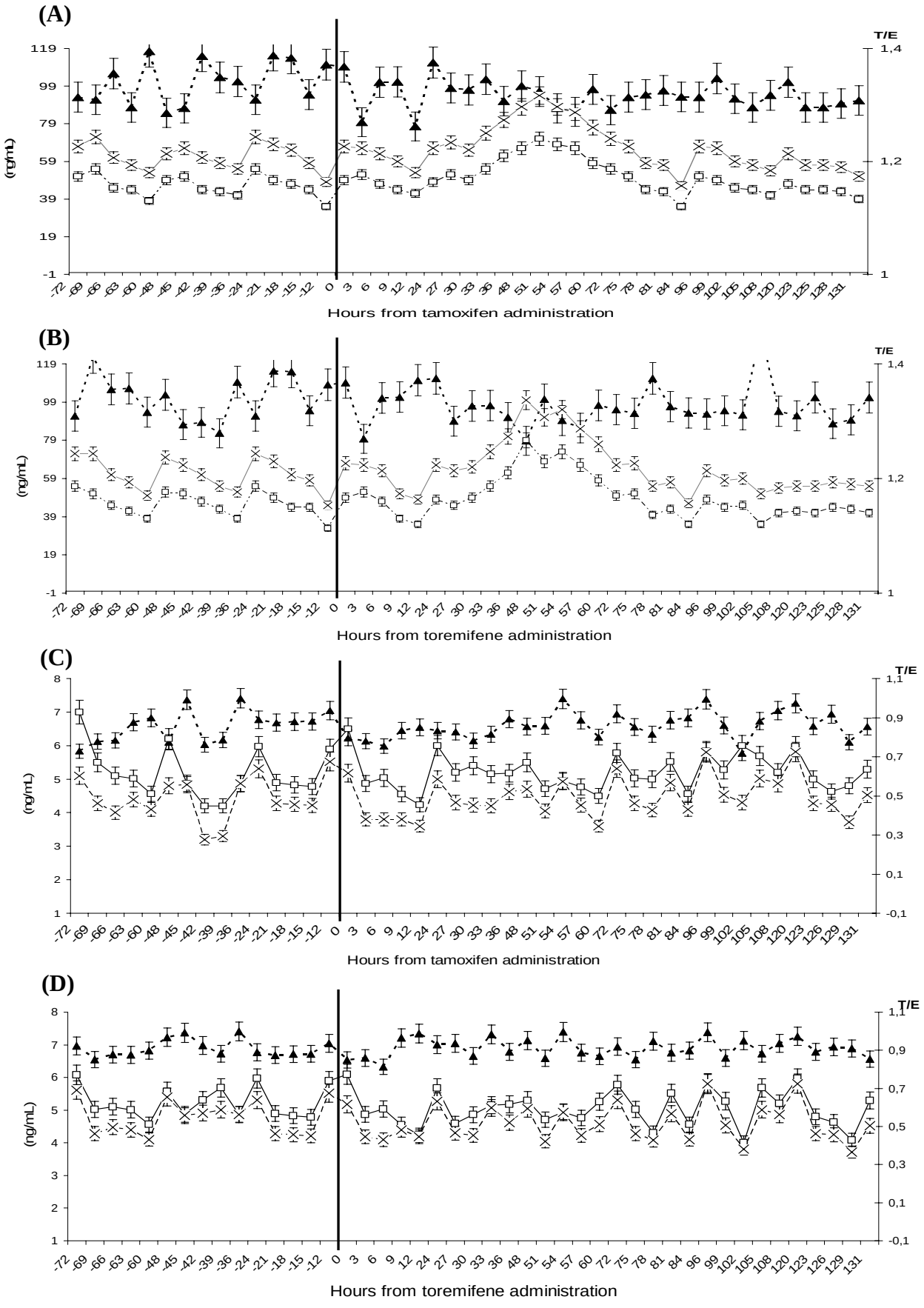

Figure 1: T (x), E (□) and T/E ratio (▲) profiles (males A, B; females C,D) after drug administration.