Journal of Chromatography, 489 (1989) 121–126
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4595

EFFECT OF EXTENDED USE OF SINGLE ANABOLIC STEROIDS ON URINARY STEROID EXCRETION AND METABOLISM

L.M. HARRISON*, D. MARTIN, R.W. GOTLIN and P.V. FENNESSEY

Department of Pediatrics, University of Colorado, Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262 (U.S.A.)

SUMMARY

Long-term use of single anabolic steroids by weightlifters and body builders at dosages ≥ 25 mg per 24 h resulted in reduced excretion of urinary androgen metabolites, androsterone and etiocholanolone, compared to values prior to anabolic use. The excretion of major urinary metabolites of glucocorticoids was not affected by anabolic use. Urinary excretion of anabolic steroids or anabolic metabolites averaged 20–25% of total anabolic steroid administered. The major excreted metabolites of methandrostenolone, nandrolone, oxandrolone and oxymetholone were identified by gas chromatography–mass spectrometry based on the major mass spectral ion peaks.

INTRODUCTION

The use of anabolic steroids for weight and strength gains by athletes has become increasingly popular [1]. However, the influence of anabolic steroids on the endocrine system is not well documented [2]. Previous studies in this laboratory investigating the influence of anabolic steroids on the endocrine system have shown that long-term use of multiple anabolic steroids resulted in reduced excretion of endogenous urinary steroid metabolites [3]. Administration of multiple anabolic steroids makes it difficult to distinguish the effect of a specific individual anabolic steroid, although there have been a number of publications dealing with chromatography of anabolic steroids [4–6]. Therefore, the present study is designed to investigate the effect of long-term use of single anabolic steroids on the excretion of endogenous urinary steroid metabolites and the pattern of excretion of anabolic steroid metabolites.

0378-4347/89/$03.50

EXPERIMENTAL

Method

Weightlifters and body builders who routinely use anabolic steroids as a part of their training regimen and who had volunteered for this study were required to abstain from anabolic steroid use for at least three weeks, while maintaining their exercise regimen (Fig. 1). After at least three weeks without anabolic use, each participant was requested to begin use of a single anabolic steroid (oxandrolone, oxymetholone, methandrostenolone or nandrolone). Dosages of anabolic steroid averaged at least 25 mg per 24 h and each individual was free to choose the form of steroid administration (oral or parenteral).

Anabolic use was discontinued subsequent to at least three weeks of administration. Each volunteer continued to train without anabolic steroid use for a minimum of three weeks after discontinuing anabolic use.

Urine was collected over a 24-h period by each volunteer on three occasions: (1) after at least three weeks 'off cycle' and just prior to initiating single anabolic use; (2) after at least three weeks of single anabolic steroid use; (3) at least three weeks after discontinuing single anabolic use.

Steroids were isolated from the urine samples and quantitated as previously reported [7]. Briefly, 5 μg of 5β-androstan-17α-ol-3-one (Steraloids, Wilton, NH, U.S.A.) were added to a 5-ml aliquot of urine to serve as internal standard. Each sample was passed through a C_{18} Sep-Pak (Water Assoc., Milford, MA, U.S.A.) and washed with water. Free and conjugated steroids were eluted with methanol. After drying off the methanol under nitrogen gas, the samples were hydrolyzed using *Helix pomatia* digestive juice (Behring Diagnostics, La Jolla, CA, U.S.A.; 12 h at 55°C). Free and deconjugated steroids were extracted using methylene chloride, then ethyl acetate. The combined extracts were washed once with saturated bicarbonate and twice with water. The sample was then evaporated by dryness and derivatized to the methoxime of ketones and trimethylsilyl ether of alcohols (Pierce, Rockford, IL, U.S.A.).

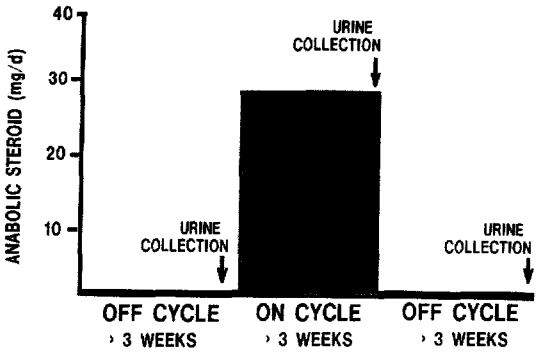


Fig. 1. Experimental procedure used for administration of anabolic steroids and collection of urine by volunteers. Each arrow indicates a 24-h urine collection period.

Gas chromatography (GC)

Quantitation of the urinary steroid extracts was achieved using a Hewlett-Packard 3710A gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) fitted with a modified Van den Berg falling-needle injector and coupled to a 30 m×0.25 mm I.D. 0.25 μm DB-1 fused-silica open tubular column (J&W Scientific, Rancho Cordova, CA, U.S.A.). The carrier gas was helium (20 cm/s) and the make-up gas was nitrogen with a flow-rate of 20 ml/min. Hydrogen and air flow-rates were 30 and 300 ml/min, respectively. The injector and flame ionization detector temperatures were 300°C.

Sample analysis was conducted with an initial column temperature of 200°C held for 4 min, then increased to 300°C at 4°C/min. The recorder chart speed was 1.25 cm/min. Quantitation of each peak was attained as previously published [7] based on the area ratio of each peak to the internal standard.

Gas chromatography–mass spectrometry

Qualitative confirmation of endogenous urinary steroid metabolites and anabolic steroids and tentative identification of anabolic steroid metabolites was accomplished using a Varian 3400 gas chromatograph (Varian Assoc., Sugar Land, TX, U.S.A.) interfaced to a Finnigan ion trap mass spectrometer (Finnigan MAT, San Jose, CA, U.S.A.). The GC column was a DB-1 fused-silica column (30 m×0.25 mm I.D., 0.25 μm) from J&W Scientific. The carrier gas was helium and no make-up gas was required as the column was inserted directly into the ion source.

Confirmation of endogenous urinary steroid identity was contingent upon having both retention time and mass spectra that were identical with authentic urinary steroid standards (Steraloids). Confirmation of the identity of excreted anabolic steroids was contingent upon both retention time and mass spectra match with the derivatized anabolic steroid standards (Steraloids). Anabolic steroid metabolites were tentatively identified based upon evaluation of the fragmentation pattern observed in the mass spectra.

Statistics

The influence of anabolic steroid use on the excretion of the major androgen metabolites (androsterone and etiocholanolone) and glucocorticoid metabolites (tetrohydrocompound 'E', THE; tetrohydrocompound 'F', THF; *allo*-tetrohydrocompound, *allo*-THF) was analyzed using the General Linear Models procedure of the Statistical Analysis System (SAS Institute, Cary, NC, U.S.A.).

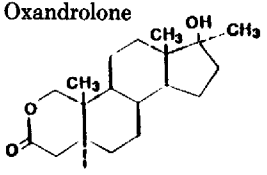
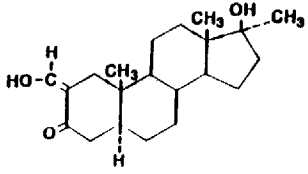
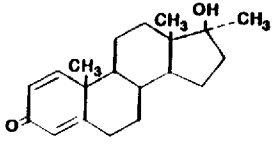
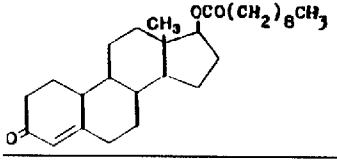
RESULTS AND DISCUSSION

Extended use of a single anabolic steroid results in reduced secretion of androsterone and etiocholanolone ($p < 0.05$, Fig. 2). Included for comparison only are androsterone and etiocholanolone values for a non-anabolic user, non-athlete group. The suppressive effect of anabolic steroids on androsterone was also maintained during the time period after anabolic steroid use had been discontinued

TABLE I

MAJOR COMPOUNDS IDENTIFIED IN THE URINE FOLLOWING ANABOLIC STEROID USE

For each derivatized metabolite (TMS, MOX) we have shown the methylene unit value (MUV), chemical structure (if known) and significant mass spectral peaks.

Anabolic agent	MUV	Major excreted compounds	Major mass spectral peaks
Oxandrolone 	29.47	Oxandrolone (17-hydroxy-17-methyl-2-oxandrostan-3-one)	378, 363, 308, 288, 143
Oxymetholone 	26.39	3,17-Dihdyroxy-17-methylandrostane	450, 435, 360, 345, 143
	27.47	2,17-Dihydroxy-17-methyl-3-oxo-2-androstene	462, 447, 372, 357, 267
	29.19	2,3,17-Trihydroxyandrostane	552, 537, 462, 143
Methandrostenolone 	26.45	3,17-Dihydroxy-17-methyl-1-androstene	448, 358, 143
	26.78	17α-Hydroxy-17-methylandrost-1,4-dien-3-one	401, 386, 370, 311, 143
	28.58	6,17-Dihyroxy-17-methyl-3-oxo-1,4-androstdiene	489, 458, 399, 368, 309, 278, 237, 143
Nandrolone decanoate 	24.67	5α-Estran-3α-ol-17-one (19-nor-5α-androstan-3α-ol-17-one)	377, 362, 346, 256, 199
	24.98	5β-Estran-3α-ol-17-one (19-nor-5β-androstan-3α-ol-17-one)	377, 362, 346, 256, 199
	26.37	4-Estren-3,17-dione (19-nor-4-androsten-3,17-dione)	330, 299, 268, 186, 143

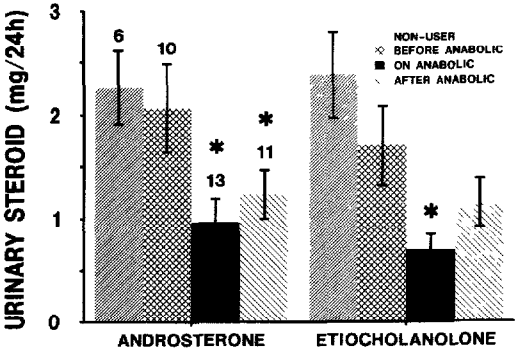


Fig. 2. Values for major urinary androgen metabolites before, during and after anabolic steroid use by weightlifters (± S.E.M.). The asterisk indicates values significantly different from the 'before anabolic' column ($p < 0.05$). Included for comparison only are values from a non-athlete, non-anabolic user group. The numbers above each bar are the number of participants at each phase of the study. Several volunteers did not complete every phase.

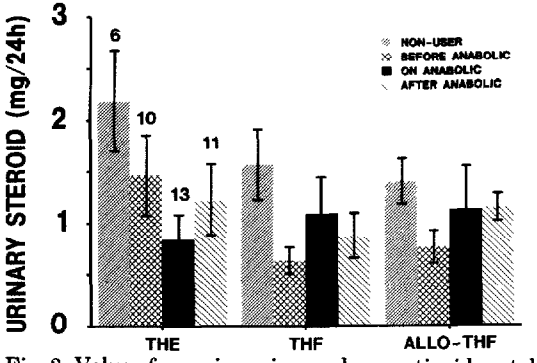


Fig. 3. Values for major urinary glucocorticoid metabolites before, during and after anabolic steroid use by weightlifters (± S.E.M.). Included for comparison only are values from a non-athlete, non-anabolic user group. The numbers above each bar are the number of participants at each phase of the study. Several volunteers did not complete every phase.

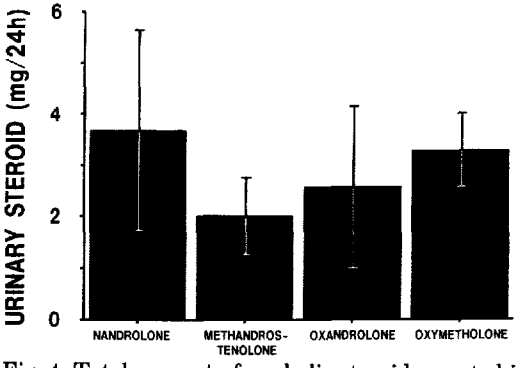


Fig. 4. Total amount of anabolic steroid excreted in the urine of participants. Values represent the summation of parent compound plus all measurable metabolites. Both oral and parenteral forms of anabolic steroids are represented.

($p < 0.05$). In contrast, single anabolic steroid use did not suppress excretion of the major glucocorticoid metabolites THE, THF and *allo*-THF in urine (Fig. 3).

An unexpected finding of this study was that only a minor amount of the anabolic steroid administered was excreted in the urine (Fig. 4), either as the parent compound or metabolites of the anabolic steroid. Out of the average 25 mg per 24 h anabolic steroid administered, less than 4 mg per 24 h could be accounted for by urinary excretion of the anabolic steroid or steroid metabolites. This observation applied to all four of the anabolic steroids used in this study regardless of whether the steroid was administered orally or parenterally. The remainder of the anabolic steroid administered is likely either sequestered within the body or excreted in the feces, although we have not investigated these possibilities.

The pattern of excretion of the major anabolic steroid metabolites following long-term use did not differ from previous observations of short-term use [6, 8, 9]. The major steroid metabolites identified and the mass spectra ion peaks are shown in Table I.

Previous studies in this laboratory found that long-term concurrent use of multiple anabolic steroids can reduce excretion of urinary steroids [3]. The results from the present study indicate single anabolic steroids can reduce excretion of androgen metabolites. Specific individuals appear to have suppressed glucocorticoid metabolite excretion following anabolic steroid use, but this observation was not sustained for the entire group studied. Further studies are currently underway to determine which (if any) specific anabolic steroid is more effective in causing the endocrine effects noted. Finally, the relatively small amount of anabolic steroid excreted in the urine has not been reported previously, to our knowledge, and the reason for this phenomenon cannot be explained by this study.

REFERENCES

1 G. Perlmutter and D.T. Lowenthal, Am. Fam. Physician, 32 (1985) 208.
2 J.D. Wilson, Endocr. Rev., 9 (1988) 181.
3 P.V. Fennessey, R.W. Gotlin, D. Martin, S. Smith and L.M. Harrison, J. Pharm. Biomed. Anal., 6 (1988) 999.
4 E. Houghton and P. Teale, Biomed. Mass Spectrom., 8 (1981) 358.
5 H.W. Dürbeck, I. Büker, B. Scheulen and B. Telin, J. Chromatogr., 167 (1978) 117.
6 G.P. Cartoni, M. Ciardi, A. Giarrusso and F. Rosati, J. Chromatogr., 279 (1983) 515.
7 A.W. Pike, C. Moynihan, S. Kibler, P.G. Marsh and P.V. Fennessey, J. Chromatogr., 306 (1984) 39.
8 H.W. Durbeck and I. Buker, Biomed. Mass Spectrom., 7 (1980) 437.
9 C.K. Halton and D.H. Caltin, Clin. Lab. Med., 7 (1987) 655.