Incidental Clostebol Contamination in Athletes after Sexual Intercourse

To the Editor:

Clostebol is a synthetic androgenic steroid with anabolic effects that is frequently used in sports to increase physical performance. Because of medical and ethical reasons, the use of clostebol is prohibited by the International Olympic Committee (IOC) (1), and its misuse would fall under the strict liability rule of the IOC and the World Antidoping Agency. It is therefore the responsibility of athletes to submit evidence contrary to any ruling issued against them by the appropriate sports body. Despite the prohibition against the use of clostebol, abuse of this steroid is increasing, mainly in Brazilian athletes. In Brazil, clostebol acetate is present in medicines for dermatologic and gynecologic treatments, whereas in the US, the Food and Drug Administration does not approve of the use of medicines that contain anabolic agents.

Our laboratory, LABDOP, is accredited by the IOC and in the past 3 years has encountered four urine samples that contained clostebol metabolites. One male athlete whose urine tested positive for traces of clostebol metabolites claimed that he was contaminated as a result of sexual intercourse with a woman taking a medication containing clostebol. The IOC did not exonerate him from the results reported by LABDOP. The remaining athletes maintained that the presence of clostebol metabolites in their urine was the result of using clostebol-containing medications. Despite this controversy, the directive from the IOC has been followed, and positive results are always enforced. A previous publication by Debruyckere et al. (2) showed the presence of clostebol metabolites in human urine after oral intake of contaminated meat, but did not mention sexual intercourse.

LABDOP undertook the present study to determine whether the urine of men exposed to intravaginal clostebol acetate during sexual intercourse contains clostebol metabolites. A gas chromatographic-mass spectrometric method (3, 4) was used to test for the presence of two metabolites of clostebol, clostebol-M1 (4-chloroandrostan-4-en-3α-ol-17-one) and clostebol-M2 (4-chloroandrost-3α-ol-17-one), and other steroids in urine samples. The procedure involves preextraction with XAD-2 resin, elution with tert-butyl methyl ether, hydrolysis with β-glucuronidase from Escherichia coli, extraction with n-pentane, and derivatization at 60°C for 60 min with a solution containing 1 mL of N-methyl-N-(trimethylsilyl)trifluoroacetamide, 2 μg of NH₄I, and 6 μL of 2-mercaptoethanol (3, 4). The analytes were monitored in selected-ion monitoring mode.

In Brazil, clostebol acetate is available for intravaginal administration. One such preparation (Trofodermin™; Searle) contains 200 mg of clostebol acetate and 200 mg of neomycin sulfate per 40-g blister. The package insert states that Trofodermin is indicated for cervicitis, postoperative vaginitis, and ulcerative vaginitis, and the recommended dose is 5 g once or twice a day. Two healthy couples (group I) and two healthy men (group II) were involved in the study. A baseline urine was obtained from all volunteers before exposure to clostebol acetate. Participants were healthy and without a history of drug use or gynecologic disease. The study was approved by the University ethics committee (protocol 168/02). Immediately after intravaginal application of 5 g of clostebol acetate, group I had sexual intercourse lasting ~20 min (experiment I). In experiment II, the men in group II applied 200 mg of clostebol acetate topically to their penis for 20 min. Urine samples were collected from all participant volunteers for the following 2 days.

The urine of the men in experiment I contained trace amounts of clostebol-M1 (0.9–3.5 μg/L) with a tₘₐₓ of 16 h. The concentration of clostebol-M1 in the urine of the females reached a maximum of 35 μg/L after 23 h. Small amounts of clostebol-M2 were also detected. The urine of the men in experiment II contained higher amounts of clostebol-M1, with a peak concentration of 22 μg/L after 3.5 h, and was detectable for 15 h. The baseline urines contained no clostebol, clostebol-M1, or clostebol-M2. The possibility of incidental contamination from sexual intercourse was confirmed, despite the fact that the amount of clostebol-M1 (long-term metabolite) was near the limit of detection (μg/L). Because the IOC does not make a distinction among circumstances or means of administration of anabolic compounds, athletes should be warned not to use clostebol-containing medications and to be aware of their partner’s medical treatments.

References
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Evaluation of the Comparability of Commercial Ghrelin Assays

To the Editor:

Several assays are available to measure human ghrelin (h-GHS), but the comparability of results among assays is unknown. As an addition to previous investigations concerning the stability of h-GHS (1), it was our objective to compare the two most commonly used commercial h-GHS RIAs, focusing on side-by-side determinations, recoveries, and assay imprecision.

The assays were purchased from Phoenix Pharmaceuticals (cat. no. RK-031-30) and Linco Research (cat. no. GHRT-89HK). Samples were stored at −80 °C and assayed as recommended by the manufacturers.

For side-by-side comparisons, we used anonymized surplus sera (n = 151) from clinical studies of lean and obese individuals [mean body mass index (BMI), 26 kg/m²; range, 17–39 kg/m²]. Separate side-by-side comparisons were performed with 68 samples from two individuals (34/profile; BMI, 19.5 kg/m²) taken at 20-min intervals to evaluate the suitability of the assays for determining intraindividual changes. All participants gave informed consent.

For recovery and imprecision studies, serum was collected from four males (27–33 years of age; no drug use; normal blood pressure; BMI, 22–24 kg/m²). Assay calibrators were added to achieve added concentrations of 100, 200, 400, or 800 ng/L. Each was measured 20 times. Recovery was calculated by subtracting the basal value of the sample. The CV was determined for each sample as well as for a Lyphochek control serum (cat. no. 40141; Bio-Rad), which was also measured 20 times. The interassay CV was calculated from repeated measurements of the Lyphochek control (n = 12) and pooled serum controls (n = 12) by the Phoenix method over a longer period. No data for the interassay CV of the Linco assay are available because it had not been used previously in our laboratories.

EVAPAK 3.0 evaluation software (Boehringer-Mannheim) was used for linear regression analysis (Passing–Bablok) (2).

Each assay uses 100 μL of sample. Linco provides ready-to-use calibrators; Phoenix provides a lyophilized preparation that must first be dissolved in assay buffer and then diluted repeatedly. Linco, in contrast to Phoenix, provides controls (two) per assay.

Both assays have a 100% cross-reactivity with h-des-octanoyl-GHS. Moreover, the Linco assay can be used for rodent and canine sera, whereas the specificity of the Phoenix antibody surprisingly changed between January 2003 (lot 500117) and August 2002 (lot 419523) to 1% cross-reactivity with non-human GHS.

Linco gives the concentrations in ng/L, whereas Phoenix gives concentrations in pg/tube, presented in a general protocol for peptide RIAs. The Phoenix calibration curve includes concentrations of 10–1280 ng/L. The calibrators of the Linco assay range from 100 to 10 000 ng/L.

In each assay, a better curve fit was obtained when the two lowest calibrators were excluded.

The side-by-side comparison yielded linear regressions of r = 0.976 (n = 151) and r = 0.890 (intraindividual profiles). The measured values obtained with the Phoenix RIA ranged from 10 to 1240 ng/L (median, 340 ng/L), whereas the measured values obtained with the Linco assay were 10-fold higher (131–11 666 ng/L; median, 3282 ng/L). The regression is shown in Fig. 1. The 10-fold difference was observed neither when the calibrator of the Linco assay was replaced by the calibrator of the Phoenix assay nor vice versa.

Recovery of h-GHS was 54–104% [mean (SD), 75 (22)%] with the Linco assay and 67–123% with the Phoenix assay, with the higher recoveries at higher concentrations of added h-GHS.

Intraassay CV for the Phoenix method were 8–12% at the lower concentrations and 7–8% at higher concentrations. The CV of the Linco assay were 7–11% in the lower range and 5–9% in the upper concentration range.

We consider intraassay CV <10%.

Fig. 1. Linear regression between h-GHS concentrations in serum samples (n = 151; r = 0.976; r = 0.805).