

Urinary Testosterone Equivalent Levels in Mature Male and Female Racing Camels.

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ABSTRACT

Testosterone, the principal androgen, is synthesized in the testes, the ovary and the adrenal cortex. It is responsible for the development and maintenance of the male secondary sex characteristics. It also exerts growth-promoting effects and important protein anabolic response to exercise. These later effects have been utilized as a doping agent. Since testosterone equivalent is present endogenously in male and female animals, a threshold level needs to be established in order to identify exogenous administration. For this reason testosterone equivalents levels were measured in the urine of 125 mature female and 56 male racing camels. Urine samples were subjected to solvolysis and testosterone equivalent levels were measured by ELISA. The data showed that testosterone equivalent levels were normally distributed in male, but not in female, camels and were therefore log transformed. A log means plus four standard deviations were proposed which should safely cover the maximum endogenous levels which are 1.6 and 0.63 ng/ml for male and female respectively. These values have been used as a cut off in this laboratory.

Key words: Testosterone, Urine, Racing, Camel.

INTRODUCTION

The use of anabolic steroids in athletics as adjuvant to improve strength and performance was first reported in the 1950s. From that time the use of anabolic steroids among athletes has increased and their misuse has reached epidemic proportion in some sporting disciplines. On the basis of sport ethics and the prospects of the adverse health effects, the International Olympic Committee (IOC)

has prohibited the use of anabolic steroids in athletics (Report of International Olympic Committee Medical Commission, Innsbruck, 1974).

The ban of anabolic steroid misuse in the athletic community has prompted several scientists to develop analytical methodologies that would allow a large number of samples to be reliable and economically screened in relatively short periods of time (Jondorf and Moss; 1978). The primary screening procedure for anabolic steroids in this laboratory is ELISA. The camel racing authorities in the United Arab Emirates (UAE) are obliged to introduce “doping control” which has been established for horses and dogs. Thus, the biological samples obtained from camels immediately after racing should be free of any exogenous drugs. Synthetic anabolic steroids in the veterinary field may be valuable in conditions associated with high rate of tissue break down, or in case of postoperative convalescence (Brander and Pugh, 1977).

Some anabolic steroids have also been evaluated for their effects on lean body masses and protein synthesis (Wynn, 1967; Forbs, 1985; Griggs, et al., 1989). Thus, anabolic steroids can improve the condition of debilitated animals and affect their weight gain and performance positively. Anabolic steroids can also affect the pack cell volumes of the blood (Wilson et al., 1993). Due to the endogenous presence of some anabolic steroids and their metabolite in the male or female camel’s urine, the detection of exogenous administration of these anabolic agents requires the determination of the threshold value.

MATERIALS AND METHODS

ELISA Kit contents

The nandrolone test commercially available from Neogen Corporation of Lexington, KY, U.S.A. which contained EIA buffer, wash buffer, K-blue substrate, nandrolone enzyme conjugate and nandrolone anti body pre-coated plate of 96 wells.

Chemicals, glassware and solvents

All glassware was siliconized with a dichlorooctamethyltetrasiloxane fluid (PIERCE) before use. Sulfuric

acid and ethyl acetate were of analytical grade. Water was purified by reverse osmosis and filtered over an Elgastat UHQ11 water purification system (Jencons) before use. Reference testosterone (4-Androsten-17 β -ol-3-one) was obtained from Sigma and kept at room temperature in the dark until use. Blank reference urine was collected from a young female camel (6 months old) as described by Wasfi, et al., (1997). Testosterone standards were prepared in methanol (10 mg/ml).

Sample preparations

Samples were prepared by solvolysis according to (Houghton et al., 1990) a hundred micro-liters of centrifuged urine sample was placed in a glass tube. Acidified ethyl acetate 900 μ l (100 μ l concentrated sulfuric acid: 50 ml ethyl acetate) was added as a supernatant and incubated overnight at 37 °C. An aliquot of (100 μ l) of the supernatant was transferred to an assay tube. The solvent was removed by evaporation under nitrogen reduced pressure (Zymark). The residue was reconstituted in 100 μ l EIA assay buffer and directly used (20 μ l) for ELISA as described by the manufacturer, Neogen Corporation of Lexington, K.Y., U.S.A; Nandrolone kit number 104615 (5 kits bulk). Each sample was analyzed in duplicate.

RESULTS

Accuracy and precision of the ELISA – intra-assay variation (coefficient of variation CV) was determined for the blank reference urine, mature male and female camels. Each sample was subjected to solvolysis once and pipette in duplicate (n = 4) onto a single plate. Mean concentrations were 0.0003 (young female, CV = 2.24%), 0.056 (mature female, CV = 9.13%) and 11.4 (mature male, CV = 3.19) ng/ml. Thus, intra-assay CV ranged from 2.24 to 9.13%.

Inter assay variation and recovery were determined by measuring blank reference urine with added testosterone at 5 concentrations (0.01, 0.1, 1, 10 and 20 ng/ml). For each concentration, 4 aliquots of blank reference urine had the corresponding amount of testosterone added, then each sample was subjected to solvolysis once and pipetted in duplicate onto a single plate. The calibration curves (2 to 4000 pg/well) were prepared in the blank reference urine to determine the recovery of added

testosterone. The mean values obtained from different assays were 0.012 ± 0.002 ng/ml (CV = 16.7%, recovery = 120.%) for 0.01 ng/ml, 0.07 ± 0.018 ng/ml (CV = 26.08%, recovery 70%) for 0.1 ng/ml, 1.006 ± 0.002 ng/ml (CV = 0.21%, recovery = 100%) for 1 ng/ml and 13.91 ± 0.6 ng/ml (CV = 4.3%, recovery = 139%) for 10 ng/ml, and 18.94 ± 1.37 ng/ml (CV = 5.1%, recovery = 94.7%) for 20 ng/ml. Thus inter assay variation ranged from 0.21 to 26.08%. The analytical method was used to determine the concentration values in post-race urine samples from 56 mature males and 125 female camels. The data showed that testosterone equivalent levels were normally distributed in males, but not in female camels (Table 1). Therefore the data was log transformed (Table 2). A log means plus four standard deviations were proposed which safely cover the maximum endogenous levels that are 1.6 and 0.63 ng/ml for male and female camels respectively. This value has been used as threshold in this laboratory.

Table 1: The means concentration of testosterone equivalent in male and female camel urine

| Camel | No. camels | Mean ng/ml | SD | SEM | Median |
|--------|------------|------------|------|--------|--------|
| Male | 56 | 12.3 | 3.72 | 0.497 | 11.5 |
| Female | 125 | 0.0879 | 0.14 | 0.0125 | 0.042 |

Table 2: The Log means concentration of testosterone equivalent in male and female camel urine

| Camels | No. Camels | Mean ng/ml | SD | SEM | Median |
|--------|------------|------------|-------|--------|--------|
| Male | 56 | 1.07 | 0.132 | 0.0177 | 1.06 |
| Female | 125 | -1.31 | 0.484 | 0.0449 | -1.31 |

DISCUSSION

Since testosterone has both androgenic and anabolic activities, its use in the field of human athletics and other sports as an anabolic agent would appear to be limited, as a large number of anabolic steroids have been developed with less androgenic activity. The anabolic effect of androgens is more pronounced in boys before puberty, women and the castrate than in normal men (Wilson et al., 1993). This observation may be significant in the field of camel racing as 100% of female camels urine is analyzed routinely in this laboratory. It is thus not surprising that racing camels may have been given the parent steroids of the proprietary veterinary anabolic preparations (testosterone, nandrolone, boldenone and trenblone). Due to cross reactivity among these four steroids, immunoassay is non specific and does not allow for the differentiation between endogenous hormones excreted in urine and the administered one or their metabolites. The antibody rose to 19-nortestosterone can be used not only for the ready detection of 19-nortestosterone itself, but it has potential for preliminary ELISA screening of related anabolic steroids in biological fluids of special interest (Jondorf, 1977).

Under normal circumstances, any endogenous steroid's hormone levels in mature male and female racing camel urine should be below the threshold reported in the present study. This screening, in conjunction with appropriate gas chromatography/mass spectrometry (GC/MS and GC/MS/MS) technique of confirmatory analysis (Houghton, 1977 and Houghton et al., 1985) gives the required degree of confidence in detecting exogenous steroids related to testosterone (Houghton, 1979) and 19 nortestosterone (Houghton, 1977).

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