Study of Endogenous Profile of hCG in Male Brazilian Athletes

Introduction

Human Chorionic Gonadotrophin (hCG) is classified in the annual WADA’s prohibited list in section S.3 as one of the peptide hormones used as doping by athletes. The misuse of this hormone by male athletes has basically two purposes: i) to stimulate the endogenous secretion of testosterone (T), maintaining the epitestosterone/testosterone (T/E) ratio unchanged and ii) regression of the testicular atrophy due to anabolic steroids misuse. Since hCG is an endogenous substance, to consolidate an adverse analytical finding (AAF), it demands a strategy which allows to differ the hCG naturally produced from the one exogenously administrated. Following the WADA’s recommendation, an hCG AAF can be established only if the analyzed urine shows that the hormone concentration is higher than the expected one. Meanwhile, in the lack of a clearer pattern of what would be an “expected level”, the doping control laboratories have been applying cut off values between 5 and 25 mIU/mL, sometimes with no clear statistic basis.

Another factor that must be considered is the diversity of available immunoassays for the detection of hCG which may use different epitopes. Such a fact creates the necessity of considering not only the population studied but also the methodology applied in the establishment of a criterion of evaluation. This way, the evidence of the exogenous misuse must fall back in the detection of levels that exceed an interval of reference previously established to a certain population when analyzed through a determined analytical method.

The aim of the actual study is the establishment of a cut off value for the characterization of an AAF through the statistical examination of the profile of urinary excretion of hCG in male athletes obtained with the usage of Microparticles Enzyme Immunoassays (MEIA - Abbott System). As the MEIA technique was originally developed...
for plasma analysis, a full validation protocol was done aiming to evaluate the performance of the assay in urine analysis.

**Experimental**

**Total Antibody β-hCG:** Anti-βC terminal + anti – β1.

**Specificity:** Analyte cleaved + intact + β free.

**Sample preparation:** Urine samples were analysed after previous centrifugation (3000 rpm during 5 min). No other procedure of manipulation was used.

**Controls:** To each analytical batch, hCG controls were analyzed in plasma concentrations 25mIU/mL and 100mIU/mL. According to the manufacturer the control should fall in an interval of 30% of the nominal value.

**Instrumental Conditions:** The analyses were accomplished after procedure of calibration and maintenance according to specifications of the manufacturer.

**Validation of the Analysis procedure:** The method was validated through the calculation of the sensitivity, specificity, linearity, exactness, repeatability and robustness according to protocol suggested by the Guideline of Validation of Analytical and Bioanalytical Methods deliberation RE 899, May 29th, 2003, National Agency of Sanitary Vigilance (ANVISA), Health Ministry³.

**Results**

The method was validated for analysis of human urine through protocol recommended by ANVISA³, obtaining repeatability lower than 10% in this matrix to the concentration level of 1 mU/mL or higher. To the level of 0.5 mU/mL the repeatability was 15%, so this concentration was considered as the quantification limit. The recovery was between 100 and 120%. Linearity was evaluated considering the concentrations 0.5, 1, 3, 5, 10, 25, 50 and 100 mIU/mL, \( y = 1.176x – 0.1074 \) obtaining the coefficient of determination \( (r^2) > 0.99 \). The robustness of the method was evaluated through urine analysis with pH and/or density altered, endogenous steroid profile demonstrating bacterium degradation and raised sedimentation. Of all the criteria evaluated, the presence of sediment showed a great interference in the results, which indicates that the centrifugation step is critical for the elimination of the noise derived from the matrix. However, considering the diversity of the Brazilian population, the sampling involved Caucasians, Afro-Americans and Indo-Americans descendents. One thousand two hundred and forty six (1,246) male athletes had their urines analyzed by the MEIA technique.
for Total hCG (Abbott IMx) after previous centrifugation. For each analytical batch, hCG controls in plasma with concentrations of 25 mIU/mL (Fig. 1) and 100 mIU/mL were tested showing a maximum variation of 30%, as specified by the manufacturer. An abnormal level of hCG resulting in some false-positive had been described for blood analysis with commercial available immunoassays, including Abbott System. Some reasons have been presented for these results as cross reactivity, lack of dilution protocol, presence in serum of auto- and heterophilic antibodies. In our hands, the presence of sediments still seem to be the most significant factor for the elevation of the background in procedures involving urine analysis with MEIA. The manufacturer stipulates 5 mIU/mL as the detection limit in plasma analysis. All the results above this value (1.25%) were reanalyzed three more times. In all those samples the obtained results were below 1 mIU/mL. The population target was evaluated for normal distribution through D’Agostino & Pearson and Shapiro-Wilk tests. Those tests demonstrated that the distribution showed a non-normal profile (p < 0.0001).

That fact leaded to the adoption of a non-parametric approach on the evaluation of the results. The estimate for a cut off value for hCG analyzed by MEIA β-hCG total technique, was obtained through two different approaches i) the inference of a far outside value and ii) the calculation of the reference interval. The calculation of the extreme value was previously used by Delbeke et al. on the analysis of the profile of hCG excretion in athletes and is based on the evaluation of the population interquartile. In the studied population, the interquartile interval was 0.32 mIU/mL. The extreme value was then calculated through the equation (percentile 75% + (3 x interquartile interval)) obtaining the value of 2.38 mIU/mL, comparable to 2.28 mIU/mL found by Delbeke et al. Independently, the reference intervals of the population were calculated through the estimate of the inter-fractal interval (95%). Once again, the non-parametric approach was utilized as suggested by the Specialists Panel in interval values of reference of IFCC and by the Committee of Patronizing Reference Values of ICSH. The reference value obtained was 3.06 mIU/mL with an interval of confidence at 90% of (2.83 – 3.87). It seems that the value 5 mIU/mL is superior either to the extreme value as well as to the reference value considering the population and the method utilized. This fact suggests 5 mIU/mL as the candidate for a cut off value, since the 10 mIU/mL could lead to an increased index of false negative results.
**Conclusion**

Male athletes use hCG aiming the increase of the production of endogenous testosterone. The evidence of the exogenous misuse must fall back in the detection of levels that exceed an interval of reference previously established to a certain population when analyzed through a determined analytical method. Two statistical approaches were used. Both traditionally applied to calculate reference intervals for clinical parameters among the human population and formerly used for doping control cut off values definition. The approach of the inference of the “far outside value” gave concentration of 2.38 mIU/mL and the calculation of the reference interval gave a value of 3.06 mIU/mL with an interval of confidence at 90% of (2.83 – 3.87). Therefore, the suggested cut off value 5 mIU/mL is suitable for determining an adverse analytical finding, considering the population of Brazilian athletes and the MEIA (Abbott System) technique.

**References**