

Endogenous steroid profiling in urine and blood for the detection of HCG abuse in sport

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Introduction

The administration of Human Chorionic Gonadotropin (HCG) is known to increase testosterone production in the testis and to reduce the LH production through a negative feedback (Fig 1). Furthermore, HCG is used to normalize testicular testosterone production that is suppressed during and after prolonged use of anabolic steroids. The intake of HCG is prohibited in sports, but there is today no efficient test to evidence its abuse.

The present study examined the possibility to use steroid profiling in urine and other hormones levels in serum to detect the misuse of HCG in a male population.

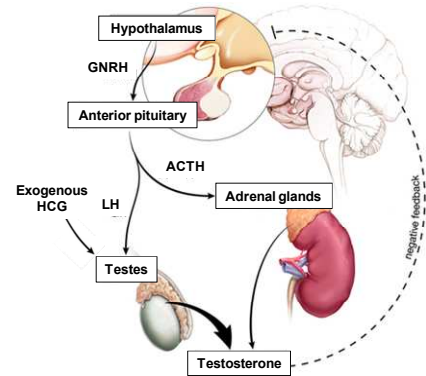


Fig 1. Effect of exogenous HCG on the production of testosterone in male (Based on Aragon-Ching JB et al. *Frontiers in Bioscience* 12, 2007)

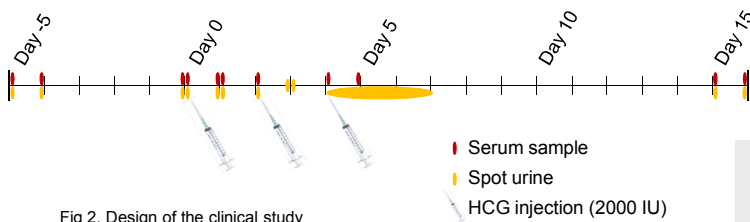


Fig 2. Design of the clinical study

Results & Discussion

Preliminary results management was performed using a profile of several hormone levels and concentration ratio over the time of the study. Since the urinary excretion of testosterone glucuronide is strongly correlated to the UGT2B17 gene polymorphism¹, the subjects were divided into two groups, with the gene (n = 8) and without (n = 2). The levels of testosterone in serum and urine increased significantly after the injection of HCG (Fig 3.a) for the 10 subjects. As the LH production is decreased by a negative feedback, the T/LH ratio increases significantly in all profiles (Fig 3.b). Finally, the ratio testosterone over androsterone (T/A) in urine seems to be a valuable biomarker of HCG misuse since this ratio was significantly influenced in 9 of the 10 volunteers' profiles (Fig 3.c). It is important to note that such biomarkers are submitted to a large inter-individual variability regarding metabolisms, particularly during the HCG treatment.

¹ Jakobsson et al. *J. Clin. Endocrinol. Metab.* 91, 2006

Method

The cohort was composed of ten males aged from 21 to 29. They were submitted to three subsequent injections of HCG (3 x 2000 IU of Choriomon[®]) on days 0, 2 and 4 of the study. Serum and urine samples were collected before, during and after the HCG treatment (Fig 2).

The steroid profile (free and glucuroconjugated forms of testosterone, epitestosterone, androsterone, etiocholanolone, α -diol, β -diol, DHEA and DHT) in urine was determined after hydrolysis and derivatization prior to GC-MS analysis. The levels of other hormones (total HCG, free β -HCG, LH, SHBG and testosterone) in serum were determined using specific kits for Immulite[®] 2000 from Siemens (Deerfield, IL, USA).

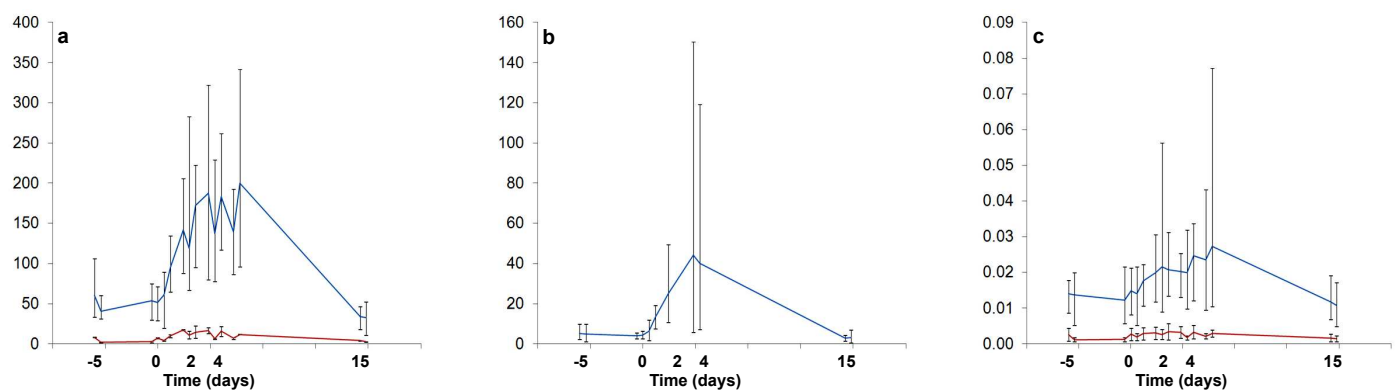


Fig 3. Mean excretion profile (n=10) for a) testosterone in urine, b) T/LH ratio in serum and c) T/A ratio in urine. The whiskers represent the minimum and maximum values at each time. Levels and ratios in urine are separated in two groups, the blue line corresponds to subjects with UGT2B17 gene and red line corresponds to subjects with an UGT2B17 deletion.

Conclusions

The misuse of HCG leads to a rapid increase of testosterone production and excretion in urine in both groups of subjects regardless the UGT2B17 polymorphism. The most discriminative biomarker appears to be the T/LH ratio in serum. In addition, the T/A ratio seems to be an interesting biomarker deduced from the steroid profile in urine. The sensitivity and specificity of these biomarkers have to be determined in further analyses using a biological passport approach in order to obtain a longitudinal follow-up of the HCG effect on selected hormone levels.