"Development of standardized methods for determination of hCG in urine"

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Project overview

Human chorionic gonadotropin (hCG) is used to restore gonadal function after use of anabolic hormones. These suppress gonadal function by inhibiting pituitary secretion of luteinizing hormone (LH) and follicle stimulating hormone. hCG stimulates steroid production in the gonads after use of anabolic steroids. hCG is available as pharmaceutical product and is used for doping.

Use of gonadotropins in sports is prohibited in males. hCG can be detected by immunological techniques in urine 7-10 days after administration. A concentration exceeding 5 IU/L is considered positive. Various hCG assays are used in anti-doping laboratories although results obtained by these differ. Urine contains degradation products of hCG, which different assays detect differently. Presently available hCG assays are clinically approved for use on serum but not for urine. The excretion rate of urine varies causing up to 10-fold variation in hCG concentration. Measuring urinary creatinine and correcting the hCG result accordingly can compensate for this, but these methods have not been validated. Furthermore, hCG immunoreactivity may be lost when urine is stored at -20 C and also by adsorption of hCG to the collection tubes.

We propose to develop standardized methods for determination of hCG in urine. The project comprises the following parts.
1.Characterization of the forms of hCG in urine after parenteral administration of hCG.
2. Development of a reference method for determination of hCG in urine including correction for variation in urine excretion rate.
3. Establishment of procedures for collection and storage of urine before assay.
4. Establishment of reference values for various forms of hCG in urine from males.
5. Comparison of the ability of selected commercial assays to identify the various forms of hCG occurring in urine.
6. Development of quality control procedures for assay of hCG in urine.

Results and Conclusions

In order to evaluate methods to be used in doping control for human chorionic gonadotropin (hCG), we have determined the urine concentrations of intact hCG and its subunits, hCGβ, hCGα and the core fragment of hCGβ (hCGβcf) in about

1000 doping control urine samples from male athletes, who agreed to the use of their samples for research purposes. In addition, hCG and hCGβwere determined in samples obtained during the first 9 days after injection of urinary or recombinant hCG to 12 male volunteers.

The results obtained by various hCG assays varied considerably, but results above 5 IU/L were observed in only 3 samples with two assays. After correction for urine density, no sample had a result above 3 IU/L in two different assays. With the present decision limit of 5 IU/L, no falsely elevated results were obtained if a positive test required elevated results with two different assays.
The problem caused by adsorption of hCG to the precipitate forming when urine is frozen needs to be taken into account when selecting methods and sample handling protocols to be used for doping control. In the present study, the samples were not frozen before analysis and thus the results for these are valid for establishment of cut-off values for doping control provided that samples are not frozen before assay. Methods to avoid formation of precipitates in frozen urine need to be developed.

Of the methods studied, the AutoDelfia and Delfia Express assays are best suited for doping control.