

S. Voss, A. Gotzmann, H. Geyer, U. Mareck, W. Schänzer

Evaluation of LH Concentration in Male and Female Urines. Effects of a Single LH Application on the Steroid Profile

Institute of Biochemistry Cologne, Carl-Diem-Weg 6, D50933 Köln, Germany

Abstract

According to rules of the World Anti-Doping Agency [WADA] lutenizing hormone [LH] belongs to the list of forbidden substances in men and since January 2005 also in women [1]. Aim of the study was to investigate urinary LH reference values for men and women. Urinary LH concentrations of 483 samples of European male and female athletes were estimated by a microparticle enzyme immuno assay [MEIA]. In addition the LH concentrations in morning urines of one man and two women, one with and one without contraceptive, were analysed over one month in order to obtain the respective LH profile. To investigate whether increased LH values after administration of Lutropin can be detected and to study possible changes in the steroid profile, urinary LH was estimated before and after the administration of a single dose of 75 IU LH (Luveris® from Serono).

Introduction

LH is a peptide hormone, which is pulsatile secreted by the pituitary gland. In men LH stimulates the leydig cells to produce testosterone. In women LH plays an important role for ovulation. In female menstrual cycle an expansive variability of LH concentrations in blood can be found. Data of urinary LH concentrations are missing. Actually there are no data to distinguish between negative samples and samples after misuse of LH in sport (adverse analytical finding). Beside this it is unknown whether the application of small doses of LH, e.g. 75 IU, will change urinary LH- or androgenic steroid concentrations [2, 3].

Experimental

The analysed urine samples were doping control samples of the Cologne anti-doping laboratory: 232 male and 251 female samples, in- and out-of competition from different national and international European sports federations. The urinary LH profiles of three volunteers, one male and two females, were estimated by analysing the respective morning urines over the period of one month. The collecting period for the females represents one

menstrual cycle. The female volunteers (study without medication), 27 and 29 years old, were healthy and active in sports. In addition the male subject, 27 years old, healthy and active in sport too, received one single injection of 75 IU of LH (Lutropin alpha, Luveris®, Serono) subcutaneously in the morning (9 a.m.). This excretion study was medically monitored. All urine samples were collected within the first 15 hours after administration. Urinary LH concentration of all samples was determined using a MEIA test [4] after a previous centrifugation step of the native urine at 1150 g for 5 minutes (Hermle, Wehingen, D). The LH reagent packs, LH controls (5, 40, 80 mIU/ml) and LH calibrators (0, 2, 10, 25, 250 mIU/ml) were obtained from Abbott (Wiesbaden, D). The sample preparation to evaluate the androgenic steroid profile was performed according to screening 4 procedure - sample preparation for anabolic androgenic steroids [5].

Results and Discussion

Figure 1 shows the distribution of urinary LH concentration in male and female under the condition of doping control. It can be observed that in 69% of the female samples LH concentrations range between 0 – 4 mIU/ml. In comparison 54% of the male samples are in the same range. In the range between 4 – 8 and 8 – 12 mIU/ml male values are dominant or even equal (35% vs 19%, 8% vs 6%). The highest LH concentration in woman is estimated to be 102 mIU/ml, while the highest value in men is 19 mIU/ml. Figure 2 and 3 show the urinary LH concentration over the period of one month. Figure 2 shows the absolute LH concentrations and figure 3 the LH excretion rates.

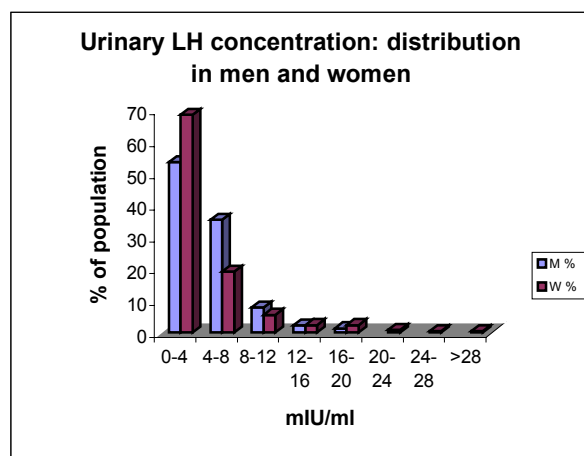


Figure 1

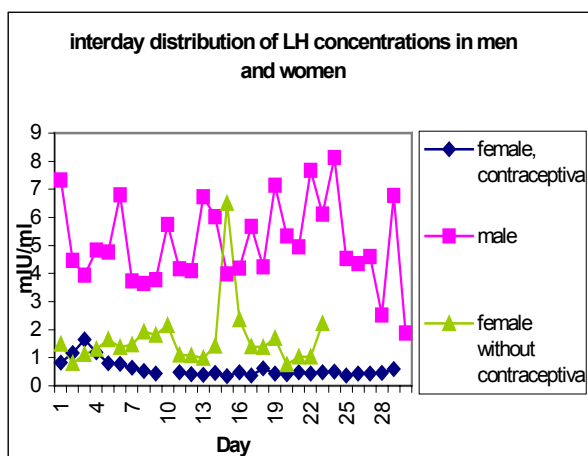


Figure 2

Observations:

1. The LH concentrations in male show a wide inter-day variability (lowest value 2 mIU/ml, highest value 8 mIU/ml).

2. The female volunteer without contraceptive shows a typical profile of the female menstrual cycle. The highest LH concentration is estimated with 7 mIU/ml and can be associated with the ovulation. A higher density of measuring points during this period might result in even higher values. The concentrations around the time of ovulation range between 0,8 mIU/ml and 2,2 mIU/ml.
3. The female volunteer with contraceptive (Lovellev®) shows lower urinary LH concentrations (lowest 0,4 mIU/ml, highest 1,7 mIU/ml). The low concentrations can be associated with a LH suppression caused by the contraceptive.

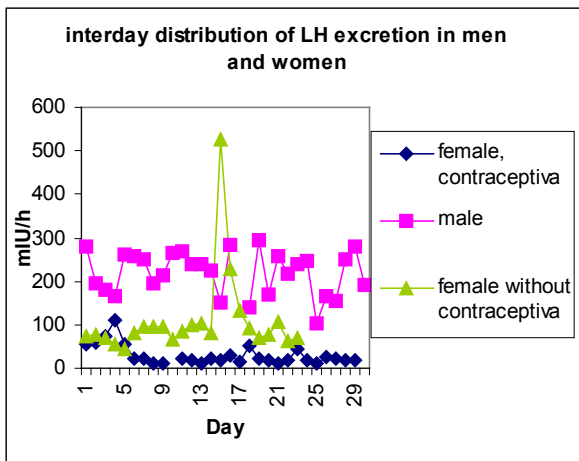


Figure 3

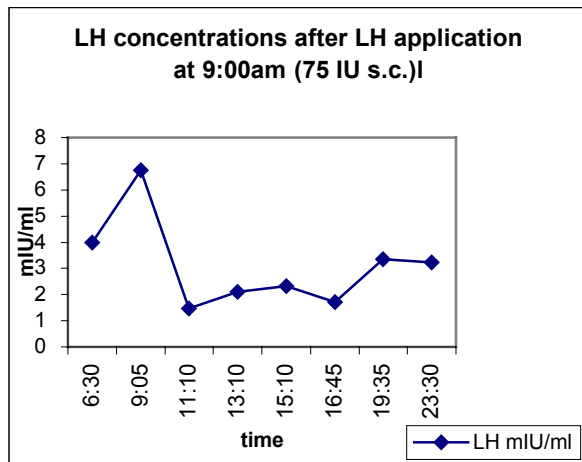


Figure 4

The LH values after application of one single dose of Luveris® do not show a significant increase in urinary LH concentrations compared to the LH concentrations estimated over one month. A detection of the exogenous LH administration is not possible by the applied method of quantitation (Figure 4). The investigations in steroid profiles after LH application do not show a significant increase in urinary testosterone or epitestosterone concentrations, but in the excretion rates of these hormones. Testosterone and epitestosterone excretion is slightly elevated for about six hours compared to normal values without medication (Figure 5,6).

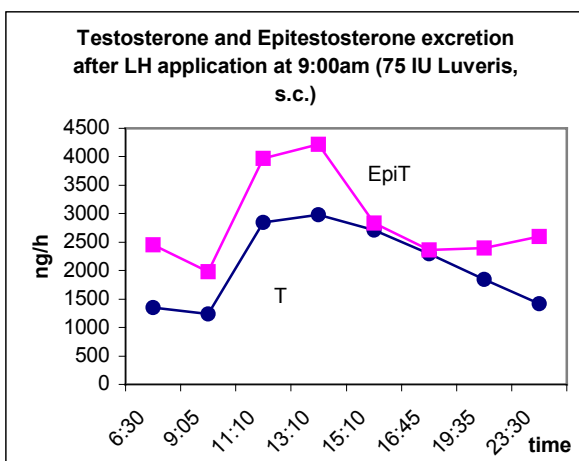


Figure 5

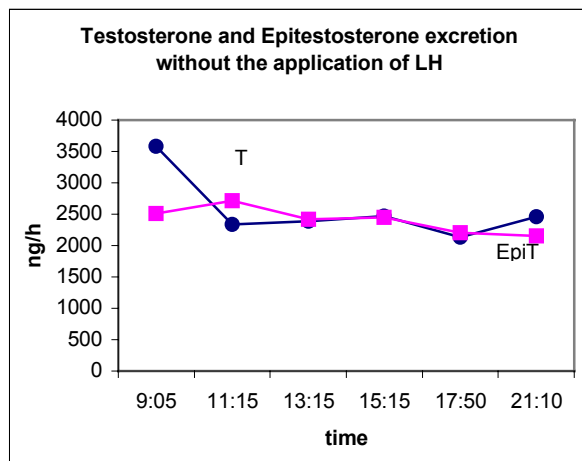


Figure 6

Conclusions

- It is not possible to detect LH misuse after one single dose by quantitation of urinary LH.
- There seems to be a stimulating effect of LH on the urinary testosterone and epitestosterone excretion, which may correspond to an increased steroid production.
- Further studies should be performed in order to proof the effect of small doses of LH on the steroid profile in healthy persons with a sufficient pool of volunteers. An application in regular intervals and the analysis of LH in blood as well as in urine would be of interest too.

Acknowledgments

We acknowledge the provision of Luveris® by Serono© and financial support by the Manfred Donike Society, Cologne.

References

- [1] World Anti-Doping Agency: The 2005 Prohibited List, Montreal 2005
- [2] Kicman, A.T.; Cowan, D.A.: Peptide hormones and sport: Misuse and detection. *Br Med Bull.* 1992, 48:496-517
- [3] Wu, M; Liu, X., Wang, S.; Shen, L.: Detection of LH (luteinizing hormone) in sport – one year statistics. In: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U. (eds.) *Recent Advances in Doping Analysis (9)*. Sport und Buch Strauß, Köln 2001, 317-320
- [4] Perry, P.J.; MacIndoe, J.H.; Yates, W.R.; Scott, D.S.; Holman T.L.: Detection of anabolic steroid administration: ratio of urinary testosterone to epitestosterone vs the ratio of urinary testosterone to luteinizing hormone. *Clin Chem.* 1997,43:731-5
- [5] Mareck, U., Thevis, M., Gotzmann, A., Bredehöft, M., Geyer, H., Schänzer, W.: Comprehensive sample preparation for anabolic steroids, glucocorticosteroids, beta-receptor blocking agents, selected anabolic androgenic steroids and buprenorphine in human urine. In: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U. (eds.) *Recent Advances in Doping Analysis (12)*. Sport und Buch Strauß, Köln 2004, 65-68