In: Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (eds.)
Recent Advances in Doping Analysis (1). Sport und Buch Strauß, Köln 1994

T.J.A. Waasdorp*, D. de Boer*, L. van Schaik*, H. Gerretsen*, P. Schoone* and J.H.H. Thijssen+.

Testosterone and Epitestosterone under certain Physiological and Pathological Conditions.

*Netherlands Institute for Drugs and Doping Research, Utrecht, the Netherlands
+Laboratory for Endocrinology, Academic Hospital Utrecht, the Netherlands

Introduction

The ratio of testosterone to epitestosterone (T/E) in urine may discriminate exogenous from endogenous testosterone[1]. Although other ratios have been suggested as additional indicators of testosterone administration, e.g. the ratio of testosterone to luteinizing hormone (T/LH)[2,3], the T/E is the only criterion officially adopted by the International Olympic Committee (IOC)[4]. In some cases, however, it may be difficult to judge on T/E ratio only. Besides the practical approach to use additional indicators like the T/LH ratio, it is worthwhile to develop fundamental knowledge about testosterone and epitestosterone. Since testosterone plays an important role in several endocrinic disorders, e.g. X-chromosomal abnormalities, a lot of research about testosterone production, metabolism and excretion has been done by clinical chemists before testosterone was used as dope agent. The knowledge of testosterone is therefore quite extensive, in contrast to that of epitestosterone. As far as the origins of these steroids concern, many have been reported for testosterone (see Table 1), but for epitestosterone only few are mentioned[5]. More information can be obtained by making measurements under specific pathological or physiological conditions. In this study, testosterone and epitestosterone were measured in follicular fluid, in urines of pregnant women and in urines of patients suffering from Addison's disease or 21-hydroxylase deficiency.

Table 1: Sources of testosterone

1. testes
2. ovaries
3. adrenal cortex
4. peripheral metabolism of androstenedione
5. peripheral metabolism of dehydroepiandrosterone
6. placenta

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Physiological conditions

Follicular fluid: Oogenesis refers to the entire sequence of events by which oogonia are transformed into ova. During early fetal life, the oogonia proliferate by mitotic division to form primary oocytes surrounded by a layer of follicular cells. These oocytes remain dormant in the ovaries until puberty. During maturation the oocyte completes the first meiotic division and the follicle increases in size. At the last stage of maturation shortly before ovulation, the antrum inside the human follicle has grown and is totally filled with fluid. This fluid, rich in steroids, is believed to play an important role in follicular maturation, oocyte development, ovulation, egg transport and possibly in implantation[6]. Follicular fluid was collected during the isolation of secondary oocytes from mature follicles of women who applied for in vitro fertilization. Epitestosterone (mainly unconjugated) was found in pooled human follicular fluid, whereas testosterone was not.

Pregnancy: Epitestosterone and testosterone were also measured in 24h urine samples of pregnant women. Of 2 women 24h urine samples were collected once a month from week 8 of pregnancy until a few weeks after delivery. It was found that the absolute excretion per 24 h of both testosterone and epitestosterone increased during pregnancy. Shortly after delivery, the excretion of epitestosterone decreased to normal, whereas the excretion of testosterone remained at a high level (see Figure 1).

Pathological conditions

Addison's disease: Addison's disease is characterized by primary adrenal insufficiency[7]. From 58 Addisonian females and from 28 Addisonian males, 24h urine samples were collected. To compare the excretion values of these patients with those of healthy people, 24h urine samples were also collected from a control group consisting of 34 males and 18 females. Both Addisonian males and females showed significantly lower testosterone and epitestosterone excretions than the respective control group. In respectively Addisonian males and females excretions were 67% and 89% lower for testosterone, and 23% and 61% lower for epitestosterone (see Figure 2). Apparently, the adrenals have a large contribution to testosterone and epitestosterone in females. In males the adrenal contribution to testosterone is obvious but to epitestosterone is rather small.

21-Hydroxylase deficiency: The absence of 21-hydroxylase leads to a deficiency of glucocorticoids. As a result, hypophyseal secretion of ACTH increases, causing enlargement of the adrenal glands and increased synthesis of pregnenolone. Eventually, this leads to virilization (see Figure 3). From a female patient one 24h urine sample and an untimed blood sample were available. The excretions of testosterone and epitestosterone per 24 hours were 5.4 µg and 2.0 µg , respectively. Compared to reference ranges as determined in the control group of females mentioned above (testosterone 3.4-68.3 µg/24h and epitestosterone 1.2-35.5 µg/24h) these excretions are rather low. High cortisol in the blood sample indicates that substitution therapy is applied. The low testosterone and epitestosterone excretions are probably caused by the negative feedback of cortisol on ACTH secretion. However, plasma DHEAS was rather high (15 µmol/L compared to a reference range of 2.1-8.8 µmol/L).

Remarks

Testosterone and epitestosterone excretions were examined under several conditions, particular physiological or specific pathological conditions. Considering the altered excretion values compared to "normal" circumstances two remarks should be taken into account according to the sources of testosterone and epitestosterone:

1. Separate sources can be different in their contribution to testosterone and epitestosterone.
2. The contribution of a source to testosterone can be different from its contribution to epitestosterone.
3. The contribution of a source to testosterone and epitestosterone is different in males and females.

References

1. Donike M., K.-R. Bärwald, K. Klostermann, W. Schänzer and J. Zimmermann. Nachweis von exogenem Testosteron. Sport: Leistung und Gesundheit/Kongreβbd. Dtsch. Sportärztekongreβ 1982 Köln.

2. Brooks R.V., S.P. Collyer, A.T. Kicman, G.J. Southan and M.A. Wheeler. HCG doping in sport and methods for its detection. In Official proceedings IInd I.A.F. world symposium on doping in sport. Eds. P. Bellotti, G. Benzi and A. Ljungqvist. pp. 37-45. London, UK: International Athletic Foundation, 1989.

3. Waasdorp T.J.A.. Evaluation of the T/LH ratio. In: Proceedings of the 10th Cologne Workshop on Dope Analysis 7th to 12th June 1992, pp. 123. Eds. M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke and S. Rauth. Köln, Sport und Buch Strauß, Edition Sport, 1993.

4. International Olympic Committee. Annex 2: List of doping classes and methods, 1993. International olympic charter against doping in sport.

5. Wilson H. and M.B. Lipsett. Metabolism of epitestosterone in man. J. Clin. Endocr. 26: 902-914, 1966.

6. Moore K.L.. The Developing Human - clinically orientated embryology, third edition. W.B. Saunders Company, 1982.

7. Nelson, D.H.. Adrenal androgens. In: The adrenal cortex: Physiological Function and Disease, pp. 102-112. Major problems in internal medicine, volume XVIII. W.B. Saunders Company, 1980.

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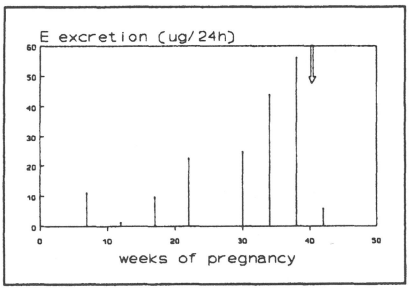
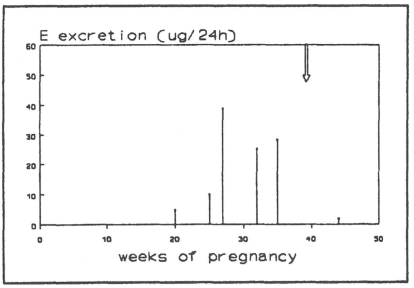


subject 1
pregnant of a boy



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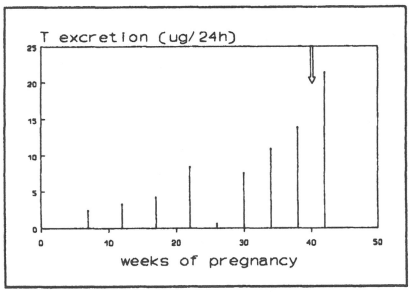

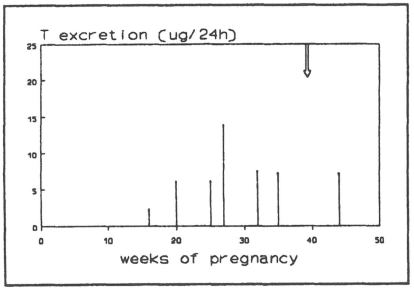

Figure 1: Testosterone and epitestosterone excretions per 24 hours in 2 subjects during pregnancy (↓ = delivery)

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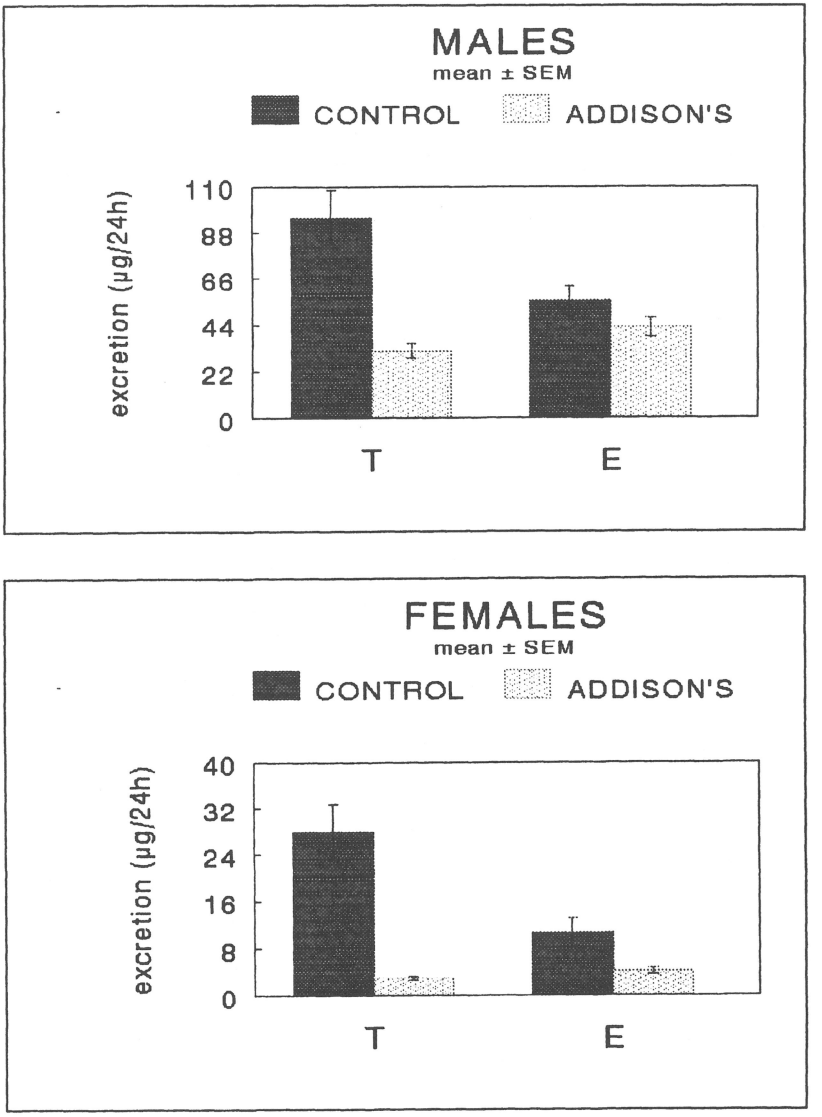

Figure 2: Testosterone and epitestosterone excretions per 24 hours of Addisor compared to controls in males and females

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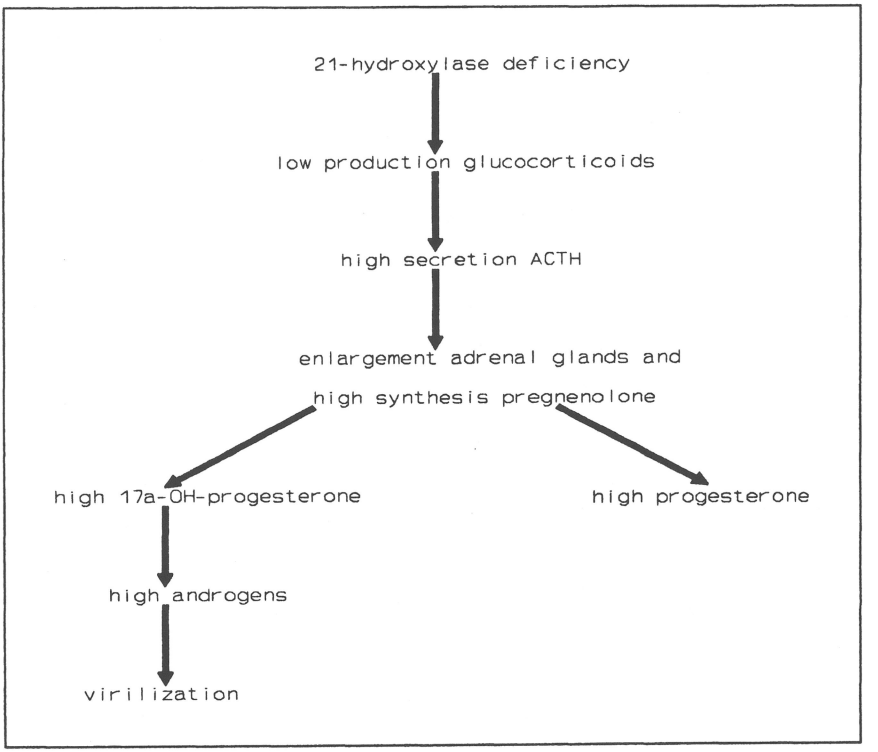

Figure 3: Scheme 21-hydroxylase deficiency

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