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RECENT ADVANCES
IN DOPING ANALYSIS
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W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

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R. KAZLAUSKAS:
Effects of Dehydroepiandrosterone on Urinary Steroids
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Dr R. Kazlauskas

EFFECTS OF DEHYDROEPANDROSTERONE ON URINARY STEROIDS

Australian Sports Drug Testing Laboratory, Sydney, Australia

Dehydroepiandrosterone (DHEA) is classified as having weak androgenic activity and is sold both as the parent substance in tablet form (25 -100mg) and as an injectable ester form, prasterone enanthate (Gynodian Depot 200mg). It is an endogenous steroid produced in high amounts (15-30mg daily). Over the past few years it has been acclaimed as having many beneficial properties and as such may be available over the counter as a health food.

DHEA is one of the main precursors for the biosynthesis of the endogenous steroids. It is metabolised via androstenedione to testosterone (T), 5α-androstan-3α,17β-diol (5α-diol), 5β-andostan-3α,17β-diol (5β-diol), androsterone (A), etiocholanolone (Etio) and many of the endogenous steroids regularly measured during dope control (see FIG 1). It therefore can be expected to effect the endogenous profile and change many of the parameters such as the Testosterone/Episteoerone (T/E) ratio and the A/Etio ratio.

![Diagram of steroid biosynthesis]

**FIG 1**

We undertook several studies to look at the effect on the steroid profile. Two of these consisted of the oral administration of DHEA as an ester (prasterone enanthate) (200mg) and of DHEA (100mg). The ester preparation was Gynodian Depot produced by Schering, 1ml containing prasterone enanthate 200mg and estradiol valerate 4mg.
Excretion studies.

One subject - male 90Kg - ingested orally the contents of a Gynodion Depot vial. No ill effects were observed. Urine was collected for a period of three days before ingestion and then for 3 days after ingestion. The S.G and pH were measured and the normal extraction for steroid screening was performed. The enzyme hydrolysis was performed by both H. pomatia enzyme at pH 5 and with E. coli enzyme at pH 7 to obtain information about both the glucuronide and the sulphate conjugates. H. pomatia hydrolysis would be expected to allow detection of free steroid, glucuronides and some sulphates whereas E. coli should only detect the free and glucuronide conjugates.

The same subject ingested 2 x 25mg tablet of DHEA and urine was collected for 3 days. The enzyme hydrolysis was performed by both H. pomatia enzyme at pH 5 and with E. coli enzyme at pH 7.

Results.

The changes over time of a number of screening parameters are shown in FIGS 2-11 for the DHEA ester. The graphs represent the measurement against time (hours).

The graphs for FIGS 12-20 are changes with time for the DHEA.

There was very little difference between the results for the ingestion of the two different preparations.

The T/E ratio (FIGS 2, 5, 12) for both the E. coli and H. pomatia hydrolysis rose to about 2 - 3 for a short period of time but was back to normal within 20hrs. Similarly the T and E concentrations are elevated for the same period with the T rising higher than the E (FIGs 10, 20). The Androsterone/Etiocholanolone ratio (A/Etio) only rose to double the resting value for less than 10hrs (FIGS 3, 6, 13) but then fell well below the normal ratio to about 0.2 giving rise to an elevated Etio/A ratio. This ratio was elevated for considerable time - up to 50hrs. The A/T and A/E ratios only gave a short but marked rise (FIG 9). A similar effect to the A/Etio changes was noticeable for the 5α/5β-diol ratio. The short elevation is shown in FIGS 4, 7, 14 with a subsequent long term reduction of this ratio. FIG 8 Shows the inverse plot which demonstrates the long term elevation of the 5β/5α-diol ratio. This effect can be seen regardless of the hydrolysis procedure and is the main feature of DHEA application.

This effect may be due to the production of sulphate esters of the 5β steroids as well as the glucuronides. The etiocholanolone concentration after H. pomatia hydrolysis (FIG 11) appears to have two peak excretion periods. The first 0-10hrs corresponds to that obtained for the E. coli hydrolysis. However a second elevation in concentration occurs between 20 and 30hrs. Androsterone only has one maximum and corresponds to that seen with E. coli. The difference between the two hydrolysis conditions may be due to glucuronide vs glucuronide plus sulphate derivatives with sulphate excretion of 5β steroids occurring at a later time to that for glucuronides.

A similar effect is seen with DHEA which is normally excreted as the sulphate. With H. pomatia hydrolysis (FIG 18), DHEA levels are, as expected, much higher than for E.
coli hydrolysis (FIG 19). The DHEA is excreted either free or as glucuronide as well as the sulphate for only a short period of 0-6hrs (as seen with E. coli hydrolysis) while the sulphate is continuously excreted for much longer after H. pomatia hydrolysis. The effect of this is quite marked when the DHEA/E or DHEA/T ratios are measured (FIGS 16, 17). The H. pomatia hydrolysis shows a bimodal excretion possibly due to some free (or glucuronide derivative) DHEA being initially excreted due to overload.

Overall the main effects seen after a single dose of DHEA in this individual are a substantial rise in androsterone for a short period; the DHEA level rises substantially; the A/Etio and 5α/5β-diol ratios change mainly giving a very low value after 20 hrs and persisting up to 50 hrs. Other parameters such as T/E are only slightly affected for a short time period only. The time of sample collection has a major bearing on the profile observed because of the inversions experienced in many of the ratio parameters. It is yet to be established if any of these changes are outside the population distribution to an extent to allow a definitive reporting of a DHEA doping case.

Considerable work needs to be done to enable these parameters to be developed. DHEA may show considerable individual variation in excretion. This has now to be determined in a large group of volunteers both male and female of different age groups and ethnic origin.