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Individual Variations of Urinary Steroid Profiles in Glucuronide and Sulfate Fractions after DHEA Oral Administration

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Abstract

Individual urinary steroid profiles are known to be stable during most of human adult life. However, these profiles display considerable inter-individual variations in many parameters, including, significantly, the extent to which steroid metabolites are distributed between the glucuronide and sulfate fractions during excretion. Short and long-term effects of DHEA oral administration on urinary steroid profiles are observed in this study, which reinforce previously reported data and provide new information especially in regards to sulfate fraction steroids. High sulfate excretors favor a 5β-steroid metabolic route for which 5-androsten-3,17-dione is suggested as intermediate metabolite, as opposed to 4-androsten-3,17-dione preferred by naturally low sulfate men. The latter demonstrate sudden appearance in urine or significant increase of DHEA sulfate and other 3β-hydroxy steroid sulfates as a result of DHEA use. All participants show an abrupt drop of androsterone to etiocholanolone ratio (A/E) in glucuronide fraction beginning 8 to 16 hours after administration. This is a long-term effect traceable for up to four days. Disturbance of A/E (0.1 and lower) is long and pronounced in high sulfate men. Overwhelming effect of DHEA also includes a short-term suppression of corticosteroid production. Rise of androsterone and simultaneous fall of corticosteroid metabolites concentrations during the initial stages of excretion indicate DHEA administration. Androstendione appears briefly only in sulfate fraction. Unusually high concentrations of epietiocholanolone (3β-hydroxy isomer) are found in sulfate fraction cleaveable mostly by solvolysis. This is a specific long-term DHEA metabolite. T/E ratio changes are inconsistent. From no change for two volunteers with initially low 0.1 and normal 0.9 T/E, to significant increase from 1.0 to 4 and from 3.8 to 20.

Introduction

Adrenally produced dehydroepiandrosterone (DHEA) and its 3β-sulfate (DHEAS) are abundant in human serum, where they are present in far higher concentrations than any other steroid (1). While individual levels of serum DHEA are stable, inter-individual differences

may be considerable (2). DHEA and its sulfate are further utilized in other hormone production including androgenic anabolic steroids. DHEA is an unregulated popular nutritional supplement in the Unites States. It is readily available and widely advertised as an anti-aging and performance enhancing agent. As a precursor to testosterone DHEA is banned by the IOC and the major sports federations.

DHEA abuse is difficult to detect in doping control, as it is for other endogenous testosterone precursors: andostendiones and androstendiols. Isotope ratio mass spectrometry (IRMS) was found useful (3) for confirming DHEA intake by isolating urinary androstandiols and 5-androstendiols, and analyzing their ^{13}C content. Conventional GC/MS steroid profiling shows signs of DHEA abuse, such as unusually low 5α/5β urinary steroids ratios: androsterone to etiocholanolone (A/E) and 5α- to 5β-androstandiols (4,5); elevation of T/E ratio (5,6) in some individuals; and elevation of 7α- and 7β-hydroxy DHEA relative to 16α-hydroxy isomer (7). However, exact criteria indicating DHEA abuse based on variations of endogenous steroids have not been established. The following presents additional data on DHEA effect on glucuronide and sulfate steroid fractions for different individuals.

Experimental

Excretion studies

DHEA gel capsules from Marathon Nutrition (Rolling Hills Estates, CA) were checked for purity by GC/MS. Excretion studies were performed with four healthy male subjects. Each took orally a single 200 mg dose of DHEA. Urine specimens were collected for one week after administration and were stored refrigerated.

Reagents and materials

β-Glucuronidase/arylsulfatase type H-2 (Cat.# G7017) from *Helix pomatia* were purchased from Sigma. β-Glucuronidase type K12 from *Escherichia coli* was supplied by Fluka (Milwaukee, WI). N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was purchased from Campbell Science Corp. (Rockton, IL), ammonium iodide 99+% from Aldrich (Milwaukee, WI). C_{18} solid phase (200 mg) extraction cartridges were purchased from Varian (Harbor City, CA).

Urine sample preparation

Sequential urine hydrolysis with *Escherichia coli* (*E.C.*) and *Helix pomatia* (*H.P.*) enzymes, intermediate clean up on C_{18} extraction cartridges, solvolysis (with 1mL of ethyl acetate and 2μL of 4M H_2SO_4 at 55°C for I hour), and separation of urinary steroid fractions were

performed as described earlier (8). The dry residues of each fraction were derivatized with 75 μL of MSTFA-NH_4I-Dithioerythritol (1000:2:3 v/w/w) for 15 minutes at 70°C. Samples were transferred into vials, 1 μL was injected into the GC/MS.

GC/MS conditions

GC/MS was Agilent Technologies 6890/5973 with 7683A autoinjector. Column: HP-1 fused silica, crosslinked methylsilicon, 16.5m, 0.2 mm i.d., 0.11 μm film thickness. Hydrogen carrier gas was supplied by Whatman 75-32-V452 hydrogen generator. Total flow was 30.1 mL/min, average carrier gas linear velocity in the column was 100 cm/sec with constant flow of 1.7 mL per minute. Splitless injection with 0.3 minutes purge time was used. Oven temperature program: hold at 160°C for 0.4 min; raise at 35°C/min to 180°C; then 5.8°/min to 215°C, and 32°C/min to 310°C, hold for 0.03 min. Injector temperature was 270°C, transfer line 280°C.

Results and Discussion

Wide diversity of human urinary DHEA excretion is well known (9). Amounts of DHEA and other 3β-hydroxy steroids in urine of different individuals may be anywhere between high (comparable to the major urinary androsterone and etiocholanolone) and low (hardly detectable). Figures 1 and 2 show selected ion chromatograms of glucuronide and sulfate steroid fractions before (on the left of each figure) and 24-28 hours after (on the right) administration of 200 mg of DHEA by two individuals with naturally high (fig. 1) and low (fig. 2) DHEA (sulfate) excretion. On both figures the upper chromatograms represent steroid glucuronides which are fully cleaved by the *E.C.* enzyme. Chromatograms in the middle show urinary steroid sulfates cleavable by *H.P.* At the bottom are steroids which can be only liberated by acidic solvolysis.

As expected (10,11) 3β-hydroxy steroids, DHEA, epiandrosterone, and 5-androsten-3β,17β-diol, appear as sulfates, whether before or after DHEA administration. They are fully liberated by *H.P.* (middle chromatograms in fig. 1 and 2) and are not detected after consequent solvolysis (bottom). Most of the androsterone, etiocholanolone, 5α- and 5β-androstandiols, all testosterone and epitestosterone are conjugated with glucuronic acid. Some 5-10% of total androsterone and etiocholanolone show up as sulfates (chromatograms in the middle row and at the bottom). Androsterone and other 3α-hydroxy 5-α steroid sulfates can not be cleaved enzymatically and require solvolysis for full liberation.

For the high sulfate person (fig. 1), 28 hours after administration DHEA concentration in urine changes very little, although between 0 and 28 hours we observed a considerable elevation of the parent drug. On the other hand parent DHEA in the low sulfate person appears much higher after administration than naturally excreted (compare two middle chromatograms in fig.2), thus making DHEA concentration in this case a parameter indicating intake.

Previously found elevation of etiocholanolone over androsterone (4,5) is the most noticeable effect of DHEA administration (compare upper chromatograms in fig. 1 and 2). The magnitude of A/E ratio disturbance and time of its onset vary considerably from one individual to another as follows from excretion curves in fig. 3, where A and B represent correspondingly low and high sulfate persons. This long-term A/E effect can be seen for about four days after DHEA administration. However, it should be remembered that similar effect on A/E ratio may result from anabolic steroid abuse, such as stanozolol (12). Similarly after administration of 19-Nor Δ^5 steroids, 19-nor-5-androsten-3,17-dione (13) and 19-nor-5-androsten-3,17-diol in this study, mostly 19-nor-etiocholanolone is produced with smaller amounts of 19-nor-androsterone, showing preference to 5β reduction. The magnitude of 5α/5β ratio is also individual dependent as shown in fig. 4.

Strong suppression of late eluting endogenous corticosteroids (in all three steroid fractions) 8 to 12 hours after DHEA administration coincides with a peak of androsterone excretion. Reduced levels of corticosteroids one day after DHEA administration compared to original levels are still seen in fig. 1 and 2.

Recently reported rise of 7-hydroxy DHEA isomers over 16-hydroxy (7) as indication of DHEA abuse is confirmed in this study. This is a short-term effect lasting for the first few hours after administration.

Epietiocholanolone (3β-hydroxy isomer) is identified as an important long-term metabolite of DHEA in the sulfate fraction obtained by solvolysis (fig. 1, the first peak in the right bottom chromatogram). Androsten-3,17-dione whether 4 or 5 (up to 400 ng/mL) briefly appears as sulfate (3-enol) in solvolysis fraction.

The effect of DHEA on the rise of the T/E ratio is also short and very individual, as shown in fig. 5. Results confirm earlier findings (5,6) that naturally high T/E persons easily exceed positive cutoff levels after DHEA administration.

This and our earlier excretion studies involving 4- (13) and 5-androstendiones (5) and their 19-nor analogs suggest that DHEA may follow both 4- and 5-androstendione paths (fig. 6)

with variations reflecting individual preferences. Δ^5 androstendione metabolizes mostly into etiocholanolone favoring 5β reduction of its double bond, as opposed to the Δ^4 isomer where both 5α and 5β reduction takes place with some preference to the androsterone (5α) route (fig. 6).

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