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DEHYDROISOANDROSTERONE SULFATE IN PERIPHERAL BLOOD OF PREMENOPAUSAL, PREGNANT AND POSTMENOPAUSAL WOMEN AND MEN

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SUMMARY

A radioimmunoassay specific for the direct measurement of dehydroisoandrosterone sulfate in 0.5 or 1 μl of human serum was developed using an antibody directed against dehydroisoandrosterone hemisuccinate coupled to thyroglobulin. Dehydroisoandrosterone sulfate levels were measured in pregnant women throughout gestation starting with the 6th week of pregnancy, and in young ovulatory women, postmenopausal women and in men. During pregnancy the concentrations of dehydroisoandrosterone sulfate in maternal serum decreased steadily with advancing gestation [1825 ± 68 ng/ml (mean and S.E.) at 6–8 weeks gestation vs 706 ± 141 ng/ml at 36–41 weeks gestation], and the most striking decrease was observed between 14 and 16 weeks of gestation. The mean concentrations of dehydroisoandrosterone sulfate were higher in men than in young nonpregnant women (2679 ± 196 ng/ml vs 2020 ± 108 ng/ml respectively). In postmenopausal women, mean serum dehydroisoandrosterone sulfate concentrations (764 ± 28 ng/ml) were considerably lower than those in young premenopausal women $P < 0.001$).

INTRODUCTION

Dehydroisoandrosterone sulfate (3β-hydroxy-5-androsten-17-one 3β sulfate, DS) circulating in human peripheral blood arises mainly by adrenal secretion [1–2]. In human pregnancy, maternal and fetal serum DS serves as a substrate in the synthesis of placental oestrogens [3–5]. The concentrations of DS in maternal serum appear to decrease in the course of advancing pregnancy [6–8] and a negative correlation has been suggested between the stage of pregnancy and maternal DS [7]. To confirm these findings, and since the degree of biological variations in DS concentrations observed among individuals is high [6–8], we measured DS concentrations in peripheral blood of a relatively large number of women throughout pregnancy, as well as of young nonpregnant and postmenopausal women, and of men. For this purpose we developed a direct and specific radioimmunoassay (RIA) for DS based on a modification of the method described by Buster and Abraham[9] and which requires only 0.5 or 1 μl of serum for assay.

EXPERIMENTAL

Reagents. Boric acid and ammonium sulfate were analytical reagents; isooctane (2,2,4-trimethylpentane) and ethyl acetate were nanograde quality (Mallinckrodt Chemical Works). Tributylamine, isobutyl chloroformate and succinic anhydride were obtained from Eastman Organic Chemicals. Bovine serum albumin (Fraction V), bovine gamma-globulin BGII (Cohn Fraction II), and bovine thyroglobulin Type I were purchased from the Sigma Chemical Company. Ethylene glycol (chromatoquality) and celite analytical filter-aid were obtained from Matheson Coleman and Bell. The celite was fired overnight in a muffle furnace at 800°C. Kimble disposable serological pipets (5 ml) were used for preparation of chromatography minicolumns [10].

Steroids. Dehydroisoandrosterone sulfate sodium salt was purchased from Amersham/Searle Corp. Cholesteryl fatty acid esters were obtained from Supelco, Inc. Dehydroisoandrosterone and other steroids used in studies of cross-reactivity were obtained from Steraloids, Inc. [7-^{3}H(N)]dehydroisoandrosterone sulfate ammonium salt (25 Ci/mmol), [1,2-^{3}H(N)]-dehydroisoandrosterone (50 Ci/mmol) and [4-^{14}C]-dehydroisoandrosterone (50 mCi/mmol) were purchased from New England Nuclear.

Hapten. Dehydroisoandrosterone (340 mg), [4-^{14}C]-dehydroisoandrosterone (1 μCi) and succinic anhydride (230 mg) were dissolved in 3 ml of pyridine and refluxed for 24 h. After evaporation of the solvent the product, [4-^{14}C]-dehydroisoandrosterone hemisuc-

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cinate, was purified by crystallization from acetone and used for coupling to thyroglobulin.

Antigen. The thyroglobulin-dehydroisoandrosterone hemisuccinate conjugate was prepared by the mixed anhydride technique [11], and was used for immunization of three 4-month old New Zealand male rabbits, as previously described [12].

Radioimmunoassay buffer. Borate buffer 0.05 M, pH 7.8, containing 0.2% bovine serum albumin, 0.1% gamma globulin, and 2% sodium chloride was stored at 4°C and used as buffer throughout (RIA Buffer).

Radioimmunoassay of dehydroisoandrosterone sulfate. Serum (10 μl) was diluted with 190 μl of RIA Buffer, and either 10 or 20 μl of the 1:20 diluted serum (corresponding to 0.5 and 1 μl of serum) was transferred to 12 × 75 mm glass tubes in triplicate. Triplicate 10 μl aliquots of 0.05 M borate buffer, pH 7.8, with 5% ethanol containing 0, 0.25, 0.5, 1.25, 2.5, 5.0, 10.0 and 20.0 ng of dehydroisoandrosterone sulfate were used for the standard curve. Radioimmunoassay buffer (0.5 ml), [1,2-^{3}H(N)]-dehydroisoandrosterone (30,000 d.p.m./ml) and antiserum (A-286) at a final dilution of 1:2,000, was added to each tube, and the samples were incubated at 4°C for 18 h. The bound steroid was precipitated with 0.5 ml of cold saturated ammonium sulfate solution, and 0.5 ml of the supernatant solution was counted, as previously described [12]. For all standard curves, an initial antibody dilution corresponding to about 65% bound [^{3}H]-dehydroisoandrosterone was selected.

Validation of the measurements of dehydroisoandrosterone sulfate. For validating the measurements of DS in serum, similar aliquots of 1:20 diluted serum (10 μl) were hydrolyzed by the method of Burstein and Lieberman[13] after addition of [7-^{3}H(N)]-dehydroisoandrosterone sulfate (~5000 d.p.m./sample). Five ml of a solution made up of 1.2 ml 5 N sulfuric acid in 80 ml of ethyl acetate was added to each sample. The mixtures were incubated at 24°C for 48 h and were washed with 0.5 ml of 5% sodium bicarbonate solution and twice with 0.5 ml of water. The solvent was evaporated to dryness with nitrogen and the residue was chromatographed on celite mini-columns to separate the dehydroisoandrosterone fraction.

Chromatography on mini-columns. Dehydroisoandrosterone and other isopolar compounds were separated from other serum steroids using celite mini-columns similar to those reported by Abraham *et al.*[9].

Radioimmunoassay of dehydroisoandrosterone. One-half ml aliquots of the chromatographic fractions containing the dehydroisoandrosterone were transferred to scintillation vials to determine recoveries, and 100 μl aliquots in triplicate were used for RIA as previously described [12]. Triplicate 10 μl aliquots of 0.05 M borate buffer, pH 7.8, with 5% ethanol containing 0, 12.5, 25, 50, 100, 250, and 500 pg of dehydroisoandrosterone were used for the standard curve. For RIA of dehydroisoandrosterone the final antiserum (A-286) dilution was 1:8000.

Extraction of dehydroisoandrosterone from human serum. One ml of human serum was used for extraction. To each sample 50 μl of an ethanolic solution containing [1,2-^{3}H(N)]-dehydroisoandrosterone (5000 d.p.m., 16 pg) was added as internal recovery standard. After mixing, the serums were extracted one time with 7 ml of ethyl ether. The solvent was evaporated to dryness under nitrogen and the residues were chromatographed on celite mini-columns.

Specificity. The relative affinity of the antibody A-286 for heterologous steroids was determined by measuring the displacement of [1,2-^{3}H(N)]-dehydroisoandrosterone from antibody A-286 at two different dilutions: 1:2000 for dehydroisoandrosterone sulfate, and 1:8000 for dehydroisoandrosterone.

Blood samples. Antecubital venous blood was obtained between 8 and 10 AM, from men, nonpregnant, pregnant and postmenopausal women. The blood was allowed to clot and the serum was separated and stored at −20°C until assayed.

RESULTS

Radioimmunoassay of dehydroisoandrosterone sulfate

The sensitivity of antibody A-286 at a dilution of 1:2000 was 110 ng DS/ml, when utilizing the equivalent of 1 μl of serum for the direct RIA. The accuracy of the method was examined throughout the range of 0 to 256 ng of DS. In Fig. 1 we present the relation of DS added to DS measured: no systematic error was observed. Intra-assay precision was determined by measuring a sample containing 1890 ± 45 ng

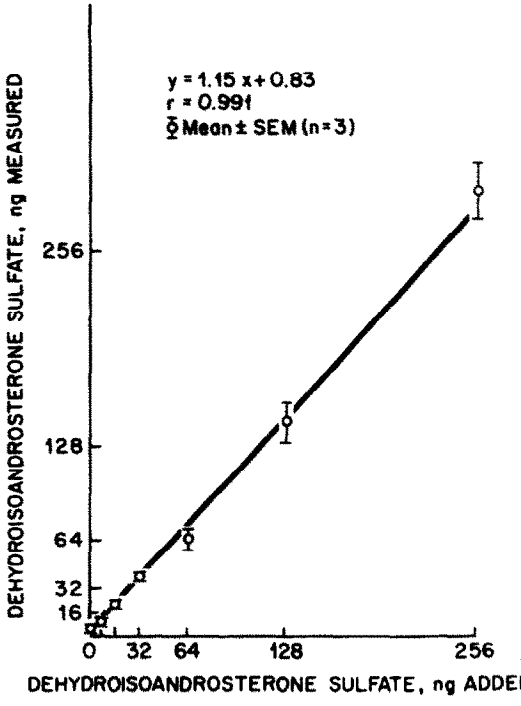

Fig. 1. Dehydroisoandrosterone sulfate measured plotted against dehydroisoandrosterone sulfate added to blood bank plasma.

Table 1. Serum concentrations of dehydroisoandrosterone sulfate in six normal men. Comparison of results obtained using 0.5 μl and 1.0 μl of serum in the direct RIA, and after hydrolysis and measurement as dehydroisoandrosterone*

Sample No.	Dehydroisoandrosterone sulfate, ng/ml Direct RIA Using 0.5 μl	Direct RIA Using 1.0 μl	Measured as dehydroisoandrosterone after hydrolysis
1	2800	3400	2898
2	4300	4200	4836
3	2800	2800	2692
4	2300	2300	2795
5	2200	2400	2276
6	2600	2750	2581

* See Materials and Methods for experimental details.

DS/ml (mean and S.E., $n = 20$) and was found to be 10%. The interassay precision of a pool containing 2424 ng DS/ml was 11% ($n = 9$), and for a sample containing 1547 ng DS/ml was 12.5% ($n = 9$).

Radioimmunoassay of dehydroisoandrosterone

The sensitivity [14] of antibody A-286 at a dilution of 1:8000 was approximately 6 pg. The blank of the assay was about 5 pg. The overall recovery of dehydroisoandrosterone after mini-column chromatography was 66.4 ± 1.9% (mean and S.E., $n = 15$). The accuracy of the RIA method was examined throughout the range of 0 to 8.0 ng of dehydroisoandrosterone. The intra-assay precision was determined by measuring the dehydroisoandrosterone in a serum sample containing 4.9 ± 0.4 ng dehydroisoandrosterone/ml (mean and S.E., $n = 5$) and was found to be 19%. The inter-assay precision of measurement of dehydroisoandrosterone in a serum sample containing 4.2 ng dehydroisoandrosterone/ml ($n = 5$) was 20.8%.

Table 2. Cross-reactions of steroids with the dehydroisoandrosterone antiserum A-286 using a final dilution of 1:2000

Steroid	Cross-reaction, %*
Dehydroisoandrosterone sulfate	100
Cholesteryl 3β-sulfate	0.80
Pregnenolone 3β-sulfate	0.22
Testosterone 17β-sulfate	0.08
Cortisol 21-sulfate	<0.01
Estrone 3-sulfate	<0.01
Estradiol 3-sulfate	<0.01
Estriol 3-sulfate	<0.01
Cholesteryl 3β-myristate	<0.01
Cholesteryl 3β-palmitate	<0.01
Cholesteryl 3β-stearate	<0.01
Cholesteryl 3β-oleate	<0.01
Cholesteryl 3β-linoleate	<0.01

* Calculated as

$$\frac{\text{nanograms of dehydroisoandrosterone sulfate at 50\% binding}}{\text{nanograms of heterologous steroid at 50\% binding}} \times 100.$$

Quantification of DS in serum by direct radioimmunoassay and as dehydroisoandrosterone after hydrolysis

From the data presented in Table 1, we conclude that there is good agreement between the results obtained by measurement of DS by direct RIA of either 0.5 or 1.0 μl of serum, with the values obtained after hydrolysis of DS and measurement of dehydroisoandrosterone following mini-column chromatography. These findings are supportive of the validity of the direct RIA technique for measuring serum DS.

Antibody A-286 had a greater specificity [15] for dehydroisoandrosterone sulfate than for any of the other steroids examined (Table 2). Unconjugated steroids cross-reacted minimally when using a 1:2000 final antibody dilution.

Concentrations of DS in human serum

The serum concentrations of DS in men, young ovulatory women and postmenopausal women are presented in Table 3. There were highly significant differences ($P < 0.001$) between the serum DS concentrations in young ovulatory women and postmenopausal women. The mean serum DS concentrations in men were higher than those found in young women.

The individual serum DS concentrations in the premenopausal, pregnant and postmenopausal women of our study are presented in Fig. 2, and the concentrations of DS in maternal serum throughout human gestation starting with the 6th week of pregnancy are presented in Table 4. A highly significant ($P < 0.001$) reduction in plasma concentrations of dehydroisoandrosterone sulfate occurs after 16 weeks gestation (mean and S.E. = 747 ± 28 ng/ml, $n = 74$) compared to values observed from 6 to 14 weeks gestation (mean and S.E. = 1696 ± 44 ng/ml, $n = 265$). The pattern of serum DS concentrations during a 24 h period in the first, second and third trimester of gestation in a normal pregnant woman is presented in Fig. 3.

DISCUSSION

A direct RIA for DS was developed using an antibody elicited in rabbits against dehydroisoandrosterone hemisuccinate-thyroglobulin. This RIA differed from that reported by Buster and Abraham[9] in that we utilized a higher concentration of antibody which

Table 3. Dehydroisoandrosterone sulfate concentrations in serum of men, young ovulatory women and post-menopausal women

Subjects	n	Dehydroisoandrosterone sulfate ng/ml (mean ± S.E.)	Range ng/ml
Men	19	2679 ± 196	1200–4200
Young ovulatory women	31	2020 ± 108	1050–3550
Post-menopausal women	263	764 ± 28	20–3150

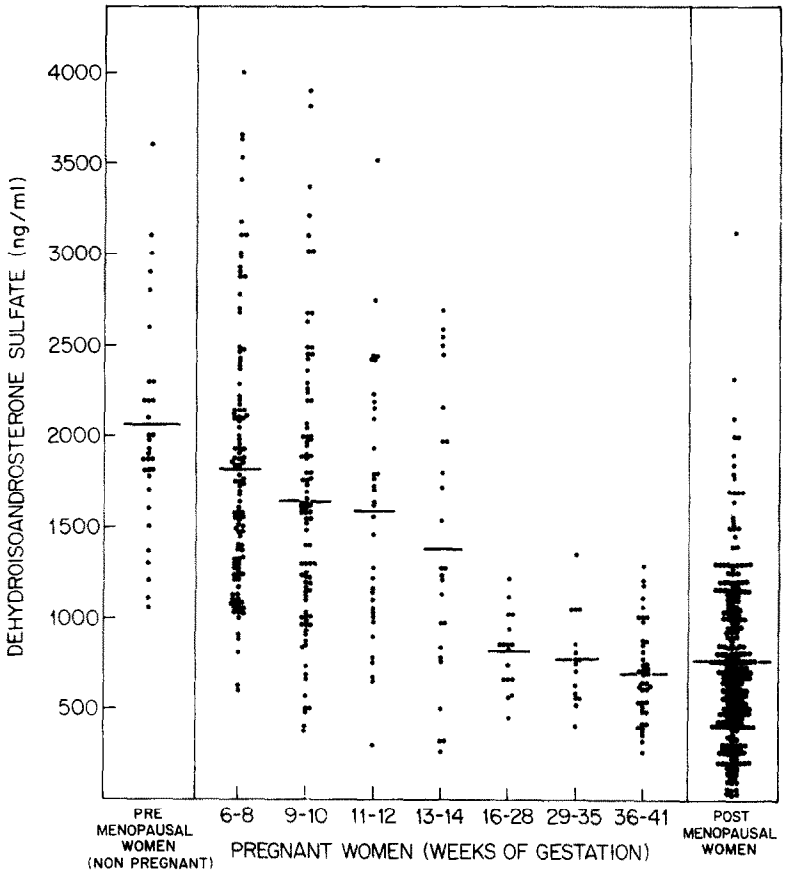

Fig. 2. Individual serum DS concentrations in the premenopausal, pregnant and postmenopausal women of this study. The mean values are indicated by the horizontal bar.

yielded a dose-responsive curve in the range of 0.2 to 10 ng per tube; moreover, we needed only 10 or 20 μl of diluted serum (1:20) or the equivalent of 0.5 or 1 μl of serum per tube for assay.

After menopause, ovarian steroidogenesis is markedly reduced. From the data presented in Table 3 it is evident that adrenal cortical function is also reduced in postmenopausal women since there is a sharp decrease in the mean serum DS concentrations in such women compared with those of young ovulatory women and men. The range of serum DS concentrations found in men, premenopausal and postmenopausal women in this study are in agreement with data reported by other investigators [2, 9, 16).

In normal pregnant women the metabolic clearance rate of DS (MCR-DS) increases progressively with advancing gestation (25 liters/24 h at 21–22 weeks gestation and 55 liters/24 h or higher at term [17]). This is in contrast to nonpregnant women where the MCR-DS is about 7 liters/24 h [18]. Irreversible metabolism and clearance of DS from maternal plasma involves two major routes: (a) the conversion of DS to oestradiol-17β in the placenta, and (b) the conversion of DS to 16α-hydroxy-DS, mainly in the maternal liver. Quantitatively minor routes for removal of DS from the plasma of pregnant women include: (a) direct urinary excretion of DS, (b) the elimination of DS *via* "neutral pathways," e.g., 17-ketosteroids, (c) loss of DS into the fetus, and (d) other excretory processes [19]. Production rates of DS during pregnancy are not greatly altered [6]. However, the serum DS concentrations decrease gradually from the 6th to the 14th week of gestation and then fall abruptly around the 16th week of pregnancy followed

Table 4. Dehydroisoandrosterone sulfate concentrations in maternal serum throughout human gestation*

Weeks of pregnancy	n	Dehydroisoandrosterone sulfate ng/ml (mean ± S.E.)	Range ng/ml
6–8	113	1825 ± 68	600–4000
9–10	93	1646 ± 78	380–3900
11–12	35	1593 ± 119	300–3530
13–14	24	1387 ± 155	260–2700
16–28	17	820 ± 51	452–1220
29–35	16	778 ± 63	408–1360
36–41	41	706 ± 41	260–1300

* The difference between the mean DS concentration values of 6 to 14 weeks and 16 to 41 weeks pregnancy serum is highly significant ($P < 0.001$).

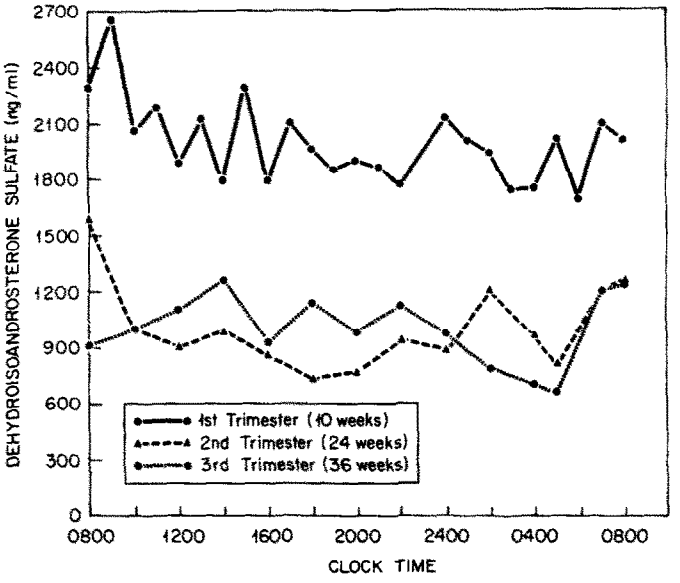

Fig. 3. Dehydroisoandrosterone sulfate concentrations measured at 1–2 h intervals over 24-hours in a normal primagravida at 10 weeks, 24 weeks and 36 weeks of gestation.

by a slow decline throughout the remainder of pregnancy (Table 4 and Figs 2 and 3). Therefore, the progressive decline in concentrations of serum DS during pregnancy is the consequence of steadily increasing MCR-DS. These observations appear to reflect physiological changes in aromatase activity of the placenta and 16α-hydroxylation in the maternal liver. In addition, the highly significant drop in serum DS concentrations observed after the 16th week of pregnancy is likely associated with the increasing utero-placental blood flow occurring about this stage of gestation. It has been shown that the placental conversion of DS to oestradiol-17β increases exponentially after the 16th week of gestation [20]. However, the striking metabolic event that leads to a reduction in maternal serum DS levels is the increase in clearance due to 16α-hydroxylation by the liver [19], although liver blood flow does not change with pregnancy [21]. While the transfer constant of conversion of maternal plasma DS to 16α-hydroxy-DS increase from 0.15 in nonpregnant women to 0.32 in late pregnancy, the clearance of DS through this metabolic route increases from approximately one liter per day to 18 liters per day. This may be the result of hyperoestrogenism [19, 22]. Whether there is greater 16α-hydroxylase enzyme in the hepatocyte is unknown. Nonethe-less, considering the enormous increase in clearance of maternal plasma DS by 16α-hydroxylation, it is likely that some alteration in the hepatocyte in response to oestrogen action takes place in late human pregnancy.

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