Disproportionate Suppression of Dehydroepiandrosterone Sulfate (DHEAS) in Treated Patients with Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency

IRAJ REZVANI,⁽³⁴⁾ LUIGI R. GARIBALDI, ANGELO M. DIGEORGE, AND HENRY G. ARTMAN

Department of Pediatrics, Temple University School of Medicine and Section of Endocrine and Metabolic Disorders at St. Christopher's Hospital for Children, Philadelphia, Pennsylvania, USA

Summary

Serum concentrations of dehydroepiandrosterone sulfate (DHEAS) were measured in 28 patients (18 females, 10 males) with congenital adrenal hyperplasia due to 21-hydroxylase deficiency who were treated with oral hydrocortisone (non-salt losers) or hydrocortisone and 9- α -fluorohydrocortisone (salt-losers). Adequacy of therapy was assessed by clinical findings, determination of bone age, urinary excretion of 17-ketosteroids, and serum concentration of 17-hydroxyprogesterone. These allowed the separation of patients into three groups: poorly controlled, adequately controlled and overtreated. Individual values for serum levels of DHEAS were compared to mean normal values for age. In the adequately controlled and overtreated patients, mean serum concentrations of DHEAS were significantly lower than normal values for age ($P < 0.05$). In the poorly treated patients, the mean serum concentration of DHEAS was not significantly different from normal values for age ($P = 0.50$).

These data indicate that the serum concentration of DHEAS is overly suppressed in treated patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. This finding suggests that measurement of the serum levels of DHEAS has limited value in assessing the adequacy of therapy in this disease.

Abbreviations

CAH, congenital adrenal hyperplasia due to 21-hydroxylase deficiency

DHEAS, dehydroepiandrosterone sulfate

17-OHP, 17-hydroxyprogesterone

SDS, standard deviation score

A major goal in long term management of patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CAH) is to suppress the effects of excessive adrenal androgens. The adequacy of suppression has been traditionally monitored by periodic measurement of 24-h urinary excretion of 17-ketosteroids and pregnanetriol. The reliability of these assays, however, is hampered by inaccuracies in collection of 24-h urine specimens. A suitable compound that can be measured in blood, therefore, has been searched for by several investigators. Dehydroepiandrosterone sulfate (DHEAS), the most abundant adrenal androgen, seems to be a likely candidate for this purpose because it is almost exclusively synthesized by the adrenal glands (17, 19). Moreover, as DHEAS has a long half-life (24, 26), its concentration in serum is less susceptible to diurnal variations (25).

Recent studies have demonstrated that serum levels of DHEAS are increased in patients with untreated CAH (11, 15, 23); however, data on the serum concentration of the hormone during therapy are very limited. Serum levels of DHEAS did not increase in six

patients with CAH when therapy was discontinued for 3 days (33). Golden *et al.* (11) found elevated levels of serum DHEAS in patients with CAH only when they were overtly undertreated, but values for serum levels of DHEAS were not correlated with the age of the patients. Because secretion of DHEAS is age dependent (8, 15, 23), its role in the management of patients with CAH cannot be quantitated unless appropriate age-matched control values are applied. The purpose of this study was to determine the usefulness of serum concentration of DHEAS for monitoring therapy in patients with CAH and to compare serum levels of the hormone to those obtained in age-matched control subjects.

MATERIALS AND METHODS

Twenty-eight patients, 18 females and 10 males with CAH (Table 1), were followed as outpatients at 3-6 month intervals. Congenital adrenal hyperplasia had been diagnosed in each case at least 2 years before this study. All patients were being treated with oral hydrocortisone twice daily at a mean dose of 17.9 mg/m²/day; 9- α -fluorohydrocortisone (50-200 μ g/day) was added only if the patient was a "salt loser." The dose of medication had remained unchanged for at least 1 year before the study.

Adequacy of treatment was assessed by the following criteria:

- (1) normal linear growth;
- (2) normal bone age and normal rate of advancement of bone age;
- (3) no evidence of ongoing inappropriate virilization such as premature appearance of pubic hair or phallic or clitoral enlargement;
- (4) urinary 17 ketosteroids at or below age-related upper limits (14);
- (5) serum concentration of 17-hydroxyprogesterone (17-OHP) below 500 ng/dl (one patient with a serum level of 17-OHP of 757 ng/dl was considered adequately controlled because the rest of the criteria were fulfilled); and
- (6) normal testicular size for age or regular menses in adolescent male and female subjects, respectively.

On the basis of these parameters, we separated the patients into three groups: (A) poorly controlled; (B) adequately controlled; and (C) overtreated.

Serum samples for determination of DHEAS and 17-hydroxyprogesterone were obtained during clinic visits, which occurred between 0900-1600 hs. The morning doses of hydrocortisone and of 9- α -fluorohydrocortisone (when required) had been administered at approximately 0730-0830 h.

Serum samples for determination of DHEAS were also obtained from 107 control subjects (64 males, 43 females, 2-23 years of age) referred to the Endocrine Clinic, who had neither an adrenal disorder nor an abnormality of pubertal development.

Serum concentrations of DHEAS were measured by radioimmunoassay (5). The coefficient of variation within the assay was less than 3%; the interassay coefficient of variation ranged from

Table 1. Clinical and laboratory data on 28 patients with congenital adrenal hyperplasia due to 21 hydroxylase deficiency¹

Group A (poorly controlled)									
Patient/Sex	Age (Years)	Salt loser	Dose of hydro-cortisone (mg/m ² /day)	Urine		Serum			
				17 KS	PT	17 OHP (ng/dl)	DHEAS (μg/dl)	DHEAS-SDS	
1	F	9.9	Yes	26.7	(mg/day)	2750	105	+2.14	
2	F	10.3	Yes	26.2	9.2	11	9150	41	-0.67
3	F	11.1	No	19.4	21.1	22	1717	56	-0.13
4	M	12.3	No	12.4	12		1120	123	+0.57
5	M	14.4	No	28.6			18000	29	-1.53
6	M	14.9	Yes	11.8	25.2	31	4806	102	-0.50
7	F	14.9	Yes	15.4	74.6	22	16000	167	+0.41
8	F	14.9	No	18.0	22.1	9	7720	252	+1.61
9	M	15.3	Yes	18.0	23.2	23	3800	142	+0.06
10	F	15.5	Yes	14.5	16.6	22	6500	16	-1.72
11	M	16.4	No	18.7	25.9	12	997	246	+0.91
Mean ± S.D.		13.6 ± 2.3	55%	19.1 ± 5.8	25.5 ± 19.2	19 ± 7.6	6596 ± 5807	116 ± 81	+0.102 ± 1.2
Group B (adequately controlled)									
1	M	2.3	Yes	20.8			410	17	+1.55
2	M	2.5	Yes	19.2			230	2	-0.65
3	F	4.2	Yes	15.6	2.8	1.3	757	14	+0.18
4	F	8.2	No	16.7	5.0	1.1	308	15	-0.83
5	F	9.1	Yes	15.0	2.6	0.3	46	6	-1.13
6	M	11.4	No	20.0			255	54	-0.20
7	F	14.0	No	13.3	2.9	4.4	440	41	-1.37
8	F	14.7	Yes	18.5			237	15	-1.73
9	F	17.0	Yes	22.5	7.2	0.6	78	11	-2.88
10	F	22	No	18.2	2.8	0.7	162	76	-1.83
Mean ± S.D.		10.5 ± 6.5	60%	18 ± 2.8	3.9 ± 1.9	1.4 ± 1.5	292 ± 206	25 ± 24	-0.89 ± 1.22
Group C (overtreated)									
1	F	2.6	Yes	18.5	1.4	0.1	9.1	<4	-0.64
2	F	3.9	Yes	16.7	0.5	0.3	14	<4	-0.60
3	M	4.0	Yes	13.3	1.5	0.1	8	<4	-0.60
4	F	6.7	Yes	23.0	2.1	<0.05		6	-1.12
5	M	6.8	Yes	11.8	5.4	0.07	9	<4	-1.42
6	F	7.0	Yes	15.6	0.8	<0.05	23	<4	-1.27
7	F	8	Yes	12.5	2.4	<0.05	10.5	6	-1.13
Mean ± S.D.		5.5 ± 2.0	100%	15.9 ± 4.0	2.0 ± 1.6	0.1 ± 0.08	12.2 ± 5.6		-0.96 ± 0.3

¹ Abbreviations: 17 KS, 17 ketosteroids; PT, pregnanetriol; 17 OHP, 17 hydroxyprogesterone; DHEAS, dehydroepiandrosterone sulfate; and SDS, standard deviation score.

6% (at a DHEAS concentration of 288 μg/dl to 10.6% (at a DHEAS concentration of 36 μg/dl). Values less than 4 μg/dl were below the sensitivity of the assay.

Serum levels of 17-OHP were measured by radioimmunoassay (1). The coefficient of variation within the assay was less than 3%; the coefficient of variation between the assays was 15.5% when the mean level of 17-OHP was 62 ng/dl.

Twenty-four h urine samples were collected at home, generally the day before or occasionally within a few days before the visit. Urinary concentrations of 17-ketosteroids (10) and pregnanetriol (3) were measured by conventional methods.

Statistical analysis. Because serum concentration of DHEAS normally changes with age, the values from individual patients were compared to the mean of serum DHEAS from age-matched control subjects. To allow for age differences in the three groups of patients, we calculated the standard deviation score (SDS) for each patient from the formula $SDS = (x - \bar{x}) \div S.D.$ where x is the value for the patient, $\bar{x}$ and $S.D.$ are the mean and standard deviation, respectively, for the age-matched control subjects (4, 31). In this way, standard deviation scores from patients of all ages can be grouped together and averaged. By using this formula, the mean SDS value for any normal population is zero regardless of age. The P value for SDS was calculated by using two-way analysis of the variance (9). Linear regression analysis was em-

ployed to test for correlation between serum levels of DHEAS and age.

RESULTS

The pertinent clinical and laboratory data of 28 patients with CAH are listed in Table 1. Patients in poor control (Group A) and adequate control (Group B) were comparable for mean age and prevalence of "salt-losers," whereas children who were overtreated (Group C) were younger and all had the "salt-losing" form of CAH. The sex ratio and the mean daily dose of hydrocortisone were comparable in the three groups. Mean serum concentrations of DHEAS were significantly lower in patients classified as adequately controlled (Group B, 25 ± 24 μg/dl) than in patients in poor control (Group A, 116 ± 81 μg/dl, $P < 0.01$).

In Figure 1, serum levels of DHEAS from individual patients were compared to those obtained in age-matched control subjects. Serum concentrations of DHEAS were low for the degree of control of the disease, as they were within 1 S.D. of the normal mean in 9 of 11 poorly controlled patients (Group A), below 1 S.D. in 6 of 10 adequately controlled (Group B), and overly suppressed in overtreated patients (Group C). Similar results were obtained when serum concentrations of DHEAS were expressed as standard deviation scores (SDS) for age (Table 1). DHEAS-SDS values

Fig. 1. Serum concentrations of dehydroepiandrosterone sulfate (DHEAS) in 107 normal subjects and 28 patients with congenital adrenal hyperplasia. The number above each bar denotes the number of normal subjects in each age group. The vertical bars denote ± 1 S.D. from the mean. The horizontal lines show the mean values.

were not significantly different from zero (*i.e.*, the mean for normal age-matched subjects) in poorly controlled patients (Group A, $P = 0.50$), whereas in adequately treated patients with CAH (Group B) DHEAS-SDS values were significantly lower than the mean for the control population ($P < 0.05$). The mean DHEAS-SDS in overtreated patients was also significantly lower than zero; this is, however, a mere approximation because most of the values in this group of patients were below the sensitivity of the assay.

The correlation between serum concentrations of DHEAS and age is shown in Figure 2. As expected, serum concentrations of DHEAS correlated significantly with age in normal subjects from 2–18 years of age ($r = 0.78$, $P < 0.001$). The correlation was still significant in adequately controlled patients with CAH ($r = 0.64$, $P < 0.05$) but the slope of the regression line in this group was significantly less steep ($P < 0.001$) than that obtained in normal subjects, thus emphasizing again the excessive suppression of serum DHEAS in adequately treated patients with CAH. In poorly controlled patients with CAH, the correlation between age and serum DHEAS concentration was not significant ($r = 0.43$, $P < 0.10$).

RESULTS

We have examined serum DHEAS concentrations in 28 patients with CAH due to 21-hydroxylase deficiency and found them to be inappropriately low for the degree of control of the disease.

Serum levels of DHEAS were significantly below the normal values for age in patients who were believed to be in adequate control, whereas they were within normal limits for age in most of the patients in poor control.

The criteria used in our study to determine the adequacy of therapy were too crude to assess optimal treatment. One may argue that the low levels of DHEAS found in the adequately controlled patients may simply be due to a mild degree of overtreatment not detected by our insensitive methods. This, however, does not seem to be the case in as much as serum levels of 17-OHP were above the upper limit of normal [200 ng/dl (13)] in most of these subjects.

Golden *et al.* (11) also measured serum levels of DHEAS during therapy in 18 patients with CAH and found elevated levels only in those patients who were grossly undertreated. Oversuppression of serum levels of DHEAS, however, cannot be discerned in this report because these authors considered serum levels of DHEAS of less than 100 µg/dl to be normal regardless of the patients' age and stage of pubertal development.

The serum concentrations of other Δ_5 androgens have been found to be low in patients with CAH treated with glucocorticoids. Korth-Schutz *et al.* (16) and Anderson (2) found low levels of serum dehydroepiandrosterone in both optimally and poorly controlled patients with CAH and concluded that dehydroepiandrosterone is not a good index of control in this condition. In another study of 21 patients who were treated for CAH (18), pregnenolone and 17-hydroxypregnenolone increased only after repeated injections of repository ACTH, but not after short term synthetic ACTH stimulation. In contrast to Δ_5 compounds, serum concentrations of some adrenal Δ_4 compounds such as 17-OHP (33), androstenedione (6, 16), and testosterone (6, 27), increase rapidly when treatment is inadequate and decrease to normal levels when therapy is optimal; thus, the ratios of dehydroepiandrosterone or DHEAS to 17-OHP, androstenedione or testosterone, respectively, are reduced below normal values in patients with CAH who are well controlled.

The reason for disproportionately low levels of serum DHEAS in treated patients with CAH remains speculative. Dissociation between the rate of secretion of adrenal androgens and glucocor-

Fig. 2. Correlation of serum concentration of dehydroepiandrosterone sulfate (DHEAS) with age. (—), Normal subjects ($y = 11.5x - 45$, $r = 0.78$, $P < 0.01$). Individual values ($n = 103$) are not plotted to avoid crowding of the figure. (---), Patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CAH) in adequate control ($y = 2.4x + 0.2$, $r = 0.64$, $P < 0.05$). (·····), Patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CAH) in poor control ($y = 15.2x - 90$, $r = 0.42$, $P < 0.1$).

ticoids has been observed in various pathologic (7, 29) and experimental (20) conditions, as well as during normal development (21, 23, 28). In fact, increase of the DHEAS/cortisol ratio is the hallmark of adrenarche in normal children (22, 32). These observations have led to the hypothesis that adrenal androgens may be controlled by another pituitary hormone in addition to ACTH (12, 20, 30). It is conceivable that this putative adrenal androgen-stimulating hormone is suppressed more readily than ACTH by exogenous cortisol in patients treated for CAH, resulting in a far greater decrease in serum levels of DHEAS than of 17-OHP. The existence of such a hormone, however, has been challenged and alternative explanations have been suggested (2, 22).

The oversuppression of serum DHEAS observed in this study limits the usefulness of the assay in evaluating the adequacy of therapy in patients with CAH. Although high levels of serum DHEAS indicate that treatment is grossly inadequate, normal or low values of serum DHEAS do not reflect necessarily adequacy of therapy.

The physiologic significance of this disproportionate suppression of DHEAS remains unclear at this time and merits further study.

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