Decreased Dehydroepiandrosterone Sulfate in Pigmented Nodular Adrenal Dysplasia

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Previous reports on patients with endogenous Cushing’s syndrome describe low concentrations of the adrenal androgen dehydroepiandrosterone sulfate (DHEA-S) in adrenal adenoma and in a case of feminizing macronodular hyperplasia. Here we present hormonal data from two adult sisters with Cushing’s syndrome as a result of pigmented nodular adrenal dysplasia. Corticotropin concentrations were in the mid-normal range, cortisol production was unaffected by administration of dexamethasone (8 mg/24 h), and baseline concentrations of DHEA-S were <0.5 μmol/L. A low concentration of DHEA-S in these and other previously reported patients with Cushing’s syndrome correctly predicts the results of dynamic testing. Decreased DHEA-S in a patient with endogenous Cushing’s syndrome can be ascertained by assay of a single sample and should prompt consideration of the diagnosis of autonomous bilateral nodular disease as well as adrenal adenoma.

Dehydroepiandrosterone sulfate (DHEA-S) is an androgen of almost exclusively adrenal origin.4 Because of its long half-life, high concentration in serum, and slight diurnal variation, a single determination of DHEA-S conveniently indicates the degree of adrenal androgen production (1).

The finding of a low DHEA-S concentration in a patient with endogenous Cushing’s syndrome suggests adrenal autonomy from pituitary regulation, because both cortisol and adrenal androgen are corticotropin-dependent. In a series of 47 patients suspected of having Cushing’s syndrome, concentrations of DHEA-S in serum <0.4 mg/L (1.1 μmol/L) were found to be diagnostic of adrenal adenoma (2). Autonomous adrenal diseases causing Cushing’s syndrome include adrenal adenoma, adrenal carcinoma, and a heterogeneous group of nodular hyperplasias and dysplasias (3–16). Patients with carcinoma usually have increased concentrations of DHEA-S. Previous investigators reporting nodular hyperplasias and dysplasias have found serum dehydroepiandrosterone or DHEA-S to be above normal (6), within normal limits (7, 10, 17), or, in a case of feminizing macronodular hyperplasia, low (16).

Here we report the finding of low DHEA-S in two sisters with adult-onset clinical Cushing’s syndrome and pigmented nodular dysplasia of the adrenals.

Materials and Methods

Free testosterone and dehydroepiandrosterone in serum were measured by radioimmunoassay by SmithKline Bio Science Laboratories, Schaumberg, IL 60196, and Damo Clinical Laboratories, Berwyn, IL 60402, respectively. Serum and urinary cortisol were measured postoperatively by Roche Biomedical Laboratories, Dublin, OH 43017. Preoperative urinary free cortisol and total testosterone and cortisol in serum were determined in-house by radioimmunoassay (18–20). Urinary 17-hydroxycorticoestroid concentrations were determined by the Porter–Silver reaction and 17-ketosteroid concentrations by the Zimmermann reaction.

Androstenedione was measured in serum by double antibody RIA adapted from a method previously described (21). The primary antibody (rabbit anti-androstenedione 11α-succinate–human serum albumin) was supplied by Pantex, Santa Monica, CA 90404, and the tracer [125I]androstenedione, by ICN Biomedicals, Carson, CA 90746. The precipitating goat anti-rabbit antibody (Scantibodies Laboratory Inc., Santee, CA 92071) was titered for optimum precipitation of rabbit gamma globulin by 50 μL of the diluted antibody. The primary antibody was titered in the absence of added analyte and under the exact conditions of the RIA procedure used, to bind approximately 50% of the tracer. The cross-reactivities of the primary antibody with other steroids at 50% displacement agreed with the values reported by the supplier. Standard and duplicate samples of controls and patients’ serum samples (300 μL) were extracted once with 7 mL of petrolem ether and centrifuged (1000 × g, 5 min). A 5-mL aliquot of the upper layer was evaporated, and the residue was dissolved in 1 mL of assay buffer (0.1 mol/L phosphate buffered saline, containing 1 g of unflavored gelatin per liter [Knox Gelatin Inc., Englewood Cliffs, NJ 07632]). In the RIA, 50 μL of the reconstituted extract was incubated overnight at room temperature with 100 μL of tracer (10 000 counts/minute) containing 13.4 μg of carrier protein 100 μL of primary antibody, and 50 μL of the precipitating antibody. The sensitivity of the assay was 0.35 nmol/L (0.1 ng/mL) and the within- and between-assay CVs at the

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4 Nonstandard abbreviations: DHEA-S, dehydroepiandrosterone sulfate; SHBG, sex-hormone-binding globulin; and ACTH, corticotropin.

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midpoint of the assay range, 14.0 nmol/L (4.0 ng/mL), were 1.5% and 6.3%, respectively. Values reported by a reference laboratory (SmithKline Bio-Science Laboratories) agreed with those obtained by this method on the same 23 samples, \( r = 0.95 \). The results of a serially diluted patient's sample, on a log-logit plot, had a slope of \(-2.02\) compared with the standard curve's slope of \(-1.99\). An average analytical recovery of 97.1% was determined by assaying aliquots of previously evaluated samples from four patients which were added equal volumes of standards at three concentrations.

DHEA-S in serum was measured by a double-antibody method developed in-house and performed, without extraction, on 100 \( \mu \)L of 400-fold-diluted samples (10 nmol/L phosphate-buffered saline as diluent with 5 g of bovine serum albumin added per liter). Primary antibody (rabbit) against DHEA-S 3\( \alpha \)-monohemisuccinate-human serum albumin and the tracer, \(^{125}\)I-labeled DHEA-S, were from ICN Biomedicals; the precipitating antibody was supplied by Scantibodies Laboratory. Both the antibodies were titrated to give about 50% binding in the absence of added analyte. Cross-reactivities of the antibody to other steroids are reported by the supplier. In the RIA procedure, 100 \( \mu \)L of diluted patient's serum was incubated for 3 h at 37°C with 100 \( \mu \)L of primary antibody containing 15 \( \mu \)g of rabbit gamma globulin (Sigma Chemical Co., St. Louis, MO 63178), 100 \( \mu \)L of tracer (10,000–12,000 counts/min), and 50 \( \mu \)L of precipitating antibody (goat anti-rabbit) titrated for optimum precipitation. The sensitivity of the assay, defined as the minimum amount of hormone that can be measured with acceptable precision (\(<10\)%), was 0.5 pmol/L (0.2 \( \mu \)g/mL). The within-assay CV was \(<7.0\)% and the between-assay CV was \(<9.0\)% at each of three concentrations of DHEA-S (3.0, 7.1, and 10.3 pmol/L). Analytical recovery averaged 98.0% when aliquots of DHEA-S standards were added to equal volumes of patients' samples before dilution and assayed. Using a log-logit transformation demonstrated parallelism for the slopes of the plots of serially diluted patients' sera and of a standard curve (\(-1.90\) and \(-1.93\), respectively). Results obtained by this method correlated well (\( r = 0.95 \)) with those reported for 27 duplicate samples by SmithKline Bio-Science Laboratories. The normal reference interval we established from data for 37 women ages 20–40 years, was 2.2–9.0 pmol/L (0.8–3.3 pg/mL); for 18 women older than 40 years, 0.5–5.7 pmol/L (0.2–2.1 pg/mL); and for 17 men, 5.2–16.3 pmol/L (1.9–6.0 pg/mL).

Plasma corticotropin (ACTH) concentrations were measured with an RIA kit from ICN Biomedicals. Blood samples were collected in ice-cold siliconized Vacutainer Tubes containing EDTA and separated in a refrigerated centrifuge within 1 h of collection. The plasma was stored at \(-30^\circ\)C in three aliquots (to preserve sample integrity for repeat determinations) until assayed. The sensitivity of the assay was established to be 2 pmol/L (10 pg/mL) and the within-assay and between-assay CV was \(<10.0\)% throughout the range of the assay, 0–220 pmol/L (0–1000 pg/mL). In a series of experiments performed on patients' samples having corticotropin concentrations ranging from 4 to 75 pmol/L, equal volumes of standards were added and assays yielded recoveries ranging from 90.5% to 110.2%. On 31 samples, 18 of which spanned our normal reference interval, assay results correlated well (\( r = 0.94 \)) with results obtained with the Allégro kit (Nichols Institute, San Juan Capistrano, CA 92675).

Concentrations of sex-hormone-binding globulin (SHBG) were determined with the Delfia SHBG kit (Electro-Nucleonics, Columbia, MD 21046–1297). Normal reference intervals established in this laboratory were 20–100 nmol/L for 31 women (ages 22–44) and 10–60 nmol/L for 33 men.

All RIA determinations were done in duplicate, and two sets of tri-level controls were included in each assay run to monitor inter-assay variability.

**Cases and Results**

Preoperative hormonal assays results are shown in Table 1.

**Case 1.** A 26-year-old cushingoid woman was referred two years after the birth of twins. She had a four-month history of severe depression requiring hospitalization, striking facial hirsutism, amenorrhea, a facial café-au-lait spot, and cushingoid features. Computerized tomography showed the presence of a left adrenal mass. Androgen concentrations in serum included 5.2 nmol/L (normal, 5.6–24.2) for dehydroepiandrosterone and 0.1 ng/dL (normal, 0.1–0.4) for free testosterone (to convert to pmol/L, multiply by 34.67). She underwent unilateral adrenalectomy and within six months regained normal menstruation, physical appearance, and mental status. Pathological examination of the 2-cm adrenal nodule showed a well-delineated non-encapsulated tumor consisting of large polygonal cells with clear to granular cytoplasm and considerable variation of the nuclei. The surrounding adrenal tissue appeared compressed, was not hyperplastic, and contained three microscopic unencapsulated nodules. The largest was 1.5 mm in diameter, composed of similar cells and containing foci of pigmentation. Three months postoperatively her SHBG concentration in serum was 25 nmol/L. Fifteen months postoperatively her serum DHEA-S was <0.5 pmol/L, her 1600 h cortisol was 300 nmol/L (normal, 60–500), and her urinary excretion of free cortisol was 100 nmol/d (normal, 100–330).

**Table 1. Familial Pigmented Nodular Adrenal Dysplasia**

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0800 h ACTH, pmol/L</td>
<td>12</td>
<td>17</td>
<td>(2–22)</td>
</tr>
<tr>
<td>0800 h cortisol, nmol/L</td>
<td>770</td>
<td>800</td>
<td>(170–660)</td>
</tr>
<tr>
<td>1600 h cortisol, nmol/L</td>
<td>770</td>
<td>770</td>
<td>(60–500)</td>
</tr>
<tr>
<td>DHEA-S, pmol/L</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>(2.2–4.9)</td>
</tr>
<tr>
<td>Androstenedione, nmol/L</td>
<td>6.0</td>
<td>2.0</td>
<td>(2.0–10.5)</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>(0.5–3.0)</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>13</td>
<td>13</td>
<td>(20–100)</td>
</tr>
<tr>
<td>Urinary cortisol, nmol/d</td>
<td>470</td>
<td>1200</td>
<td>(70–340)</td>
</tr>
<tr>
<td>17-Hydroxycorticosteroids, ( \mu )mol/d</td>
<td>41</td>
<td>51</td>
<td>(8–17)</td>
</tr>
<tr>
<td>17-Ketosteroids, ( \mu )mol/d</td>
<td>26</td>
<td>34</td>
<td>(21–52)</td>
</tr>
<tr>
<td>Creatinine, mmol/d per 1.73 m²</td>
<td>5.7</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone, 2 mg/24 h</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0800 h ACTH, pmol/L</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone, 8 mg/24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800 h cortisol, nmol/L</td>
<td>800</td>
<td>880</td>
<td></td>
</tr>
<tr>
<td>1600 h cortisol, nmol/L</td>
<td>800</td>
<td>770</td>
<td></td>
</tr>
<tr>
<td>Urinary cortisol, nmol/d</td>
<td>1130</td>
<td>1540</td>
<td></td>
</tr>
<tr>
<td>17-Hydroxycorticosteroids, ( \mu )mol/d</td>
<td>54</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>17-Ketosteroids, ( \mu )mol/d</td>
<td>33</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Creatinine, mmol/d per 1.73 m²</td>
<td>8.6</td>
<td>7.9</td>
<td></td>
</tr>
</tbody>
</table>

*Baseline studies were obtained before administration of oral dexamethasone, 0.5 mg every 6 h for three days, followed by 2.0 mg every 6 h for three days.

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Case 2. The 35-year-old sister of the first patient was referred for cushingoid features with a one-year history of hirsutism requiring electrolysis, amenorrhea, and a recent hospitalization for severe depression. An eyelid nodule had been surgically removed in the past. She had one 10-year-old child. There were no lateralizing findings on adrenal computerized tomography. After bilateral adrenalectomy her depression improved, menses returned, and cushingoid features receded. Gross inspection at the operating table revealed multiple dark nodules on the surfaces of the uncut adrenals. Microscopically, multiple nodules were noted, some containing clear cells and others consisting of cells with deeply eosinophilic cytoplasm. Some of the eosinophilic cells contained pigment and very large nuclei but no mitotic figures. Ten months postoperatively her ACTH concentration at 1500 h was 12 pmol/L.

Echocardiography was negative for atrial myxoma in both cases.

Discussion

Reporting of adrenal androgen concentrations may help establish a classification of nodular hyperplasia and dysplasias according to pathogenesis. Heterogeneity clearly exists among patients having bilateral adrenal nodularity and Cushing's syndrome. Some sporadic cases of multinodular disease may have been ACTH-dependent at the outset (8, 13). In familial pigmented multinodular adrenocortical dysplasia, the presence of nodular nonencapsulation and internodular atrophy suggests a primary adrenal propensity to autonomous or hamartomatous development (7). An autoimmune basis for this disorder has been proposed (11, 14). Catheterization studies (22) have suggested pluriopotentiality of steroidogenesis in patients with adrenal adenoma, but few data are available from multinodular disease. The future use of tissue-culture techniques may further differentiate these diseases (23). The observation of decreased concentration of DHEA-S in our patients suggests either that cortisol production is autonomous from pituitary ACTH or that a specific defect of steroidogenesis exists in the hamartomatous tissue of our patients, affecting delta-S androgen production.

The ACTH concentrations observed in these patients are unexplained. The decreased concentration of DHEA-S in our patients might imply suppression of integrated daily production of bioactive ACTH. If so, the DHEA-S results present less ambiguity than the measured concentrations of radioimmunoassayable ACTH and arguably predicted the observed findings of both dexamethasone-nonsuppressibility and nodular disease.

Despite normal or low measured androgen concentrations, our patients had striking facial hirsutism. Possible explanations include suppression of SHBG concentrations resulting from Cushing's syndrome, excess of other unmeasured or free androgens, hair follicle hypersensitivity to androgen, or ovarian androgen overproduction before the onset of clinical illness. The androstenedione may be of ovarian origin in these patients. A less probable explanation of their hirsutism is that adrenal androgen overproduction might have occurred during an unobserved antecedent phase of their disease, with subsequent loss of androgen-synthetic capacity by formerly pluripotent cell lines.

Dynamic endocrine testing and radiographic detection of one large adrenal nodule in case 1 led to a mistaken preoperative diagnosis of solitary adenoma. The patient probably will demonstrate cortisol excess in the future, necessitating contralateral adrenalectomy. We question whether delayed postoperative androgen recovery in her case and other cases of apparently solitary adenoma (24) might be due to abnormalities of diurnal rhythm or subtle cortisol overproduction by a micronodular contralateral adrenal.

The detection of low concentrations of DHEA-S facilitates the clinical recognition of a solitary adenoma causing Cushing's syndrome (2). Recognition of bilateral autonomous nodular adrenal disease is important because (a) the treatment of choice is bilateral adrenalectomy rather than unilateral adrenal resection or pituitary surgery, (b) nodular dysplasia may be familial, and (c) some cases involve associated disorders such as cardiac myxomata, spindle cell tumors, cutaneous lentigines, and Sertoli cell tumors of the testis (10). Preoperative diagnostic findings include high-dose dexamethasone nonsuppressibility, unresponsiveness to metyrapone, unresponsiveness of ACTH to administration of corticotropin-releasing hormone, characteristic findings on adrenal scintigraphy (25), and normal or low concentrations of measured ACTH. To this list should be added, in at least some cases, the finding of low concentrations of adrenal androgen.

References

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Effect of Glycation of Low-Density Lipoprotein on the Immunological Determination of Apolipoprotein B

Richard D. Press and Peter Wilding

Non-enzymatic glycation of low-density lipoprotein (LDL) may contribute to the premature atherogenesis of patients with diabetes mellitus. To assess whether glycation of apolipoprotein B, the predominant protein of LDL, interferes with the ability to immunologically quantify this protein, we prepared and purified glycated LDL by incubating normal plasma samples with high concentrations of glucose. Although both the plasma and the LDL specimens incubated with glucose contained significantly more glycated protein than control specimens, the quantitative interaction of an apolipoprotein B-specific antibody with glycated vs nonglycated LDL was not significantly different. We conclude that apolipoprotein B can be accurately quantified immunologically despite the presence of clinically excessive degrees of LDL glycation.

Additional Keyphrases: diabetes mellitus · atherogenesis · immunonephelometry

Non-enzymatic glycation of proteins is thought to be a primary initiating event in the pathogenesis of many of the clinical complications of long-standing diabetes mellitus (1). In particular, glycation of apolipoprotein B, the major apolipoprotein of low-density lipoprotein (LDL) has been shown to occur in vitro (2, 3) and in vivo (3) after prolonged hyperglycemia. Involvement of this glycated LDL in the accelerated atherogenesis characteristic of prolonged diabetes has been suggested by reports of both impaired recognition of glycated LDL by the LDL receptor (4) and increased cholesterol ester accumulation by monocyte/macrophage cells exposed to glycated LDL (5). Because concentrations of apolipoproteins A and B in serum may be better predictors of atherogenic risk than is the typical cholesterol or lipoprotein analysis (6), more clinical laboratories have been offering apolipoprotein measurements, predominantly by immunological methods with use of commercial apolipoprotein-specific antibodies.

To assess whether non-enzymatic glycation of LDL apolipoprotein B had any quantitative effect on the interaction of this protein with the antibody used for its clinical determination, we used an immunonephelometric assay to compare the amounts of apolipoprotein B in LDL purified from plasma samples that had been pre-incubated with or without high concentrations of glucose. We found that, although the LDLs from the plasma specimens incubated with glucose were significantly more glycated than the control LDLs, this degree of glycation had no significant effect on the quantitative immunological determination of apolipoprotein B.

Materials and Methods

Specimens: Plasma was sampled from 10 donors with no clinical evidence of diabetes. These samples included five outdated units of fresh-frozen plasma, three fresh specimens obtained after therapeutic plasma exchange from patients with chronic inflammatory demyelinating polyneuropathy, one fresh specimen obtained after therapeutic plasmapheresis from a patient with polycthemia vera, and one fresh specimen from a healthy laboratory technologist. After dialysis against isotonic saline containing 1 mmol of EDTA per liter to remove the preservatives and anticoagulants required for plasma storage, the 10 plasma specimens were each divided into two identical 15-mL aliquots. To one of each pair of 10 specimens we then added concentrated glucose (210 g/L solution) to give a final concentration of 9.0 g/L; the other received no addition. All speci-