Abstract
The impact of the synthetic glucocorticoid prednisone on adrenal steroid hormone production was examined using 24-hour urinary steroid hormone profiling. Five women, who were chronically taking low-dose prednisone, were tested, and the relevant literature was reviewed. As expected, adrenal glucocorticoid production, measured by urinary terminal cortisol and cortisone metabolites, was markedly suppressed compared to reference range values ($p=0.03$). Urinary cortisol and cortisone, reflecting circulating glucocorticoids, were decreased to a lesser extent than their terminal metabolites. Urinary dehydroepiandrosterone (DHEA) excretion was dramatically suppressed ($p=0.03$), while the downstream androgen metabolites androsterone and etiocholanolone were suppressed to a lesser extent. Aldosterone and tetrahydrocorticosterone production demonstrated modest suppression after prednisone administration, but allo-tetrahydrocorticosterone, which is highly sensitive to adrenocorticotropic hormone (ACTH) secretion, was suppressed to a greater extent. Prednisone administration results in a decrease in ACTH secretion by the anterior pituitary, suppressing synthesis of glucocorticoids, DHEA, and DHEA metabolites. Decreased glucocorticoid synthesis is adaptive, because prednisone is active at the glucocorticoid receptor, but suppression of DHEA synthesis is not mitigated by prednisone. DHEA is an important sex hormone precursor, neurosteroid, and endocrine and immune modulator; therefore, DHEA depletion may have significant adverse consequences in terms of sex hormone production, bone health, endocrine and immune system function, and neuropsychiatric status. Studies of DHEA replacement in patients taking prednisone for lupus demonstrate amelioration of some of these adverse effects. (Altern Med Rev 2006;11(1):40-46)

Introduction
The synthetic glucocorticoid prednisone is used for its anti-inflammatory properties in the treatment of many different conditions, including rheumatologic, hormonal, allergic, respiratory, and other disorders. Prednisone administration results in numerous side effects, a number of which are the result of its suppression of adrenal cortex function. After prednisone administration, adrenal production of the endogenous glucocorticoids, cortisol and cortisone, declines. This can result in marked hypocortisolism after discontinuation of high-dose prednisone therapy, especially in seriously ill patients. Because prednisone suppresses adrenal function by reducing adrenocorticotropic hormone (ACTH) secretion by the anterior pituitary gland,
other adrenal hormones normally produced after ACTH stimulation are also vulnerable as a consequence of prednisone therapy.\(^1\) After ACTH administration to healthy adults, plasma and urinary concentrations of glucocorticoids, dehydroepiandrosterone (DHEA), and aldosterone all increase.\(^2\) This suggests that patients treated with prednisone may experience not only declines in glucocorticoids but also declines in aldosterone and DHEA and its metabolites. The limited data available in the literature suggest DHEA may be even more sensitive to suppression by prednisone than the glucocorticoids,\(^3\) while aldosterone is somewhat less sensitive, probably because its secretion is mediated by multiple mechanisms.

In the course of reviewing routine urinary steroid hormone profiles conducted on a large number of patients for diverse clinical purposes, the authors noticed a striking and consistent pattern in the results for the small number of patients tested who were taking prednisone. This article describes this pattern, reviews its mechanisms and the relevant literature, and discusses its clinical and research implications.

### Methods

Five women who were chronically taking prednisone had 24-hour urinary steroid hormone profiles conducted at Meridian Valley Laboratory between February and June 2005. Patients submitted a questionnaire on the use of hormones, other medications, and symptoms, with their urine specimens. The questionnaires were used as part of the laboratory quality assurance program, for review of test results, and for consultation with physicians who ordered the tests. The median age was 41 years (span 32-60); one woman had regular menstrual cycles, two had irregular cycles, and two were postmenopausal. Prednisone dose was 5 mg/day for two patients, 10 mg/day for one, and not known for two, although use of prednisone was confirmed on the questionnaire.

Steroid analysis was performed as described previously.\(^4\) Steroids were isolated from urine by solid phase extraction (C18 columns; United Chemical Technologies; Bristol, PA), eluted with methanol, and the methanolic extract was evaporated to dryness. The residue was reconstituted in acetate buffer, hydrolyzed overnight with sulfatase-beta-glucuronidase, and extracted with ethyl acetate after internal standard addition. The ethyl acetate extract was evaporated to dryness, and the MOX/TMS derivatives of the steroids were prepared. The final derivatized extracts were dissolved in hexane, washed with de-ionized water, and an aliquot of the hexane phase was injected into the gas chromatograph-mass spectrometer (GC-MS).

The GC-MS system included an Agilent 6890 GC with a 7683 Autosampler, an Agilent 5973N MSD, and an Enhanced MSD Chemstation data system. A 30 m x 0.25 mm ID, 0.25-micron film thickness dimethylpolysiloxane column was used, with helium (1.9 mL/min) as the carrier gas. Analytes were separated during a 32-minute temperature program, and the steroids were identified and quantified using selected ion monitoring. Calibration was performed with derivatized standards prepared from pure analytical reference materials (Steraloids; Newport, RI).

Chromatograms for all five cases were reviewed for accuracy as follows. Prednisone is a synthetic analog of cortisone that differs from cortisone by the presence of a double-bond rather than a single-bond between carbons 1 and 2 in the A-ring. Similarly, prednisone's metabolite prednisolone differs from cortisol only in the presence of the same double-bond in the same location. Thus, the molecular ions for prednisone and prednisolone have a mass number two less than those of cortisol and cortisone. Prednisone and prednisolone were not fully resolved chromatographically from cortisol and cortisone, but cortisol and cortisone analysis was possible because of the mass difference of the molecular ions.

Aldosterone was analyzed by radioimmunoassay (Diagnostic Products Corporation; Los Angeles, CA).

The completeness of 24-hour urine collection was assessed by measurement of creatinine (reference range 0.5-2.0 g/24 hours).

Reference ranges for the majority of analytes were taken from the literature.\(^5\) Reference ranges for cortisol, cortisone, and DHEA were determined by an in-house study of 30 healthy young adults. Reference ranges for cortisol and cortisone are similar to those in other studies using similar methodology.\(^6\) Urinary DHEA excretion declines with age; the lower limit for the DHEA reference range in this report is higher than some others reported in the literature, primarily
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Figure 1. Metabolic Pathways for Adrenal Steroids

Mineralocorticoid Pathway

- Pregnenolone
  - \( 3\beta \text{ dehydrogenase} \)
  - Progesterone
    - \( 21\)-hydroxylase
    - 11-Deoxy-Corticosterone
      - \( 11\)-hydroxylase
      - Corticosterone
        - Aldosterone
          - Tetrahydrocorticosterone
            - Tetrahydrocortisol
              - Tetrahydrocortisone

Corticosteroid Pathway

- 17-OH Pregnenolone
  - 17-OH Progesterone
    - \( 21\)-hydroxylase
    - 11\(\beta\)-Deoxycorticisol
      - \( 11\)-hydroxylase
      - Cortisol
        - Cortisone
          - Tetrahydrocorticosterone
            - Tetrahydrocortisol
              - Tetrahydrocortisone

Sex Hormone Pathway

- DHEA
  - Androstenedione
    - Testosterone
      - Estradiol
        - Androsterone
          + Etiocholanolone

Compounds measured for this study are in bold type, and synthetic enzymes are in italics.

Because this laboratory’s reference range reflects values found in young adults. The reference range for aldosterone was taken from the radioimmunoassay kit package insert and is specific for individuals with a normal sodium intake. Statistical analysis was conducted using the sign test.

Results

All five patients had normal creatinine excretion (median 1.1 g/24 hours, span 0.8-1.8 g/24 hours; reference range 0.5-2.0 g/24 hours).

Urinary adrenal steroid hormones are uniformly lower in women taking prednisone than in unmedicated patients of similar age. Steroid hormone data for the patients taking prednisone were organized into three groups: glucocorticoids, sex hormones, and mineralocorticoids (Figure 1). As expected, glucocorticoid production was suppressed, as shown in Table 1.

Results for pregnanetriol, which is elevated in adrenal hyperplasia, were within the reference range (100-1500 \( \mu \)g/24 hours) in three patients and below the lower limit in two.
Table 1. Glucocorticoid Levels in Subjects on Prednisone

<table>
<thead>
<tr>
<th>Glucocorticoid</th>
<th>Reference Range (µg/24 hr)</th>
<th>Women taking Prednisone (median, span)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>30-170</td>
<td>25 (8-38)</td>
</tr>
<tr>
<td>Cortisone</td>
<td>31-209</td>
<td>18 (9-56)</td>
</tr>
<tr>
<td>Tetrahydrocortisone</td>
<td>1700-4200</td>
<td>191 (24-1628)*</td>
</tr>
<tr>
<td>Tetrahydrocortisol</td>
<td>900-2600</td>
<td>116 (17-579)*</td>
</tr>
</tbody>
</table>

*p=0.03 (patient values compared to lower limit of reference range)

DHEA was excreted at remarkably low levels; two terminal DHEA metabolites, androsterone and etiocholanolone, were consistently below reference range values as well, as summarized in Table 2. Androsterone and etiocholanolone results for one patient were excluded from Table 2 because she was taking testosterone. In this case, urinary testosterone excretion was 66 µg/24 hr (reference range 5-35 µg/24 hr), DHEA 1 µg/24 hr, androsterone 456 µg/24 hr, and etiocholanolone 530 µg/24 hr. Androsterone and etiocholanolone are metabolites of testosterone as well as DHEA. Because of the exclusion of one patient’s values for androsterone and etiocholanolone, statistical comparisons of patient values and the lower limit of the reference range were significant at the p=0.06 level.

Steroids derived from corticosterone demonstrated a milder pattern of suppression after prednisone administration (Table 3). Results for aldosterone and tetrahydrocorticosterone were within the reference range for a majority of patients, but results for allo-tetrahydrocorticosterone were below the lower limit of the reference range for all but one patient.

The results in the tables are summarized in Figure 2, in which a vertical bar represents the span of values for each steroid; a horizontal bar intersecting the span represents the median.

Table 2. DHEA and Metabolites in Subjects on Prednisone

<table>
<thead>
<tr>
<th>Androgen</th>
<th>Reference Range (µg/24 hr)</th>
<th>Women taking Prednisone (median, span)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA</td>
<td>100-2000</td>
<td>7 (1-8)*</td>
</tr>
<tr>
<td>Androsterone</td>
<td>500-3200</td>
<td>160 (30-333)</td>
</tr>
<tr>
<td>Etiocholanolone</td>
<td>500-5000</td>
<td>177 (70-384)</td>
</tr>
</tbody>
</table>

*p=0.03 (patient values compared to lower limit of reference range)
Table 3. Corticosterone Metabolites in Subjects on Prednisone

<table>
<thead>
<tr>
<th>Corticosterone Metabolite</th>
<th>Reference Range (μg/24 hr)</th>
<th>Women taking Prednisone (median, span)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>6-25</td>
<td>6 (4-18)</td>
</tr>
<tr>
<td>Tetrahydrocorticosterone</td>
<td>30-240</td>
<td>66 (6-111)</td>
</tr>
<tr>
<td>allo-Tetrahydrocorticosterone</td>
<td>130-600</td>
<td>38 (6-218)</td>
</tr>
</tbody>
</table>

*p=0.03 (patient values compared to lower limit of reference range)

Discussion

The suppression of ACTH secretion after prednisone administration has a profound impact on adrenal steroid production. The cortisol metabolites tetrahydrocortisone, tetrahydrocortisol, and allo-tetrahydrocortisol account for approximately 50 percent of daily cortisol biosynthesis. The marked decline of these metabolites in these women taking prednisone reflects the decrease in cortisol production that results from prednisone administration. Urinary cortisol and cortisone, which reflect circulating glucocorticoids, were also diminished after prednisone, but not to the same extent as the terminal metabolites (Figure 2).

DHEA excretion was very low in this group of women, which is consistent with the ACTH mediation of DHEA production. One potential confounding factor is that DHEA production declines with age. The subjects ranged in age from 32-60 years, and the laboratory reference range lower limit in this report (100 μg/24 hr) reflects values for young adults. A comprehensive study by Weykamp et al determined urinary DHEA excretion rates across the lifespan in males and females. They reported a reference range lower limit of 58 μg/24 hr for women ages 17-50 years and the same lower limit for women ages 51-70 years. Even if this somewhat lower reference range limit is used, the urinary DHEA values for subjects in this study are remarkably low (1-8 μg/24 hr). The downstream DHEA metabolites androsterone and etiocholanolone were also consistently below the reference range lower limit, although not to the same degree as DHEA values (Figure 2).

A second potential confounding factor is that depressed DHEA synthesis in the women studied could have been caused by their medical condition, rather than prednisone administration. The medical complaints included a diagnosis of fibromyalgia in two patients and "severe aches and pains" in two patients. Four patients also complained of headaches - two severe, one moderate, and one mild. However, other studies of prednisone administration have also demonstrated the same decline in DHEA production. For example, a study of daily versus alternate-day, low-dose prednisone in hirsute women found that, after both regimens, basal serum DHEA and DHEA-sulfate concentrations were suppressed to a greater degree than serum cortisol concentrations.

Studies in adolescents and adults, but not young children, demonstrate marked suppression of DHEA synthesis after prednisone administration. The prednisone-associated reduction in ACTH release by the anterior pituitary offers a compelling mechanism for the decline in DHEA production.

The mineralocorticoid steroids aldosterone and tetrahydrocorticosterone demonstrated modest suppression after prednisone administration. This is consistent with the reports that aldosterone production is mediated by multiple mechanisms, with ACTH stimulation responsible for approximately one-half of aldosterone output. The results suggest that suppression of aldosterone synthesis after prednisone administration may only be of concern in patients consuming a low-salt diet, when demands for aldosterone synthesis are high. Allo-tetrahydrocorticosterone production was suppressed to a greater extent than...
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Figure 2. Urinary Steroids in Patients on Prednisone

Vertical bars represent the span of values for the steroids measured in five women taking prednisone, as a percentage of the lower limit of the reference range. Horizontal bars intersecting the spans indicate the median value, as a percentage of the lower limit of the reference range.

The suppression of glucocorticoid synthesis after prednisone administration is an appropriate adaptive response, because prednisone binds to the glucocorticoid receptor and elicits physiological effects that are qualitatively similar to those of cortisol (although prednisone has lower relative mineralocorticoid potency). In contrast, the marked suppression of DHEA synthesis after prednisone administration results in an uncompensated loss of a key sex steroid precursor, neurosteroid (produced in the CNS, independent of the adrenals), and endocrine- and immune-system modulator. Decreased sex hormone production may be a factor in the development of osteoporosis. Low DHEA concentrations are found in patients with depression, and may also be associated with the development of insulin resistance, hyperlipidemia, and age-related declines in immune system function. This raises the question of whether DHEA supplementation might benefit patients who...
take prednisone chronically. Two studies in women with lupus who were taking prednisone have shown DHEA supplementation often allows patients to reduce prednisone dosage and may protect against the bone loss associated with chronic prednisone administration. In another study, women with adrenal insufficiency treated with 50 mg/day DHEA experienced improvement in mood, sense of well-being, and sexuality. The subjects described in this article represent a small sample of women taking prednisone. Larger, more systematic studies would be more definitive. Nevertheless, the distinctive pattern of urinary steroid excretion in these women was noteworthy, consistent with previous reports, and is explained by well-established mechanisms. Future research should determine how rapidly DHEA suppression occurs after commencement of prednisone administration and how rapidly DHEA synthesis returns after prednisone discontinuation. Urinary steroid hormone profiling provides a comprehensive and cost-effective means to study these changes. Continued research on DHEA replacement in patients taking prednisone is also strongly supported by these findings.

In conclusion, 24-hour urinary steroid hormone profiling demonstrates that chronic prednisone administration suppresses the production of adrenal steroids, especially the glucocorticoids and DHEA, and that DHEA synthesis is the most severely affected. Although prednisone is capable of replacing the activity of the suppressed glucocorticoids, the loss of DHEA is uncompensated and may have significant adverse consequences in terms of sex hormone production, bone health, endocrine- and immune-system function, and neuropsychiatric status.

References