Elevated Salivary Cortisol in the Evening in Healthy Elderly Men and Women: Correlation With Bone Mineral Density

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Background. Aging is associated with a loss of bone mineral density (BMD) in men and women. Loss of BMD can also be caused by hypercortisolemia in men or women at any age. This study measured salivary cortisol at 2300 h and 0700 h as indices of cortisol secretory activity in 228 elderly, community-dwelling subjects. Salivary cortisol results were correlated with BMD. We hypothesized that salivary cortisol is elevated at 2300 h in elderly people, and that salivary cortisol will correlate negatively with BMD.

Methods. Saliva was sampled at 2300 h (nadir in circadian rhythm) and 0700 h (peak in circadian rhythm) in 130 men (70.7 ± 0.4 years old) and 98 women (70.0 ± 0.4 years old); approximately half of the women were receiving hormone replacement therapy (HRT). BMD was measured by dual energy x-ray absorptiometry.

Results. Salivary cortisol at 2300 h was significantly elevated in men (2.3 ± 0.1 nmollL) and women (2.1 ± 0.1 nmollL) as compared to 73 younger controls (1.2 ± 0.1 nmollL; 37 ± 1 year old). Salivary cortisol at 0700 h was not different between older subjects and younger controls. There was a significant negative correlation of lumbar (L2-4) BMD and 2300 h salivary cortisol in older women (r = -0.20, p = .05; n = 98); this correlation was significant only in women not on HRT. There was a highly significant negative correlation of lumbar (L2-4) BMD and 0700 h salivary cortisol in older men (r = -0.31, p = .003).

Conclusions. Salivary cortisol is a simple, nonstressful method for assessing activity of the hypothalamic-pituitary-adrenal (HPA) axis in the elderly population. A major finding was an elevation in the late night nadir in cortisol secretion. We also suggest that elevated cortisol secretion in elderly people may contribute to the age-related loss in bone mineral density and that this effect is prevented by HRT.

Aging in humans is associated with many changes in body habitus and composition. Of particular interest are the declines in bone mineral density and muscle mass that occur with aging (1–3). Losses of bone mineral density and muscle mass are also strongly associated with endogenous hypercortisolemia or with administration of exogenous glucocorticoids, regardless of age (4–10).

Humans demonstrate significant circadian rhythmicity in endogenous cortisol secretion with a nadir around midnight and a peak around 0800 h (11). Several studies have demonstrated age-related increases in plasma cortisol in normal volunteers, which is characterized by a higher nocturnal nadir in cortisol with an essentially normal morning peak (12–17). Although the increases in nocturnal cortisol in older subjects are relatively small compared to maximum cortisol secretion, a significant increase in nocturnal glucocorticoid activity spread over a long period of time is likely to exert significant biological effects (18).

Most of the earlier circadian rhythm studies of elderly subjects required hospitalization with placement of indwelling catheters to sample blood at frequent intervals (12–16). We recently evaluated the sampling of salivary cortisol as a noninvasive, simple method for assessing circulating cortisol activity (19). Salivary cortisol is in equilibrium with plasma cortisol and represents the unbound, biologically active fraction (~4%) of plasma cortisol (19). Plasma and salivary cortisol correlate extremely well both in younger adults (19) and elderly human subjects (20).

This study takes advantage of the simplicity of salivary sampling for the measurement of endogenous cortisol activity in a group of elderly, community-dwelling subjects already part of an extensive longitudinal study (21,22). We hypothesized that (a) salivary cortisol is elevated in the evening in elderly subjects compared to a group of younger normal subjects previously reported (19), and (b) salivary cortisol will correlate with indices of bone density and muscle mass within the elderly study group.

Methods
The study was approved by the institutional review boards of the Medical College of Wisconsin, Froedtert Memorial Lutheran Hospital, and the Veterans Affairs Medical Center, Milwaukee, Wisconsin. Subjects had already been recruited as part of a longitudinal study (21,22) and had signed informed consent.
consent. Independent, community-dwelling men and women aged 60 years or older (except for those with a symptomatic chronic disease other than hypertension, osteoarthritis, cataracts, or glaucoma) were included. Exclusion criteria included body weight less than 85% or greater than 120% of desirable weight and medications other than laxatives, antacids, estrogen/progesterone, nonnarcotic analgesics, and anti-hypertensives.

A total of 228 patients completed the study. There were 130 men (aged 70.7 ± 0.4) and 98 women (aged 70.0 ± 0.4). Of the women, 52 (aged 69.3 ± 0.6) were on hormone replacement therapy (HRT), 45 (aged 71.1 ± 0.7) were not on HRT, and 1 was not sure at the time of study.

All subjects underwent an initial interview and physical examination. Blood was drawn for complete blood count, chemistry and lipid profile, insulin-like growth factor 1 (IGF-1), free and total testosterone, and dehydroepiandrosterone sulfate (DHEA-S) as described previously (21). Bone mineral density (BMD) was measured using dual energy X-ray absorptiometry (DXA; Norland XR26, Fort Atkinson, WI). Body mass index (BMI) and waist-to-hip ratio (WHR) were assessed as described previously (21). Finally, the Geriatric Depression Scale (23) was administered; this uses a 30-point scale to screen for depression in elderly people (a score of >10 suggests significant depression).

Subjects collected saliva samples at home at 2300 h and the following morning at 0700 h. Saliva was sampled using a Salivette (Sarstedt, Newton, NC), which involves chewing on a cotton tube for 2–3 minutes. Salivettes were returned for processing on the same day. Saliva was separated from the cotton tube by centrifugation and frozen. Cortisol concentration within the saliva was measured by radioimmunoassay (19). The younger control group for salivary cortisol (n = 73; age 37 ± 1, 35 male/38 female) was selected from the same community; the results of this group have been reported previously (19). The younger group was assayed approximately 6 months before the older group. However, the same batch of assay quality controls and pools was run throughout the measurement of samples from both groups. The intraassay coefficient of variation was 3.0% (n = 15), and the interassay coefficients of variation were 6% and 12% (high [n = 16] and low [n = 23] pools, respectively).

Statistical analysis.—Analysis of variance was used to analyze quantitative variables. When relevant, age was used as a covariant in the model. Newman-Keuls multiple comparison test was used to compare 2300 h and 0700 h salivary cortisol in the old versus young control group. Regression analysis was used to examine the relationships of 2300 h and 0700 h salivary cortisol with other study variables. Tests of significance for correlation coefficients were made using Fisher’s z transformation. When relevant, the correlation coefficients were examined with the effect of WHR or BMI partialled out. Data are presented as mean ± standard error of the mean (SEM).

RESULTS

Table 1 shows the results of salivary cortisol in the elderly subjects as compared to a younger control group. Both groups demonstrated a significant diurnal rhythm in cortisol with low levels at 2300 h and high levels at 0700 h. Older subjects had significantly elevated salivary cortisol at 2300 h. However, salivary cortisol at 0700 h was not different between older and younger subjects. There was no difference between male and female subjects of either age group. Furthermore, salivary cortisol was not different between the three different age groupings of older subjects.

Table 2 shows the results of the other pertinent variables measured, which are as expected in this population (21,22). All BMDs, IGF-1, and serum androgens were significantly greater in men compared to women. Men had higher BMI with greater lean body mass and WHR. Finally, although women scored higher than men on the Geriatric Depression Scale, they were well below a score of 10, the cutoff for clinical depression.

Figure 1 shows a significant negative correlation of lumbar (L2–4) bone mineral density with 2300 h salivary cortisol in women. Women receiving hormone replacement therapy did not show a significant correlation (p = .34; n = 52), whereas

Table 1. Salivary Cortisol in Younger Control Group and Older Subjects

<table>
<thead>
<tr>
<th></th>
<th>2300 h</th>
<th>0700 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger Subjects*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n = 35)</td>
<td>1.2 ± 0.1</td>
<td>15.6 ± 1.3</td>
</tr>
<tr>
<td>Women (n = 58)</td>
<td>1.2 ± 0.1</td>
<td>13.6 ± 1.0</td>
</tr>
<tr>
<td>Older Subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n = 130)</td>
<td>2.3 ± 0.1</td>
<td>14.5 ± 0.8</td>
</tr>
<tr>
<td>Women (n = 98)</td>
<td>2.1 ± 0.1</td>
<td>15.2 ± 0.7</td>
</tr>
<tr>
<td>60–64 yrs (n = 72)</td>
<td>2.0 ± 0.2</td>
<td>14.5 ± 1.0</td>
</tr>
<tr>
<td>65–70 yrs (n = 90)</td>
<td>2.3 ± 0.2</td>
<td>15.1 ± 1.0</td>
</tr>
<tr>
<td>&gt;70 yrs (n = 66)</td>
<td>2.3 ± 0.2</td>
<td>15.1 ± 1.0</td>
</tr>
</tbody>
</table>

*Source of data, Raff et al. (19).
†Significantly greater than younger subjects of same gender by Newman-Keuls test (p < .001).

Table 2. Body Composition, Hormonal, and Other Variables Studied in 228 Elderly Community Dwellers

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men</th>
<th>Women</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD femoral (g/cm²)</td>
<td>0.84 ± 0.01</td>
<td>0.73 ± 0.01</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMD troc (g/cm²)</td>
<td>0.79 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMD Ward’s (g/cm²)</td>
<td>0.58 ± 0.01</td>
<td>0.53 ± 0.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>BMD L2 – 4 (g/cm²)</td>
<td>1.17 ± 0.02</td>
<td>0.95 ± 0.02</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMD Rad (g/cm²)</td>
<td>0.79 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>IGF-1 (mg/ml)</td>
<td>153 ± 3.6</td>
<td>114 ± 4.4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Free test (ng/ml)</td>
<td>126 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total test (mg/dl)</td>
<td>409 ± 8</td>
<td>22 ± 9</td>
<td></td>
</tr>
<tr>
<td>DHEA-S (µg/dl)</td>
<td>89.6 ± 5.6</td>
<td>51 ± 6.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>51.3 ± 0.4</td>
<td>30.7 ± 0.5</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 ± 0.3</td>
<td>25.2 ± 0.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>1.3 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Notes: BMD femoral = femoral neck bone mineral density, BMD troc = trochanter BMD, BMD L2-4 = lumbar spine BMD, BMD Rad = BMD of distal 1/3 site of the radius, BMD Ward’s = Ward’s triangle BMD, IGF-1 = insulin-like growth factor 1, Free test = serum free testosterone, Total test = serum total testosterone, DHEA-S = serum dehydroepiandrosterone sulfate, LBM = lean body mass, WHR = waist to hip ratio, BMI = body mass index.
women not receiving HRT demonstrated a significant negative correlation between lumbar BMD and 2300 h salivary cortisol (Figure 2). There were no other significant correlations between other BMD sites (femoral neck, trochanter, Ward's triangle, and at the distal radius) and 2300 h salivary cortisol in women (Table 3). In all women there was a significant negative correlation of 0700 h salivary cortisol with BMD at the distal radius, which did not partition by the use of HRT.

There was a highly significant negative correlation of 0700 h salivary cortisol and lumbar BMD in men (Figure 3, Table 3). In contrast, there was a positive correlation of 2300 h salivary cortisol and BMD in the trochanter and radius in men (Table 3). This positive correlation remained significant even when adjusted for WHR or BMI. No other BMD sites (femoral or Ward's triangle) correlated with salivary cortisol in men.

Table 3 shows the other significant correlations with salivary cortisol. In men, free testosterone was positively correlated with 2300 h salivary cortisol, and DHEA-S was positively correlated with 0700 h salivary cortisol (Table 3). There were no other significant correlations.

**DISCUSSION**

This study demonstrated elevated salivary cortisol in the late evening in older subjects as compared to a younger control group. There were no differences between gender and between different age cohorts within the older subjects. There was a significant negative correlation between 2300 h salivary cortisol and lumbar BMD in women. However, this correlation only held true in women not on HRT. There was a highly significant negative correlation of 0700 h salivary cortisol and lumbar BMD in men. Quite surprisingly, there was a positive correlation between 2300 h salivary cortisol and BMD in trochanteric and radial bone in men.

This study confirms prior intensive circadian rhythm blood sampling studies, which demonstrated an elevated nocturnal nadir in plasma cortisol and, therefore, a damped amplitude in the normal circadian rhythm of cortisol (12-16). The lack of a difference between different age cohorts within the elderly subjects confirms a 6-year longitudinal study that found significant heterogeneity in cortisol circadian rhythms within subjects as they aged (24). Our study clearly demonstrated that measurement of salivary cortisol is an excellent and appropriate method.

Table 3. Significant Correlations of 2300 h (PM) and 0700 h (AM) Salivary Cortisol Versus Other Variables Measured

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>PM Salivary Cortisol versus:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD troch</td>
<td>.18</td>
<td>.04*</td>
</tr>
<tr>
<td>BMD L2-4</td>
<td>.08</td>
<td>.36</td>
</tr>
<tr>
<td>BMD Rad</td>
<td>.21</td>
<td>.02*</td>
</tr>
<tr>
<td>Free test</td>
<td>.20</td>
<td>.02*</td>
</tr>
<tr>
<td>AM Salivary Cortisol versus:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD L2-4</td>
<td>-.31</td>
<td>.0003*</td>
</tr>
<tr>
<td>BMD rad</td>
<td>.06</td>
<td>.48</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>.20</td>
<td>.02*</td>
</tr>
</tbody>
</table>

Notes: BMD troch = trochanter bone mineral density, BMD L2-4 = lumbar spine BMD, BMD Rad = distal 1/3 radius site BMD, Free test = serum free testosterone, DHEA-S = serum dehydroepiandrosterone sulfate.

*p ≤ .05.
Every evening over a protracted period of time, one can suppose logical (free) glucocorticoid activity. Furthermore, if this occurs a decrease in glucocorticoid activity, a doubling of late evening salivary cortisol (from 1 to 2 nmol/L) represents a doubling of biologically active glucocorticoid activity. Therefore, whereas a 1 nmol/L increase in plasma cortisol yields only a small increase in glucocorticoid activity, a doubling of late evening salivary cortisol (from 1 to 2 nmol/L) represents a doubling of biological (free) glucocorticoid activity. Furthermore, if this occurs every evening over a protracted period of time, one can suppose that a significant increase in glucocorticoid effects in elderly people would accumulate. Van Cauter's group (13–15) has suggested that this small increase in nocturnal cortisol may partially account for sleep disorders common in the elderly population.

Even more compelling is the significant negative correlation of late-evening salivary cortisol and BMD in the lumbar spine in women. It is well known that glucocorticoid excess causes a more dramatic loss of trabecular bone as compared to cortical bone (5,26). Furthermore, lumbar spine compression fractures are a major complication of glucocorticoid-mediated osteoporosis (4). Interestingly, osteoporosis due to endogenous glucocorticoid excess is amenable to treatment by alendronate (6). That the negative relationship between salivary cortisol and lumbar BMD was only present in women not on hormone replacement therapy confirms the protective effect of estrogen on trabecular bone regardless of the cause of osteoporosis (27).

The correlation of BMD and salivary cortisol in men was quite interesting. The highly significant negative correlation of morning salivary cortisol and lumbar BMD suggests that loss of BMD with aging in men may also involve, in some way, cortisol secretion. It is not clear why lumbar BMD in women correlated with late evening salivary cortisol while, in men, it correlated with morning salivary cortisol. The positive correlation of trochanteric and radius BMD with late evening salivary cortisol in men was surprising. At first, we thought that this could be accounted for by increased abdominal obesity and/or BMI, which can be positively correlated with bone density and with free cortisol in men (8,28–31). However, this positive correlation remained even after the analysis was adjusted for BMI or for WHR. At this point, we are not certain of the mechanism for or significance of this positive correlation. That the negative correlation of lumbar BMD and morning cortisol was highly significant suggests that this effect probably is dominant and more physiologically compelling than the positive correlation with late evening salivary cortisol in men.

Elevated glucocorticoids are known to alter growth hormone secretion, testosterone secretion, body habitus, and fat distribution, as well as mood (8). The positive correlation of salivary cortisol with DHEA-S and free (biologically active) testosterone in men is interesting and can be attributed to parallel, feed-forward, stress-induced perturbation of the HPA and gonadal axes (31). The lack of a correlation between other variables and salivary cortisol suggests that either they are less sensitive to the effects of cortisol or the variability in these measurements obviated a subtle but significant correlation. It is likely that significant loss of muscle mass had already occurred in our study population (2,3,21). Lack of correlation of lean body mass and salivary cortisol within our study group does not prove that prior increases in late-evening salivary cortisol did not cause significant loss of muscle mass. This notion is confirmed by a study that demonstrated that glucocorticoids induce a more rapid loss of muscle mass in old rats (32). It was not surprising that there was no correlation of salivary cortisol with the Geriatric Depression Scale, as all subjects were well below the cutoff for clinical depression (23,33). It is also not surprising that women had significantly higher Geriatric Depression Scale scores (more symptoms of depression), because men consistently score lower on self-report depression scales (33).

This study demonstrated an elevated biologically active cor-
Salivary cortisol and bone density

SALIVARY CORTISOL AND BONE DENSITY

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REFERENCES


