Higher Levels and Blunted Diurnal Variation of Cortisol in Frail Older Women

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Background. Frailty is an important geriatric condition with increased vulnerability to stressors (e.g., infection and injury) and for developing functional dependence and mortality. Impairments in signaling pathways, including neuroendocrine alterations, are thought to be involved in the etiology of frailty, but have not been well characterized to date. We evaluated whether higher levels and blunted diurnal variation of salivary cortisol are cross-sectionally associated with frailty burden.

Methods. Two hundred fourteen community-dwelling women, 80–90 years old, from the Women’s Health and Aging Study participated in this study between 2004 and 2005. Seven saliva samples were collected for cortisol measurement over a 24-hour period. Main outcomes were awakening, evening, and overall mean cortisol; diurnal amplitude; and rate of decline of cortisol level during morning hours. All cortisol concentrations were log-transformed. Frailty burden was calculated, based on a previously validated tool, as the number (0–5) of the following criteria present: weakness, exhaustion, weight loss, slowness, and inactivity.

Results. Significant positive associations were found between frailty burden and evening cortisol ($\beta = 0.11, p = .04$), and between frailty burden and 24-hour mean cortisol ($\beta = 0.07, p = .03$). Increasing frailty burden was significantly associated with smaller declines in cortisol during morning hours ($\beta = 0.04, p = .02$). Frailty burden of $\geq 2$ was associated with a smaller diurnal amplitude ($\beta = -0.34, p = .03$). Awakening cortisol was not significantly associated with frailty burden ($\beta = 0.01, p = .8$). All analyses included adjustments for several important confounders.

Conclusions. Our findings provide the first epidemiological evidence that higher levels and blunted diurnal variation of cortisol may be involved in the vulnerability and clinical presentation observed in frail older women.

Key Words: Frailty—Salivary cortisol—Diurnal rhythm—HPA axis—Evening cortisol.

A major goal of geriatrics is to prevent disability and to enhance active life expectancy. Geriatricians have long recognized that disease prevention alone is not sufficient to accomplish this (1). Age-oriented and disease-oriented approaches do not adequately capture the complexity and variability of the functional health of older adults. Frailty has recently been proposed as a new geriatric paradigm that shifts the focus away from disease-specific pathologies toward understanding the core biological dysregulation and homeostatic impairments that may contribute to the decline in functional health of older adults (1–6). Clinically, frailty has been characterized by muscle wasting, weight loss, fatigue, and decreased balance and activity. Physiologically, it has been conceptualized as a state of impaired homeostatic regulation with increased vulnerability to stressors (e.g., infection and injury) and for developing functional dependency and mortality.

Activation of the hypothalamic–pituitary–adrenal (HPA) axis to produce cortisol is a fundamental endocrine response to situations that threaten homeostasis. In addition to its well-characterized roles of increasing glucose availability and inhibiting the inflammatory cascade, cortisol also mediates numerous other functions essential for stress response and its aftermath (7). Cortisol levels are generally thought to increase with age, although the evidence is somewhat equivocal (8,9). There is greater consensus that the reactivity of the HPA axis to external stressors generally increases with age (9). Elevated diurnal cortisol and impaired HPA axis response to stressors have been hypothesized to initiate or amplify alterations in many other important physiologic systems, including endocrine, metabolic, cardiovascular, immune, skeletal muscle, and skeletal systems (10–12). Chronically elevated cortisol has been implicated in the pathogenesis of a number of age-related psychiatric and somatic disorders including depression, memory deficits, cognitive impairment, obesity, cardiovascular disease (CVD), diabetes, and osteoporosis (13–15).

Impairments in signaling pathways, including neuroendocrine alterations, are thought to be involved in the etiology of frailty, but have not been well characterized to date (1,2,4,16). In this study, we sought to examine how the dynamics of diurnal activity of the HPA axis are altered in frailty. In particular, we tested the following hypotheses: (i) elevated diurnal cortisol levels are associated with increasing frailty burden and (ii) the diurnal variation of cortisol is blunted in frail older adults.
METHODS

Study Population

We measured salivary cortisol levels in 214 women (80–89 years) who participated in the sixth evaluation for the Women’s Health and Aging Study II (WHAS II) between 2004 and 2005. This study was established in 1994 as a companion study to the WHAS I study of the evolution of disability in the one-third most disabled older women. WHAS II participants were 70–79 years old at the time the study was established, and were recruited from among the two-thirds least disabled population in this age group (17). In all rounds of data collection, standardized information on functioning, health and social status, performance, and biologic measurements were collected. The larger WHAS II study and the salivary cortisol study were institutional review board approved. All participants included in these analyses signed informed consent for these data as well as salivary cortisol measurement. Of the 238 women who participated in the sixth evaluation, 214 provided saliva samples.

Cortisol Data Collection

During the sixth examination, salivary swabs were taken at five time points: (i) upon arrival at the clinic in the morning around 9 AM (t1), (ii) before starting cognitive evaluations (t2 = t1 + 30 minutes), (iii) at the conclusion of cognitive evaluations and before the evaluation of physical performance (t3), (iv) at the conclusion of physical evaluations (t4), and (v) prior to discharge after a period of rest (t5 = t4 + 45 minutes). The entire study period was slightly less than 3 hours on average. Participants were then asked to collect two saliva samples at home: one evening sample before bedtime and at least an hour after dinner (same evening), and another within 30 minutes of awakening the next morning and before brushing teeth. The exact times of collection for these two samples were not recorded. The participants then mailed these two samples to the laboratory. Sarstedt Salivettes (Newton, NC) were used. Samples were processed and frozen at −80°C, and cortisol was measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit from Diagnostic Systems Laboratory, Inc. (Webster, TX). Assay sensitivity was 0.11 μg/mL, and intra- and inter-assay coefficients of variation were 8.22% and 5.02%, respectively.

Outcome Measures and Primary Independent Variable

All salivary cortisol concentrations were log-transformed in the following analyses. Five measures of diurnal cortisol profile were used as outcomes: awakening cortisol, evening cortisol, 24-hour mean cortisol, diurnal amplitude, and rate of decline of cortisol during morning hours. Twenty-four-hour mean cortisol was calculated as the average of the seven log-transformed cortisol values collected over the 24-hour period. Amplitude of diurnal cortisol rhythm was defined as the ratio of awakening to evening cortisol. Rate of decline was estimated as the slope of a linear regression of logarithm of the cortisol values from t1 to t5 versus time of sample collection. Awakening, evening, and 24-hour mean cortisol are different measures of diurnal activity of the HPA axis. Diurnal amplitude and the rate of decline of cortisol during morning hours are measures of the diurnal variation of cortisol, and also of the resiliency of the HPA axis, with smaller values indicating blunted diurnal variation and decreased resiliency.

Frailty has been theorized to be a clinical geriatric syndrome resulting from dysregulation of multiple physiologic systems and is characterized by vulnerability to stressors, manifested as decreased ability to achieve homeostatic balance and leading to high risk of adverse outcomes including mortality and disability (2,3,5). We have identified key clinical manifestations of loss of muscle mass, weakness, low exercise tolerance (“exhaustion”), weight loss, slowness, and inactivity (4,5,18). A clinical screening approach to identifying persons with any of the latter five manifestations has been developed and validated (5,18). In this approach, persons with a critical mass of criteria present (≥3) were defined as presenting with a clinical syndrome of being frail; persons with none of the criteria were defined as nonfrail; others, with 1 or 2 criteria, were defined as prefrail, based on evidence showing that the presence of 1 or 2 criteria predicts future development of ≥3 criteria (5). In our study, however, one of the screening variables, “exhaustion,” was not measured in the sixth evaluation, making it impossible to determine exact frailty status. Therefore, as a type of sensitivity analysis, we characterized frailty status in two different ways: (i) define a woman to be frail if she has ≥2 criteria (those with 0 or 1 are nonfrail), and (ii) define a continuous variable called “frailty burden,” which is the number of frailty components present in an individual (i.e., 0, 1, 2, or ≥3). The first measure of frailty status is binary (yes/no), whereas the second measure, frailty burden, provides a more continuous representation of frailty. We used the binary frailty status variable primarily for exploring whether frail women had pronounced alterations in cortisol diurnal patterns. We used frailty burden as the primary predictor variable in the regression models.

Statistical Methods

We used two statistical methods to examine the association between various cortisol measures and frailty burden: (i) the Jonckheere–Terpstra test (JT test) of monotonic trend in cortisol markers as a function of frailty burden, and (ii) regression modeling to estimate the independent association of frailty burden (treated as a continuous variable) with cortisol markers, adjusting for potential measured confounders. The JT test is a distribution free, rank-based procedure for testing monotonic trend in the median of cortisol measures as a function of the frailty burden. It extends the two-sample Mann–Whitney test, for testing ordered alternatives for more than two groups (19). The null (H0) and the alternate (Hα) hypotheses for the two-sided JT test are:

\[ H_0: \tau_1 = \ldots = \tau_K \]

\[ H_α: \text{Either } \tau_1 \leq \tau_2 \leq \ldots \leq \tau_K, \text{ or } \tau_1 \geq \tau_2 \geq \ldots \geq \tau_K, \text{ with at least one strict inequality,} \]

where \( \tau_1, \ldots, \tau_K \) denote median of cortisol distributions for the \( K (=4) \) groups of frailty burden. The Spearman rank
correlation statistic was used to describe the pair-wise correlations between frailty burden and various demographic and health-related variables and frailty burden, as well as between 24-hour mean cortisol and those variables.

In the regression models, we adjusted for the following variables in our models: age, race (white/non-white), education (number of years of schooling, dichotomized at 12 years), smoking (yes/no), depressive symptoms (score on the Geriatric Depression Scale [GDS], dichotomized using a cutoff at 10), cognitive function (Mini-Mental State Examination [MMSE] score), diabetes (yes/no), and CVD (yes/no). We used flexible additive linear regression models using penalized regression splines to account for potential nonlinear relationships that might exist between cortisol measures and continuous confounders. We examined the residuals to ensure that model assumptions were not violated. Regression analyses were conducted using the mgcv package in R version 2.3.1 (20,21).

### RESULTS

Mean values of demographic and other confounder variables from the sixth examination are displayed in Table 1 by frailty status. Age, body weight, and depressive symptoms were significantly associated with frailty burden \((p < .05)\). Age, education, race, depressive symptoms, and CVD prevalence were associated with 24-hour mean cortisol.

Figure 1 summarizes the primary findings of this study. It depicts the average diurnal variation in salivary cortisol for frail and nonfrail older women. Cortisol at each time point was computed as the geometric mean of cortisol across individuals within each group. Frail and nonfrail women were not significantly different in their awakening cortisol (see Figure 2a for the distribution of awakening cortisol). However, the two cortisol profiles start diverging after awakening, with the frail women having higher cortisol throughout. The largest difference between the groups is observed in the evening sample (see Figure 2b for the distribution of evening cortisol).

Mean values of diurnal cortisol parameters across frailty burden, and unadjusted (JT rank correlation) and adjusted (regression coefficient) measures of association between cortisol measures and frailty burden, are provided in Table 2. There was no trend in awakening cortisol as a function of frailty burden (JT rank correlation \(\rho = 0.04, p = .65\)). Frailty burden was not independently associated with awakening cortisol \((\beta = 0.011, p = .84)\). Morning cortisol declined more slowly versus time (smaller negative slope) with increasing frailty burden (rank correlation \(\rho = 0.17, p = .02\)). Increasing

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Table 1. Mean Values of Important Potential Confounders Across Frailty Burden and Their Correlations With Frailty Burden and Cortisol

<table>
<thead>
<tr>
<th>Variables</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>(\geq 3)</th>
<th>With Frailty Burden</th>
<th>With 24-Hour Mean Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>N*</td>
<td>67</td>
<td>76</td>
<td>45</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>83.4</td>
<td>83.5</td>
<td>84.4</td>
<td>85.5</td>
<td>0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>Less than high-school graduate, %</td>
<td>18</td>
<td>16</td>
<td>28</td>
<td>25</td>
<td>0.09</td>
<td>0.20</td>
</tr>
<tr>
<td>White, %</td>
<td>86.6</td>
<td>85.3</td>
<td>76.1</td>
<td>90.0</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>10.5</td>
<td>5.3</td>
<td>17.4</td>
<td>20.0</td>
<td>0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td>GDS &gt; 10, %</td>
<td>4.5</td>
<td>6.8</td>
<td>11.0</td>
<td>10.0</td>
<td>0.29</td>
<td>0.16</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.2</td>
<td>28.1</td>
<td>27.4</td>
<td>25.5</td>
<td>-0.25</td>
<td>-0.10</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>3.0</td>
<td>2.7</td>
<td>6.5</td>
<td>15</td>
<td>0.13</td>
<td>-0.01</td>
</tr>
<tr>
<td>CVD, %</td>
<td>52</td>
<td>55</td>
<td>67</td>
<td>65</td>
<td>0.11</td>
<td>0.17</td>
</tr>
<tr>
<td>Weight (lb)</td>
<td>140</td>
<td>152</td>
<td>150</td>
<td>152</td>
<td>0.16</td>
<td>-0.02</td>
</tr>
<tr>
<td>Blood pressure, systolic</td>
<td>148</td>
<td>150</td>
<td>151</td>
<td>155</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Blood pressure, diastolic</td>
<td>75</td>
<td>74</td>
<td>77</td>
<td>76</td>
<td>-0.01</td>
<td>-0.02</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>36</td>
<td>38</td>
<td>37</td>
<td>36</td>
<td>0.08</td>
<td>-0.09</td>
</tr>
<tr>
<td>Total bone mineral density, g/cm²</td>
<td>1.05</td>
<td>1.07</td>
<td>1.08</td>
<td>1.05</td>
<td>-0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Notes: *Frailty information was unavailable for six women.  
1\(p < .05\) (based on Spearman’s rank correlation \(\rho\) statistic).  
GDS = Geriatric Depression Scale; MMSE = Mini-Mental State Examination; CVD = cardiovascular disease.

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Figure 1. Mean diurnal profiles of cortisol during a 24-hour period, comparing frail and nonfrail older women.
frailty burden was also independently associated with a smaller rate of decline of morning cortisol \((p = .02)\). Evening cortisol increased significantly with increasing frailty burden (rank correlation \(= 0.17, p = .02\)). Frailty burden was independently and positively associated with evening cortisol, with each additional frailty criterion present contributing a 12% increase (standard error \(= 5.3\%), p = .04\). The 24-hour mean cortisol increased significantly with increasing frailty burden (rank correlation \(= 0.19, p < .01\)). Frailty burden was independently and positively associated with 24-hour mean cortisol, with each additional frailty criterion present contributing a 7% increase (standard error \(= 3.2\%), p = .03\). Diurnal amplitude (ratio of awakening to evening cortisol) exhibited a nonlinear association with frailty burden: Women with \(\geq 2\) frailty criteria had a markedly blunted diurnal rhythm compared to that of women with 0 or 1 frailty burden (result not shown). The JT test was partially able to capture this negative association (rank correlation \(= -0.13, p = .09\)). In the regression models, however, frailty burden was negatively, but not independently associated with diurnal amplitude \((p = .28)\). However, when frailty was used as a binary variable, it was significantly associated with a smaller diurnal amplitude \((\beta = -0.34, p = .03)\).

**Discussion**

We provide here the first scientific evidence demonstrating the link between cortisol, an important hormone involved in the signaling pathways of stress response, and the geriatric syndrome of frailty. The rate of decline through the day from the peak was smaller, and the evening cortisol was meaningfully higher, in women with a greater frailty burden (even after adjusting for several of the potentially important confounders). A frailty burden of \(\geq 2\) was also associated with a smaller diurnal amplitude of cortisol. However, awakening cortisol was not associated with frailty burden, and there was no difference in the awakening cortisol between women with \(\geq 2\) frailty criteria and those with 0 or 1 criterion. Together, these results suggest that frail older women have chronically elevated diurnal cortisol levels.

There are many potential etiologies of elevated cortisol that could explain our findings. Chronic disease burden and age may contribute to the alterations in cortisol diurnal pattern, although these factors were insignificant in the regression models. Chronically elevated cortisol in frailty may be due to weakened negative feedback of cortisol on the hypothalamus and pituitary, potentially resulting from life-long cumulative effects of allostasis (the wear and tear of maintaining homeostasis) (14,22,23). Chronically increased inflammatory load, an example of allostatic load, could also result in elevated cortisol, as cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-alpha are well-known activators of the HPA axis (24–26). Because chronic inflammation is thought to play a central role in the pathogenesis of frailty (27,28), it could be an important link between elevated cortisol and frailty. Increased activity of the HPA axis in the evening hours has been shown in major depression, especially among women (29). However, our analysis showed that frailty was independently associated with elevated evening and 24-hour mean cortisol, even after adjustment for depressive symptoms. Variation in the quality and quantity of sleep may also partly explain elevated evening cortisol.

**Table 2. Mean Values of Diurnal Cortisol Parameters Across Frailty Burden, and Unadjusted (Jonckheere–Terpstra [JT] Test Rank Correlation) and Adjusted (Regression Coefficient) Measures of Association Between Cortisol Measures and Frailty Burden**

<table>
<thead>
<tr>
<th>Cortisol Measure</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>(\geq 3)</th>
<th>JT Rank Correlation ((p\ Value))</th>
<th>Regression Coefficient ((p\ Value))*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awakening, nmol/L</td>
<td>21.8</td>
<td>23.0</td>
<td>19.0</td>
<td>26.7</td>
<td>0.04 (.65)</td>
<td>0.011 (.84)</td>
</tr>
<tr>
<td>Evening, nmol/L</td>
<td>8.3</td>
<td>7.8</td>
<td>10.3</td>
<td>12.4</td>
<td>0.17 (.02)</td>
<td>0.11 (.04)</td>
</tr>
<tr>
<td>24-h geometric mean, nmol/L</td>
<td>12.8</td>
<td>13.9</td>
<td>14.6</td>
<td>17.5</td>
<td>0.19 (&lt;.01)</td>
<td>0.071 (.03)</td>
</tr>
<tr>
<td>Diurnal amplitude</td>
<td>2.7</td>
<td>3.0</td>
<td>1.8</td>
<td>2.4</td>
<td>-0.13 (.09)</td>
<td>-0.083 (.28)</td>
</tr>
<tr>
<td>Rate of decline per hour</td>
<td>-0.25</td>
<td>-0.20</td>
<td>-0.16</td>
<td>-0.12</td>
<td>0.17 (.02)</td>
<td>0.039 (.02)</td>
</tr>
</tbody>
</table>

*Note: *An analyses were adjusted for age, race, education, smoking, cardiovascular disease, diabetes, Mini-Mental State Examination score, and depressive symptoms score.
cortisol, although the causal direction between sleep and cortisol is unclear (30–32). This association could not be evaluated because information regarding sleep was not collected in the study. It is also possible that some of the differences in the evening and awakening cortisol could be due to differences in the time of sampling, although it is unlikely that the sampling times would vary monotonically, and in a particular direction, with frailty burden (i.e., the evening sampling times would be earlier for women with a greater frailty burden).

The strengths of this study include the number of data points collected, the long history of biological and functional information available in this population, and rigorous statistical methods. Despite these strengths, it is impossible to determine from these data whether frail older women have elevated cortisol at baseline and in nonstressful settings or if this elevation is the result of impaired ability to terminate a stress response. It is possible that the experience of traveling to and from the research site, and the examination itself, actually constituted a stressor to which the frail women were sensitive, resulting in their blunted diurnal variation in cortisol. Therefore, these results could be interpreted as indicating that (i) the afternoon/evening basal HPA axis activity is chronically higher in frail compared to nonfrail older women or (ii) the reactivity and resilience of the HPA axis to stressors is altered in frailty, with greater increase and/or a longer recovery period after a stressor. In either case, these results provide clear evidence for an association between HPA axis function and clinical frailty. Such a connection is biologically plausible, because chronic exposure to elevated cortisol is known to magnify its catabolic effects (10,33,34), leading to loss of muscle mass and strength, loss of appetite, weight loss, and decreased energy, all of which are classic symptoms of frailty (4,35). Indeed, hypercortisolism may trigger a vicious cycle (a positive feedback loop) in which chronic exposure to elevated cortisol concentrations induces catabolic injury, which in turn stimulates more cortisol production, ultimately resulting in frailty in aging. Future studies should focus on both baseline cortisol secretion and secretion in response to stress to identify more specific patterns of dysregulation in the HPA axis.

Summary

Our findings provide the first epidemiological evidence that higher levels and a blunted diurnal rhythm of cortisol may be involved in the vulnerability and clinical presentation observed in frail, older women. Efforts to thoroughly characterize the causal web of interactions relating alterations in physiological levels of cortisol to frailty could lead to a better understanding of the basic biology that underlies the multisystem dysregulation observed in frailty and to improved diagnostic and treatment efforts for frail older adults.

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