Neuroendocrine and Immunological Correlates of Chronic Stress in ‘Strictly Healthy’ Populations

Cristina M. Moriguchi Jeckel a, b  Rodrigo P. Lopes a  Maria Cristina Berleze a  Clarice Luz d  Leandro Feix c  Irani Iracema de Lima Argimon c  Lilian M. Stein c  Moisés E. Bauer a

a Laboratory of Cellular and Molecular Immunology, Instituto de Pesquisas Biomédicas and Faculdade de Biociências, b Faculdade de Farmácia, c Faculdade de Psicologia, Pontifical Catholic University of the Rio Grande do Sul, d LabVitus, Porto Alegre, Brazil

Key Words
Chronic stress · Lymphocytes · Glucocorticoids · Psychoneuroimmunology

Abstract

Background: Chronic stress has been associated with detrimental or maladaptive neuroendocrine and immunological changes. Objectives: We assessed the neuroendocrine and immunological correlates of a realistic chronic stress experienced by strictly healthy caregivers of Alzheimer’s disease patients and age-matched controls. Methods: We screened 330 caregivers and 206 non-caregivers according to the ‘strictly healthy’ conditions established by the SENIEUR protocol. Forty-one strictly healthy caregivers (60.56 ± 16.56 years) and 33 non-stressed controls (60.27 ± 14.11 years) were selected for this study. Salivary cortisol and dehydroepiandrosterone sulfate (DHEAS) were assessed at multiple points by radioimmunoassay. Peripheral T cell proliferation and cellular sensitivity to glucocorticoids (corticosterone and dexamethasone, DEX) were evaluated by colorimetric assays. We also examined the hypothalamic-pituitary-adrenal (HPA) axis response to the administration of a low-dose DEX in vivo. Results: The caregivers were significantly more stressed, anxious and depressed than non-caregivers (all p < 0.0001), in contrast to similar cortisol levels. Caregivers had reduced DHEAS levels (~32%, p < 0.0001), an increased cortisol/DHEAS ratio (39.7%, p < 0.0001) and impaired HPA axis response to DEX intake. Caregivers had a higher T cell proliferation (p < 0.0001) and increased sensitivity to glucocorticoids in vitro (p < 0.01) as compared to non-stressed controls. Conclusions: Our results suggest that the maintenance of health in chronically stressed populations may be associated with both protective and detrimental neuroendocrine and immunological changes.

Introduction

It is recognized that those caring for demented relatives can be used as a model to study the impact of chronic stress in elderly populations. Care of the chronically ill is a demanding task that is associated with increased stress, depression and poorer immune function [1]. Furthermore, providing care for a relative with dementia...
typically falls on the partners who are themselves elderly and often ill prepared for the physical and emotional demands placed upon them [2–4].

Several studies have implicated caregiving stress as an important risk factor for elderly populations. Compared with non-caregivers, subjects who provide care to a spouse who has suffered a stroke or who has dementia report more infectious illness episodes [4], they have poorer immune responses to influenza virus [3, 5] and pneumococcal pneumonia vaccines [6], they present slow wound healing [7], are at greater risk for developing mild hypertension [8], and they may be at greater risk for coronary heart disease [9]. In addition, a prospective longitudinal study found that the relative risk for mortality among caregivers was significantly higher (63%) than non-caregiving controls [10]. A recent study indicates that a pro-inflammatory cytokine (IL-6) may be involved with this increased morbidity in caregiving populations [11].

The daily stress experienced by the caregivers of Alzheimer patients may lead to premature aging of neuroendocrine and immune systems. Healthy aging has been associated with neuroendocrine and immunological changes including hypothalamic-pituitary-adrenal (HPA) axis activation with increasing cortisol [12] and blunted cell-mediated immunity [13]. The caregivers of demented patients had further reduced T cell function as well as impaired steroid immunoregulation in association with increased cortisol levels compared to non-stressed elderly controls [2, 14]. These data suggest that chronic stress or chronic glucocorticoid exposure would be associated with premature aging of the immune system (immunosenescence). Giving support to this hypothesis, it has recently been observed that psychological stress (both perceived stress and chronicity of stress) was significantly associated with higher oxidative stress, lower telomerase activity and shorter telomere length, which are known determinants of cell senescence and longevity [15]. Although chronic stress is a risk factor for bad health outcomes in the elderly, some individuals may preserve their healthy status during such conditions.

In the present article, we assessed the neuroendocrine and immunological correlates of chronic stress in populations judged to be ‘strictly healthy’ by the SENIEUR protocol. In particular, we analyzed the impact of chronic stress exposure on HPA axis function, investigated both HPA axis and lymphocyte sensitivity to glucocorticoids as well as assessed the T cell proliferation as an index of cell-mediated immunity.

**Methods**

**Subjects**

The 545 subjects (330 caregivers and 215 controls) were part of a project on chronic stress and health in adults. The spousal dementia caregivers were recruited from the Alzheimer’s Disease Association support groups of the Hospital de Clínicas (Porto Alegre, Brazil) and Hospital São Lucas (Porto Alegre, Brazil). All subjects were screened according to the strictly healthy criteria established by the SENIEUR protocol [16]. The health conditions were checked according to previous work [12, 13] and were based on accurate clinical investigations and hematological and biochemical parameters. Forty-one (32 females) strictly healthy and primary caregivers (mean ± SD age: 60.56 ± 11.56 years) and 33 strictly healthy (26 females) and non-stressed controls (mean ± SD age: 60.27 ± 14.10 years) were included in the study. Non-caregiver participants were recruited through a variety of sources including university advertisements and referrals from other participants. The current research was approved by the Pontifical Catholic University of Rio Grande do Sul ethics committee. Written informed consent was obtained from all participants.

The exclusion criteria were: infections (during the previous 2 weeks); acute or chronic inflammation; autoimmune diseases; heart, renal, gastrointestinal, liver, neurological, lung, neurodegenerative, hematological or endocrine diseases; under-nourishment; immunodeficiencies; mood disorders; caregiving (for non-caregivers); neoplasias; smoking (more than 25 cigarettes/day) and drugs (alcohol, antidepressants, immunosuppressants, anti-coagulants and steroids).

**Assessment of Stress, Anxiety and Depressive Symptoms**

The psychological status was assessed by means of structured clinical interviews and performed by a trained investigator. The stress levels were assessed by the Inventory of Stress Symptoms Lipp for adults (ISSL) [17]. The ISSL identifies the presence of stress symptoms, type of symptoms (somatic and psychological) and stress phase, including: alarm, adaptation, quasi-exhaustion and exhaustion. It includes 37 items of somatic symptoms and 19 items of psychological symptoms. The scoring was performed by means of 3 different stages related to the duration (Q1 = last 24 h, Q2 = last week, and Q3 = last month) and intensity of stress symptoms. The sum of all physical as well as psychological symptoms within each stage resulted in a score that is related to the presence of stress according to the following criteria: Q1 > 6 (alarm phase) or Q2 > 3 (adaptation phase) or Q3 > 8 (exhaustion phase). The duration of administration was 10 min.

The State Trait Anxiety Inventory (STAI) was employed as self-report generic measures of anxiety [18]. STAI is a 20-item assessment device that includes separate measures of state and trait anxiety. The duration of administration was 10 min. Scores on the STAI have a direct interpretation: high scores (80 = highest) on their respective scales mean more trait or state anxiety and low scores mean less. It has been found to be a sensitive indicator of changes in transitory anxiety experienced by patients in counseling, psychotherapy and behavior-modification programs.

The Beck Depression Inventory was employed as self-report scale for assessing depression levels [19]. The inventory consists of 21 items to assess the intensity of depression in clinical and normal patients. Each item is a list of 4 statements arranged in increasing severity about a particular symptom of depression.
CO₂ atmosphere. DEX (a selective glucocorticoid receptor agonist) and fetal calf serum 10%; all from Sigma) for 96 h at 37 °C in 5% CO₂ supplemented with gentamicin 0.5%, glutamine 1%, Hepes 1%, RPMI-1640 (Gibco, Carlsbad, Calif., USA) in complete culture medium (RPMI-1640 with 10% fetal calf serum) supplemented with gentamicin 0.5%, glutamine 1%, Hepes 1%, and fetal calf serum 10%; all from Sigma) for 96 h at 37°C in 5% CO₂ atmosphere. DEX (a selective glucocorticoid receptor agonist) and corticosterone (which binds to both glucocorticoids and mineralocorticoid receptors) were added in duplicate (50 µl/well; both water-soluble substances purchased from Sigma) to mitogen-stimulated lymphocyte cultures (PHA 1%). Glucocorticoid concentrations were used in a range (10⁻⁹–10⁻⁶ M) that free glucocorticoids would reach during resting state (10⁻⁸ M) or stress (10⁻⁶ M). Data are presented as percentage of basal proliferation (stimulated without steroids). The proliferative responses were determined by a modified colorimetric assay (MTT) as previously described [13]. The optical density (OD) was determined using a Bio-Rad ELISA plate reader at wavelengths of 570 and 630 nm. Proliferation/viability was expressed as OD of stimulated – OD of non-stimulated cultures.

**Statistical Analysis**

All variables were tested for normality of distribution by means of the Kolmogorov-Smirnov test. Proliferation and cortisol data were analyzed by repeated measures ANOVA that included 1 between-subjects variable (caregivers vs. controls) and 1 within-subjects variable (mitogen or cortisol levels). Multiple comparisons among levels were checked with Tukey post-hoc test. Differences between variables were also assessed by Student’s t test or Mann-Whitney test, when indicated. Differences in proportions between groups were compared by means of χ² test. The area under the curve (AUC) of hormonal data and in vitro DEX responses was estimated using the trapezoidal rule. Relationships between variables were assessed by means of Pearson’s product moment correlations. Data are expressed as mean ± SE in all figures and tables. All significance levels were 2-tailed. Statistical analyses were performed with the SPSS 15.0 for Windows (SPSS Inc., Chicago, Ill., USA).

**Results**

**Sociodemographic and Health-Related Data**

The present study employed a stringent protocol to recruit extremely healthy subjects and thus enabled us to control for diseases that would interfere with the biological responses to stress exposure. Based on these criteria of being ‘strictly healthy’, only 12.4% of caregivers and 15.34% of non-caregivers were included in this study. The sample characteristics are shown in table 1. Caregivers and non-caregivers were homogeneous, as shown by similar sociodemographic and health-related profiles. The caregiver and control cohorts did not differ in the proportion of women (χ² = 0.006, p = 0.94), age (t = 0.10, p = 0.92), ethnicity (χ² = 3.52, p = 0.17), religious beliefs (χ² = 6.89, p = 0.14) or education level (t = 2.23, p = 0.05). The mean education level of both groups was partial college. The caregivers were spending a mean ± SD of 16.0 ± 5.73 h per day in caregiving-related activities and reported they had been providing care for 4.03 ± 2.89 years. The majority of the caregivers’ spouses had received a diagnosis of Alzheimer’s disease (95.12%). The remainder had a diagnosis of Lewy Body dementia (2.44%) or inconclusive diagnostic (2.44%). The caregiver and non-caregiver cohorts were very similar as regarding the various...
biochemical and hematological data (table 1). All non-caregivers had partners who were alive at the time of recruitment and who did not have a diagnosis of dementia. Furthermore, non-caregivers did not have any other caregiving duties, such as an elderly parent or disabled child. These criteria ensured that only individuals in the experimental group were exposed to chronic caregiver stress.

**Psychological Distress and Autonomic Function**

The psychological variables evaluated here confirm that chronic caregiving stress is associated with significant psychosocial distress (table 2). The caregivers reported greater anxiety (STAI trait: t = 8.50, p < 0.0001; STAI state: t = 6.94, p < 0.0001), depression (t = 4.20, p < 0.0001) and stress (Q1: t = 2.78, p < 0.001; Q2: t = 4.57, p < 0.0001; Q3: t = 4.11, p < 0.0001) than non-caregivers. Age was not correlated with psychological assessments. In agreement with the psychological distress, the caregivers had signs of low-grade autonomic activation, as shown by significantly increased diastolic blood pressure (U = 480.50, p < 0.05), heart rate (U = 387.00, p < 0.05) and systolic blood pressure (U = 527.50, p = 0.21), although the latter did not reach statistical significance (table 2). Interestingly, the number of hours spent in caregiving-related activities was correlated with systolic blood pressure (r = 0.46, p < 0.004). In accordance with the majority of caregiving studies [5], caregivers’ depressive symptoms, anxiety or stress symptoms were not reliably correlated with caregiving variables such as years spent caregiving or the number of hours per day consumed by caregiving activities.

**Salivary Cortisol and DHEAS Levels**

The salivary cortisol was evaluated here as an important estimate of adrenal function. The cortisol levels differed significantly over the 3 sampling times $F_{2,140} = 319.30$, $p < 0.0001$ (fig. 1a), presenting a regular circadian rhythm: peak levels in the morning and nadir in the evening. No changes in cortisol levels were observed in the caregivers compared to non-stressed controls ($F_{1,70} = 1.86$, $p = 0.18$; fig. 1a). In agreement with these results, the caregivers had similar integrated (AUC) cortisol levels compared to non-caregivers: 98.12 ± 3.64 versus 103.05 ± 3.92 nmol/l/h, respectively (t = 0.92, $p = 0.36$). There was an interaction between the variables group × cortisol levels, indicating lower cortisol levels at 8 a.m. in the caregiver cohort compared to non-caregivers ($p < 0.05$). Age was not correlated with cortisol assessments. We also measured morning salivary DHEAS levels as an important marker of adrenal function and because it can antagonize many glucocorticoid-related changes. The caregivers had significantly lower DHEAS levels ($t = 6.1$, $p < 0.0001$; fig. 1b) and increased cortisol/DHEAS ratio ($t = 8.50$, $p < 0.0001$).

### Table 1. Health-related, biochemical and hematological data

<table>
<thead>
<tr>
<th></th>
<th>Caregivers (n = 41)</th>
<th>Non-caregivers (n = 33)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60.56 ± 11.56</td>
<td>60.27 ± 14.11</td>
<td>0.92</td>
</tr>
<tr>
<td>Education, years</td>
<td>9.95 ± 2.67</td>
<td>11.48 ± 3.12</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>26.38 ± 5.23</td>
<td>26.38 ± 5.64</td>
<td>0.99</td>
</tr>
<tr>
<td>Sleep, h</td>
<td>6.78 ± 1.68</td>
<td>7.25 ± 0.86</td>
<td>0.18</td>
</tr>
<tr>
<td>Smokers/non-smokers</td>
<td>83/3</td>
<td>72/6 ± 3.12</td>
<td>0.80</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>105.05 ± 4.23</td>
<td>93.56 ± 2.37</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>24.22 ± 1.12</td>
<td>24.17 ± 1.05</td>
<td>0.97</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>26.20 ± 1.55</td>
<td>24.04 ± 2.25</td>
<td>0.42</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.94 ± 0.02</td>
<td>0.98 ± 0.04</td>
<td>0.31</td>
</tr>
<tr>
<td>RBC, 10^6/mm³</td>
<td>4.55 ± 0.07</td>
<td>4.38 ± 0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>WBC, mm³</td>
<td>6,514.63 ± 331.88</td>
<td>6,281.20 ± 314.94</td>
<td>0.62</td>
</tr>
<tr>
<td>Neutrophils, mm³</td>
<td>3,788.92 ± 237.57</td>
<td>3,685.48 ± 238.37</td>
<td>0.78</td>
</tr>
<tr>
<td>Lymphocytes, mm³</td>
<td>1,978.83 ± 92.28</td>
<td>1,862.61 ± 102.81</td>
<td>0.42</td>
</tr>
<tr>
<td>Monocytes, mm³</td>
<td>551.17 ± 344.04</td>
<td>488.80 ± 161.52</td>
<td>0.40</td>
</tr>
<tr>
<td>Eosinophils, mm³</td>
<td>210.84 ± 25.20</td>
<td>188.30 ± 60.92</td>
<td>0.70</td>
</tr>
<tr>
<td>Basophils, mm³</td>
<td>37.57 ± 4.20</td>
<td>30.48 ± 3.67</td>
<td>0.26</td>
</tr>
</tbody>
</table>

ALT = Alanine transaminase; AST = aspartate transaminase; BMI = body mass index; RBC = red blood count; WBC = white blood count.

### Table 2. Psychosocial characteristics and autonomic function

<table>
<thead>
<tr>
<th></th>
<th>Caregivers (n = 41)</th>
<th>Non-caregivers (n = 33)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>3.37 ± 2.71</td>
<td>1.75 ± 2.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Q2</td>
<td>5.24 ± 3.48</td>
<td>2.06 ± 2.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Q3</td>
<td>5.27 ± 4.07</td>
<td>1.94 ± 2.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>STAI trait</td>
<td>44.78 ± 5.16</td>
<td>31.25 ± 8.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>STAI state</td>
<td>48.73 ± 6.51</td>
<td>36.38 ± 8.70</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BDI</td>
<td>10.61 ± 8.79</td>
<td>3.55 ± 4.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>75.42 ± 8.46</td>
<td>71.35 ± 8.71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mm Hg</td>
<td>13.60 ± 1.75</td>
<td>12.94 ± 1.81</td>
<td>0.18</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>8.33 ± 1.28</td>
<td>7.79 ± 0.89</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Q1 = Stress last 24 h; Q2 = stress last week; Q3 = stress last month; STAI = State Trait Anxiety Inventory; BDI = Beck Depression Inventory.
Sensitivity of HPA Axis to Glucocorticoids

In order to evaluate the sensitivity of the HPA axis to the steroid feedback, we examined the HPA axis response in suppressing cortisol levels following administration of a synthetic glucocorticoid (DEX) in vivo. This test enabled us to distinguish HPA axis non-suppressors from suppressors. There was a significantly higher proportion of non-suppressors in the caregiver cohort compared to non-caregivers (29.3 vs. 3.2%, respectively; $\chi^2 = 8.69, p = 0.004$). The suppressors and non-suppressors had similar basal cortisol levels in the caregiving cohort: AUC cortisol, 97.67 \pm 4.00 nmol/l/h versus 99.21 \pm 8.10 nmol/l/h, respectively.

T Cell Proliferation and Peripheral Sensitivity to Glucocorticoids

We evaluated the mitogen-induced T cell proliferation/viability in vitro as an index of non-specific cell-mediated immunity. The caregivers had a significantly higher cell proliferation/viability compared to non-caregivers ($F_{1,55} = 15.17, p < 0.0001$; fig. 2a). There was an interaction between the variables mitogen \times group, indicating the proliferation was particularly high in the caregiver cohort at PHA 2%. The same pattern was observed when proliferation/viability data was analyzed co-varying for age.

The measurement of peripheral hormones may not be sufficient to finally determine the functional hormonal action in target tissues. Here, we investigated the peripheral lymphoid sensitivity to steroids by analyzing the
ability of glucocorticoids to suppress T-cell proliferation in vitro. Repeated measures ANOVAs revealed that caregivers had increased cellular sensitivity to both DEX (F1, 58 = 7.08, p < 0.01) and corticosterone (F1, 57 = 8.18, p = 0.006; fig. 2b and 2c).

**Discussion**

This study reported the impact of a realistic chronic stress exposure on several neuroendocrine and immunological functions in strictly healthy individuals. The rigorous screening for health conditions (SENIEUR protocol) restricted us to recruit approximately 12–15% of caregivers and age-matched controls, controlling for diseases and health-related behaviors that would interfere with the biological correlates of stress exposure. Caregivers were consistently more distressed than non-caregivers, but they did not present common stress-related physiological changes, such as increased cortisol levels or blunted lymphocyte proliferation. On the other hand, the healthy caregivers showed some potentially damaging effects of chronic stress including a higher cortisol/DHEAS ratio and resistance to glucocorticoid effects in the central nervous system. This is the first study that shows the endocrine-immunological status of chronically stressed strictly healthy (SENIEUR) subjects.

**Stress without Corresponding Hormonal Changes**

In accordance with previous studies, we demonstrated that the caregivers were significantly more stressed than age-matched non-caregivers, confirming the chronic
stress model in adult volunteers [2, 11, 21, 22]. In line with these results, the caregivers showed a low-grade autonomic activation, as shown by increased diastolic pressure and heart rate. The assessment of cortisol in saliva has proven to be a stress-free procedure and reliable measure of unbound steroid in the circulation [23]. However, the distressed caregivers had similar salivary cortisol levels as non-stressed controls. In fact, morning cortisol levels were significantly lower in caregivers than in non-caregivers. These data are in partial contrast with previous work, considering that some studies reported increased [2, 3] or lower cortisol levels [22] and others observed no hormonal changes [21]. These discrepancies could be explained by differences in health-related behaviors, social support or coping skills experienced by the caregivers [24]. We hypothesize that good health conditions are associated with better resilience to stress and thus a lower allostatic load [25]. On the other hand, the low-grade autonomic activation could have been implicated with altered neuroendocrine response. Indeed, a recent study observed that hypertensives had lower cortisol awakening responses and reduced suppression of morning cortisol levels after DEX administration [26]. Taken together, these data suggest that an increased cardiovascular response would negatively impact the caregiver’s health without necessarily activating the HPA axis [27, 28].

We also measured salivary DHEAS levels as an important marker of adrenal function. Dehydroepiandrosterone (DHEA) is the major secretory product of the human adrenal glands. The hormone is uniquely sulfated (DHEAS) before entering the plasma, and this hormone is converted to DHEA and its metabolites in various peripheral tissues [29]. Serum DHEA levels decrease by the second decade of life, and reach about 5% of their original level in the elderly [30]. The morphological correlates of the age-related changes of DHEAS/DHEA secretion are progressive atrophy of the zona reticularis of the adrenal glands [31]. DHEA and its metabolites have been reported to have enhancing immunomodulatory properties [32–34] and may antagonize many glucocorticoid-related changes. We observed that caregivers had significantly lower DHEAS levels and a significantly increased cortisol/DHEAS ratio compared to non-caregivers. In view of the well-known inhibitory effects of pro-inflammatory cytokines on adrenal steroidogenesis and that therapy with anti-IL6 receptor favors androgen secretion in patients with rheumatoid arthritis [35], the possibility of decreased DHEAS levels being due to an immune-mediated mechanism should be considered.

The impaired DHEAS secretion, together with the increase of cortisol, results in an enhanced exposure of various tissues (including the brain and immune system) to the cytotoxic/immunomodulatory effects of glucocorticoid [36]. In spite of an increased cortisol/DHEAS ratio, the strictly healthy caregivers showed no signs of the premature immunosenescence usually seen in chronically stressed subjects. Therefore, some subjects could have developed resilience factors (discussed below) enabling them to buffer some potentially damaging physiological changes.

**Maintenance of Cell-Mediated Immunity**

The mitogen-induced T cell proliferation/viability was evaluated here as an index of non-specific cell-mediated immunity. In contrast to previous studies [2, 4, 37], the strictly healthy caregivers had increased cell proliferation/viability as compared to non-caregivers. These data are also in contrast to previous findings of lower NK cell activity, lower IL-2 production [2] or increased production of immune-regulatory cytokines (IL-10 and TNF-α) in caregivers of Alzheimer’s patients [37] as compared to age-matched controls. In addition, caregiving has been associated with low-grade inflammation, as shown by increased serum IL-6, C-reactive protein and D-dimer levels in elderly stressed caregivers [11, 38]. Although not investigated here, these changes are commonly observed during human aging and it was thus suggested that chronic stress would be associated with premature aging of the immune system [14, 39]. Considering that these inflammatory markers have been associated with increased morbidity and frailty during aging, it could be speculated that previous studies included subjects who had subclinical diseases or who were not completely healthy. In fact, none of them included strictly healthy individuals. In addition, previous work included subjects significantly older than our samples, suggesting that chronic stress would particularly suppress cell-mediated immunity in elderly people. Here, no differences were observed when proliferation/viability data was analyzed co-varying for age. Further studies should address the inflammatory status of strictly healthy individuals under stress.

**Altered Glucocorticoid-Immune Signaling**

The measurement of peripheral hormones may not be sufficient to finally determine the functional hormonal action in target tissues. Therefore, to further examine the cross talk between peripheral hormones and the immune system, we also investigated the lymphocyte sen-
sensitivity to low concentrations of synthetic (DEX) and naturally occurring steroid (corticosterone). Glucocorticoid immunomodulation is orchestrated by specific binding of glucocorticoids to 2 distinct cellular receptors: mineralocorticoid and glucocorticoid receptors. Although mineralocorticoid receptors have higher affinity for circulating glucocorticoids than do glucocorticoid receptors, most (if not all) effects on the immune system are mediated via the latter type of receptor [40]. Cells from caregivers were more sensitive to both DEX- and corticosterone-related suppressive effects on cellular proliferation/viability than controls. These data are in contrast to previous work reporting that chronic stress [2] or major depression [20, 41] were associated with relative resistance to the DEX effects on lymphoid cells. It should be noted that the slope of suppression observed in the control group is compatible with previous data reporting reduced cellular sensitivity to DEX in strictly healthy aging [13]. Our immunological findings are in line with the cortisol data presented here (i.e. low morning cortisol), suggesting that cells would up-regulate glucocorticoid receptors during lower levels of endogenous ligand. These findings would be of protective value for the caregiver.

Impaired HPA Axis Response to DEX in vivo

The HPA axis sensitivity to glucocorticoids was assessed by the response in suppressing endogenous cortisol levels following DEX administration. In contrast to increased cellular sensitivity to glucocorticoids in vitro, the great majority of caregivers failed to show suppression of salivary cortisol after DEX intake in vivo, which may suggest lower negative feedback sensitivity of the HPA axis. This impaired response was not related to cortisol levels at baseline. Further studies are required to determine why a defect in HPA axis functionality is not associated with reciprocal changes in basal cortisol levels or reciprocal impaired sensitivity in the periphery. Furthermore, suppressors and non-suppressors showed similar psychological morbidity, T cell proliferation and cellular sensitivity to glucocorticoid in vitro (data not shown). Although the DEX suppression test provides cogent information on the central nervous system response to glucocorticoids, it is not specific for any stress-related pathology. Indeed, a reduced functionality of the HPA axis feedback inhibition has been described in various pathologies, including major depression [20], psychotic depression [42], borderline personality disorder [43], anxiety disorders [44], chronic inflammatory diseases [45], dementias [46] and during human aging [47]. Here, the low-grade autonomic activation could be related to impaired HPA responses since hypertension has been associated with blunted cortisol response to awakening and lower negative feedback sensitivity [26].

Potential Factors Involved with Better Resilience to Stress

To date, the mechanisms underlying the resilience to chronic stress of some extremely healthy subjects are largely unknown. However, some potential variables may be involved including adequate health engagement control strategies [48], social support [49], personality traits and coping strategies [49, 50]. Distressed subjects are more likely to have health habits that put them at greater risk for diseases, including poorer sleep, a greater propensity for alcohol and drug abuse, poorer nutrition and less exercise, and these health behaviors have cardiovascular, immunological and endocrinological consequences [49]. However, research also suggests that older adults who engage in active behaviors (health engagement control strategies) to overcome their physical problems do not experience enhanced levels of emotional distress [51] and have attenuated secretion of salivary cortisol [48]. Personality and coping styles reflect individual differences in appraisal and response to stressful situations, and both have been associated with the onset and course of chronic and progressive health problems, including cardiac morbidity and mortality [49]. A potent resilience factor for health outcomes may be the induction and maintenance of a positive emotional state through personality and coping styles. Older patients have been shown to be more vulnerable to negative emotions as they have smaller social support networks [52]. Caregivers of dementia patients who reported lower levels of social support and higher levels of distress on study entry showed the greatest negative changes in immune function 1 year later [4]. Conversely, stressed individuals with better social support developed stronger immune responses to vaccination [53]. Here, preliminary data from our laboratory indicated that healthy caregivers used different coping strategies from non-caregivers, including seeking social support and accepting responsibility (unpubl. data). Therefore, several resilience factors could be associated with better neuroendocrine and immune responses observed in strictly healthy subjects exposed to chronic stress.

In summary, the findings of the present study lead us to conclude that well preserved health conditions may spare the stressed caregiver from damaging effects on...
some cogent neuroendocrine and immune functions. Future studies should address whether other systems are also spared from the lack of cortisol signaling, such as brain areas specially targeted during stress exposure and involved with cognition. It is also necessary to investigate whether extremely healthy caregivers show better resilience to acute stress exposure.

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