Physiology & Behavior 106 (2012) 40–45

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Does cellular aging relate to patterns of allostasis? An e`xamination of basal and stress reactive HPA axis activity and telomere length

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ARTICLE INFO

Article history:
Received 19 August 2011
Received in revised form 9 November 2011
Accepted 16 November 2011

Keywords:
Cortisol
Allostasis
Allostatic load
Telomere
Cellular aging
Stress
HPA axis

ABSTRACT

Long-term exposure to stress and its physiological mediators, in particular cortisol, may lead to impaired telomere maintenance. In this study, we examine if greater cortisol responses to an acute stressor and/or dysregulated patterns of daily cortisol secretion are associated with shorter telomere length. Twenty-three postmenopausal women comprising caregivers for dementia partners (n = 14) and age- and BMI-matched non-caregivers provided home sampling of cortisol–saliva samples at waking, 30 min after waking, and bedtime, and a 12-hour overnight urine collection. They were also exposed to an acute laboratory stressor throughout which they provided saliva samples. Peripheral blood mononuclear cells were isolated from a fasting blood sample and assayed for telomere length. As hypothesized, greater cortisol responses to the acute stressor were associated with shorter telomeres, as were higher overnight urinary free cortisol levels and flatter daytime cortisol slopes. While robust physiological responses to acute stress serve important functions, the long-term consequences of frequent high stress reactivity may include accelerated telomere shortening.

1. Introduction

How does psychological stress "get under the skin" to cause deleterious health outcomes? McEwen and colleagues have put forth a helpful model describing how the wear-and-tear of repeated physiological responses to psychological stress that are part of allostasis, over time, lead to allostatic load (damage due to these fluctuations) and eventually poor health outcomes. Much population and experimental data support this model [1,2], and it is relatively well established that chronic psychological stress is harmful to health. Stress has been linked longitudinally to disease states such as metabolic syndrome, cardiovascular disease, diabetes, and other diseases of aging [3–5]. These general and pervasive effects of stress may be linked to changes proximal to the stress response, such as changes in regulation of the HPA axis, but also may have common cellular mechanisms. Telomeres may be one of the common cellular mechanisms linking chronic stress to diseases of aging. Telomeres are DNA–protein complexes that protect chromosomal DNA from damage. As mitotic cells divide, telomeres get shorter, leaving the cell vulnerable to genomic instability, end-to-end chromosome fusion, less efficient mitosis, and loss of ability for cell replenishment and thus tissue replenishment [6,7]. Even non-mitotic cells may develop shortened telomeres when chronically exposed to oxidative stress [8]. When telomeres shorten to a certain point, cells undergo senescence. Telomere shortening, therefore, represents both a *marker* and *mechanism* of biological aging [9], as the progression toward senescence can be monitored by telomere shortness and telomere dysfunction activates p53-mediated cellular damage [10].

Chronic caregiving stress has been related to shorter telomere length in both young women [11] and in older men and women [12]. Similar findings have emerged in experimental models of stress in mice [13]. Thus, telomere shortening may be one important pathway by which stress gets under the skin to promote early aging. Shorter telomere length has been related to conditions of chronic adversity, such as longer working hours [14], being single [15], lower socioeconomic status [16],

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doi:10.1016/j.physbeh.2011.11.016

major depression [17] and duration of depression [18], and childhood trauma [19,20].

Given that telomere length is associated with a variety of stressor exposures, here we conceptualize telomere length as a potential molecular-level measure of allostatic load. Allostatic load incorporates dysregulation across multiple systems and telomere length may provide an index of cumulative inputs from multiple regulatory systems. Shorter telomere length is associated with worse function across multiple regulatory systems, including greater inflammation, oxidative stress, and insulin resistance [21–23]. Moreover, the cumulative inflammatory load of being high on both interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) was related to shorter telomere length than being high on just one individually [21]. For these reasons we conceptualize telomere length as a potential summary measure of total cumulative biochemical stressor exposures [24]—in a sense a molecular measure of allostatic load.

Psychological stress activates the hypothalamic-pituitary-adrenocortical (HPA) axis, and the end product, cortisol, can be used as one index of stress reactivity. *In vitro* studies have demonstrated that the application of high doses of hydrocortisone to lymphocytes lowers telomerase [25], the enzyme primarily responsible for telomere maintenance. Telomerase elongates telomeric DNA to counteract shortening of telomeres and thus protects them [26]. The allostatic load model predicts that multiple patterns of altered responses to stress (strained allostasis) can contribute to allostatic load. Given the consistent associations of stress with telomere shortness, it follows that certain stress-related patterns of allostasis may also lead to telomere shortening. Relevant here, an individual might: (1) experience exaggerated responses to repeated "hits" of acute stress and therefore have excessive exposure to physiological stress mediators; or (2) have a dysregulated diurnal rhythm of stress mediators, in particular flat slope or high evening levels of cortisol.

In the current study, we tested whether patterns of HPA axis dysregulation that are hypothesized to contribute significantly to development of allostatic load are associated with immune cell aging as measured by telomere shortness. We assessed the magnitude of diurnal cortisol slope as well as several other patterns that may indicate altered allostasis in the form of exaggerated reactivity or slow recovery from daily stress arousal. We exposed participants to an acute psychological stressor in the laboratory and measured their salivary cortisol response. Exaggerated response may indicate greater stress responding to the minor hassles of daily life. We also measured nocturnal cortisol output to assess basal activity during the quiescent period of the diurnal rhythm. We predicted that greater reactivity to acute psychological stress and signs of diurnal dysregulation such as a flatter rhythm or greater nocturnal output of cortisol would be associated with shorter telomere length.

2. Materials and methods

2.1. Participants

To draw from a population experiencing a wide range of chronic psychological stress, the study sample was drawn from a community sample and comprised 14 women caring for a partner with dementia and 9 non-caregiving women of similar age and BMI. Caregivers were recruited via flyers and ads in the community, from the University of California, San Francisco (UCSF) Memory and Aging Clinic, and from community organizations for dementia. Their matched non-caregivers were recruited through flyers and ads in the community and referrals of friends from caregiver participants. The resulting sample did in fact have a wide range of stress as measured by the Perceived Stress Scale [27]. Normal values for adults from a poll of a representative U.S. sample are available [28]. The stress score for a typical sample of adult women ranges from 7.1 to 20.3. In this sample, the stress score ranged from 7.6 to 23. To control for effects of menopausal status on cortisol reactivity, all participants were postmenopausal, and the women ranged in age from 51 to 79 ($M = 62$, $SD = 6.46$). Other exclusion criteria included the presence of major medical conditions such as heart disease, cancer, or diabetes, use of medications containing agents known to affect cortisol levels (e.g., hydrocortisone, DHEA), and regular smoking. Three women were on SSRI medication; the pattern of results shown below do not change with the subtraction of these women. The sample was predominantly white (82%), with the remaining self-reporting as Asian (11%), black (5%) and Latina (2%). The educational attainment was 38% college educated, 29% advanced degree, 19% some college or technical school, 9% high school and 5% an AA degree.

2.2. Procedures

All procedures were approved by the University of California, San Francisco Committee on Human Subjects Research. After providing informed consent, participants provided a fasting morning blood sample for separation of peripheral blood mononuclear cells (PBMCs) and measurement of telomere length. Participants were weighed and measured to obtain Body Mass Index (BMI), calculated as weight (kg) divided by height (meters) squared. Participants were then instructed to provide three saliva samples per day at waking, waking + 30 min, and bedtime for the next three consecutive days to measure diurnal cortisol rhythm. They were also instructed to collect their urine on the last night of saliva sampling. They were then scheduled to return approximately one week later for the laboratory stress session.

2.2.1. Urine sampling

Urine was collected over 12 h. Participants were instructed to void to clear their bladder at the beginning of the collection (usually 20:00). They then voided into a specimen hat (and saved this in a bottle in a cold cooler) at all later times before bed and during the night when they naturally woke to urinate, and in the morning, until 12 h had passed (usually until 08:00). The urine was either collected by study staff or delivered by the participant the following morning. It was immediatey aliquoted and frozen at −80 C for batch analysis. Total free cortisol was used as the measure of overnight urinary cortisol secretion. Participants had to provide at least 200 mL of urine to be included for data analysis, and the urinary cortisol values were adjusted for flow-rate (volume).

2.2.2. Saliva sampling

Each saliva sample was collected in 2 mL SaliCaps tubes (IBL Hamburg, Germany) via the passive drool method. Participants were instructed to collect the first sample while still in bed and to not eat, drink, brush their teeth, or engage in vigorous activity between the first two morning samples and 20 min prior to the bedtime sample. They were also requested to refrain from alcohol prior to and for the duration of each sampling day.

The saliva samples were stored in the participant's freezer at 4 º C until the kit was returned to study staff on ice, where it was stored at −80 º C. Samples were shipped for analysis on dry ice and assayed in batch at Dr. Kirscbaum's lab at Dresden University of Technology in Germany.

2.2.3. Acute lab stressor

In the laboratory testing session, all participants were run between 1400 and 1700 hrs to control for the diurnal rhythmicity of cortisol. There, participants were exposed to a modified Trier Social Stress Test [TSST; 29]. This is a widely used, standardized laboratory stressor designed to elicit psychological stress and cortisol responses. The 20-minute stress task was comprised of four 5-minute stressful periods, including introduction to evaluators and task instructions, speech preparation period, a videotaped public speaking task in