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Telomere Length is Inversely Correlated with Urinary Stress Hormone Levels in Healthy Controls but not in Un-medicated Depressed Individuals- Preliminary Findings

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Abstract

Objective—Leukocyte telomere length (LTL) is a biomarker of cellular aging affected by chronic stress. The relationship of LTL to the stress hormones, cortisol and catecholamines, is unclear, as are possible differences between healthy controls (HC) and individuals with Major Depressive Disorder (MDD). This small pilot study is the first to examine the relationship between cortisol, catecholamines and LTL specifically in un-medicated MDD in comparison with HC.

Methods—Participants included 16 un-medicated MDD subjects and 15 HC for assay of LTL, 12-hour overnight urinary free cortisol and catecholamine levels.

Results—LTL, cortisol and catecholamine levels did not significantly differ between groups. In HC, a hierarchical regression analysis indicated that higher levels of cortisol were correlated with shorter LTL (p=.003) above and beyond age and sex. Higher catecholamine levels were nearly-significant with shorter LTL (p=.055). Neither hormone was correlated with shorter LTL in MDD (p's >.28). To assess a possible cumulative effect of stress hormone activation, a summary score was calculated for each subject based on the number of stress hormone levels above the median for

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that group (HC or MDD). A significant inverse graded relationship was observed between LTL and the number of activated systems in HC (p=.001), but not in MDD (p=.96).

Conclusion—This pilot study provides preliminary evidence that stress hormone levels, especially cortisol, are inversely related to LTL in HC, but not in un-medicated MDD. Clarification of these relationships in larger samples could aid in understanding differential mechanisms underlying stress-related cellular aging in healthy and depressed populations.

Keywords

catecholamines; cellular aging; cortisol; depression; stress hormones; telomere length

Introduction

Shortened leukocyte telomere length (LTL) is an index of biological aging [1] associated with disease and mortality risk [2–4]. Accelerated LTL shortening is reported in several psychiatric illnesses and stress-related conditions [5], although underlying mechanisms are unknown [1,6]. Telomere shortening may occur with repeated mitoses, inflammation or oxidative stress [7] in the absence of adequate telomerase activity [1]. Elevated cortisol and catecholamine levels may be related to telomere shortening, but this is poorly studied, and data are conflicting [3,7–9]. Despite reports of altered LTL and stress hormones in major depressive disorder (MDD) [1], differences between healthy controls (HC) and MDD individuals have been infrequently assessed [7], and have never been evaluated exclusively in un-medicated MDD participants. Determining the relationship of LTL to stress hormones could clarify mediators of the relationship of LTL with chronic stress and psychiatric illness. We hypothesized that elevated cortisol and catecholamine levels would be correlated with shorter LTL. Since this has not previously been tested in MDD vs. controls, we set out to explore whether differences between groups might obtain. However, in the absence of prior data, we posited the null hypothesis of no differences between groups; we, therefore, assessed between-group differences in an exploratory manner.

Methods

Subjects

Sixteen un-medicated MDD (11 females, 5 males) and 15 HC (9 females, 6 males) subjects, between 25–65 y.o., participated. These subjects represent a subset of those who participated in a study of cell aging in MDD with different hypotheses tested [10]. The present sample includes all subjects from that larger study who had LTL, cortisol, and catecholamine assay results available. One HC subject had cortisol but not catecholamine data available, leaving 14 HC for the catecholamine analysis in the present study. This study was approved by the University of California, San Francisco, Committee on Human Research.

Subjects were diagnosed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). MDD subjects met DSM-IV-TR criteria for non-psychotic MDD (DSM 5 was not in use during the conduct of this study), and had 17-item Hamilton Depression Rating Scale [11] ratings   17. HC subjects had no lifetime Axis I disorder history. Exclusion criteria included: positive urine toxicology or pregnancy test, uncontrolled

medical illnesses and interfering medications (including antidepressants and other psychotropics) within 6 weeks of the study (except infrequent short-acting benzodiazepines for sleep, but not within one week of the study). Depressed subjects were further excluded for: history of mania/hypomania or psychosis; eating disorders or post-traumatic stress disorder (PTSD) (month); and substance abuse (6 months). Co-morbid anxiety disorders (except PTSD) were allowed if the MDD diagnosis was primary.

Specimen Collection and Assays

Telomere Length: Whole blood was drawn at approximately 8:00am after fasting (except water) since 10:00pm the night before, and leukocytes were saved for LTL assay. DNA was prepared using commercial reagents (Gentra Puregene Blood Kit, Qiagen, Valencia, CA). LTL was assayed by real-time PCR, as described previously (Wolkowitz et al., 2011). The inter-assay CV was 4%. LTL is reported in base pairs (bp)=3,274+2,413*(T/S).

Urinary Cortisol and Catecholamines: Subjects collected 12-hour urine samples from 8:00pm until 8:00am the following day. With one exception, blood for LTL was collected within one day of urine sampling. Sensitivity analysis showed that excluding this one subject did not alter the findings. Urine containers were kept chilled, and sodium metabisulfite was used as a catecholamine preservative. Urine logbooks indicated excellent compliance. Catecholamines (epinephrine and norepinephrine) were assayed using ELISA (American Laboratory Products Company [Alpco], Windham, NH). Intra- and inter-assay CV's were 10.2 and 15.3%. Cortisol was measured by a radioimmunoassay; intra- and inter-assay CV's were 9.3 and 5.4%. Creatinine was measured spectrophotometrically by the Jaffe reaction at 490 nm after extraction of the samples with ethyl ether.

Statistics

Baseline characteristics (Table 1) were calculated using T-tests, univariate analyses and partial correlations. Age and sex were covaried in all analyses; covarying for ethnicity and tobacco did not alter results. The non-normal distribution of cortisol was transformed using square roots (SQRT). Stress hormone concentrations are reported as ratios to urinary creatinine concentrations (ug cortisol or catecholamine/g creatinine) determining adequacy of the collection. Hierarchical multiple linear regression assessed the relationship between cortisol and catecholamines vs. LTL.

To assess cumulative effects of both cortisol and catecholamine systems, a relative hormone activation (RHA) score was calculated. The RHA score was determined using hormone values above the median for the separate MDD and HC groups. RHA scores could equal 0 (low cortisol and catecholamine), 1 (high cortisol *or* catecholamine) or 2 (high cortisol *and* catecholamine). A related approach, using a tertile split, was previously used in a study of LTL relationships with stress markers [7]; however, due to sample size, a median split was used.

Results

Demographics

There were no significant between-group differences in age, sex, BMI, education, or current tobacco use (Table 1).

Relationship of Telomere Length to Urinary Free Cortisol and Catecholamines

There were no significant between-group differences in cortisol and catecholamine concentrations or LTL (Table 1). Across all subjects, there was no correlation between LTL and cortisol ($r = -.096$, $p = .62$) or catecholamines ($r = -.21$, $p = .28$). In HC subjects, after accounting for sex and age, cortisol levels were significantly inversely associated with LTL ($\beta = -.76$, $p = .003$), and catecholamines showed a near-significant trend towards an inverse relationship with LTL ($\beta = -.52$, $p = .055$) (Figure 1). In MDD subjects, no significant relationships were found between LTL and cortisol ($\beta = .069$, $p = .786$) or catecholamines ($\beta = -.31$, $p = .29$) (Figure 1b).

Relationship of Telomere Length and Relative Hormone Activation (RHA)

In HC subjects, higher RHA scores were associated with shorter LTL ($\beta = -.77$, $p = .001$). *Post-hoc* testing showed significantly shorter LTL in HC subjects with both high cortisol and catecholamine levels compared to those with low hormones ($p = .047$, 4881 vs. 5289 bp) (Figure 1c). There was no significant relationship between LTL and the RHA score in the MDD subjects ($\beta = -.012$, $p = .96$)

Discussion

This pilot study suggests activated stress systems are correlated with shorter LTL in HC subjects, but not in un-medicated individuals with MDD, which is partially consistent with, and extends, previous reports [3,7,8]. Similar to our results in HC, one previous study found, in primarily a non-MDD population, that LTL was inversely correlated with 12-hour overnight urinary cortisol, epinephrine, and norepinephrine [3]. Another study, in a mixed sample of women, found complicated relationships: a trend towards shorter LTL in individuals with high and low levels of cortisol compared to those with moderate cortisol levels; further, urinary catecholamines were inversely correlated with LTL in women with moderate-to-high levels of perceived stress [8].

The largest study to date, including a mixed group of HC and current or remitted MDD and anxiety disorder individuals, found, across all subjects, that shorter LTL was significantly associated with higher cortisol awakening responses, increased heart rates, and long cardiac pre-ejection period [7]. Although, these associations did not differ between HC and patients, their findings are mixed, as other measures indicative of cortisol and catecholamines were not significantly correlated with LTL [7]. Similar to the present findings with cortisol and catecholamine system activations in HC, that study found that the number of stress dysregulations (increased cortisol awakening response, elevated interleukin-6 levels, elevated C-reactive protein levels and increased heart rate) predicted shorter LTL in a graded

manner [7]. Multiple dysregulations or activations may create an increased allostatic load triggering accelerated cellular aging as marked by shortened LTL [7].

The mechanisms underlying the link between stress hormones and LTL in HC are undefined, but may be due to effects of the stress hormones on LTL mediated by effects of inflammation, oxidative stress, cell turnover [1] or changes in "metabolic strategies" [12]. *In vitro* studies are somewhat inconsistent, with one showing that exposure to cortisol resulted in reduced telomerase activity, which could result in LTL shortening [13], while another found that exposure to cortisol increased telomere length [6]. In one of the few *in vivo* studies, four weeks of corticosterone exposure was found to significantly shorten telomeres in mice [12].

The reasons for the lack of a significant relationship between LTL and stress hormones in MDD are unknown. Chronically elevated concentrations of stress hormones could lead to down-regulation of certain catecholamine receptors (e.g., cardiac beta-receptors) [7] and glucocorticoid receptors [14]. However, we did not find differences in basal catecholamine and cortisol concentrations in our sample. Alternatively, MDD could be associated with increased telomerase activity [15], which could mitigate telomere attrition related to cortisol or catecholamines. This null finding in MDD could be the result of a Type 2 error, due to our small sample size, although both groups were of similar size. To further test whether the two groups are significantly different from one another, a moderation analysis would be useful (with group as the moderator); however, our small sample size precluded a meaningful analysis of this sort. Such an analysis should be conducted in future larger studies.

Strengths of this study include: physically healthy, well-characterized un-medicated subjects and 12-hour overnight urine samples (a cumulative assessment of stress hormone excretion). Limitations include: small sample size, lack of longitudinal data, lack of phenotyping of specific leukocyte subtypes, and the lack of assessment of daytime or diurnal stress hormone levels. This study provides preliminary evidence that elevated cortisol, and possibly catecholamines, are correlated to shorter LTL in HC, but not in unmedicated MDD. The lack of a significant correlation between LTL and stress hormones in un-medicated subjects with MDD is a novel finding. These results emphasize the need to examine controls separately in neuroendocrine and biomarker studies, since their regulation may differ.

Acknowledgments

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Highlights

- TL was inversely correlated with cortisol and catecholamines in HC.

- In MDD, no sig. correlations were found between TL and cortisol or catecholamines.

- In HC, the activation score showed a significant inverse relationship with TL.

- TL in HC with 2 activated systems were sig. shorter than those with 0 activation.

- There was no significant relationship in MDD with activation scores.
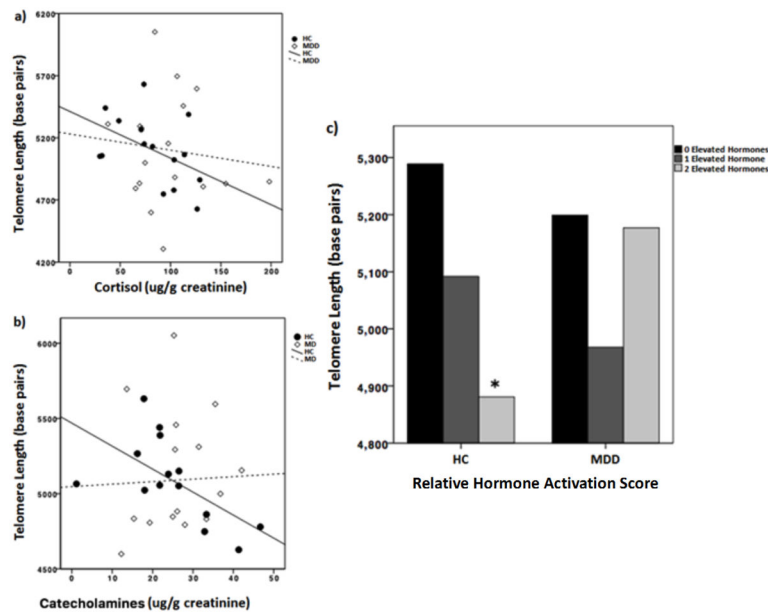
Figure 1. Relationships between Leukocyte Telomere Length and Urinary Cortisol and Catecholamines

Relationships between leukocyte telomere length and (a) urinary cortisol levels and (b) urinary catecholamine levels, in healthy controls (HC) and major depressive disorder (MDD) subjects. Analyses adjusted for age and sex. In HC, TL was significantly inversely associated with cortisol ($\beta = -.76$, $p = .003$), and near-significantly with catecholamines ($\beta = -.52$, $p = .055$). In MDD, TL was not significantly associated with cortisol ($\beta = .069$, $p = .79$) or with catecholamines ($\beta = -.31$, $p = .29$). (c) The Relative Hormone Activation Score (RHA) could equal 0 (low cortisol and catecholamine), 1 (high cortisol *or* catecholamine) or 2 (high cortisol *and* catecholamine). The RHA score showed a significant inverse relationship with telomere length in HC ($\beta = -.77$, $p = .001$) but not in MDD ($\beta = -.012$, $p = .96$). In HC, having both cortisol and catecholamine activation (score= 2) was significantly different than having no activation (score=0) (4881 vs. 5289 bp, $p = .047$).

Table 1

Demographics: Means and Standard Deviation

Variable	MDD (n=16)	HC (n=15[*])	Statistical Test, p value; Cohen's D MDD vs. HC
Age (years)	37.4±11.9	34.3 ±7.8	$t(29) = -.85$, $p =$ NS, $d = .31$
Sex (% female)	69%	60%	$t(29) = .49$, $p =$ NS, $d = .18$
BMI (kg/m2)	24.12± 3.93	25.02 ±4.07	$t(29) = .62$, $p =$ NS, $d = .22$
Current Tobacco Use (% Yes)	25%	20%	$t(29) = -.24$, $p =$ NS, $d = .088$
Education (years)	17.13± 2.06	16.67 ±3.04	$t(19)= -.49$ [**], $p =$ NS, $d= .46$
Quick Inventory of Depressive Symptomatology	13.69 ±4.36	2.40 ±1.72	$t(19)= -9.35$ [**], $p< .001$, $d= 3.4$
Telomere Length (base pairs)	5091.00 ±450.38	5103.60 ±278.54	$F(1,29)= .093$, $p =$ NS, $d =.034$
Cortisol/Creatinine (mcg/g)	100.42 ±39.07	82.17 ±34.17	$F(1,29)= 1.91$, $p =$ NS, $d =.50$
Catecholamines/Creatinine (mcg/g) [*]	26.74 ±8.50	24.98 ±11.26	$F(1,28)= .24$, $p =$ NS, $d =.18$

[*] Catecholamine values were only available for 14 of the healthy controls.

[**] Equal variances not assumed