Cell Aging in Relation to Stress Arousal and Cardiovascular Disease Risk Factors

Psychoneuroendocrinology (2006) 31, 277–287

PNEC

www.elsevier.com/locate/psyneuen

2005 CURT P.RICHTER AWARD WINNER

Cell aging in relation to stress arousal and cardiovascular disease risk factors

Elissa S. Epel[a,*], Jue Lin[b], Frank H. Wilhelm[c], Owen M. Wolkowitz[a], Richard Cawthon[e], Nancy E. Adler[a], Christyn Dolbier[d], Wendy B. Mendes[f], Elizabeth H. Blackburn[b]

[a]*UCSF Department of Psychiatry, San Francisco, CA, USA*
[b]*UCSF Department of Biochemistry and Biophysics, San Francisco, CA, USA*
[c]*Department of Clinical Psychology and Psychotherapy, University of Basel, Basel, Switzerland*
[d]*Department of Psychology, East Carolina University, Greenville, NC, USA*
[e]*Department of Human Genetics, University of Utah, Salt Lake City, UT, USA*
[f]*Department of Psychology, Harvard University, Cambridge, MA, USA*

Received 10 August 2005

KEYWORDS
Telomerase;
Telomere length;
Stress;
Heart rate variability;
Stress hormones;
Metabolic syndrome

Summary We previously reported that psychological stress is linked to and possibly accelerates cellular aging, as reflected by lower PBMC telomerase and shortened telomeres. Psychological stress is a major risk factor for cardiovascular disease (CVD), with multiple behavioral and physiological mediators. Telomere shortness has been associated with CVD, but the relationship between low telomerase activity, a potential precursor to telomere shortening, and CVD risk factors has not been examined in humans. Here we examine whether telomere length and telomerase in leukocytes are associated with physiological signs of stress arousal and CVD risk factors in 62 healthy women. Low telomerase activity in leukocytes was associated with exaggerated autonomic reactivity to acute mental stress and elevated nocturnal epinephrine. Further, low telomerase activity was associated with the major risk factors for CVD —smoking, poor lipid profile, high systolic blood pressure, high fasting glucose, greater abdominal adiposity—as well as to a composite Metabolic Syndrome variable. Telomere length was related only to elevated stress hormones (catecholamines and cortisol). Thus, we propose that low leukocyte telomerase constitutes an early marker of CVD risk, possibly preceding shortened telomeres, that results in part from chronic stress arousal. Possible cellular mechanisms by which low telomerase may link stress and traditional risk factors to CVD are discussed.

Abbreviations BMI, body mass index; CVD, cardiovascular disease; HRV, heart rate variability; PBMC, peripheral blood mononucleocytes; ANCOVA, analysis of covariance.

* Corresponding author. UCSF Department of Psychiatry, 3333 California Street, Suite 465, San Francisco, CA, USA. Tel.: +1 415 476 7648; fax: +1 415 476 7744.
E-mail address: epele@healthpsych.ucsf.edu (E.S. Epel).

0306-4530/$ - see front matter
doi:10.1016/j.psyneuen.2005.08.011

These findings may implicate telomerase as a novel and important mediator of the effects of psychological stress on physical health and disease.

1. Introduction

We recently reported that chronic psychological stress was associated with both shorter telomere length and lower telomerase activity in leukocytes (Epel et al., 2004). Telomeres are DNA-protein complexes that cap chromosomal ends and promote chromosomal stability. Telomere length is the downstream output of the actions of both lengthening and shortening activities in the cell (Blackburn, 2000). Most notably, the cellular enzyme telomerase adds telomeric repeat sequences to the chromosomal DNA ends, preserving not only telomere length but also healthy cell function, prolonged stem cell proliferation, and long-term immune function, independently of telomere length (Kim et al., 2003; Ly et al., 2005; Marrone et al., 2005).

Stress arousal is likely to be one pathway through which chronic stress may impact cellular aging (Sapolsky, 2004). Chronic life stress can lead to overexposure to stress responsive adrenal hormones, primarily cortisol and catecholamines, or to hyporesponsiveness, also reflecting dysregulation (McEwen, 1998). In addition to adrenal hormones, autonomic nervous system reactivity to stress, particularly heart rate variability, can serve as a physiological index of stress vulnerability, and is related to anxiety (Porges, 1995; Friedman and Thayer, 1998). We thus examined whether telomerase levels and telomere length in peripheral blood mononucleocytes (PBMCs) are associated with two indices of stress arousal—nocturnal excretion of stress hormones (catecholamines and cortisol) and autonomic nervous system stress reactivity to a standardized stressor (vagal tone or heart rate variability).

Psychological stress has recently emerged as one of the major risk factors for CVD. The largest epidemiological study of risk factors for CVD to date, with almost 30,000 participants, across 52 countries, identified the top six modifiable risk factors: cigarette smoking, poor lipid profile, high blood pressure, diabetes, abdominal obesity, and an index of psychosocial stress (Yusef et al., 2004). The cellular mechanisms by which many of these physiological and behavioral risk factors, particularly stress, can lead to disease are not well understood.

However, there is now converging evidence from basic and clinical studies that telomere maintenance plays an important role in organismal longevity. Shorter telomeres in human leukocytes have been linked to age-related conditions and diseases including hypertension, greater pulse pressure, hypercholesterolemia (reviewed in Serrano and Andres, 2004), and increases in insulin resistance (Gardner et al., 2005). Moreover, in the only prospective study of telomere length and longevity, telomere length predicted earlier mortality, particularly from infections and CVD (Cawthon et al., 2003). It is unknown exactly how telomere maintenance might influence CVD processes. Telomerase increases telomere length. Although telomerase has not been examined in relation to CVD risk in humans, telomerase insufficiency leads to bone marrow failure (Marrone et al., 2005), while in animal studies and in vitro, telomerase insufficiency has been linked to cardiovascular pathobiology (Yang et al., 1999; Oh, 2003). These findings raise the possibility that telomerase may play an important role in early development of CVD in humans.

Given the inter-relationships between stress, telomere length and CVD risk factors, in the present study we also examined the relationship between cellular aging and CVD risk factors. We tested whether telomere length and telomerase were related to early development of the top CVD risk factors, in a sample of healthy young women (i.e. those without frank disease). Further, many CVD risk factors tend to be correlated, representing underlying insulin resistance, labeled as the Metabolic or Insulin Resistance Syndrome (Reaven, 1988). The Metabolic Syndrome tends to accrue with aging and is a well-established precursor to chronic diseases, most notably CVD (Lakka et al., 2002). Therefore, we examined whether cell aging was linked to this underlying syndrome, in addition to individual risk factors. We specifically tested the hypothesis that women with greater levels of cellular aging (i.e. shorter PBMC telomere length and lower telomerase activity) would have greater stress arousal and CVD risk factors, including level of Metabolic Syndrome.

2. Materials and methods

2.1. Study overview

Sixty-two healthy women (all mothers), aged 20–50, were assessed on mood, life stress, acute mental

stress reactivity in response to a standardized laboratory stressor, and health parameters, including a blood draw. Within 1 week after the laboratory assessment, participants completed questionnaires assessing stress (Perceived Stress Scale) (Cohen and Williamson, 1988), tendency to experience negative mood (Positive and Negative Assessment Scale) (Watson et al., 1988), years of education completed, and health behaviors, and completed an overnight collection of urine from home, to be assayed for stress hormones.

2.2. Participants and recruitment

Participants were initially recruited through their child's health care professional in clinics in Bay Area hospitals, or by public postings. To capture a range of stress levels, the sample included both mothers of chronically ill children and mothers of healthy children. All women had at least one biological child, and of these participants, 40 were mothers of children with a neurological or gastrointestinal disorder, or a pervasive development disorder (i.e. autism), and 22 had healthy children (for more detail, see (Epel et al., 2004).This is the same sample of study participants for which relationships between chronic psychological stress and telomere length, telomerase and oxidative stress were previously reported(Epel et al., 2004), (with the addition of four additional participants who had measures of telomerase and other parameters but not telomere length). Telomerase activity and telomere length in PBMCs were measured as described previously (Epel et al., 2004). The 62 participants were free of any acute or chronic health conditions by self report, with the exception of controlled hypertension ($n=2$) and controlled hypothyroidism ($n=1$). Participants did not take any other regular medications, except oral contraceptives ($n=19$, 30.6%), and antidepressants ($n=4$). All women were currently menstruating. Nine percent currently smoked ($n=5$), but refrained from smoking the morning of the blood draw. Participants did not consume more than seven alcoholic beverages per week, or exercise more than 1 h a day. They refrained from alcohol the day before the study and during the study day.

2.3. Laboratory session

The laboratory sessions took place at the Oakland Children's Hospital Pediatric Clinical Research Center. Efforts to minimize hormonal variability from menstrual cycle stages included timing sessions as follows: Sessions occurred during the first 7 days of the follicular phase of the menstrual cycle, when estrogen and progesterone are lowest. For those taking oral contraceptives, in the majority of cases, women participated during the 'placebo week' when hormone levels are also lowest.

Participants fasted for 10 h, starting from after dinner the previous night, and had a fasting blood draw around 0800 h on the test day. Additional assessments included smoking status, resting hemodynamics, adiposity, blood insulin, glucose and lipid profile. PBMCs were isolated immediately from the blood, and frozen at $-80\,^{\circ}C$. Telomerase activity levels and telomere length were measured from frozen cells ($>80\%$ viability; telomerase activity levels measured are shown corrected to arbitrary units per viable cell). All participants were then exposed to a standardized laboratory stressor, while autonomic nervous system reactivity (cardiac vagal control or high frequency heart rate variability) was measured repeatedly. Detailed procedures are described below.

2.4. Standardized stressor

Participants were exposed to an adapted version of the standardized Trier Social Stress Test (Kirschbaum et al., 1993). They first rested for 30 min before sitting baseline measurements of autonomic arousal were taken for 5 min. Participants were given task instructions, and then exposed to 15 min of stressful tasks, including a 5-min speech preparation period, deliverance of a 5-min videotaped speech with a live audience of two people, and a 5-min performance of serial subtraction of a prime number from a high number. They performed these tasks while seated to limit movement that could affect the psychophysiology recordings.

2.4.1. Cardiovascular and autonomic reactivity

Cardiovascular beat-to-beat data were assessed at baseline, during mental stress, including the anticipation/preparation period, speech stressor, and arithmetic stressor. An automatic arm cuff inflation blood pressure monitor (model SD-700S, IBS, Waltham, MA) was triggered 4 min after onset of each task, and systolic and diastolic values were recorded on a log sheet. A customized computer program written in MATLAB (Wilhelm et al., 1999) was used for computation of heart rate and high frequency HRV (also termed respiratory sinus arrhythmia, RSA, an index of cardiac vagal control). The beat-to-beat values of inter-beat intervals (IBI) were edited for outliers due to artifacts or ectopic myocardial activity, linearly interpolated, and

converted into instantaneous time series with a resolution of 4 Hz. IBI series were linearly detrended, and the power spectral densities derived for each experimental period using the Welch algorithm, which ensemble averages successive periodograms (a total of 9 240-point segments, overlapping by 50%, were Hanning windowed, zero padded to length 256, and subjected to Fast Fourier transform; estimates of power were adjusted to account for attenuation produced by the Hanning window). High-frequency HRV was computed by summation of power spectral density values in the 0.15–0.50 Hz frequency range, and resulting values were normalized using the natural logarithm.

2.5. Urine sampling

A subsample of 41 participants collected a 12 h nocturnal urine sample which was assayed for stress hormones (cortisol, epinephrine, and norepinephrine) and creatinine on one night in the week following the session, typically from 2000 to 0800 h (to assess resting arousal, minimizing contributions from daytime activity levels). Selection of the subsample was based simply on the timing of implementation of the urine collection (the participants run in the first few months did not collect urine). The urine was kept in a cooler with ample ice blocks for the duration of the collection, and picked up promptly the next morning. Aliquots were taken and frozen at −80°C until batch analysis (to minimize interassay variation). The range of collection times was from 11.75 to 12.75 h, with 90% of the sample collecting for a full 12 h. Urinary hormones were analyzed as ng/mg creatinine, to adjust for differences in durations of collections and in body mass. Participants who had collection volumes less than 300 ml ($n=4$) were determined to have incomplete collections and their urinary hormone values were excluded from analyses, as done in other studies with 12 h urine collections (e.g. Greendale et al., 1999). Values were normally distributed, and results were similar when using a natural log transformation (raw values are shown). Assays were done in the laboratory of Dr Robert Chatterton, Northwestern University. Urinary epinephrine and norepinephrine were assayed with kits provided by American Laboratory Products Company (Alpco), Windham, NH. The sensitivity is 0.33 and 1.33 ng/ml, respectively, for epinephrine and norepinephrine. For epinephrine the intra- and interassay coefficients of variation (CV's) were 7.2 and 15.4%, respectively. For norepinephrine, the intra- and interassay CVs were 10.2 and 15.0%, respectively. Cortisol was measured by a radioimmunoassay. The sensitivity of the method is 10 nml. Intra- and interassay CVs were 9.3 and 5.4%, respectively. Creatinine was measured spectrophotometrically by the Jaffe reaction at 490 nm after extraction of the samples with ethyl ether to remove interfering substances.

2.6. Cardiovascular disease risk and metabolic syndrome composite

Weight was measured using a balance beam scale, and height with a stadiometer. BMI was calculated as weight (kg)/height (m)2. Saggital diameter was measured with a meter stick with a leveler on the top, as the widest horizontal diameter from the lower lumbar to the belly, a measure which is highly correlated with visceral fat from CT scans (Kvist et al., 1988).

Lipid levels were analyzed in triplicate in the Core Laboratory of the GCRC at the Medical College of Wisconsin (RR00058). Fasting plasma triglyceride and cholesterol levels were determined spectrophotometrically using kits from Stanbio Laboratory, Inc. (San Antonio, TX), and Roche Diagnostics (Indianapolis, IN), respectively. HDL-cholesterol was determined after phosphotungstic acid/MgCl2 precipitation (Sigma Diagnostics, St Louis, MO). The respective intraassay and interassay coefficients of variation for the lipid analyses were 4.7 and 5.3% for triglyceride, 5.5 and 6.7% for cholesterol, and 5.7 and 6.1% for HDL-cholesterol. LDL cholesterol level was derived with the formula Total Cholesterol−(0.20*Triglyceride+HDL). Insulin and glucose were measured in the Cornell GCRC, using standard methods with commercial kits.

The 'National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII)' definition for Metabolic Syndrome includes scoring in the elevated range on at least three of the following symptoms: abdominal obesity, triglycerides, low HDL, high blood pressure, and high fasting glucose (NCEP, 1999). To examine the subclinical level of cumulative risk factors in a healthy sample who would not meet threshold for full blown Metabolic Syndrome, we examined the level of Metabolic Syndrome by creating a continuous measure of how high one is on each of these five factors. Each factor, waist circumference, HDL cholesterol, triglycerides, systolic blood pressure, and glucose levels, was standardized, and a mean was taken. Higher mean z scores represent a greater extent of Metabolic Syndrome.

3. Statistical analyses

Our primary goal was to test whether women with differing levels of cellular aging differed in stress arousal and CVD risk factors. Participants were categorized into high and low telomerase activity groups, and high and low telomere length groups, based on mean splits. *T*-tests, with 1-tailed significance tests for a priori hypotheses, were performed to test for any differences between these cell aging groups. To follow up these analyses, we tested whether findings were independent of the two major confounding factors, age and BMI, by performing analyses of covariance (ANCOVAs) adjusting for these factors with these covariates. There were no significant differences in telomerase activity or telomere length overall between the caregiving vs. control groups, and thus these women were examined as one group. All variables were examined for normal distributions, and transformed when appropriate (i.e. telomerase was slightly positively skewed, and thus log transformed).

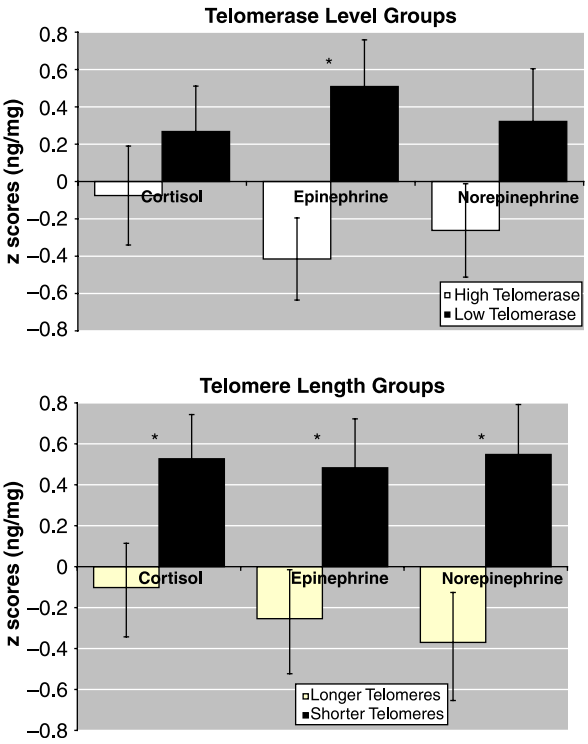

Figure 1 Nocturnal urinary stress hormones (ng/ml creatinine, standardized values), adjusted for BMI and age. (A) Low and high telomerase activity groups. (B) Short and long telomere length groups. *$=p<.05$, levels are higher compared to levels of the same hormone in the other (high/long) group.

4. Results

4.1. Stress arousal

We found that lower telomerase levels were related to neuroendocrine and psychosocial data indicative of greater life stress. ANCOVAs showed that the women with low telomerase had significantly higher excretion of nocturnal epinephrine ($p<.01$), and marginally higher norepinephrine ($p<.08$) but similar levels of cortisol (See Fig. 1 for standardized values, adjusted for BMI and age). The low telomerase group was not significantly different in negative affect, although when examined as a continuous variable, telomerase (log transformed) was correlated with trait negative mood (using the PANAS) ($r=-.31$, $p<.02$), and as reported elsewhere, with perceived stress and chronicity of stress (Epel et al., 2004). Low telomerase was also associated with fewer years of education (Table 1), which is itself a risk factor for CVD, in part through conferring greater exposure to chronic stress (Wamala et al., 1999).

To examine autonomic reactivity to stress (heart rate, blood pressure, and HRV), repeated measure ANCOVAs were performed, controlling for baseline values of each dependent variable, BMI, and age. Groups were similar in blood pressure and heart rate reactivity. However, the low telomerase group responded to stress with greater decreases in HRV, compared to the high telomerase group (Fig. 2). Average HRV during stress controlling for baseline HRV, age, and BMI was lower in the low telomerase group: $F(1,40)=3.85$, $p<.028$).

Telomere length, unlike telomerase, was not related to negative mood, education, or HRV. Shorter telomere length was, however, significantly related to higher levels of all three stress hormones measured (cortisol, epinephrine, and norepinephrine), after controlling for BMI and age (p's$<$ .05, see Fig. 1 for standardized levels).

4.2. Cardiovascular disease risk

As shown in Table 1, *t*-tests reveal that although age was similar between the high and low telomerase groups, the low telomerase group had significantly greater BMI and abdominal adiposity (saggital diameter), and higher resting heart rate, systolic blood pressure, fasting glucose, LDL and total cholesterol, total/HDL cholesterol ratio, and a greater tendency toward lower resting HRV. They were also more likely to be smokers; although there were only five current smokers, all were in the low

Table 1 Raw means and standard errors of age, education, and CVD risk factors in high and low telomerase groups.

	High telomerase M (SE) $N=29$	Low telomerase M (SE) $N=33$	t-test values, significance (1-tailed)	Significance after adjusting for BMI, age (ANCOVA)
Age (years)	39.4 (1.0)	37.2 (1.2)	-1.41, $p<.14$	N/A
Education (years)	15.50 (0.35)	14.5 (0.33)	-2.02, $p<.025$	N/A
Adiposity				
Body mass index (BMI; kg/m^2)	23.99 (0.68)	27.44 (1.12)	2.55, $p<.007$	N/A
Sagittal diameter (cm)	19.22 (0.88)	22.09 (1.01)	2.12, $p<.019$	N/A
Resting cardiovascular activity[a]				
Systolic blood pressure (mm Hg)	105.04 (1.79)	109.83 (1.60)	2.00, $p<.025$	$p<.150$
Diastolic blood pressure (mm Hg)	64.96 (1.85)	68.10 (1.53)	1.36, $p<.09$	$p<.330$
Heart rate (bpm)	76.56 (1.90)	82.58 (1.85)	2.27, $p<.015$	$p<.041$
High frequency heart rate variability (ms^2)	6.40 (0.11)	6.15 (0.09)	-1.67, $p<.051$	$p<.054$
Cholesterol levels				
HDL (mg/dl)	50.11 (1.96)	45.93 (2.55)	-1.30, $p<.095$	$p<.300$
LDL (mg/dl)	119.11 (6.39)	142.97 (9.46)	2.37, $p<.011$	$p<.012$
Total (mg/dl)	184.14 (7.0)	203.31 (8.56)	1.72, $p<.045$	$p<.016$
Total/HDL ratio	3.77 (0.17)	4.83 (0.34)	2.73, $p<.005$	$p<.017$
Fasting insulin (uIU/mL)	12.33 (1.56)	11.81 (1.29)	-0.26, $p<.395$	$p<.463$
Fasting glucose (mg/dL)	84.33 (0.94)	88.69 (1.40)	2.55, $p<.007$	$p<.008$
Metabolic syndrome (mean z-score)	$-.21$ (.09)	.13 (.09)	2.65, $p<.006$	$p<.09$

N/A = not applicable.
[a] Cardiovascular results are after excluding the two subjects on antihypertensive medications.

telomerase group (Chi square of telomerase group by smoking group: $X^2(1)=4.77$, $p<.015$), falling within the bottom 15% of all telomerase values (Fig. 3).

Because obesity both may be linked to low telomerase (via unknown pathways) and also could generate the other risk factors, BMI (and age) were controlled for using ANCOVAs. As shown in Table 1, differences between the low and high telomerase groups in systolic blood pressure and Metabolic Syndrome composite became non-significant and thus were partially mediated via BMI (age was not different between groups, only BMI). In contrast, heart rate, lipids, and fasting glucose differences remained significant and hence were independent of BMI. We performed the same analyses with telomere length groups but did not observe associations between telomere length and CVD risk.

5. Discussion

Chronic psychological stress has been associated both with signs of accelerated leukocyte aging in our current sample (Epel et al., 2004) and with CVD in large samples (e.g. Yusef et al., 2004), but the intermediate mediators of these relationships have not been characterized. The present study represents a first test of whether leukocyte aging might potentially underlie the relationship between stress and CVD risk factors. We found that telomere shortness and low telomerase are both associated with stress arousal (nocturnal stress hormones), and low telomerase is associated with several risk factors for CVD, as discussed below. It is likely that stress hormones play an important role in the pathway to cellular aging. These findings support a tentative role for telomerase in the pathway between stress and disease. Further research is needed to determine the mechanisms of how the telomerase/telomere maintenance system might play a causal role in CVD, whether stress is an early precursor, and how genetic predispositions might promote vulnerability to these related factors (stress arousal, cellular aging).

5.1. Stress arousal

This is the first clinical study to our knowledge to document relationships between stress hormones

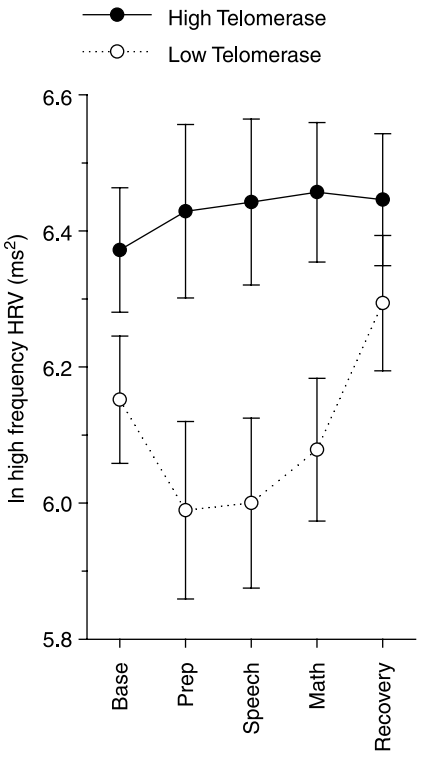

Figure 2 High frequency HRV by telomerase groups. Autonomic nervous system function at baseline and during stressful tasks for low and high telomerase groups. As shown above, across all timepoints, a quadratic model best fits the data, $F(1,41)=7.81$, $p<.004$, showing lower baseline but also a decrease in HRV during the stressor in the low telomerase group only. Two subjects with controlled hypertension were excluded (all values adjusted for age and BMI). Base, baseline; Prep, preparation/anticipation of speech; Speech, speech task; Math, math task; Recovery, rest period.

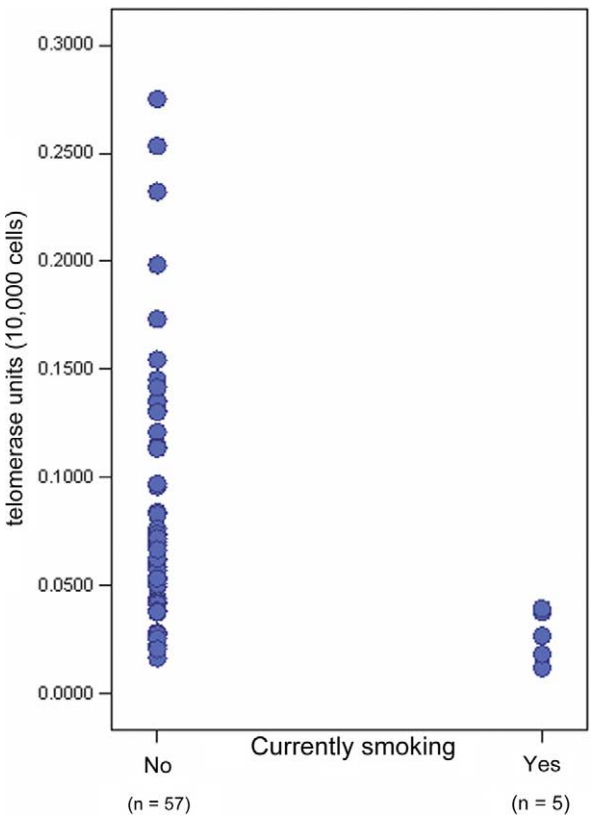

Figure 3 Telomerase levels among smokers and non-smokers. The five smokers had significantly lower average telomerase than the non-smokers, t-test$=3.26$, $p<.002$.

with any markers of cellular aging in people (or in vitro). Telomerase status and telomere length are well established indices of cellular health and longevity (Blackburn, 2000). Here we show that low telomerase and short telomeres are both related to elevations in nocturnal excretion of epinephrine (and short telomeres to elevations in norepinephrine and cortisol). These results are consistent with our earlier findings that greater perception and duration of psychological stress are related to shorter telomere length and decreased telomerase activity (Epel et al., 2004).

While both low telomerase and short bulk telomere length were related to higher stress hormones, mainly epinephrine, the remaining tests showed significant associations for telomerase, but not for telomere length. There is a consistent pattern linking telomerase activity to additional indices of stress and stress arousal (negative mood, lower education, autonomic arousal), as well as CVD risk factors, and a lack of similar associations for telomere length. In terms of autonomic arousal, women with lower telomerase activity levels tended to have higher resting heart rate and somewhat lower resting cardiac vagal control and greater vagal suppression (decreases in high frequency HRV) in response to a standardized laboratory stressor, all findings independent of age and BMI. Chronically suppressed vagal control may indicate impaired allostasis and restorative processes, and increased risk for CVD, whereas vagal suppression in response to acute stressors is a physiological indicator of psychological stress vulnerability, at least in infants (Porges, 1995; Carney et al., 2001). Since parasympathetic nervous system activity controls restorative processes, it may be particularly important in promoting longevity.

The direction of causality for the present findings is unknown. Stress and associated stress arousal may contribute to decreased telomerase activity, since the opposite direction of causality seems less probable. In vitro, catecholamines can reduce

oxidative stress in certain neurons (Troadec et al., 2001) but, more relevant to this study, peripheral infusions of catecholamines increase oxidative stress in rats (Aizawa et al., 2002).

Glucocorticoids are linked to neuronal cell death (Sapolsky, 1996), in part through greater oxidative stress damage, by increasing intra-neuronal levels of glutamate and calcium and impairing antioxidant defenses (McIntosh and Sapolsky, 1996). Glucocorticoid administration increases plasma markers of oxidative stress in mammals (Orzechowski et al., 2002; Lin et al., 2004). It is unclear how these stress hormones might affect telomerase and telomeres in human PBMCs. The relationships between stress hormones and telomerase activity in vitro are currently under investigation (J.L. and E.H.B., unpublished results).

5.2. Telomerase and CVD risk

Low telomerase activity was consistently associated with proxy markers for the top six major risk factors for CVD (Yusef et al., 2004): smoking, abdominal adiposity, lipids, fasting glucose, blood pressure, and psychosocial stress. Telomerase was also associated with a Metabolic Syndrome cluster, although this relationship was diminished after controlling for BMI, and became of borderline significance.

Low telomerase, through an unidentified pathway, could plausibly lead to increases in the Metabolic Syndrome, the Metabolic Syndrome factors could lead to decreased telomerase, or, most likely, bidirectional pathways exist, as described below. Finally, both may be independently related to a third factor.

5.2.1. Lower telomerase contributing to CVD processes

Low telomerase and its predicted eventual outcome, shortened bulk telomeres, potentially prevent the long-term tissue replenishment necessary for endothelial and smooth muscle cardiovascular cells, contributing to CVD risk. Low telomerase in leukocytes may reflect low telomerase in cardiovascular cells as well, although this has not been explored. Prior evidence, although limited to animal or in vitro studies, suggests that low telomerase is associated with cardiovascular cell viability. In cardiomyocytes, overexpression of the protein component of telomerase (hTERT) inhibits apoptosis in culture (Oh, 2003). In vascular smooth muscle cells, telomerase is related to cell division, whereas telomerase inhibition is related to reduced cell growth (Minamino and Kourembanas, 2001). Telomerase regulates mitosis and longevity of endothelial and progenitor endothelial cells (Yang et al., 1999; Murasawa et al., 2002). Telomerase could also play a significant role in early disease processes independently of telomere length. Telomerase allows cells to grow healthily, even those with short average telomere length (Kim et al., 2003). Telomerase is protective of telomeric DNA even for long telomeres, preventing them from occasional catastrophic shortening that can cause cell growth arrest or genomic instability (Chan and Blackburn, 2003). Telomerase promotes stem cell proliferation even in the absence of any telomerase enzymatic functional capability (Sarin et al., in press).

5.2.2. Telomerase reflecting CVD processes

Telomerase could also be a marker, rather than causal factor, of CVD risk. Low PBMC telomerase activity could reflect overall organismal low telomerase activity, including low telomerase in cardiovascular tissue. Thus, low telomerase may simply be an early cellular indicator of disease processes. Early factors, such as prenatal stress and nutrition, can pre-program aspects of physiology (obesity, insulin resistance, hypothalamic pituitary adrenal responsiveness), causing later Metabolic Syndrome (McMillen and Robinson, 2005). Such early developmental factors might also affect trajectories of cellular aging as well, by limiting the total replenishment capabilities of cells. Such limitation is seen in patients haploinsufficient for telomerase, who show premature bone marrow stem cell exhaustion (Vulliamy et al., 2001; Marrone et al., 2005).

5.2.3. CVD processes decreasing telomerase

The Metabolic Syndrome, through obesity or insulin resistance, may accelerate cell aging processes by causing, for example, greater exposure to hyperglycemia (Blazer et al., 2002), insulin (Gardner et al., 2005) and oxidative stress associated with insulin resistance (Davi et al., 2005). Given the relations we report here between one-time measurement of leukocyte telomerase activity level and stable factors such as one's body mass and cholesterol, it appears as if snap-shot telomerase levels reflect relatively stable levels over time. This may be especially true in the current study given that no participants were ill, and that the collection conditions were highly standardized, in terms of time of day, fasting state, and stage of menstrual cycle. Prolonged relative elevations of blood glucose levels, even within the normal non-diabetic range

(range of 71–107 in this sample), might down-regulate telomerase activity or expression, and hence promote cell senescence, regardless of telomere length (Chan and Blackburn, 2003; Kim et al., 2003). Thus low telomerase may be part of the cellular events that promote both early disease as well as explain how later these risk factors lead to frank CVD. Prospective cohort studies are needed to indicate whether low leukocyte telomerase activity should be included among markers for CVD risk.

5.2.4. Telomere length and CVD risk: null findings

We found no relationship between mean bulk telomere length (long vs. short telomere groups, or by using correlations) and CVD risk. This might be because of the relatively young healthy sample analyzed in our study, or because telomere length is associated with CVD through non-traditional pathways (i.e. independent of common CVD risk factors). In one study, short telomere length conferred a three-fold risk of premature myocardial infarction, but this relationship was independent of established risk factors (blood pressure, smoking, lipids, and inflammation) (Brouilette et al., 2003). Lastly, the failure to discern a relationship could be due to methodological limitations for telomere length measurement. The critical shortening of even one telomere may trigger premature senescence of a cell. Hence, average bulk telomere length in a relatively young and healthy sample may not be sensitive enough to detect early changes, such as single strand DNA breaks in the telomere or rare catastrophic shortening events that occur when telomerase is deficient (Chan and Blackburn, 2003), which would affect a cell's longevity. In addition, most risk factors in this sample, such as blood pressure and fasting glucose, were in the subclinical range, as might be expected given the young age and health of the sample. Epidemiological studies are needed to determine whether our null findings are due to the lack of clinical morbidity or relatively young age of the current sample. Since telomere length was not correlated with CVD risk factors over the whole sample, telomerase may be a separate indicator from telomere length, or more likely, an earlier indicator than telomere length; of cell aging.

Eventually, with increasing age, if telomerase activity indeed reflects stable levels, telomere length may become shortened in the low telomerase activity group. Supporting this hypothesis, telomerase and telomere length were marginally related ($r = +.23$, $p < .06$) in the 30–50 year old participants ($n = 47$), but they were not related among those under 30 years old ($r = -.15$, ns) ($n = 9$). If it is true that telomerase is related to shorter telomeres mainly with advancing age, then over time, we hypothesize that insufficient telomerase will lead to shorter telomeres, reduced replenishment of tissues, decreased efficiency of endothelial and vascular functioning, and frank disease.

6. Summary

Our results show, for the first time, that lower levels of PBMC telomerase activity are associated with increased excretion of stress hormones and well-established physiological and behavioral risk factors for CVD in a healthy sample. While in vitro studies have suggested a crucial function of telomerase in the health of cardiovascular-related cells, this is the first human study to show links between telomerase and major CVD risk factors. Interestingly, telomere length was not associated with CVD risk factors in our relatively young cohort. Telomere length is driven upward by telomerase activity. Our finding that low telomerase is associated with the major risk factors for CVD, prior to any onset of clinically manifested CVD or telomere shortening, suggest that the telomere shortness previously reported in CVD (Serrano and Andres, 2004) may be a direct result of previously lower telomerase activity. Thus, in younger individuals, such as the 20–50 year-old cohort we studied, telomerase activity may be an earlier marker of preclinical disease risk than telomere length, possibly acting as a harbinger of subsequent telomere shortening that manifests later in life.

These findings offer an important clue into mechanisms—both of how telomere shortening is linked to stress and CVD, and possibly of how traditional risk factors promote CVD. It may be highly clinically significant that obesity and smoking are linked to decreased telomerase. These common risk factors could impair telomerase enzyme function or interfere with its expression. Perhaps most significantly, our findings suggest early precursors of low telomerase activity—neuroendocrine and autonomic stress arousal. These findings also open up the possibility that telomerase may be a useful marker for monitoring health, such as a barometer for examining effects of lifestyle and psychosocial interventions on cardiovascular health. There is yet much to be learned about human health from

inquiring about state of mind, stress homeostasis, and cellular events.

Acknowledgements

This study was supported by the John D. and Catherine T. MacArthur Foundation Network on Socioeconomic Status and Health; the Hellman Family Fund; and the Oakland Children's Hospital Pediatric Clinical Research Center (under the auspices of National Institute of Mental Health Grant M01-RR01271), National Institutes of Mental Health Award K08 MH64110-01A1, and a National Alliance for Research on Schizophrenia and Depression Young Investigator's Award (to E.S.E.); the Steven and Michele Kirsch Foundation and National Institutes of Health Grant GM26259 (to E.H.B.), and grant 105311-105850 by the Swiss National Science Foundation (to F.H.W).

References

Aizawa, T., Ishizaka, N., et al., 2002. Angiotensin II and catecholamines increase plasma levels of 8-epi-prostaglandin F(2alpha) with different pressor dependencies in rats. Hypertension 39 (1), 149–154.

Blackburn, E.H., 2000. Telomere states and cell fates. Nature 408 (6808), 53–56.

Blazer, S., Khankin, E., et al., 2002. High glucose-induced replicative senescence: point of no return and effect of telomerase. Biochem. Biophys. Res. Commun. 296 (1), 93–101.

Brouilette, S., Singh, R.K., et al., 2003. White cell telomere length and risk of premature myocardial infarction. Arterioscler. Thromb. Vasc. Biol. 23 (5), 842–846.

Carney, R.M., Blumenthal, J.A., et al., 2001. Depression, heart rate variability, and acute myocardial infarction. Circulation 104 (17), 2024–2028.

Cawthon, R., Smith, K., et al., 2003. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361, 393–395.

Chan, S.W., Blackburn, E.H., 2003. Telomerase and ATM/Tel1p protect telomeres from nonhomologous end joining. Mol. Cell 11 (5), 1379–1387.

Cohen, S., 1988. Perceived Stress in a Probability Sample of the United States. The Social Psychology of Health: Claremont Symposium on Applied Social Psychology. S.S.S. Oskamp Sage, Newbury Park, CA.

Davi, G., Falco, A., et al., 2005. Lipid peroxidation in diabetes mellitus. Antioxid. Redox Signal 7 (1-2), 256–268.

Epel, E., Blackburn, E., et al., 2004. Accelerated telomere shortening in response to exposure to life stress. PNAS 101, 17312–17315.

Friedman, B.H., Thayer, J.F., 1998. Anxiety and autonomic flexibility: a cardiovascular approach. Biol. Psychol. 49 (3), 303–323.

Gardner, J.P., Li, S., et al., 2005. Rise in insulin resistance is associated with escalated telomere attrition. Circulation 111 (17), 2171–2177.

Greendale, G., Unger, J., et al., 1999. The relation between cortisol excretion and fractures in healthy older people: results from the MacArthur studies. J. Am. Geriatr. Soc. 47, 799–803.

Kim, M., Xu, L., et al., 2003. Catalytically active human telomerase mutants with allelespecific biological properties. Exp. Cell Res. 288 (2), 277–287.

Kirschbaum, C., Pirke, K., et al., 1993. The Trier Social Stress Test–A tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.

Kvist, J., Chowdhur, B., et al., 1988. Predictive equations of total and visceral adipose tissue volumes derived from measurements with computed tomograpy in adult men and women. Am. J. Clin. Nutr. 48, 1351–1361.

Lakka, H.M., Laaksonen, D.E., et al., 2002. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. Jama 288 (21), 2709–2716.

Lin, H., Lin, H., Decuypere, E., et al., 2004. Oxidative stress induced by corticosterone administration in broiler chickens (Gallus gallus domesticus) 1. Chronic exposure. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 139 (4), 737–744.

Marrone, A., Walne, A., et al., 2005. Dyskeratosis congenita: telomerase, telomeres and anticipation. Curr. Opin. Genet. Dev. 15 (3), 249–257.

McEwen, B., 1998. Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171–179.

McIntosh, L.J., Sapolsky, R.M., 1996. Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin-induced toxicity in neuronal culture. Exp. Neurol. 141 (2), 201–206.

McMillen, I.C., Robinson, J.S., 2005. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol. Rev. 85 (2), 571–633.

Minamino, T., Kourembanas, S., 2001. Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. Circ. Res. 89 (3), 237–243.

Murasawa, S., Llevadot, J., et al., 2002. Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. Circulation 106 (9), 1133–1139.

NCEP, 2001. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 285 (19), 2486–2497.

Oh, H., et al., 2003. Telomere attrition and Chk2 activation in human heart failure. Proc. Natl Acad. Sci. USA 100, 5378–5383.

Orzechowski, A., Ostaszewski, P., et al., 2002. Rats with a glucocorticoid-induced catabolic state show symptoms of oxidative stress and spleen atrophy: the effects of age and recovery. J. Vet. Med. A Physiol. Pathol. Clin. Med. 49 (5), 256–263.

Porges, S., 1995. Cardiac vagal tone: a physiological index of stress. Neurosci. Biobehav. Rev. 19, 225–233.

Reaven, G.M., 1988. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37 (12), 1595–1607.

Sapolsky, R.M., 1996. Why stress is bad for your brain. Science, 749–750. [August 9].

Sapolsky, R.M., 2004. Organismal stress and telomeric aging: an unexpected connection. Proc. Natl Acad. Sci. USA 101 (50), 17323–17324.

Serrano, A.L., Andres, V., 2004. Telomeres and cardiovascular disease: does size matter? Circ. Res. 94 (5), 575–584.

Troadec, J.D., Marien, M., et al., 2001. Noradrenaline provides long-term protection to dopaminergic neurons by reducing oxidative stress. J. Neurochem. 79 (1), 200–210.

Vulliamy, T., Marrone, A., et al., 2001. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature 413 (6854), 432–435.

Wamala, S.P., Mittleman, M.A., et al., 1999. Potential explanations for the educational gradient in coronary heart disease: a population-based case-control study of Swedish women. Am. J. Public Health 89 (3), 315–321.

Watson, D., Clark, L., et al., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. J. Pers. Social Psychol. 54, 1063–1070.

Wilhelm, F.H., Grossman, P., et al., 1999. Analysis of cardiovascular regulation. Biomed. Sci. Instrum. 35, 135–140.

Yang, J., Chang, E., et al., 1999. Human endothelial cell life extension by telomerase expression. J. Biol. Chem. 274 (37), 26141–26148.

Yusef, S., Hawken, S., et al., 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 364.