



Increased telomerase activity and comprehensive lifestyle changes: a pilot study

Dean Ornish, Jue Lin*, Jennifer Daubenmier*, Gerdi Weidner, Elissa Epel, Colleen Kemp, Mark Jesus M Magbanua, Ruth Marlin, Loren Yglecias, Peter R Carroll, Elizabeth H Blackburn

Summary

Background Telomeres are protective DNA–protein complexes at the end of linear chromosomes that promote chromosomal stability. Telomere shortness in human beings is emerging as a prognostic marker of disease risk, progression, and premature mortality in many types of cancer, including breast, prostate, colorectal, bladder, head and neck, lung, and renal cell. Telomere shortening is counteracted by the cellular enzyme telomerase. Lifestyle factors known to promote cancer and cardiovascular disease might also adversely affect telomerase function. However, previous studies have not addressed whether improvements in nutrition and lifestyle are associated with increases in telomerase activity. We aimed to assess whether 3 months of intensive lifestyle changes increased telomerase activity in peripheral blood mononuclear cells (PBMC).

Methods 30 men with biopsy-diagnosed low-risk prostate cancer were asked to make comprehensive lifestyle changes. The primary endpoint was telomerase enzymatic activity per viable cell, measured at baseline and after 3 months. 24 patients had sufficient PBMCs needed for longitudinal analysis. This study is registered on the ClinicalTrials.gov website, number NCT00739791.

Findings PBMC telomerase activity expressed as natural logarithms increased from 2.00 (SD 0.44) to 2.22 (SD 0.49; $p=0.031$). Raw values of telomerase increased from 8.05 (SD 3.50) standard arbitrary units to 10.38 (SD 6.01) standard arbitrary units. The increases in telomerase activity were significantly associated with decreases in low-density lipoprotein (LDL) cholesterol ($r=-0.36$, $p=0.041$) and decreases in psychological distress ($r=-0.35$, $p=0.047$).

Interpretation Comprehensive lifestyle changes significantly increase telomerase activity and consequently telomere maintenance capacity in human immune-system cells. Given this finding and the pilot nature of this study, we report these increases in telomerase activity as a significant association rather than inferring causation. Larger randomised controlled trials are warranted to confirm the findings of this study.

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Introduction

Telomeres are protective DNA–protein complexes at the end of linear chromosomes that promote chromosomal stability. Telomere maintenance is required for the complete replication of DNA, protecting chromosomes from nuclease degradation, from end-to-end fusion, and from cellular senescence.¹ Telomere length and rate of telomere shortening are indicators of mitotic cell age, because telomeres shorten during normal cell divisions. Telomere shortening is counteracted by the cellular enzyme telomerase.

Telomere shortness in humans is emerging as a prognostic marker of disease risk, progression, and premature mortality. The aspect of cellular ageing that is conferred by diminished telomere maintenance seems to be an important precursor to the development of many types of cancer.^{2,3} Shortened telomeres predict poor

clinical outcomes, including increased risk of metastasis in patients with breast cancer,⁴ increased risk of bladder, head and neck, lung, and renal-cell cancers,⁵ worse progression and prognosis of patients with colorectal cancer,⁶ prostate-cancer recurrence in patients undergoing radical prostatectomy,⁷ and decreased survival in patients with coronary heart disease and infectious disease.⁸

Although telomere length predicts clinical outcomes and mortality, cells with shortened telomeres can remain genetically stable if the telomere maintenance system, which includes telomerase, is fully functioning.⁹ Telomerase adds telomeric repeat sequences to the chromosomal DNA ends, preserving not only telomere length, but also healthy cell function and long-term immune function.¹⁰

Telomerase is expressed at low concentrations in peripheral-blood mononuclear cells (PBMCs).¹¹ Until

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*These authors contributed
equally

Department of Medicine

(Prof D Ornish MD,
J Daubenmier PhD),

Department of Biochemistry
and Biophysics (J Lin PhD,
Prof E H Blackburn PhD),

Department of Psychiatry

(E Epel PhD), Department of
Urology, The Helen Diller

Family Comprehensive Cancer
Center, School of Medicine
(M J M Magbanua PhD,

Prof P R Carroll MD), University
of California, San Francisco, CA,
USA; and Preventive Medicine

Research Institute, Sausalito,
CA, USA (Prof D Ornish,

G Weidner PhD, C Kemp MSN,
R Marlin MD, L Yglecias BA)

Correspondence to:

Prof Dean Ornish, Preventive
Medicine Research Institute,
Sausalito, CA 94965, USA
dean.ornish@pmri.org

now, few studies have studied telomerase activity in these cells because of the high detection threshold. In previous studies, we adapted the standard telomerase enzymatic activity assay¹² to quantify the low activity of telomerase in normal unstimulated human PBMCs.¹³ In these studies, and in the current study, telomerase activity was measured as telomerase enzymatic activity per viable cell in PBMC samples.

Decreased telomere maintenance capacity has also been linked to increased risk of cardiovascular disease, independent of chronological age. In a study of healthy women, we reported that telomerase activity in PBMCs, although not telomere length, was inversely associated with six major cardiovascular disease risk factors.¹⁴ These findings suggested that telomerase activity might be a more direct and potentially earlier predictor than telomere length of long-term cellular viability or genomic stability (or both) and disease processes.

Lifestyle factors known to promote cancer and cardiovascular disease might also adversely affect telomerase and, eventually, telomere length. For example, increases in obesity and insulin resistance over 10–13 years are associated with decreases in telomere length.¹⁵ However, previous studies have not addressed whether improvements in nutrition and lifestyle are associated with increased telomerase activity.

In previous randomised controlled trials, we reported that interventional comprehensive lifestyle changes (improved nutrition, moderate exercise, stress management techniques, and increased social support) beneficially affected the progression of both coronary heart disease^{16,17} and early-stage prostate cancer.¹⁸ In the Gene Expression Modulation by Intervention with Nutrition and Lifestyle (GEMINAL) study,¹⁹ a prospective single-arm pilot clinical intervention study in men with indolent low-risk prostate cancers, we reported that such comprehensive lifestyle changes were associated with modulations of gene expression profiles in healthy prostate tissue. Two-class paired analysis of global gene expression by use of significance analysis of microarrays detected 48 up-regulated and 453 downregulated transcripts after 3 months of lifestyle intervention. Pathway analysis identified substantial beneficial modulation of biological processes that have crucial roles in tumorigenesis, including protein metabolism and modification, intracellular protein traffic, and protein phosphorylation (all $p < 0.05$). Because these patients chose active surveillance for reasons unrelated to both the GEMINAL study and the current study, it was possible to assess the association between changes in lifestyle and changes in telomerase without confounding interventions such as radical prostatectomy, radiation, or chemotherapy.

In the current study, we aimed to assess the hypothesis that in the same cohort of patients in the GEMINAL study, this behavioural intervention might be associated with increased PBMC telomerase activity after 3 months.

Methods

Patients

Men with low-risk prostate cancer willing to make comprehensive lifestyle changes gave written informed consent under a protocol approved by the University of California San Francisco Institutional Review Board. These patients chose active surveillance rather than conventional treatments for prostate cancer for reasons unrelated to this study (eg, advice from their physician, concerns about side-effects of treatment). Eligibility criteria included: pathology-confirmed prostate cancer, prostate-specific antigen (PSA) concentration 10 ng/mL or lower (or <15 ng/mL if there was documented benign prostatic hyperplasia or prostatitis) at the time of screening, Gleason score of 6 or lower, stage T1 or T2a tumour (according to the Tumour, Nodes, Metastases staging system), 33% or less of biopsy cores positive for the presence of adenocarcinoma, and 50% or less of the length of a tumour-core positive for the presence of adenocarcinoma.

A detailed description of patient recruitment has been reported elsewhere.¹⁹ Standard clinical methods were used for waist circumference, weight, height, blood pressure, serum lipids, C-reactive protein, and PSA.

Lifestyle intervention

A 3-month comprehensive lifestyle modification was comprised of a 3-day intensive residential retreat, followed by an outpatient phase where participants met with staff for 4 hours per week and had weekly telephone contact with a study nurse. Lifestyle modifications included a low-fat (10% of calories from fat), whole foods, plant-based diet high in fruits, vegetables, unrefined grains, legumes, and low in refined carbohydrates; moderate aerobic exercise (walking 30 min/day, 6 days/week); stress management (gentle yoga-based stretching, breathing, meditation, imagery, and progressive relaxation techniques 60 min/day, 6 days/week), and a 1-h group support session once per week.²⁰ The diet was supplemented with soy (one daily serving of tofu plus 58 g of a fortified soy protein powdered beverage), fish oil (3 g daily), vitamin E (100 IU daily), selenium (200 µg daily), and vitamin C (2 g daily).^{21,22} Participants were provided with all of their food during the intervention period. A registered dietician, exercise physiologist, clinical psychologist, nurse, and stress management instructor were available for education and counselling. Adherence was assessed with self-reported questionnaires which were used to compute a mean adherence score.^{16–18} The decision to use a 3-month duration was arbitrary and was based on the resources available.

Measurement of telomerase activity

Changes in telomerase activity were measured in PBMC samples by comparing telomerase activity per viable cell in PBMC samples at baseline and at 3 months. PBMCs were separated from serum and red blood cells by

gradient density centrifugation by use of Ficoll-Paque (GE Healthcare, Piscataway, NJ, USA). PBMCs (buffy coats) were stored in a cryopreservation media composed of 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St Louis, MO, USA), and Roswell Park Memorial Institute 1640 (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Hyclone, Logan, UT, USA). All samples were stored at -80°C until further processing. Telomerase activity in PBMCs was assayed at the beginning and the end of the 3-month intervention study period (baseline and 3 months).^{13,14}

Telomerase activity was assayed by use of the TRAPeze kit (Chemicon, Upstate/CHEMICON, Temecula, CA, USA) with modifications and optimisations as follows: cryopreserved cells (1 mL) were thawed by incubating the vials at 37°C for 2 min and immediately transferred to 10 mL of cold Dulbecco's phosphate-buffered saline (DPBS; ie, PBS without Mg^{2+} and Ca^{2+} ; Invitrogen, Carlsbad, CA, USA). Cells were spun at 1500 rpm (486 g) at 10°C for 10 min in a Sorvall Legend RT tabletop centrifuge swing-out rotor (Thermo Fisher Scientific, Waltham, MA, USA) and supernatant was removed. Cells were washed in 10 mL of cold DPBS one more time and then resuspended in 1 mL of cold DPBS. Live cells were then counted with a haemocytometer (Bright-Line haemocytometer, Reichert, Buffalo, NY, USA) by use of Trypan blue (Invitrogen) exclusion criteria. Between 5×10^5 and 1×10^6 cells were pelleted and lysed with 1xCHAPS [3-(3-cholamidopropyl) dimethylammonio-1-propanesulfonate] buffer as directed by the manual for the TRAPeze kit. The thawing and extract preparation process took about 90 min and set-up of the telomerase activity assay reactions about 60 min. For each PBMC sample, an extract corresponding to 1000 cells/mL was made and two concentrations, corresponding to 5000 and 10000 cells, were assayed for each sample to ensure the assay was in the linear range. The reaction was done according to the manual for TRAPeze kit and run on a 10% polyacrylamide-8M urea sequencing gel (National Diagnostics, Atlanta, GA, USA). The gel was exposed to a phosphorimager plate overnight and scanned on STORM 860 (GE Healthcare). As positive control standards, 293T human tumour cells were used and telomerase activity was expressed as the equivalent of number of 293T cells. Telomerase activity was quantified by use of ImageQuant 5.2 software (GE Healthcare). Briefly, signals from the product ladders on the gels were added and normalised against the signal from internal control band for the same lane to get the product divided by the internal control value. For each sample, the product divided by the internal value was divided by the product divided by the internal control value of 20 293T cells and then multiplied by 20 to obtain the final telomerase activity unit, defined as 1 unit equals the amount of product from one 293T cell or from 10000 PBMCs. Inter-assay variability was established to be 7% (webtable 1). The baseline and 3-month samples were treated identically at all steps. For all telomerase

activity (TRAP) assays, each sample was labelled as being a baseline or 3-month sample of a given participant, but was blinded with respect to all other information about the participant.

In previous studies,^{13,14,23} measures of telomerase have been treated as a quantitative parameter without separating PBMCs into different cell types. We have done so in this study to enable us to compare our longitudinal study findings with previous cross-sectional study reports.

Baseline and 3-month samples for the same participant were assayed in the same batch and run on the same gel to eliminate any differences caused by reaction or procedural batch-to-batch variations. Additionally, the same reagent batch number of the TRAPeze telomerase detection kit was used for all samples to eliminate measurement shift caused by different reagent batch numbers. In addition to the 293T cancer-cell extract as the standard for telomerase activity quantification, we also included two control extracts from resting PBMCs in each run. Cell viability was established after thawing and telomerase activity was calculated on a per viable cell basis. The viability of the PBMC cell samples in this cohort fell within the normal range for samples we have previously used for telomerase assays. A paired sample *t* test showed no significant difference between the percentage of viable cells available for pre-intervention and post-intervention assays. Natural logarithm transformations were done for baseline and 3-month telomerase activity values to achieve normal distributions, as reported previously for PBMC telomerase,¹³ and these transformed values were used for statistical analysis.

Psychosocial measures

Psychological distress was assessed by use of the Impact of Event Scale, a well-validated measure of distress associated with a traumatic event.²⁴ Most of our patients learned they had prostate cancer several months before the beginning of this study, so their level of distress was at a chronic steady-state level. The mean duration from time of prostate-cancer diagnosis to entry into this study was 12.3 months, the median was 7.5 months (SD 9.3); the minimum was 2.5 months; maximum was 34.0 months. The Medical Outcomes Study Short Form, 36 item, Health Status Survey, version 2 (SF-36v2) was used to measure general health-related quality of life.²⁵

Adherence questionnaire

The lifestyle index, based on a formula validated in our previous research, measured overall adherence to intervention guidelines and was calculated as the mean percentage of adherence to guidelines for each lifestyle behaviour. Exercise and stress management adherence logs were completed by participants at baseline and after each week during the 3 months of intervention. Adherence to the exercise guidelines was ascertained by dividing the self-reported minutes of exercise each week by 180.

See Online for webtable 1

Adherence to the stress management guidelines was calculated by dividing the self-reported minutes of stress management each week by 420. The percentage of calories from fat and the daily intake of dietary cholesterol (mg) were measured by use of a validated semiquantitative food-frequency questionnaire administered at baseline and at 3 months. Adherence to the dietary guidelines of 10% of total calories from fat and 0 mg of dietary cholesterol was computed and averaged by use of the formula: $(1 - 1/40 [\% \text{fat} - 10] + [1 - 1/1000 (\text{mg cholesterol})]) / 2$, where 50% of calories from fat and 1000 mg of dietary cholesterol represented 0% adherence. A lifestyle index score of 1.0 suggested 100% adherence, and scores could exceed 1.0 for patients who completed more hours of exercise and stress management than required.

Statistical analyses

We used paired *t* tests (two-tailed) to compare baseline values to those obtained 3 months after the intervention began. As in our previous study, because values for telomerase activity and C-reactive protein were not normally distributed, these values were first converted by use of a natural logarithm transformation which yielded distributions that did not significantly deviate from normality.¹³ Paired *t* tests were then done.

The associations of changes in coronary heart disease risk factors and psychological parameters to changes in telomerase activity were assessed. The residuals of changes in telomerase activity after adjustment for

baseline telomerase activity were used because baseline adjustment increases statistical power and accounts for the possible effect of regression to the mean.²⁶ We postulated that participants who had the largest decreases in coronary heart disease risk factors and distress would have the greatest increases in telomerase activity. Thus, we did secondary analyses to test these a-priori hypotheses by use of one-tailed *t* tests. $p < 0.05$ was used as the level of significance.

Since we could not assume independent effects of these factors, we did a multiple regression analysis by use of the two predictors which showed some association with change in telomerase activity (changes in intrusive thoughts about prostate cancer and changes in low-density lipoprotein [LDL] cholesterol) and changes in telomerase as the predicted variable to ascertain whether the effects were independent or because of shared variance, controlling for telomerase activity per viable cell at baseline. This study is registered on the ClinicalTrials.gov website, number NCT00739791.

Role of the funding source

The sponsors approved the study design and provided funding. The sponsors had no role in the collection, analysis, or interpretation of the data, or in the writing of the report. All authors had access to the raw data; the sponsors did not. The corresponding author had full access to all of the data and had the final responsibility to submit for publication.

	Mean at baseline (SD)	Mean at 3 months (SD)	Mean change (SD)	95% CI of change	p*
Cardiovascular risk factors					
BMI (kg/m ²)	26.5 (3.6)	23.9 (3.0)	-2.6 (1.2)	-3.0 to -2.2	0.001
Systolic blood pressure (mm Hg)	129.5 (15.1)	120.3 (12.6)	-9.2 (12.3)	-13.8 to -4.6	0.001
Diastolic blood pressure (mm Hg)	68.2 (10.7)	62.8 (10.3)	-5.4 (8.1)	-8.4 to -2.3	0.002
LDL (mmol/L)	3.2 (0.7)	2.3 (0.7)	-0.9 (0.5)	-1.1 to -0.7	0.001
HDL (mmol/L)	1.3 (0.3)	1.0 (0.3)	-0.2 (0.2)	-0.3 to -0.1	0.001
Total cholesterol (mmol/L)	5.0 (0.9)	3.8 (0.8)	-1.2 (0.6)	-1.4 to -0.9	0.001
LDL/HDL ratio	2.7 (1.0)	2.3 (0.8)	-0.4 (0.6)	-0.6 to -0.1	0.002
Triglycerides (mmol/L)	1.2 (0.8)	1.1 (0.5)	-0.2 (0.6)	-0.4 to 0.1	0.158
Waist circumference (cm)	97.2 (11.1)	89.5 (9.2)	-7.7 (4.1)	-9.1 to -6.2	0.001
C-reactive protein (natural logarithm)	0.1 (1.1)	-0.1 (1.3)	-0.3 (1.0)	-0.6 to 0.12	0.168
Psychological functioning					
SF-36					
Mental Component Summary	49.7 (11.8)	56.2 (5.5)	6.5 (10.4)	2.6 to 10.3	0.003
Physical Component Summary	54.7 (4.9)	55.4 (5.5)	0.8 (6.0)	-1.5 to 3.0	0.490
Impact of Event Scale					
Intrusive thoughts	6.2 (5.6)	3.7 (4.8)	-2.5 (4.6)	-4.2 to -0.8	0.010
Avoidance	10.1 (6.8)	7.1 (5.8)	-3.0 (7.8)	-5.9 to -0.1	0.043
PSA tests					
Total PSA (ng/mL)	4.8 (3.9)	4.6 (3.4)	-0.2 (1.9)	-0.5 to 1.0	0.480
Percent of free PSA (ng/mL)	17.5 (7.4)	18.9 (8.3)	1.3 (3.6)	-0.3 to 2.7	0.055

LDL=low-density lipoprotein. HDL=high-density lipoprotein. *Paired samples *t* test, two-tailed.

Table 1: Cardiovascular risk factors and psychological functioning at baseline and at 3 months

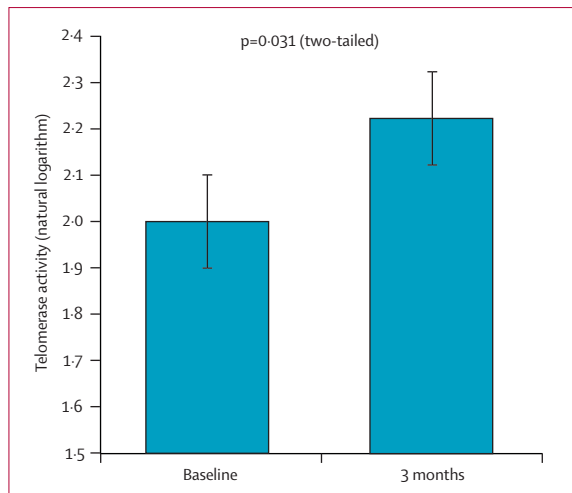


Figure 1: Mean telomerase activity in peripheral-blood mononuclear cells at baseline and at 3 months
Vertical lines represent 1 SEM.

Results

273 men were screened. 96 men declined to participate after learning more about the study, and 147 did not meet inclusion criteria (45 had treatment for prostate cancer, 16 had high Gleason scores or tumour stages, 15 had high PSA concentrations, 40 had atypia or no cancer, 19 lived out of the area, 4 did not want an additional biopsy, 2 were already making comprehensive lifestyle changes, 1 was not able to stop taking aspirin, 2 had serious psychiatric illness, and 3 did not return telephone calls), leaving 30 enrolled patients with a mean age of 62.2 years (SD 7.5), aged 49–80 years.

Of the 60 samples analysed from the 30 study participants (one baseline and one 3-month was required from each participant), seven samples (from 6 patients) did not yield enough cells to assay; thus, both baseline and 3-month telomerase activity data were obtained for 24 patients (no patients were leucopenic or had any known bone-marrow involvement).

Patients were able to adhere closely to the lifestyle recommendations. After 3 months, they reported consuming a mean of 11.6% (SD 3.0) of calories from fat per day, exercising for a mean of 3.6 h (SD 1.5) each week and practising stress management for a mean of 4.5 (SD 2.0) h each week. The dosage of all medications remained stable through the 3-month assessment, with the exception of one participant whose dosage of a statin drug was decreased.

Substantial improvements in risk factors for cardiovascular disease were noted, including decreases in body-mass index (BMI), systolic and diastolic blood pressure, and lipids (table 1). Waist circumference decreased from 97.2 cm (SD 11.1) to 89.5 cm (9.2) ($p=0.001$). Triglycerides and C-reactive protein decreased, although these changes did not reach statistical significance. Total PSA did not change significantly (from

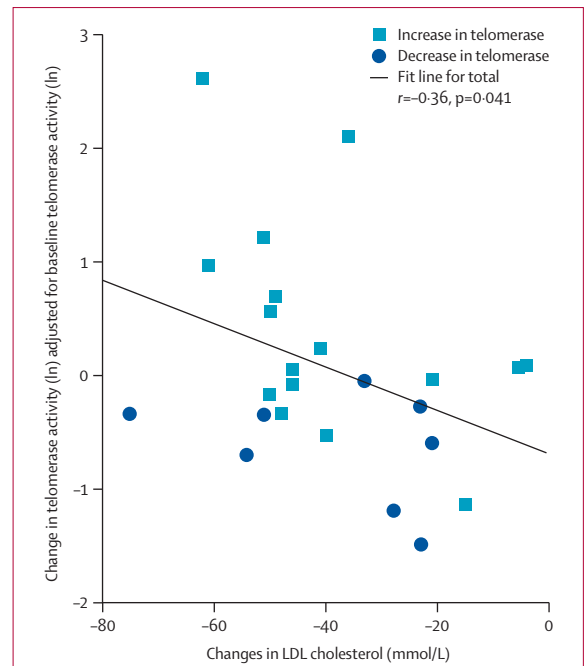


Figure 2: Association of 3-month changes in telomerase activity (adjusted for baseline telomerase activity) with 3-month changes in low-density lipoprotein (LDL) cholesterol
ln=natural logarithm. $R^2=0.131$.

4.8 ng/mL [SD 3.9] to 4.6 ng/mL [3.4], $p=0.48$), although percent of free PSA improved from 17.5 (SD 7.4) to 18.9 (8.3), $p=0.055$, and there was no clinical evidence of disease progression in these patients. Patients reported substantial decreases in psychological distress, as shown by lower scores on the intrusive and avoidant thoughts subscales of the Impact of Event scale (table 1). Mental health-related quality of life also improved, with increases in the Mental Component Summary score of the SF-36, and physical health-related quality of life remained stable (table 1).

Complete (baseline plus 3-month) telomerase data were available for 24 participants. The baseline and changes in cardiovascular risk factors and psychological measures for these 24 patients did not differ significantly from those without complete telomerase data (webtable 2). In the 24 participants, PBMC telomerase activity expressed as a natural logarithm increased from 2.00 (SD 0.44) to 2.22 (0.49, $p=0.031$; figure 1). Raw values of telomerase increased from 8.05 (SD 3.50) standard arbitrary units to 10.38 (6.01) standard arbitrary units.

Decreases in LDL cholesterol were significantly associated with increased telomerase activity ($r=-0.36$, $p=0.041$; figure 2). Changes in BMI, blood pressure, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, LDL/HDL ratio, and C-reactive protein (natural logarithm) were not significantly associated with changes in telomerase activity (data not shown).

See Online for webtable 2

Decreases in one measure of psychological distress (intrusive thoughts) were significantly associated with increased telomerase activity ($r=-0.35$, $p=0.047$; figure 3). Changes in avoidance and changes in mental and physical health-related quality of life were not associated with changes in telomerase activity (data not shown).

Findings of the multiple regression analysis confirmed that changes in intrusive thoughts and LDL cholesterol remained significant after accounting for baseline telomerase activity ($B=-0.046$, $p=0.03$; $B=-0.008$, $p=0.05$), suggesting that changes in the number of intrusive thoughts and changes in the amounts of LDL cholesterol are each independently associated with changes in telomerase activity. We did not find a significant correlation between adherence to the lifestyle intervention and changes in telomerase activity (data not shown). The changes in individual patients are shown in table 2.

Discussion

To our knowledge, we have reported here the first longitudinal study showing that comprehensive lifestyle changes—or any intervention—are significantly associated with increases in cellular telomerase activity and telomere maintenance capacity in human immune system cells. Telomerase activity increased 29.84% during the course of the 3-month intervention; however, because of the relatively small number of patients, these findings need to be viewed as preliminary. While increases in telomerase are considered beneficial, the precise biological importance of this extent of increase in telomerase is not fully known.

These findings are biologically plausible, new, and consistent with earlier studies showing decreases in telomerase in response to life stress. In an earlier cross-sectional study, both the perception and the chronicity of emotional stress were significantly associated with lower telomerase activity and shorter telomere length in healthy women.¹³ In the current study, we noted that decreasing psychological distress was associated with increases in telomerase activity over a 3-month period. Also, our finding that decreases in LDL cholesterol over time were associated with increases in telomerase activity is consistent with findings from an earlier cross-sectional study.¹⁴ We did not find a significant correlation between adherence to the intervention and changes in telomerase activity. In part, this might be because we provided take-home meals for these patients and met with them frequently, so adherence to the intervention was quite high. Consequently, there was not much variability in adherence, and a certain amount of variability is usually needed to show correlations between adherence and outcomes. Additionally, given the limitations of self-reported data and the relatively small sample, it would be unlikely to find significant correlations even if there was an association between adherence and changes in these measures.

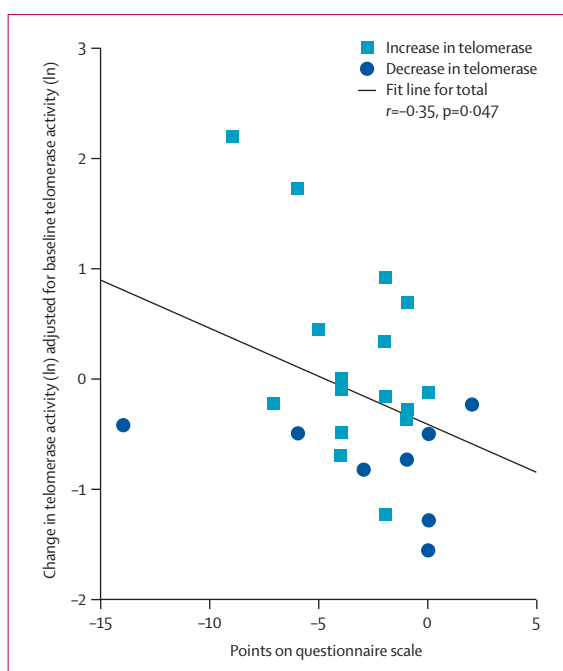


Figure 3: Association of 3-month changes in telomerase activity (adjusted for baseline telomerase activity) with 3-month changes in intrusive thoughts about prostate cancer
ln=natural logarithm. $R^2=0.123$.

We were interested to know if these lifestyle changes affect telomere maintenance over time. However, the estimated rate of telomere loss per year based on several cross-sectional studies (averaging around 30–60 base pairs per year) suggests that detectable changes in telomere length in human beings will not occur in 3 months, but at least a year might be needed. By contrast, changes in physiological measures such as telomerase, an enzyme, might occur quickly. Future studies that are of longer duration with a randomised control group for comparison should assess both telomerase and telomere length.

Increasing evidence suggests that PBMC telomere shortness and low telomerase activity might be important risk factors for cancer and cardiovascular disease. Upregulating telomerase *in vitro* promotes cell longevity and genomic stability.²⁷ Thus, increasing telomerase activity in healthy PBMCs as a result of comprehensive lifestyle changes might have important clinical relevance. Our findings suggest, but do not prove conclusively, the possibility that some or all aspects of the combined lifestyle changes in this study might be responsible for the recorded increase in telomerase activity. If subsequent randomised controlled trials substantiate that comprehensive lifestyle changes might increase telomerase activity and telomere length, then this might be a powerful motivator for many people to beneficially change their diet and lifestyle. Also, this finding might stimulate new research to establish whether increases in telomerase activity and telomere length might be associated with increases in life span and

	Time since death (months)	Baseline telomerase unit activity*	3-month telomerase unit activity*	Baseline BMI	3-month BMI	Baseline LDL (mmol/L)	3-month LDL (mmol/L)	Baseline IES: intrusion subscale	3-month IES: intrusion subscale	Baseline SF-36 Physical Component score	3-month SF-36 Physical Component score	Baseline SF-36 Mental Component score	3-month SF-36 Mental Component score	Baseline lifestyle adherence score	3-month lifestyle adherence score
1	7.5	1.18	1.82	22.71	22.47	1.76	1.22	8	1	58.2	59.4	54.0	56.0	0.97	1.06
2	5.5	1.75	1.84	25.57	24.07	3.11	2.07	7	3	54.0	62.0	51.6	49.2	0.51	0.74
3	5.0	1.84	2.09	27.72	23.69	2.72	1.53	1	0	43.1	53.5	60.2	63.1	0.42	1.07
4	22.0	1.88	2.16	23.27	21.94	2.41	1.22	3	1	57.3	56.3	60.6	63.7	0.29	1.15
5	7.5	1.45	1.81	25.08	22.08	3.68	2.43	17	13	55.5	57.2	48.5	53.3	0.71	1.06
6	22.0	2.17	2.58	27.28	23.28	3.21	1.94	13	8	53.9	54.2	57.0	57.3	0.41	0.81
7	6.0	2.53	2.85	36.90	33.93	3.60	2.02	1	0	58.0	58.9	62.0	61.5	0.39	0.96
8	4.5	2.18	2.37	22.36	20.15	2.82	1.76	4	0	60.8	42.3	34.9	54.7	0.19	0.77
9	23.0	1.72	1.41	26.41	24.11	3.16	2.56	0	0	55.2	46.9	36.7	51.7	0.31	0.88
10	24.5	1.60	1.94	26.07	24.12	3.55	2.25	5	4	56.4	59.6	60.6	60.9	0.99	1.27
11	23.0	1.28	1.37	24.09	22.34	2.69	2.31	5	3	60.6	66.9	56.0	39.1	0.45	1.08
12	6.5	1.40	2.19	29.19	26.03	5.44	4.14	3	1	53.8	56.3	41.0	51.8	0.79	0.93
13	5.0	2.49	2.95	24.09	21.15	2.85	1.53	19	17	47.7	53.2	46.4	52.7	0.55	0.99
14	3.5	2.12	2.09	24.21	23.38	2.82	1.50	6	0	58.9	53.4	25.3	51.7	0.27	1.06
15	4.0	1.86	2.16	29.18	26.08	2.36	2.23	2	2	45.4	43.2	63.1	64.1	0.36	0.69
16	2.5	2.17	2.15	26.76	24.03	2.95	2.36	18	4	55.0	58.5	53.5	59.7	0.53	0.87
17	16.5	1.90	1.89	27.52	23.55	3.19	2.64	1	0	52.1	48.8	59.5	64.2	0.62	0.45
18	3.0	2.12	1.93	24.29	22.23	3.86	2.46	8	5	62.5	55.8	34.8	53.0	0.15	0.73
19	4.5	1.79	3.03	28.31	24.08	3.16	2.23	7	1	51.8	54.3	51.6	53.0	0.15	0.96
20	8.0	2.37	2.40	27.96	25.30	2.87	2.77	6	2	53.2	60.6	60.1	56.0	0.83	1.17
21	27.0	2.76	1.99	30.75	28.00	2.69	1.97	1	1	50.6	58.9	52.3	61.5	0.33	0.83
22	6.5	2.65	2.32	37.35	31.21	4.30	2.36	1	1	49.2	57.7	49.6	57.2	0.14	1.09
23	34.0	2.09	3.40	23.14	19.93	3.96	2.36	12	3	58.4	58.1	57.4	62.3	0.80	1.12
24	8.0	2.60	2.43	29.33	26.41	3.91	3.06	3	5	53.8	47.0	58.2	57.9	1.31	1.31

BMI=body-mass index. LDL=low-density lipoprotein. IES=Impact of Event Scale. SF-36=Medical Outcomes Study Short Form 36 item. *Per 10 000 viable cells.

Table 2: Changes in individual patients

decreases in the incidence or severity of chronic diseases, because lifestyle factors known to promote cancer and cardiovascular disease might also adversely affect telomerase activity and telomere length. A power calculation based on the noted changes in telomerase activity was done as follows: estimated effect size=mean/SD=0.22/0.47=0.47. If the effect size is 0.5, to have a 90% power to detect a difference by use of p=0.05, then n=68 patients per group would be needed. If the effect size is 0.4, the value for 90% power to detect a difference by use of p=0.05, then n=110 patients per group would be needed. This number of patients is realistic, therefore, this information might motivate other researchers to design a randomised controlled trial with 68–110 patients in each group (Mendell N, SUNY Stony Brook, Stony Brook, NY, USA, personal communication).

PBMC telomerase activity is an important known determinant of telomere length. Telomerase activity measured in clinical PBMC samples constitutes a relatively new parameter that, to date, has been measured in very few studies in human beings. From previous data, changes in PBMC mean telomere length are not expected to be detectable over the short time period of the present

study (3 months), so we did not measure it in this study. Also, studies with patients and animal models bearing telomerase mutations, and with human cells in culture, suggest that telomerase status might be associated with healthy immune function independent of telomere length.^{28–30}

Since this was a pilot study, we did not have the resources to recruit or test a usual-care control group; as such, we report these increases in telomerase as a significant association rather than inferring causation. It is possible that other factors besides changes in lifestyle might have contributed to the increase in telomerase activity after 3 months. Because the men in the study all chose active surveillance for reasons unrelated to this study, it was possible to assess the association between changes in lifestyle and changes in telomerase activity without confounding interventions such as radical prostatectomy, radiation, or chemotherapy.

We considered factors that might confound interpretation of the findings. First, the possibility that changes in telomerase activity over the 3-month period were a result of inter-assay variability is unlikely because blood samples were drawn in an identical manner, at the

same location, and at the same time of day at both time intervals, and baseline and 3-month samples for each participant were assayed together within the same batch in the same laboratory. Also, the finding that not all men showed increases in telomerase activity decreases the likelihood that a systematic error affected all samples.

We also considered the possibility that the noted increases in telomerase activity could be as a result of the stress of making comprehensive lifestyle changes or the stress of having been diagnosed with prostate cancer. However, participants reported that their stress levels decreased rather than increased. Also, as described earlier, the mean duration from time of prostate cancer diagnosis to enrolment in the current study was 12.3 months, therefore decreases in psychological stress are unlikely to be accounted for by decreases in acute stress resulting from a recent diagnosis of prostate cancer. PSA concentrations remained stable, and free PSA improved substantially, therefore it is unlikely that the telomerase activity in PBMCs increased because of progression of prostate cancer.

Another potential explanation we considered was that telomerase activity per cell in PBMCs became upregulated as part of the immune response to infections. However, none of the participants developed infectious diseases during the study intervention period. Also, concentrations of C-reactive protein, a marker of inflammation and infection, decreased significantly ($p < 0.039$ for the 24 patients with complete telomerase data) rather than increased during this time period.

Only 30 of 126 eligible patients volunteered; this proportion (while not unusually low in clinical trials) could affect the generalisability of the findings because substantial motivation is needed to make comprehensive lifestyle changes. The exact mechanism of the recorded telomerase activity increase in this study and its association with decreases in LDL cholesterol and psychological distress is unknown. However, a few possibilities can be suggested. Decreases in physiological stress might have a role. Chronic psychological distress has been associated with both shorter telomeres^{14,31-33} and lower telomerase activity in PBMCs.^{13,14} Overexposure to stress-related hormones (catecholamines and cortisol) might increase oxidative damage to cells, compromising the telomere maintenance system. Studies in animals suggest that norepinephrine and cortisol increase oxidative stress.³⁴⁻³⁶ Healthy women with lower telomerase activity in PBMCs (below the mean) had significantly greater chronic stress and negative mood, along with greater oxidative stress and nocturnal excretion of urinary epinephrine compared with those with higher telomerase activity.^{13,14} Furthermore, an in-vitro study recorded that cortisol exposure led to lower telomerase activity in T cells.³⁷

In a study of healthy women, we reported that telomerase activity in PBMCs, although not telomere length, was inversely related to six major risk factors for

cardiovascular disease.¹⁴ These findings suggest that telomerase activity levels in unstimulated cells might be a more direct and potentially earlier predictor than telomere length of long-term cellular viability or genomic stability (or both).

In addition to occurring in normal cell division, telomere shortening is accelerated by oxidative stress. In-vitro studies have suggested that a portion of the oxidative damage sustained by telomeres remains unrepaired and affects the amount of shortening in the next cell division.^{38,39} Furthermore, oxidative stress seems to downregulate telomerase activity in vascular smooth muscle cells⁴⁰ and endothelial cells.⁴¹ An association between oxidative stress and telomere length has been reported in vivo too.^{42,43}

Inflammation is another factor thought to contribute to telomere attrition in cells of the immune system by promoting leucocyte turnover.^{44,45} Oxidised LDL is inversely associated with telomerase activity in PBMCs⁴⁶ and activates monocytes and macrophages by upregulating adhesion molecules and increasing production of inflammatory cytokines.⁴⁷ The increased production of certain cytokines has been shown to adversely affect telomerase activity.⁴⁸ These oxidative stress and inflammatory processes potentially pose threats to the telomere maintenance system.

The most common causes of oxidative stress and inflammation stem from unhealthy nutrition, little exercise, obesity, metabolic syndrome, and chronic emotional stress. In the Harvard Nurses' Health Study, higher intakes of red and processed meats, sweets, desserts, French fries, and refined grains increased blood markers of inflammation, whereas higher intakes of fruit, vegetables, legumes, fish, poultry, and whole grains decreased blood markers of inflammation.⁴⁹ Therefore, changes in diet, exercise, and stress management might decrease markers of oxidative stress and inflammation.^{50,51}

The diet used in this study might have beneficial effects on many chronic diseases associated with older age. This diet is low in components (eg, saturated fats, cholesterol) that have been implicated in the development of diseases associated with ageing (including some types of cancer, cardiovascular diseases, diabetes, age-related macular degeneration, dementia) and high in several dietary constituents (eg, lycopene, antioxidants, carotenoids, zinc, fibre, folate) that might decrease the risk of these conditions.⁵¹

Obesity, insulin resistance, and related cardiovascular disease processes, which are related to oxidative stress and inflammation,^{52,53} have all been linked to shorter telomeres.^{54,55} Thus, improvements in these health conditions might favourably affect the regulation of telomerase activity.

In a study that followed individuals over a 10-year period (but did not intervene in changing their weight), decreases in obesity were associated with increases in bulk telomere length, suggesting that shortening

telomere length associated with obesity might be partially reversible.⁵⁵ Also, in-vitro studies have shown that antioxidants reverse oxidative telomeric damage, prolonging the replicative lifespan and slowing telomere shortening.³⁹

In summary, lifestyle factors known to promote cancer and cardiovascular disease processes might also adversely affect the telomere maintenance system, including telomerase. However, previous studies have not addressed whether telomerase activity might be associated with interventions of diet and lifestyle. Clearly, larger studies with a randomised control group are needed. This pilot study has provided not only new findings but also data for power calculation estimates needed to carry out larger clinical trials with sufficient power to detect differences due to the intervention, the relative contribution of each component, duration of intervention, rates of recruitment, possible mechanisms involved, and if telomere length changes over a longer period of time.

Contributors

DO, JL, JD, GW, EE, RM, PC, and EHB designed the study. DO, JL, JD, CK, MJMM, RM, PC, and EHB undertook the research. JL, JD, GW, EE, LY, and EB analysed the data. DO, JL, JD, GW, EE, CK, PC, and EHB wrote the report.

Conflicts of interest

DO is a consultant for Safeway (Pleasanton, CA, USA), PepsiCo (Purchase, NY, USA) and Mars (Hackettstown, NJ, USA) on manufacturing healthy foods, and is chair of Google Health Advisory Council. The other authors declared no conflicts of interest.

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References

- Blackburn EH. Switching and signaling at the telomere. *Cell* 2001; **106**: 661–73.
- DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular mechanisms of prostate carcinogenesis: implications for diagnosis, detection, prevention, and treatment. *J Cell Biochem* 2004; **91**: 459–77.
- Rodier F, Kim SH, Nijjar T, Yaswen P, Campisi J. Cancer and aging: the importance of telomeres in genome maintenance. *Int J Biochem Cell Biol* 2005; **37**: 977–90.
- Griffith JK, Bryant JE, Fordyce CA, Gilliland FD, Joste NE, Moyzis RK. Reduced telomere DNA content is correlated with genomic instability and metastasis in invasive human breast carcinoma. *Breast Cancer Res Treat* 1999; **54**: 59–64.
- Wu X, Amos CI, Zhu Y, et al. Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Inst* 2003; **95**: 1211–18.
- Gertler R, Rosenberg R, Stricker D, et al. Telomere length and human telomerase reverse transcriptase expression as markers for progression and prognosis of colorectal carcinoma. *J Clin Oncol* 2004; **22**: 1807–14.
- Fordyce CA, Heaphy CM, Joste NE, Smith AY, Hunt WC, Griffith JK. Association between cancer-free survival and telomere DNA content in prostate tumors. *J Urol* 2005; **173**: 610–14.
- Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003; **361**: 393–95.
- Blackburn EH. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett* 2005; **579**: 859–62.
- Blackburn EH. Telomere states and cell fates. *Nature* 2000; **408**: 53–56.
- Weng NP, Levine BL, June CH, Hodes RJ. Regulated expression of telomerase activity in human T lymphocyte development and activation. *J Exp Med* 1996; **183**: 2471–79.
- Kim NW, Wu F. Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). *Nucleic Acids Res* 1997; **25**: 2595–97.
- Epel ES, Blackburn EH, Lin J, et al. Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci USA* 2004; **101**: 17312–15.
- Epel ES, Lin J, Wilhelm FH, et al. Cell aging in relation to stress arousal and cardiovascular disease risk factors. *Psychoneuroendocrinology* 2006; **31**: 277–87.
- Gardner JP, Li S, Srinivasan SR, et al. Rise in insulin resistance is associated with escalated telomere attrition. *Circulation* 2005; **111**: 2171–77.
- Ornish D, Brown SE, Scherwitz LW, et al. Can lifestyle changes reverse coronary heart disease? The Lifestyle Heart Trial. *Lancet* 1990; **336**: 129–33.
- Ornish D, Scherwitz LW, Billings JH, et al. Intensive lifestyle changes for reversal of coronary heart disease. *JAMA* 1998; **280**: 2001–07.
- Ornish D, Weidner G, Fair WR, et al. Intensive lifestyle changes may affect the progression of prostate cancer. *J Urol* 2005; **174**: 1065–69; and discussion 1069–70.
- Ornish D, Magbanua MJM, Weidner G, et al. Changes in prostate gene expression in men undergoing an intensive nutrition and lifestyle intervention. *Proc Natl Acad Sci USA* 2008; **105**: 8369–74.
- Ornish D. *The Spectrum*. New York: Ballantine Books, 2008.
- Gunawardena K, Campbell LD, Meikle AW. Combination therapy with vitamins C plus E inhibits survivin and human prostate cancer cell growth. *Prostate* 2004; **59**: 319–27.
- Wang MH, Abreu-Delgado Y, Young CY. Effects of vitamin C on androgen receptor mediated actions in human prostate adenocarcinoma cell line LAPC-4. *Urology* 2003; **62**: 167–71.
- Damjanovic AK, Yang Y, Glaser R, et al. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J Immunol* 2007; **179**: 4249–54.
- Horowitz M, Wilner N, Alvarez W. Impact of event scale: a measure of subjective stress. *Psychosom Med* 1979; **41**: 209–18.
- Ware JE, Kosinski M. How to Score Version 2 of the SF-36 Health Survey (Standard and Acute Forms). Quality Metric Inc, Lincoln: 2002.
- Vickers AJ, Altman DG. Statistical notes: analysing controlled trials with baseline and follow up measurements. *BMJ* 2001; **323**: 1123–24.
- Zhu J, Wang H, Bishop JM, Blackburn EH. Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening. *Proc Natl Acad Sci USA* 1999; **96**: 3723–28.
- Finkel T, Serrano M, Blasco MA. The common biology of cancer and ageing. *Nature* 2007; **448**: 767–74.
- Sarin KY, Cheung P, Gilson D, et al. Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature* 2005; **436**: 1048–52.
- Cao Y, Li H, Deb S, Liu JP. TERT regulates cell survival independent of telomerase enzymatic activity. *Oncogene* 2002; **21**: 3130–38.
- Simon NM, Smoller JW, McNamara KL, et al. Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biol Psychiatry* 2006; **60**: 432–35.
- Damjanovic AK, Yang Y, Glaser R, et al. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J Immunol* 2007; **179**: 4249–54.
- Lung FW, Chen NC, Shu BC. Genetic pathway of major depressive disorder in shortening telomeric length. *Psychiatr Genet* 2007; **17**: 195–99.

- 34 Aizawa T, Ishizaka N, Usui S, Ohashi N, Ohno M, Nagai R. Angiotensin II and catecholamines increase plasma levels of 8-epi-prostaglandin F(2alpha) with different pressor dependencies in rats. *Hypertension* 2002; **39**: 149–54.
- 35 Orzechowski A, Ostaszewski P, Wilczak J, et al. 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* 2002; **49**: 256–63.
- 36 Lin H, Decuypere E, Buyse J. Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*) 1. Chronic exposure. *Comp Biochem Physiol B Biochem Mol Biol* 2004; **139**: 737–44.
- 37 Choi J, Fauce SR, Effros RB. Reduced telomerase activity in human T lymphocytes exposed to cortisol. *Brain Behav Immun* 2008; **22**: 600–05.
- 38 Richter T, Zglinicki T. A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Exp Gerontol* 2007; **42**: 1039–42.
- 39 von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002; **27**: 339–44.
- 40 Matthews C, Gorenne I, Scott S, et al. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. *Circ Res* 2006; **99**: 156–64.
- 41 Haendeler J, Hoffmann J, Diehl JF, et al. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. *Circ Res* 2004; **94**: 768–75.
- 42 Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. *Diabetes Care* 2006; **29**: 283–89.
- 43 Adaikalakoteswari A, Balasubramanyam M, Mohan V. Telomere shortening occurs in Asian Indian Type 2 diabetic patients. *Diabet Med* 2005; **22**: 1151–56.
- 44 Bekaert S, De Meyer T, Rietzschel ER, et al. Telomere length and cardiovascular risk factors in a middle-aged population free of overt cardiovascular disease. *Aging Cell* 2007; **6**: 639–47.
- 45 Aviv A, Valdes A, Gardner JP, Swaminathan R, Kimura M, Spector TD. Menopause modifies the association of leukocyte telomere length with insulin resistance and inflammation. *J Clin Endocrinol Metab* 2006; **91**: 635–40.
- 46 Tsiropoulou G, Chatzipanagiotou S, Boufidou F, et al. Serum oxidized low-density lipoprotein is inversely correlated to telomerase activity in peripheral blood mononuclear cells of haemodialysis patients. *Nephrology* 2006; **11**: 506–09.
- 47 Pasini AF, Anselmi M, Garbin U, et al. Enhanced levels of oxidized low-density lipoprotein prime monocytes to cytokine overproduction via upregulation of CD14 and toll-like receptor 4 in unstable angina. *Arterioscler Thromb Vasc Biol* 2007; **27**: 1991–97.
- 48 Xu D, Erickson S, Szeps M, et al. Interferon alpha down-regulates telomerase reverse transcriptase and telomerase activity in human malignant and nonmalignant hematopoietic cells. *Blood* 2000; **96**: 4313–18.
- 49 Lopez-Garcia E, Schulze MB, Fung TT, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* 2004; **80**: 1029–35.
- 50 Roberts CK, Vaziri ND, Barnard RJ. Effect of diet and exercise intervention on blood pressure, insulin, oxidative stress, and nitric oxide availability. *Circulation* 2002; **106**: 2530–32.
- 51 Dewell A, Weidner G, Sumner MD, et al. Relationship of dietary protein and soy isoflavones to serum IGF-1 and IGF binding proteins in the Prostate Cancer Lifestyle Trial. *Nutr Cancer* 2007; **58**: 35–42.
- 52 Keaney JF Jr, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 2003; **23**: 434–39.
- 53 Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004; **25**: 4–7.
- 54 Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet* 2005; **366**: 662–64.
- 55 Gardner JP, Li S, Srinivasan SR, et al. Rise in insulin resistance is associated with escalated telomere attrition. *Circulation* 2005; **111**: 2171–77.