



Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study

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Summary

Background Telomere shortness in human beings is a prognostic marker of ageing, disease, and premature morbidity. We previously found an association between 3 months of comprehensive lifestyle changes and increased telomerase activity in human immune-system cells. We followed up participants to investigate long-term effects.

Methods This follow-up study compared ten men and 25 external controls who had biopsy-proven low-risk prostate cancer and had chosen to undergo active surveillance. Eligible participants were enrolled between 2003 and 2007 from previous studies and selected according to the same criteria. Men in the intervention group followed a programme of comprehensive lifestyle changes (diet, activity, stress management, and social support), and the men in the control group underwent active surveillance alone. We took blood samples at 5 years and compared relative telomere length and telomerase enzymatic activity per viable cell with those at baseline, and assessed their relation to the degree of lifestyle changes.

Findings Relative telomere length increased from baseline by a median of 0.06 telomere to single-copy gene ratio (T/S) units (IQR -0.05 to 0.11) in the lifestyle intervention group, but decreased in the control group (-0.03 T/S units, -0.05 to 0.03, difference $p=0.03$). When data from the two groups were combined, adherence to lifestyle changes was significantly associated with relative telomere length after adjustment for age and the length of follow-up (for each percentage point increase in lifestyle adherence score, T/S units increased by 0.07, 95% CI 0.02–0.12, $p=0.005$). At 5 years, telomerase activity had decreased from baseline by 0.25 (-2.25 to 2.23) units in the lifestyle intervention group, and by 1.08 (-3.25 to 1.86) units in the control group ($p=0.64$), and was not associated with adherence to lifestyle changes (relative risk 0.93, 95% CI 0.72–1.20, $p=0.57$).

Interpretation Our comprehensive lifestyle intervention was associated with increases in relative telomere length after 5 years of follow-up, compared with controls, in this small pilot study. Larger randomised controlled trials are warranted to confirm this finding.

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Introduction

Telomeres are protective DNA and protein complexes at the end of linear chromosomes that promote chromosomal stability. Telomere maintenance is required for the complete replication of DNA and protection of chromosomes from nuclease degradation, end-to-end fusion, and cellular senescence.¹ Telomeres typically shorten during normal cell divisions and, therefore, telomere length and rate of shortening are indicators of mitotic-cell age.² Telomere shortening is counteracted by the cellular enzyme telomerase.³ In human beings, telomere shortening is a potential prognostic marker for disease risk and progression and for premature death.⁴

Cellular ageing conferred by diminished telomere maintenance seems to be an important precursor to the development of many types of cancer.⁵ Shortened

telomeres have been associated with poor clinical outcomes, including increased risk of prostate-cancer recurrence after radical prostatectomy.⁶ Poor outcomes are also reported in patients with coronary heart disease (shortened survival)⁷ and infectious diseases.⁸

Short telomere length in peripheral-blood mononuclear cells (PBMCs) is associated with ageing and ageing-related diseases, such as cancer, stroke, vascular dementia, cardiovascular disease, obesity, osteoporosis, and diabetes.^{1,9} Telomere attrition has been proposed as a potential mechanism that triggers the chromosomal rearrangement characteristic of prostate cancer.¹⁰ Men with variable telomere length in prostate-cancer cells and short telomere length in prostate-cancer-associated stromal cells are at a substantially increased risk of metastasis or dying from prostate cancer.¹¹

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In the GEMINAL study¹² we investigated the effects of comprehensive lifestyle changes (plant-based diet, moderate exercise, stress management, and increased social support) on telomerase activity in men with low-risk prostate cancer. After 3 months of intervention, telomerase activity was significantly increased in PBMCs. However, although changes in physiological features, such as telomerase activity, can be seen quickly, changes in relative telomere length might not be detectable in such a short period. Therefore, we investigated whether long-term lifestyle changes would affect relative telomere length, telomerase activity, or both, and whether the degree of lifestyle change would alter the extent of the effects.

Methods

Patients

We enrolled all participants between 2003 and 2007. Participants in the intervention group came from the GEMINAL study¹² and those in the external control group from the University of California, San Francisco (UCSF) MENS study.^{13,14} Eligible participants were men with low-risk prostate cancer who had chosen active surveillance rather than conventional treatment (for reasons unrelated to the study), so it was possible to assess the association between changes in lifestyle and changes in telomerase activity and telomere length without potentially confounding treatments. Both groups were recruited with the same selection criteria, with the a priori intention of comparing them. Inclusion criteria were pathology-confirmed prostate cancer, prostate-specific antigen (PSA) concentration 10 µg/L or lower (or lower than 15 µg/L if patients had benign prostatic hyperplasia or prostatitis), Gleason score of 6 or lower, a stage T1 or T2a tumour (according to the tumour, node, metastasis staging system), and 33% or less of biopsy cores and 50% or less of the length of a tumour-core positive for adenocarcinoma. The protocol for this study was approved by the UCSF institutional review board. All participants provided written informed consent.

Interventions

Participants underwent active surveillance plus a lifestyle-change intervention (intervention group) or active surveillance alone (control group). The lifestyle intervention included the following four components: diet high in whole foods, plant-based protein, fruits, vegetables, unrefined grains, and legumes, and low in fat (approximately 10% of calories) and refined carbohydrates (take-home meals were provided to patients for the first 3 months of the intervention); moderate aerobic exercise (walking 30 min per day on 6 days per week); stress management (gentle yoga-based stretching, breathing, meditation, imagery, and progressive relaxation for 60 min daily); and increased social support (60 min support-group sessions once per week).¹⁵

At each weekly session patients did an additional 1 h of moderate exercise, supervised by an exercise physiologist,

1 h of stress-management techniques, supervised by a certified stress-management specialist, and 1 h of support group, led by a clinical psychologist, and attended a 1 h lecture during dinner, generally from a dietitian, registered nurse, or physician. All members of the intervention staff were available to answer patients' questions and to provide counselling at the weekly support sessions. Spouses and partners were encouraged to attend support sessions but were not required to do so.

After the first 3 months meetings were not compulsory, but patients could continue to meet on their own for two 4 h meetings per month for the duration of the study. A physician or nurse was on site during meetings and patients could request that other clinical staff attend or be available by telephone at these times, as needed.

Peripheral blood mononuclear cells

30 mL blood was drawn from participants into heparin tubes at baseline and at 5 years. The samples were anonymised so that laboratory technicians were unaware of treatment group and collection time. PBMCs were purified within 1 h of blood draw with Ficoll gradient (Ficoll-Paque PLUS, GE Healthcare, Pittsburgh, PA, USA) according to the manufacturer's protocol. Viable PBMCs were counted with the Trypan blue exclusion method (0.4% Trypan blue stain, Invitrogen, Grand Island, NY, USA) and cryopreserved in 90% fetal bovine serum (Thermo Scientific, Waltham, MA, USA) and 10% dimethyl sulphoxide (Hybrimax, Sigma, St Louis, MO, USA) at the concentration of 10⁷ cells per mL. Cells were transferred to cryogenic vials (Corning, Corning, NY, USA) and stored in a Mr Frosty freezing container (Nalgene, Rochester, NY, USA) at -80°C for up to 1 week, after which PBMCs were transferred to liquid nitrogen until analysis.

Before assay, the tubes containing PBMCs were removed from the liquid nitrogen and placed on dry ice. PBMCs were thawed in a 37°C water bath and placed on ice until being transferred to 15 mL tubes with 10 mL cold Dulbecco's phosphate-buffered saline and being centrifuged at 3000 rpm (2012 g) in a Sorvall Legend XTR table top centrifuge (Thermo Scientific, Asheville, NC, USA) at 10°C for 10 min. After the supernatant was aspirated, PBMCs were resuspended in 2 mL cold Dulbecco's phosphate-buffered saline. Viable cells were counted using the Trypan blue exclusion method to obtain 10⁶ cells/mL and placed into two 1.5 mL Eppendorf tubes. After centrifugation at 7000 rpm (5024 g) in an Eppendorf refrigerated microcentrifuge 5417R (Eppendorf, Hauppauge, NY, USA) at 4°C for 5 min, supernatant was aspirated off. The cell pellets for assessment of relative telomere length were stored at -80°C for batch DNA purification later. 200 µL of 1X CHAPS buffer from the TRAPeze telomerase detection kit (Merck Millipore, Billerica, MA, USA) was added to the cell pellets to be assessed for telomerase activity and the tubes were placed on ice for 30 min to lyse the cells. To best ensure assay reproducibility, buffer from the

same batch was used in all tubes. Cell lysate was centrifuged at 14000 rpm (20817 g) in an Eppendorf refrigerated microcentrifuge 5417R at 4°C for 20 min. The extracts were placed in 0.5 mL Eppendorf tubes and stored at -80°C until all samples had been processed.

Measurement of relative telomere length

Quantitative PCR (qPCR) was used to assess telomere length relative to a standard reference DNA, expressed as telomere to single-copy gene ratio (T/S).^{16,17} The telomere qPCR primers were tel1b (5'-CGGTTT[GTTTGG]₅GTT-3'), used at a final concentration of 100 nmol/L, and tel2b (5'-GGCTTG[CCTTAC]₅CCT-3'), used at a final concentration of 900 nmol/L. Single-copy gene (human β globin) qPCR primers were hbg1 (5'-GCTTCTGACACAACCTGTGTTCACTAGC-3'), used at a final concentration of 300 nmol/L, and hbg2 (5'-CACCAACTTCATCCACGTTCCACC-3'), used at a final concentration of 700 nmol/L. The final reaction mix was 20 mmol/L Tris-hydrochloride, pH 8.4, 50 mmol/L potassium chloride, 200 μ mol/L of each deoxyribonucleotide triphosphate, 1% dimethyl sulphoxide and 0.4x SYBR green I, 2 ng/ μ L *Escherichia coli* DNA, 0.04 U/ μ L platinum Taq DNA polymerase (Invitrogen), and 1.5–20.0 ng genomic DNA.

For the telomere qPCR reaction, the thermal cycling profile was 1 min at 94°C followed by 25 cycles of denaturing at 96°C for 1 s and annealing or extension at 54°C for 60 s with fluorescence data collection. The single-copy gene qPCR profile was 1 min at 94°C followed by eight cycles of denaturing at 95°C for 15 s, annealing at 58°C for 1 s, and extension at 72°C for 20 s, followed by 29 cycles of denaturing at 96°C for 1 s, annealing at 58°C for 1 s, extension at 72°C for 20 s, and hold at 83°C for 5 s, with data collection. All samples were run twice and the mean T/S was calculated. Within each run, each sample was run in triplicate and averaged. The between-plate variability of the quality-control samples for telomere length measurement was 7.6% and the within-plate variability was 7.1%.

Tubes containing 8.75, 2.9, 0.97, and 0.324 ng reference DNA from HeLa cells were included in each qPCR run so that the quantity of targeted templates in each research sample could be determined relative to the reference DNA sample by the standard curve method. Additionally, four control genomic DNA samples from cancer-cell lines were used to calculate a normalising factor for each run. In each batch, the T/S of each control DNA was divided by the average ratio for the same DNA from ten runs to obtain a normalising factor. The average normalising factor was used to correct the participant DNA samples to calculate the final T/S.

Assessment of telomerase activity

The cryopreserved PBMCs were thawed and viable cells were counted with the Trypan blue method.¹⁸ Telomerase activity reaction was assessed with the TRAPeze kit and

run on an 8% polyacrylamide-8M urea sequencing gel. The gel was exposed to a phosphorimager plate overnight and scanned with a STORM 860 molecular imager (GE Healthcare, Piscataway, NJ, USA). We used human embryonic kidney 293T cells as a positive control; telomerase activity is expressed as the equivalent of the number of 293T cells per 10000 PBMCs. Telomerase activity was quantified with ImageQuant (version 5.2) as previously described.¹⁷ The lowest quantifiable level of telomerase activity is 0.5 units. In the Blackburn laboratory, the average within-assay variability of PBMC samples (assayed in triplicate) is 8.0% and the between-assay variability (assayed on two different days) is 6.7%.

Lifestyle-index scores

Lifestyle-index scores were calculated at baseline and 5 years with established lifestyle adherence formulas that have been applied to men with early-stage prostate cancer previously to assess changes in coronary atherosclerosis (stenosis) measured with quantitative coronary arteriography,^{19,20} PSA concentration, and in-vitro LNCaP cell growth.²¹ We calculated the mean percentage of adherence according to the following formula:

$$\frac{t + ((u / 7 + v / 420) / 2) + ((x / 7 + y / 180) / 2) + z}{4}$$

where t indicates support-group attendance (% sessions attended in 1 year), u indicates stress-reduction activity days per week, v stress-reduction activity minutes per week, x exercise days per week, y exercise minutes per week, and z adherence to diet. Adherence to exercise and stress management was assessed by dividing the self-reported number of minutes per week by the average number of minutes requested in the intervention per week (180 and 420, respectively). Dietary adherence was calculated in the following way:

$$z = \frac{(1 - 1 / 40[\%fat-10]) + (1 - 1 / 995[mg\ cholesterol])}{2}$$

Increases in scores (mean percentage of adherence) reflect improved adherence to the recommended four components of the intervention—nutrition, stress management, exercise, and social support. We included dietary fat and cholesterol as surrogate measures of dietary adherence because they can be quantified with as continuous variables and because dietary guidelines suggested obtaining only 10% of calories from fat. We used a semiquantitative food frequency questionnaire to assess consumption of other recommended foods.²²

A lifestyle index score of 1.0 suggested 100% adherence. Scores could exceed 1.0 for individuals who completed more hours of exercise and stress management than required.

Statistical analysis

The main outcomes of interest were changes from baseline in relative telomere length, telomerase activity, lifestyle index score, and PSA concentration at 5 years. We calculated differences between the intervention and control groups with the non-parametric Wilcoxon's rank sum test. This approach avoided the necessity of making assumptions about the distributions of variables in a small study sample. As a sensitivity analysis we used parametric *t* tests. We also analysed the data after exclusion of any

patients who received treatment for their prostate cancer during the observation period, to assess the effect of such treatment on results.

We also explored whether there was an association between adherence to lifestyle recommendations and relative telomere length and telomerase activity. We combined data for all participants irrespective of group assignment and did regression analyses with the generalised estimating equations approach to account for the likelihood that measurements within an individual will correlate more strongly than those between individuals. Generalised estimating equations take increased correlation into account in the calculation of SEMs of the regression results by estimating the within-person correlation. Thus, SEMs of the estimates are corrected, while the findings are averaged across all participants. Because telomerase activity in our cohort did not seem to be normally distributed, we did unlogged and logged analyses on the outcome. All models were checked for goodness of fit with residual analysis and plots.

We deemed *p* values of 0.05 or less to be significant. All statistical analyses were done with Stata (version 12) software.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

11 (37%) of the original 30 patients from the lifestyle intervention group had sufficient baseline samples of PBMCs to assess telomerase activity and relative telomere length. However, one patient died after an accident before the end of the study. The lifestyle intervention group, therefore, included ten patients. Of 34 controls enrolled, 25 (74%) had sufficient baseline and available 5-year blood samples for assessment and were included in the analyses. Baseline characteristics of patients were similar in the two groups, although in view of the size of the groups there was not sufficient power to detect small differences between them (table 1). The mean time between baseline and follow-up blood sample collection was 5.2 (SD 0.3) years for the lifestyle intervention group and 4.1 (SD 1.1) years for the control group.

In the lifestyle intervention group participants' telomere length had increased by a median of 0.06 T/S units (IQR -0.05 to 0.11) after 5 years. By contrast, in the control group a decrease in length was seen (median -0.03 T/S units, IQR -0.05 to 0.03). The difference in changes between groups was significant (*p*=0.03; table 2). In the control group, relative telomere length decreased in 16 (64%) of 25 patients, compared with three (30%) of ten in the lifestyle intervention group.

	Control group (n=25)	Lifestyle intervention group (n=10)	<i>p</i> value*
Age (years)	63.2 (56.1-69.5)	61.8 (54.8-67.9)	0.94
Weight (kg)	89.4 (78.0-94.8)	82.9 (73.6-94.5)	0.22
BMI (kg/m ²)	26.7 (24.0-29.5)	25.3 (23.9-28.0)	0.29
Blood pressure (mm Hg)			
Systolic	137 (125-148)	140 (124-154)	0.77
Diastolic	75 (69-83)	75 (62-76)	0.27

BMI=body-mass index. *Calculated with Wilcoxon's rank sum test.

Table 1: Median (IQR) baseline characteristics

	Control group (n=25)	Lifestyle intervention group (n=10)	<i>p</i> value*
Relative telomere length (T/S)	-0.03 (-0.05 to 0.03)	0.06 (-0.05 to 0.11)	0.03
Telomerase activity†	-1.08 (-3.25 to 1.86)	-0.25 (-2.25 to 2.23)	0.64
Score for adherence to lifestyle changes	-0.06 (-0.17 to 0.08)	0.22 (0.13 to 0.36)	0.02
PSA concentration (µg/L)	0.7 (-0.40 to 2.80)	1.05 (-7.00 to 5.30)	0.93

Data are median (IQR). T/S=telomere to single-copy gene ratio units. PSA=prostate-specific antigen. *Calculated with Wilcoxon's rank sum test. †Expressed as equivalent of the number of human embryonic kidney 293T cell control per 10 000 peripheral blood mononuclear cells.

Table 2: Changes in outcome measures from baseline after 5 years

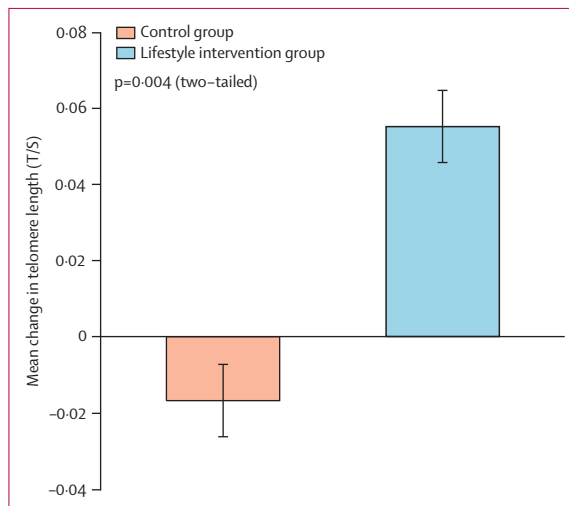


Figure 1: Mean change in relative telomere length over 5 years with lifestyle intervention compared with control
Vertical lines represent 1 SEM. T/S=telomere to single-copy gene ratio units.

Three patients in the lifestyle intervention group underwent conventional treatment during the study: two patients underwent brachytherapy (one 17 months and one 24 months after enrolment), and another had hormonal treatment 25 months after enrolment. Two patients in the control group underwent conventional treatment during the study: one had a radical prostatectomy 15 months after enrolment and the other underwent brachytherapy after 39 months. When the analysis was repeated without these five patients, the increase in relative telomere length in the lifestyle intervention group was slightly smaller (0.05 T/S units) but remained significant compared with the length in the control group ($p=0.05$).

The difference in mean relative telomere length between groups was similar to the median change. In the lifestyle intervention group, mean relative telomere length increased from 0.63 (SD 0.09) T/S units at baseline to 0.69 (SD 0.15) T/S units at 5 years. In the control group, it decreased from 0.71 (SD 0.12) T/S units at baseline to 0.69 (SD 0.15) T/S units at 5 years (difference between groups $p=0.004$; figure 1). Individual changes are shown in figure 2 and non-parametric results for this and other comparisons, done because of small sample size, are shown in table 2.

As expected, participants in the lifestyle intervention group made more lifestyle changes than those in the control group. The median lifestyle score increased by 0.22 units (IQR 0.13 to 0.36) in the lifestyle intervention group compared with a decrease of 0.06 units (-0.17 to 0.08) in the control group ($p=0.02$, table 3). The relative telomere length of three participants in the lifestyle intervention group decreased. The median lifestyle score change for these three patients was 0.13 units (IQR 0.03 to 0.34), compared with 0.29 units (0.21 to 0.56) for those whose relative telomere length increased.

When the data for the two groups were combined to take account of control group participants making some degree of lifestyle changes based on general information obtained outside of the study, we found a significant dose-response relation between the degree of lifestyle change and the extent of change in telomere length. For each percentage point increase in adherence score, the average relative telomere length increased by 0.07 T/S units (95% CI 0.02–0.12, $p=0.005$) after adjustment for age at end of study and length of follow-up.

Age at the end of the study was independently predictive of a shortening in relative telomere length. Each additional year of age was associated with a mean decrease in relative telomere length of 0.005 T/S units ($p=0.007$, table 4). The significance of the association between relative telomere length and adherence to lifestyle intervention in the adjusted model, however, indicates that this variable functions independently of age and in the opposite direction. Postestimation residual analysis showed that the residuals were normally distributed around zero, which supports the fit of the

model to the data. The average coefficient of variation for samples tested for relative telomere length was 3.7%.

The median change in PBMC telomerase activity at 5 years did not differ significantly between the two groups in the non-parametric or parametric analyses (table 2). Because the sample size is small, however, this finding cannot entirely rule out the presence of a difference that our study was underpowered to detect. Residual analysis of the telomerase activity model revealed a poor fit with data on the original scale, which was improved by using the natural log of the outcome. The relative risk for the effect of adherence to the intervention on telomerase activity was non-significant (table 5).

Although PSA values increased slightly in both groups over the 5-year period, the difference in the changes between the two groups was not significant (table 2).

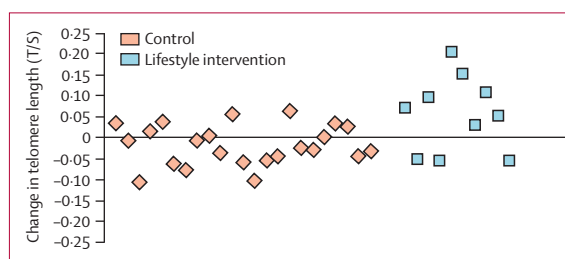


Figure 2: Changes in telomere length for individual participants in the lifestyle intervention and control groups
T/S=telomere to single-copy gene ratio units.

	Control group (n=25)	Lifestyle intervention group (n=10)
Adherence score at baseline		
Mean (SD)	0.60 (0.27)	0.56 (0.27)
Median (IQR)	0.60 (0.32 to 0.72)	0.58 (0.31 to 0.79)
Range	0.25 to 1.21	0.15 to 1.21)
Adherence score at 5 years		
Mean (SD)	0.59 (0.25)	0.78 (0.28)
Median (IQR)	0.55 (0.42 to 0.80)	0.84 (0.51 to 0.96)
Range	0.18 to 1.08	0.35 to 1.20
Change in adherence score		
Mean (SD)	-0.05 (0.30)	0.23 (0.32)
Median (IQR)	-0.06 (-0.17 to 0.08)	0.22 (0.13 to 0.36)
Range	-0.78 to 0.57	-0.44 to 0.70

Table 3: Comparison of adherence to lifestyle changes between groups

	Coefficient (95% CI)*	p value
Adherence to lifestyle changes score	0.07 (0.02 to 0.12)	0.005
Age at end of study	-0.005 (-0.009 to -0.001)	0.007
Length of follow-up	-0.00003 (-0.0001 to -0.00007)	0.61

*Each one-unit change in the predictor indicates an increase or decrease in relative telomere length by the amount of the coefficient, with other predictors held constant.

Table 4: Multivariable regression results for relative telomere length

	Relative risk (95% CI)*	p value
Adherence to lifestyle changes score	0.93 (0.72–1.20)	0.57
Age at end of study	1.00 (0.99–1.01)	0.3
Length of follow-up	1.17 (0.63–2.18)	0.24

*Because the log of telomerase activity was modelled, coefficients are exponentiated and interpreted as relative risks.

Table 5: Multivariable regression for telomerase activity

No patients in either group died as a result of their disease during the 5-year study period. One patient in the lifestyle intervention group died in an accident. No metastases were detected during the observation period.

Discussion

After 5 years, relative telomere length had increased in the lifestyle intervention group and decreased in the control group; the difference between the two groups was significant. We also found a correlation between the degree of adherence to the lifestyle changes and the extent of change in relative telomere length. To our knowledge, this is the first time that any intervention has been associated with significant increases in telomere length when compared with a non-intervention group selected with the same criteria. Our findings are biologically plausible and consistent with earlier studies (panel). Nevertheless, in view of the small sample size and this being a pilot study, we report increases as associations without necessarily proving causation. Additionally, although increases in relative telomere length are thought to be beneficial, the full biological implications remain to be determined in large randomised, controlled trials.

Lifestyle changes in control groups have been important confounders in other studies, such as the Women's Health Initiative.²⁹ Although some participants in the control group in our study did make lifestyle changes based on general information obtained outside the study, the degree of change in the lifestyle intervention group was much greater than that in most other studies. The degree of control group contamination, therefore, was correspondingly lower. Our primary analysis showed a significant difference between the lifestyle intervention and control groups in relative telomere length after 5 years. Additionally, the median lifestyle index score increased in the intervention group over the 5-year period, whereas it decreased in the control group. The lifestyle changes in the control group were, therefore, not sufficient in number or degree to dilute the between-group differences.

We have reported previously that our lifestyle intervention was associated with an increase in telomerase activity after 3 months.¹² At the time we presumed this effect was a beneficial finding because patients showed clinical improvement. We were, however, unable to rule out the possibility that telomerase

activation over such a short time was a compensatory mechanism induced by adverse conditions (eg, a worsening of prostate cancer). An increase in telomerase activity in one context could be viewed as beneficial because it might lead to an increase in relative telomere length, although increases in telomerase activity in normal human cells might be harmful; the combination of increased telomerase activity and short relative telomere length has been correlated with multiple risk factors and, therefore, could be a compensatory reaction to shortened relative telomere length.^{23,30–33} Our finding of increased relative telomere length in the lifestyle intervention group, however, supports the theory of a beneficial rather than compensatory effect.

Increased relative telomere length in longitudinal studies has been proposed to be due mainly to measurement error.³⁴ We agree that the appearance of telomere lengthening can be an artifact in cross-sectional studies, in which measurement is made at only one time point, or in studies that have no control groups for comparison. We, however, included a control group recruited with the same selection criteria as the patients in the lifestyle intervention group and for which laboratory assessment was blinded. Any sources of measurement error, therefore, would probably have affected both study groups equally. A high degree of measurement error would have made it difficult to show significant differences between groups, especially in our small sample, but the average coefficient of variation of the samples in this study was 3.7%. The fact that we found significant differences between groups with parametric and non-parametric tests further decreases the likelihood that the increased relative telomere length in the intervention group was an artifact of measurement error.

Telomere length is controlled by multiple mechanisms, but the lack of significant change in telomerase activity over 5 years in this study suggests it is not one of them. Relative telomere length is maintained in about 85% of all cancers by increases in telomerase activity, but in other cases it is maintained by a mechanism called alternative lengthening of telomeres.³⁵ We did not measure this alternative mechanism, and further research might be warranted. Another possible explanation for the lack of effect on telomerase activity is that telomere maintenance and lengthening might be triggered in the short term by comprehensive lifestyle changes, but once telomere lengthening has occurred, telomerase activity might not need to remain raised. Further exploration of such an effect is needed.

Genetic and non-genetic factors affect the length of telomeres in human beings. Relative telomere length is inversely correlated with age, but substantial variation between individuals of the same age has been reported. Single nucleotide polymorphisms in genes involved in regulation of telomerase activity and relative telomere length have been linked to increased risk of telomere

shortening in white blood cells and to disease risks.^{9,36} Non-genetic factors that affect relative telomere length include nutrition, psychological stress, exercise, and other health-related behaviours.³³ Severe or chronic psychological stress accelerates biological ageing, although the underlying mechanisms are unclear. For instance, perceived chronic emotional stress in mothers has been correlated with short relative telomere length and low telomerase activity in PBMCs.²⁴ In this study, raised concentrations of stress hormones, such as epinephrine, norepinephrine, and cortisol, were associated with short relative telomere length in PBMCs, which supports previous findings.³⁷

Stress-management techniques have been associated with an increased capacity for telomere maintenance. We have previously shown significantly increased telomerase activity after a 3-month meditation retreat at 3 months when compared with matched controls.³⁸ In a cross-sectional study, participants who cited poor concentration (described as mind-wandering) had shorter telomeres across immune cell types (granulocytes and lymphocytes) than did those who reported better concentration, even after adjustment for stress.³⁹ In another cross-sectional study, Hoge and colleagues⁴⁰ compared relative telomere length between men and women who had practiced daily meditation for at least 4 years and in a control group of non-meditators matched for age, sex, education, and history of depression. A trend towards longer relative telomere length in the meditation group than in the control group was seen, although the difference between groups was not significant. In a secondary analysis of women only, they also found significantly longer relative telomere lengths in meditators than in non-meditators.⁴⁰ A study of telephone counselling to lessen stress in survivors of cervical cancer showed a long-term association between patient-reported distress and telomere length. Reduction in distress was associated with increased relative telomere length when women who received counselling and controls were combined, but not when the two groups were compared.⁴¹

The Finnish Diabetes Prevention Study, a longitudinal, randomised, multi-institutional study of a lifestyle intervention for patients with diabetes, showed increased relative telomere length in around two-thirds of participants in the lifestyle intervention group and usual-care control group after an average of 4.5 years.⁴² This study shows the importance of having a control group. Without one the findings could have suggested that the lifestyle intervention caused an increase in telomere length, when, in fact, increases were seen in both groups.

Unhealthy lifestyle factors related to diet and nutrition (including smoking, consumption of processed meat, and high body-mass index) correlate with short relative telomere length.⁴³ By contrast, circulating vitamin D, multivitamin supplementation, and dietary intake of vitamins C and E have been associated with increased relative telomere length.⁴⁴ In a longitudinal study,

Panel: Research in context

Systematic review

At the start of this study, in 2006, we searched PubMed and Google Scholar with the terms, "telomere", "intervention", "clinical trials", "exercise", "stress", "nutrition", "diet", "meditation", and "social support", without limits on date or language of publication. The studies we identified suggested that lifestyle factors affect relative telomere length. The studies retrieved, however, were case reports, cross-sectional studies (which present data for only one specific time point each), and longitudinal studies (without control groups selected with the same criteria as patients in the intervention groups). Nevertheless, in disorders other than prostate cancer, such as Alzheimer's disease and stress-related illness, the findings suggested associations with short telomere lengths.²³ The perception of stress was more strongly correlated with shorter telomere length than objectively measured values.²⁴ Obesity negatively affected relative telomere length in women.²⁵ By contrast, leucocyte telomere length was positively associated with increased physical activity in leisure time, even after adjustment for age, sex, body-mass index, smoking, socioeconomic status, and physical activity at work.²⁶ The association was confirmed in twin pairs discordant for physical activity level: telomeres in active twins were a mean of 88 nucleotides longer than those in less active twins.²⁷ Relative telomere length was significantly shorter in lower than in higher socioeconomic status groups.²⁸ We previously reported that 3 months of comprehensive lifestyle changes were associated with significantly increased telomerase activity in human immune-system cells,¹² which we presumed at the time to be a beneficial finding because of clinical improvements. We could not, however, rule out the possibility that, in view of the short time frame, telomerase activation was a compensatory mechanism induced by adverse conditions (ie, worsening of prostate cancer). Therefore, we investigated the long-term effects of the lifestyle intervention on relative telomere length.

Interpretation

Our findings are consistent with those of earlier studies, but to our knowledge this is the first study of any intervention that has shown a significant change in relative telomere length in human immune-system cells over time when compared with a non-intervention group. We noted a correlation between the degree of positive lifestyle change and increase in telomere length when all participants were assessed together, which supports the internal validity of this study. Although our sample size was small and all participants had early-stage cancer with low risk of metastasis, we believe large, randomised trials to test the validity and applicability of our results are warranted.

decreases in psychological distress, cortisol, dietary fat intake, and glucose were associated with increases in telomerase activity.⁴⁵ In a non-intervention cohort of patients with coronary artery disease, an inverse relationship was found between baseline concentrations of marine ω -3 fatty acids in blood and rate of telomere shortening over 5 years.⁴⁶ A randomised trial of supplementation with ω -3 fatty acids compared with placebo showed no significant differences between groups for telomerase activity or relative telomere length in the primary analysis.⁴⁷ In a secondary analysis, however, the ratio of ω -6 (which is associated with inflammation and oxidative stress) to ω -3 fatty acids in blood was inversely correlated with relative telomere length across both groups.

Oxidative stress preferentially damages telomeric regions over other genomic DNA regions and inhibits telomerase activity *in vitro* in various cell types.⁴⁸ The effects of biochemical mediators on telomerase activity

might contribute to associations between lifestyle factors and relative telomere length.³⁰ In a prospective study of 100 000 people of different ethnic origins with a mean age of 63 years, shorter relative telomere length was associated with death, even after adjustment for demographic and behavioural factors, such as education, smoking, and alcohol consumption. All analyses were controlled for age and sex.⁴⁹ Relative telomere length was positively correlated with level of education and body-mass index and negatively correlated with cigarette smoking and alcohol consumption.⁴⁹

In a study of Mediterranean diet, high baseline relative telomere length significantly predicted notable decreases in bodyweight, body-mass index, waist circumference, and waist-to-height ratio.⁵⁰ Additionally, changes in relative telomere length during the 5-year intervention were inversely associated with changes in these four indices.

Our study is limited by the small sample size and because it was not randomised, which increases the possibility of unknown sources of bias. We kept sampling bias to a minimum as far as possible by use of the same selection criteria for the lifestyle intervention and control groups. The assays for relative telomere length and telomerase activity were done in the same laboratory by staff unaware of patients' group assignments. The groups were similar at baseline for all measured variables. The significant difference in relative telomere length and the significant correlation between adherence to intervention and relative telomere length across groups at 5 years support the internal validity of the study. The average follow-up period for the lifestyle intervention group was longer than in the control group (5.2 vs 4.1 years). Decreases in relative telomere length might have been expected, therefore, because telomere length usually decreases over time. Nevertheless, when we combined groups and adjusted for length of follow-up, duration was not associated with telomere length. The generalisability of our results must also be considered. As our participants had localised, early-stage prostate cancer, deaths or metastasis were not expected and were not seen. However, we measured relative telomere length and telomerase activity in PBMCs rather than in prostate tissue and, therefore, these findings might be applicable to other groups, including men who do not have prostate cancer and women. Five participants received surgery or radiation treatment during the 5-year follow-up period. However, all measurements were made before treatment.

In conclusion, our comprehensive lifestyle intervention was associated with significant increases in relative telomere length in men with early-stage prostate cancer, compared with active surveillance alone. Adherence to these healthy behaviours was also associated with increased relative telomere length when all study participants were assessed together. These results add to existing data (panel) and suggest that further investigation in randomised trials in larger and different populations would be useful.

Contributors

DO, JL, JMC, EE, GW, RM, SJF, JD, NC-W, PRC, and EHB designed the study. DO, JL, JMC, CK, RM, SJF, MJMM, JD, IE, NC-W, PRC, and EHB undertook the research. JL, JMC, EE, GW, SJF, JD, IE, NKH, NC-W, and EHB analysed the data. All authors were involved in the writing of the report.

Conflicts of interest

JL, EE, and EHB were co-founders of Telome Health Inc, a diagnostic company that assesses telomere biology. DO and CK work with Healthways Inc to educate and support people in improving health-related behaviours. The other authors declare that they have no conflicts of interest.

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