1

A natural product telomerase activator as part of a health maintenance program

B. Metabolic and cardiovascular response

Calvin B. Harley[1], Weimin Liu[2], Peter L. Flom[3] and Joseph M. Raffaele[4]

[1]Consultant to TA Sciences.

[2]TA Sciences, Inc., New York, NY.

[3]515 West End Ave., New York, NY, 10024

[4]PhysioAge Systems, LLC, New York, NY.

Word Count: 3600 excluding references/legends/abstract)

Address for correspondence and reprints:

Calvin B. Harley, Ph.D.

1177 Sandalwood Drive,

Murphys, CA, USA 95247

charley@telomehealth.com

Phone #: 650-289-0990

Key Words: telomerase, telomere, health maintenance, TA-65

Abbreviated Title: A Telomerase Activator in Health Maintenance

ABSTRACT

Short average telomere length is associated with low telomerase activity and certain degenerative diseases. Studies in animals and with human cells confirm a causal mechanism for cell or tissue dysfunction triggered by critically short telomeres, suggesting telomerase activation may be an approach to health maintenance. We previously reported on positive immune remodeling in humans taking a commercial health maintenance program, PattonProtocol-1, composed of TA-65® (a natural product-derived telomerase activator) and other dietary supplements (1). In over a 5 year period and an estimated 7000 person-years of use, no adverse events or effects have been attributed to TA-65 by physicians licensed to sell the product. Here we report on changes in metabolic markers measured at baseline (n=97-107 subjects) and every 3-6 months (n=27-59 subjects) during the first 12 months of study. Rates of change per year from baseline determined by mixed effects ANOVA were -3.72 mg/dL for fasting glucose (p=.02), -1.32 mIU/mL for insulin (p=0.01), -13.2 and -11.8 mg/dL for total- and LDL-cholesterol (p=0.002, p=0.002, respectively), -17.3 and -4.2 mm Hg for systolic and diastolic blood pressure (p=0.007 and 0.001, respectively), and -3.6 umole/L homocysteine (p=0.001). In a subset of individuals with bone mineral density (BMD) measured at baseline and 12 months, density increased 2.0% in the spine (p=0.003). We conclude that in addition to apparent positive immune remodeling, PattonProtocol-1 may improve markers of metabolic, bone and cardiovascular health.

228 words

Introduction

Telomeres, the structures that protect the ends of our chromosomes, are fascinating components of our genome. They are complex, dynamic structures composed of DNA, RNA, and protein that play a key role in aging and age-related disease, including cancer (2-4). At a biochemical level, telomeres protect chromosome ends from degradation or recombination events, and facilitate faithful segregation of chromosomes during cell division. Early work over 20 years ago showed that average length of human telomeres in normal somatic (non-tumor, non-germline) cells is a measure of the remaining replicative capacity of those cells (5,6) and that short telomeres are associated with disease, including cardiovascular disease, cancer, HIV/AIDS and Downs Syndrome (6-11). Telomere length in general becomes shorter with each cell division, until one or more telomeres become critically short, triggering cell senescence, loss of normal cell function, genomic instability, and possibly cell death or tumor initiation (12-16).

Telomerase, the enzyme that synthesizes telomeric DNA (17), can slow or even reverse telomere shortening in normal human cells in culture (18,19), and contribute to slowing or reversal of degenerative, age-related disease in animals (14,20-22). In almost all large scale cross-sectional studies in humans, telomere length is inversely correlated with chronological age, but with the recent interest in longitudinal tracking of telomere length in humans, evidence for possible telomere lengthening within an individual is emerging (23-31). Because telomere length can vary within different cell lineages, average TL is a function of the distribution of different cell types and their telomere lengths at any given time. Thus, for

complex tissues, one cannot easily distinguish between true telomere length changes versus a shift over time in subsets of cells in the sampled tissue(s).

In the late 1990s we (Harley and colleagues at Geron Corporation and the Hong Kong University of Science and Technology) conducted a screening program for telomerase activators, which led to the discovery that certain small molecule components of Huang Xi, a traditional Chinese medicine reputed to maintain health, were activators of telomerase (1,22,32-34). Definitive pharmacodynamic studies and randomized, placebo controlled studies with these molecules in humans have not been completed, but the majority of studies to date have suggested that they modestly activate telomerase and/or help maintain telomere length *in vitro* and *in vivo,* and that they have positive functional effects on human cells. In addition, a number of recent studies have suggested that telomerase activation in mice with shortened telomeres and signs of engrained age-related disease can be rescued in part through telomerase activation without signs of increased global cancer risk (14,20-22). Thus, telomerase activation is gaining support as a potential treatment or prevention of degenerative diseases using drugs, and for health maintenance using dietary supplements.

In our first analysis of Patton-Protocol-1, a commercial health maintenance program composed of TA-65® (a natural product-derived telomerase activator), other dietary supplements, and physician counseling), we reported positive immune remodeling over a one-year period relative to baseline values (1). These changes included significant declines in senescent cytotoxic (CD8$^+$/CD28$^-$) T cells, particularly in CMV seropositive subjects. In addition, we found that TA-65 alone increased telomerase in human cells in culture, and in aged mice with shortened telomeres, TA-65 reduced the percentage of cells with short telomeres and

improved the structure of multiple tissues (22). A recent study on the association between telomere length and experimentally induced upper respiratory track viral infection in healthy adults (35) underscored the predictive value of short telomeres for viral infections and clinical illness, especially when telomere length was measured in CD8+/CD28- cells.

Here we report on observed changes from baseline in metabolic and cardiovascular markers, including bone mineral density, from the same set of subjects consuming TA-65 for a one-year period.

Methods

PattonProtocol-1

PattonProtocol-1 was launched in January 2007 by TA Sciences (New York) as a commercial age-management product composed of a natural product-derived telomerase activator (TA-65®, described below), a dietary supplement pack, laboratory testing, and physician counseling. Further details are found in Harley et al (1). All subjects signed a comprehensive Customer Acknowledgement Form. Baseline assays (Table 1) indicated that most individuals were within the normal ranges (data not shown). The consulting physician prescribed new medications for only 3 subjects. There was no qualitative change in the overall conclusions whether these subjects were included or excluded from the analyses. The number of subjects at 3, 6, 9, and 12 months for most tests was 43, 59, 27, and 37, respectively. The age and gender frequencies of the subset at each time-point were similar to those of the total baseline population (n=114; 63±12 years, 72% male).

TA-65®

TA-65® was exclusively licensed from Geron Corporation (Menlo Park) to TA Sciences (NY). It is a single molecule entity with 95% purity by HPLC derived from a proprietary extract of the dried root of Astragalus *membranaceus.* All data in this report are from the original TA-65 product. Post 2011, the purity of the manufactured product has improved from roughly 95% to >98%.

Clinical Laboratory Assays

At baseline and each time-point after initiation of the product blood samples were drawn from each subject (for plasma or serum assays) and shipped the same day at ambient temperature to appropriate commercial, academic, or contract laboratories. Assays for standard blood counts, blood chemistry (including glucose, insulin, hemoglobin A1C, and serum vitamins), specialized immune subsets (CD8$^+$, CD28$^-$ and CD95, both gated on CD8$^+$), CMV antibody titer, and inflammation markers (homocysteine and cardio C-reactive protein) were all conducted at a standard clinical laboratory (either Quest Diagnostics, Bio-Reference Laboratory, or UCLA Clinical Laboratories and Pathology Services). Data related to the immune markers have been reported previously [1].

Bone Mineral Density

Bone mineral density (BMD) was analyzed at baseline and at 12 months on the first available 31 subjects at PhysioAge Medical Group (New York, NY) to reach the 12 month time-point. Full AP spine L1-L4 and dual proximal femur (neck, trochanter, and dual mean) bone density data was generated using a DEXA (dual-energy x-ray absorptiometry) instrument (GE Healthcare).

7

Statistics

Since data were collected primarily as a hypothesis-generating exercise and the clients were not participating in a controlled clinical study, statistical analysis was not formally defined *a priori*. Baseline data was primarily analyzed for cross-sectional donor age effects to compare results with the "PattonProtocol-1" population. Where significance of linear regression lines is reported for cross-sectional analysis, the F-distribution was used to calculate the probability that the observed correlation occurred by chance and p-values are reported. ANOVA modeling of the 1 year change in values used a multilevel or mixed model with random intercepts and slopes. Each person has an individual starting point and an individual slope for change over time on treatment.   For each dependent variable, we first graphed the data, looking for outliers and for points that indicated the presence of disease. Erroneous data entries were corrected, as were entries indicating either a failure to follow protocol (e.g., glucose over 125 or insulin over 20 indicating non-fasting). SBP over 160 or DBP over 100 was taken to indicate hypertension. These relatively high cutoffs for SBP and DBP were chosen so as not to exclude those individuals with possible metabolic syndrome but not currently carrying a diagnosis of hypertension or on anti-hypertensive medication. Graphs were examined for nonlinear trends and multilevel models were used to account for dependence in the data. For each dependent variable we tested a relatively full model that included fixed effects of time, time squared, baseline age, sex, telomere length and CD8+CD28- T-cells using unstructured covariance. Time, age and sex were kept in all models. Other variables were eliminated if the parameter estimate was close to 0 and the p value above 0.10. The initial model included random effects for intercept, slope and slope squared. The random effect of slope squared was deleted if the fixed effect for time squared was deleted. The resultant model was tested with three different covariance

structures, unstructured, compound symmetry, and auto-regressive, and the choice made was based on the Akaike Information Criterion.

Results and Discussion

Key Baseline Observations

Most subjects for this study were relatively affluent individuals motivated to maintain personal health, and thus may not reflect the general population. Table 1 shows mean values, standard deviations, count, slope and R^2 from linear regression on subject age, and the statistical significance of the slope for the baseline tests investigated in this report. As expected, this population showed a statistically significant increase as a function of client age in fasting blood glucose (0.33 mg/dL/year), systolic blood pressure (0.51 mmHg/year), and homocysteine (0.066 µmol/L/year), and a significant decrease in DHEA sulfate (-2.6 mcg/dL/y). The increase in serum folate (0.12 ng/ml/y) was unexpected but may reflect the relatively greater use of folic acid containing multivitamins in older compared to younger prior to starting the Protocol.

As described previously (1), by cross-sectional analysis, telomere length declined in a characteristic manner in this population as a function of client age in both lymphocytes and granulocytes (55 and 34 bp/year; p=10^{-15} and 10^{-8}, respectively).

Changes from Baseline While on PattonProtocol-1

The baseline analysis confirmed that test data from TA Sciences subjects were similar to those reported in other elderly groups and provided a benchmark (36) against which we could plot relative changes with time on the PattonProtocol-1. As discussed earlier, it is important to

note that we cannot determine the contribution of any single component of the PattonProtocol-1 to the observed changes. Changes from baseline that are unusual in comparison to other dietary supplement studies could be due to TA-65® and its effects on telomeres, but it is also possible that placebo effects or the unique combination of ingredients in the TA Sciences dietary supplement pack also play a role. Medical/lifestyle questionnaires at baseline and post the 12 month period for each client were used to record changes in medications, diet or exercise while on the PattonProtocol-1. No clients reported significant changes in diet or exercise, but where clients reported a change in drug therapy, the test results which the physician felt could be significantly impacted by the medication change were excluded from the analysis. Since this study did not have a control group, seasonal effects could also be a factor, but since the 114 clients studied here were enrolled over a 2.5 year period, it is unlikely that seasonal effects influenced the observed changes from baseline. With these caveats in mind, we summarize the most interesting findings below.

Observed Changes from Baseline: Blood sugar, Cholesterol, Blood Pressure, Inflammatory Markers, Vitamin levels, and Bone Mineral Density

Fasting glucose and insulin levels both declined with time in clients on the PattonProtocol-1 (at a rate of -3.72 mg/dL and -1.32 mIU/mL per year, respectively; Table 2). Decreases of this magnitude for fasting glucose are significant relative to the mean baseline values and the donor-age dependent change per year in the baseline population (98 mg/dL and +0.34 mg/dL/year, respectively, from Table 2). In the general population, Sehl et al. (36) reports 0.5-1.5 mg/dL//year increase in fasting glucose. On a relative basis, PattonProtocol-1 apparently reversed ≈11 years of mean increase in fasting glucose. The concomitant reduction

in fasting glucose and insulin is indicative of an improvement in insulin sensitivity. Shorter leukocyte telomere length has been associated with insulin resistance, oxidative stress, and metabolic syndrome (37-39). It is possible that the combination of antioxidants and increased telomerase activation improved insulin sensitivity in our subjects, though the exact mechanism is unknown.

Total and LDL cholesterol also decreased significantly. At 12 months, the 13.2 mg/dL and 11.8 mg/dL decreases in total and LDL cholesterol, respectively, were statistically significant (p=0.002 for both,) and large compared to the small decrease associated with client age in this population (-0.29mg/dL/y and -0.37 mg/dL/y, respectively). Cholesterol generally increases with age (40), possibly due to reduced clearance of LDL from circulation and reduction in conversion of cholesterol to bile acids, but some studies have suggested a flat profile with aging or even a reduction in total cholesterol with age attributed to poor health(41). Since the baseline average total cholesterol in the PattonProtocol-1 clients was borderline high (mean 188mg/dL, Table 2), we consider the declines of ≈10mg/dL in total and LDL cholesterol to be beneficial as it is comparable to what has been achieved in successful diet and exercise programs (42). The mechanism of the reduction in cholesterol is unclear, but given no subjects reported changes in their exercise or dietary habits, it is possible that increased telomerase activation from the PattonProtocol1 contributed. A small study demonstrated that increased telomerase activity in PBMCs is significantly associated with a decrease in LDL cholesterol (43).

Systolic and diastolic blood pressure declined 17.8 mm/Hg and 4.2 mm/Hg, respectively over the 1 year period (Table 2). In the baseline population, DBP was relatively flat but SBP increased 0.51 mm Hg/year (Table 2), comparable to that seen in other studies (44) suggesting

11

that the PattonProtocol-1 may have reversed >30 years of increase in SBP. The decrease in SBP

is comparable to first line therapy with diuretics which confer a significant reduction in

cardiovascular events. Using the Framingham 10-year CVD event calculator, these reductions

would cause a 25% reduction in 10 year risk for males (from 8% to 6%). Mechanisms are

difficult to determine given the observational nature of the study, but telomere attrition has

been associated with higher blood pressure and increased aldosterone production(45) which in

turn can increase oxidative stress and blood pressure by reducing nitric oxide production. If the

reduction in percent short telomeres and senescent cytotoxic T-cells we reported previously

also resulted in decreased inflammatory cytokines (such as TNF-α and IL-6), this process could

have been reversed in our cohort and explain the reduction in blood pressure. This reversal of

inflammation along with increased telomerase activation could rescue presenescent

endothelial cells and increase the production of nitric oxide which could reduce SBP. The

supplement packs could also have contributed.

The changes we observed could not continue year after year. To explore whether the

slope of observed trends is constant regardless of the starting level of the dependent variable

we generated sub-group spline graphs. Figure 1A shows a graph of fasting glucose levels over

time. Levels of glucose at baseline were divided into three groups: Low (below 88), high (above

102) and middle (the middle 50%,). Splines were fit to each group. The overall trend was for

those in the highest group to have relatively large declines, those in the mid-range smaller

declines, and those in the lowest group a slight increase. Figure 1B is similar for insulin, except

here groups were set based on medical expertise: High (above 10), low (below 5) and middle

and patterns were similar to those for glucose. These patterns suggest an improvement in

insulin sensitivity. Insulin resistance is a cardinal element of the metabolic syndrome and

thought to be a result of increased inflammation which the PattonProtocol1 might have reduced. From a clinical standpoint, this pattern of effectiveness is similar to metformin, a first-line therapy for pre-diabetes.

The same pattern is demonstrated by the spline graphs for LDL cholesterol (Figure 1C) and systolic BP (Fig 1D). The cholesterol groups were divided by the treatment categories defined by the National Cholesterol Education Program: Low (< 100) middle (100-130) and high > 130), and the SBP groups were tertiles, where high was above 138, low below 120, and middle 120-138. The moderating trends show biomarker improvements in those that need it, with little or no change in those within the normal biomarker range. The moderating trend for SBP fits with the mechanisms of SBP reduction discussed above in which a dysfunctional endothelium (in those with higher baseline SBP) is repaired rather than a direct antihypertensive effect which could result in hypotension. One might see these moderating trends as regression to the mean, but the decreasing variance over time that is evident from all four of the spline graphs argues against this.

Homocysteine and C-reactive protein (CRP) are significant markers of inflammation and are associated with arterial dysfunction and cardiovascular disease risk (16,46). We observed significant reductions in both homocysteine (Table 2) and CRP (not shown) while clients were on the PattonProtocol-1, but for CRP, a number of individuals had acute spikes in CRP, possibly related to infections, which confounded the analysis. The 3.6 μmol/mL decrease in homocysteine in clients on the Protocol (p=<0.001) is dramatic compared to the general increase with age (0.066 μmol/mL/y). It is likely that folate and vitamins B12 and B6 in the supplement packs played a role in the decline in homocysteine, but a number of individuals

13

who were taking potent supplement packs before initiation of the protocol also showed

reductions in homocysteine, including some subjects whose folate levels actually decreased

(data not shown). It is also possible that other components of the Protocol reduced

inflammatory response and improved health through elongation of short telomeres and

remodeling of the immune system. If reduction in inflammation and cardiovascular risk could

be achieved through PattonProtocol-1 without high levels of folate found in some dietary

supplement programs, this would be beneficial since supra-physiological levels of folate could

be detrimental (47,48).

Finally, bone mineral density was measured at baseline and 12 months in an essentially

random subset of individuals (n=31). Of the 31 subjects, 26 were either not on hormone

therapy or made no change in hormone therapy during the one year period. The remaining 5

subjects who changed therapy were excluded from the analysis. Of the 26 analyzed subjects,

one had osteoporosis and 4 others had varying degrees of osteopenia. In the overall group,

there was a 2.0% increase in AP spine L1-L4 BMD (p=0.003), Table 3. This is as large an increase

in BMD as seen in a three year study of calcium and vitamin D therapy in comparable subjects

without osteoporosis(49) and certainly contrasts with the expected ≈ 0.5% decrease per year.

There were no significant changes in hip BMD (data not shown). Moreover, because the

average baseline vitamin D levels were well into the normal range, it is less likely that the

approximately 20% increase in vitamin D contributed significantly to the BMD increase.   A

plausible alternative explanation for this improvement in BMD could be related to the emerging

understanding of the relationship among hyperlipidemia, T-cells, and bone density (50). The

reduction in senescent suppressor cells we previously reported (1) combined with the reduction

in LDL cholesterol could decrease the number of activated T-cells in the bone marrow and

thereby reduce activated RankL and other inflammatory cytokines. This would reduce

osteoclast activity and potentially explain the improved bone density.

Discussion and Conclusions

This study adds to the observations from 2011 that PattonProtocol-1, containing

conventional supplements plus TA-65, a small molecule telomerase activator, has a positive

impact on biomarkers of aging or age-related disease. The initial observational study of roughly

50 subjects on product(1) focused on results from independent diagnostic laboratory testing of

immunological markers over a one-year period, while here we focus on similar laboratory

testing of metabolic biomarkers and bone mineral density in the same cohort over the same 1

year period. We found reductions in fasting blood sugar, insulin, cholesterol, blood pressure,

and homocysteine, and increases in bone mineral density, all considered positive health

changes. These data suggest that PattonProtocol-1 (TA-65 in combination with other

supplements and physician counseling), improves health and may reduce risk of morbidity and

mortality.

The major limitation of these observational studies is that they are not randomized,

placebo-controlled trials with defined dosing of TA-65. Such a study is in progress, but the

observation that TA-65 was primarily beneficial to CMV+ humans who were "blind" to their

CMV status(1), and that TA-65 had positive effects when given to aged telomerase-positive but

not telomerase negative mice (51), suggests that TA-65 is an active molecule that should be

further investigated.

Because telomerase may extend the lifespan of relatively rare pre-malignant cells which

otherwise might die due to critical shortening of telomeres, it is possible that a telomerase

activator could increase cancer risk in some individuals. However, prevention of critical shortening of telomeres in multiple tissues throughout an aging human by telomerase activation could be a net tumor suppressive mechanism by reducing genomic instability and maintaining health of normal tissues. Much larger controlled studies will be needed to assess the potential risk and benefits of TA-65 in humans. Subjects taking TA-65 or any dietary supplement should consult their doctor and carefully consider the product's potential risks and benefits. There had been approximately 260 person-years of TA-65 dosing, mainly in the 5-50 mg/day range through June 2010, with no reports of new diagnoses of cardiovascular disease or cancer. This represented age-adjusted incidence rates significantly below the average US rates for CVD and cancer. As of June 2013, there are now an estimated 7000+ person-years of TA-65 exposure at an average 50mg TA-65 dose equivalence. Disease and mortality was not formally tracked, but TA Sciences reports few if any adverse events, and no cases of adverse events being attributed to TA-65 by the subjects' doctors. In the overall (all ages) US population, 7000 person years of life would entail about 30 new diagnoses each for cancer and CVD, and about 60 deaths, based on data from the Centers for Disease Control and Prevention. Because of lack of matching demographics for the study population, as well as lack of exact disease and mortality data, we cannot make a reliable comparison of incidence rates in the study population versus the general population, but the data to date do not point to increased risk of morbidity or mortality in subjects taking TA-65. In conclusion, TA-65, a moderate telomerase activator, is a novel dietary supplement which may enhance one's healthspan.
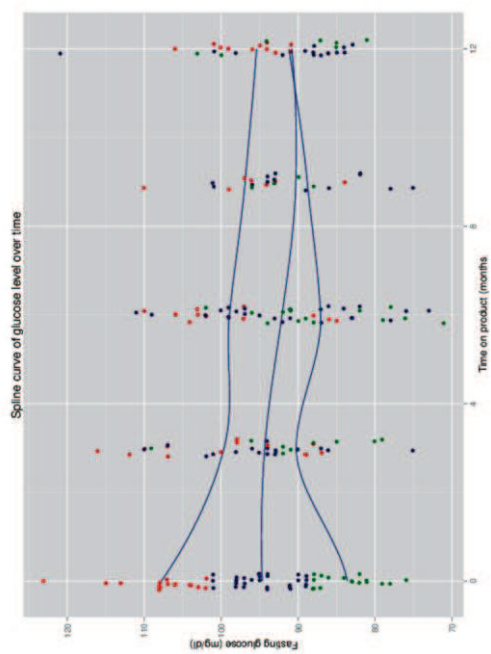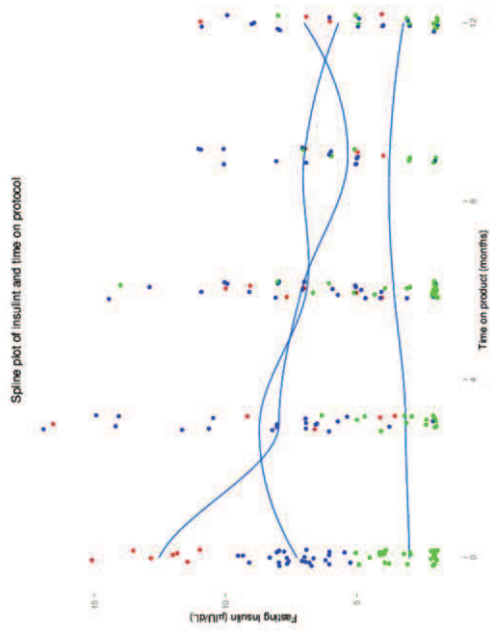
Acknowledgements

Conflicts of interests: CBH is one of the inventors of TA-65, is a paid consultant to TA Sciences, and is the Chief Scientific Officer of Telome Health, Inc, which has a license arrangement with TA Sciences. CBH owns stock and stock options in Telome Health. WL is an employee of TA Sciences. JMR offers TA-65 in his office and is on TA Sciences Scientific Advisory Board. PLF declared no conflict of interest.

17

Figure Legends

Spline curve of glucose level over time

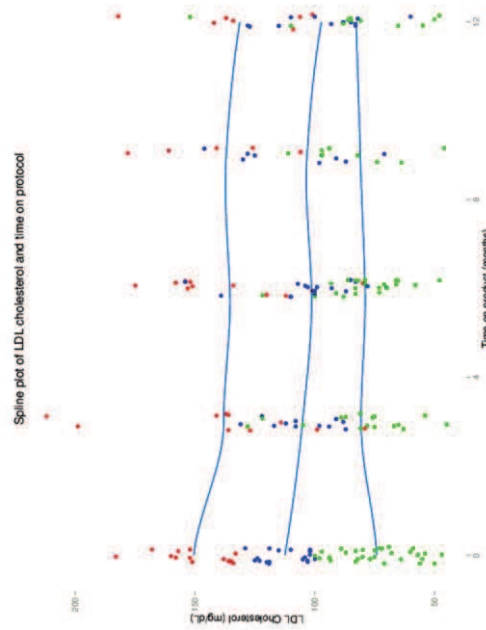Spline plot of insulint and time on protocol

Figure 1. Spline graphs based on the multi-level ANOVA model, representing the change over time (baseline to 12 months) for (A) fasting glucose, (B) serum insulin, (C) LDL cholesterol, and (D) systolic blood pressure. In each panel, dots represent subjects on PattonProtocol-1. To avoid overplotting of symbols, a small amount of random noise (jitter) was added to each plot. Subjects with low, mid, and high baseline values are colored green, blue, and red, respectively. The cut-off values for these subgroups are given in the text. The shaded regions around each spline represent the 95% confidence intervals.

REFERENCES

1. Harley, C.B., Liu, W., Blasco, M., Vera, E., Andrews, W.H., Briggs, L.A. and Raffaele, J.M. (2011) A natural product telomerase activator as part of a health maintenance program. *Rejuvenation research*, **14**, 45-56.

2. Lin, J., Epel, E. and Blackburn, E. (2012) Telomeres and lifestyle factors: roles in cellular aging. *Mutation research*, **730**, 85-89.

3. Kropski, J.A., Lawson, W.E., Young, L.R. and Blackwell, T.S. (2013) Genetic studies provide clues on the pathogenesis of idiopathic pulmonary fibrosis. *Disease models & mechanisms*, **6**, 9-17.

4. Aubert, G., Baerlocher, G.M., Vulto, I., Poon, S.S. and Lansdorp, P.M. (2012) Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. *PLoS genetics*, **8**, e1002696.

5. Harley, C.B., Futcher, A.B. and Greider, C.W. (1990) Telomeres shorten during ageing of human fibroblasts. *Nature*, **345**, 458-460.

6. Allsopp, R.C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E.V., Futcher, A.B., Greider, C.W. and Harley, C.B. (1992) Telomere length predicts replicative capacity of human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 10114-10118.

7. Vaziri, H., Dragowska, W., Allsopp, R.C., Thomas, T.E., Harley, C.B. and Lansdorp, P.M. (1994) Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9857-9860.

8. Vaziri, H., Schachter, F., Uchida, I., Wei, L., Zhu, X., Effros, R., Cohen, D. and Harley, C.B. (1993) Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. *American journal of human genetics*, **52**, 661-667.

9. Counter, C.M., Avilion, A.A., LeFeuvre, C.E., Stewart, N.G., Greider, C.W., Harley, C.B. and Bacchetti, S. (1992) Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *The EMBO journal*, **11**, 1921-1929.

10. Counter, C.M., Hirte, H.W., Bacchetti, S. and Harley, C.B. (1994) Telomerase activity in human ovarian carcinoma. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 2900-2904.

11. Counter, C.M., Botelho, F.M., Wang, P., Harley, C.B. and Bacchetti, S. (1994) Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed human B lymphocytes. *Journal of virology*, **68**, 3410-3414.

12. Perera, S.A., Maser, R.S., Xia, H., McNamara, K., Protopopov, A., Chen, L., Hezel, A.F., Kim, C.F., Bronson, R.T., Castrillon, D.H. *et al.* (2008) Telomere dysfunction promotes genome instability and metastatic potential in a K-ras p53 mouse model of lung cancer. *Carcinogenesis*, **29**, 747-753.

13. Artandi, S.E. and DePinho, R.A. (2010) Telomeres and telomerase in cancer. *Carcinogenesis*, **31**, 9-18.

14. Sahin, E. and Depinho, R.A. (2010) Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*, **464**, 520-528.

15. de Lange, T. (2009) How telomeres solve the end-protection problem. *Science*, **326**, 948-952.

16. Ridker, P.M., MacFadyen, J., Cressman, M. and Glynn, R.J. (2010) Efficacy of rosuvastatin among men and women with moderate chronic kidney disease and elevated high-sensitivity C-reactive protein: a secondary analysis from the JUPITER (Justification for the Use of Statins in Prevention-an Intervention Trial Evaluating Rosuvastatin) trial. *Journal of the American College of Cardiology*, **55**, 1266-1273.

17. Blackburn, E.H., Greider, C.W. and Szostak, J.W. (2006) Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nature medicine*, **12**, 1133-1138.

18. Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S. and Wright, W.E. (1998) Extension of life-span by introduction of telomerase into normal human cells. *Science*, **279**, 349-352.

19. Jiang, X.R., Jimenez, G., Chang, E., Frolkis, M., Kusler, B., Sage, M., Beeche, M., Bodnar, A.G., Wahl, G.M., Tlsty, T.D. *et al.* (1999) Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. *Nature genetics*, **21**, 111-114.

20. Jaskelioff, M., Muller, F.L., Paik, J.H., Thomas, E., Jiang, S., Adams, A.C., Sahin, E., Kost-Alimova, M., Protopopov, A., Cadinanos, J. *et al.* (2011) Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*, **469**, 102-106.

21. Bernardes de Jesus, B., Vera, E., Schneeberger, K., Tejera, A.M., Ayuso, E., Bosch, F. and Blasco, M.A. (2012) Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO molecular medicine*, **4**, 691-704.

22. Bernardes de Jesus, B.B., Schneeberger, K., Vera, E., Tejera, A., Harley, C.B. and Blasco, M.A. (2011) The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging cell*, **10**, 604-621.

23. Svenson, U., Nordfjall, K., Baird, D., Roger, L., Osterman, P., Hellenius, M.L. and Roos, G. (2011) Blood cell telomere length is a dynamic feature. *PloS one*, **6**, e21485.

24. Epel, E.S., Merkin, S.S., Cawthon, R., Blackburn, E.H., Adler, N.E., Pletcher, M.J. and Seeman, T.E. (2009) The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging*, **1**, 81-88.

25. Jacobs, T.L., Epel, E.S., Lin, J., Blackburn, E.H., Wolkowitz, O.M., Bridwell, D.A., Zanesco, A.P., Aichele, S.R., Sahdra, B.K., MacLean, K.A. *et al.* (2011) Intensive meditation training, immune cell telomerase activity, and psychological mediators. *Psychoneuroendocrinology*, **36**, 664-681.

26. Farzaneh-Far, R., Lin, J., Epel, E.S., Harris, W.S., Blackburn, E.H. and Whooley, M.A. (2010) Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. *JAMA : the journal of the American Medical Association*, **303**, 250-257.

27. Kiecolt-Glaser, J.K., Epel, E.S., Belury, M.A., Andridge, R., Lin, J., Glaser, R., Malarkey, W.B., Hwang, B.S. and Blackburn, E. (2013) Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: A randomized controlled trial. *Brain, behavior, and immunity*, **28**, 16-24.

28. Ho, R.T., Chan, J.S., Wang, C.W., Lau, B.W., So, K.F., Yuen, L.P., Sham, J.S. and Chan, C.L. (2012) A randomized controlled trial of qigong exercise on fatigue symptoms, functioning, and telomerase activity in persons with chronic fatigue or chronic fatigue syndrome. *Annals of behavioral medicine : a publication of the Society of Behavioral Medicine*, **44**, 160-170.

29. Wolkowitz, O.M., Mellon, S.H., Epel, E.S., Lin, J., Reus, V.I., Rosser, R., Burke, H., Compagnone, M., Nelson, J.C., Dhabhar, F.S. *et al.* (2012) Resting leukocyte telomerase activity is elevated in major depression and predicts treatment response. *Molecular psychiatry*, **17**, 164-172.

30. Lavretsky, H., Epel, E.S., Siddarth, P., Nazarian, N., Cyr, N.S., Khalsa, D.S., Lin, J., Blackburn, E. and Irwin, M.R. (2013) A pilot study of yogic meditation for family dementia caregivers with depressive symptoms: effects on mental health, cognition, and telomerase activity. *International journal of geriatric psychiatry*, **28**, 57-65.

31. Epel, E. (2012) How "reversible" is telomeric aging? *Cancer prevention research*, **5**, 1163-1168.

32. Fauce, S.R., Jamieson, B.D., Chin, A.C., Mitsuyasu, R.T., Parish, S.T., Ng, H.L., Kitchen, C.M., Yang, O.O., Harley, C.B. and Effros, R.B. (2008) Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. *Journal of immunology*, **181**, 7400-7406.

33. Yung, L.Y., Lam, W.S., Ho, M.K., Hu, Y., Ip, F.C., Pang, H., Chin, A.C., Harley, C.B., Ip, N.Y. and Wong, Y.H. (2012) Astragaloside IV and cycloastragenol stimulate the phosphorylation of extracellular signal-regulated protein kinase in multiple cell types. *Planta medica*, **78**, 115-121.

34. Zhu, J., Lee, S., Ho, M.K., Hu, Y., Pang, H., Ip, F.C., Chin, A.C., Harley, C.B., Ip, N.Y. and Wong, Y.H. (2010) In vitro intestinal absorption and first-pass intestinal and hepatic metabolism of cycloastragenol, a potent small molecule telomerase activator. *Drug metabolism and pharmacokinetics*, **25**, 477-486.

35. Cohen, S., Janicki-Deverts, D., Turner, R.B., Casselbrant, M.L., Li-Korotky, H.S., Epel, E.S. and Doyle, W.J. (2013) Association between telomere length and experimentally induced upper respiratory viral infection in healthy adults. *JAMA : the journal of the American Medical Association*, **309**, 699-705.

36. Sehl, M.E. and Yates, F.E. (2001) Kinetics of human aging: I. Rates of senescence between ages 30 and 70 years in healthy people. *The journals of gerontology. Series A, Biological sciences and medical sciences*, **56**, B198-208.

37. Sahin, E., Colla, S., Liesa, M., Moslehi, J., Muller, F.L., Guo, M., Cooper, M., Kotton, D., Fabian, A.J., Walkey, C. *et al.* (2011) Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature*, **470**, 359-365.

38. Haendeler, J., Drose, S., Buchner, N., Jakob, S., Altschmied, J., Goy, C., Spyridopoulos, I., Zeiher, A.M., Brandt, U. and Dimmeler, S. (2009) Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. *Arteriosclerosis, thrombosis, and vascular biology*, **29**, 929-935.

39. Rentoukas, E., Tsarouhas, K., Kaplanis, I., Korou, E., Nikolaou, M., Marathonitis, G., Kokkinou, S., Haliassos, A., Mamalaki, A., Kouretas, D. *et al.* (2012) Connection between telomerase activity in PBMC and markers of inflammation and endothelial dysfunction in patients with metabolic syndrome. *PloS one*, **7**, e35739.

40. Berns, M.A., de Vries, J.H. and Katan, M.B. (1988) Determinants of the increase of serum cholesterol with age: a longitudinal study. *International journal of epidemiology*, **17**, 789-796.

41. Volpato, S., Zuliani, G., Guralnik, J.M., Palmieri, E. and Fellin, R. (2001) The inverse association between age and cholesterol level among older patients: the role of poor health status. *Gerontology*, **47**, 36-45.

42. Kelley, G.A., Kelley, K.S., Roberts, S. and Haskell, W. (2012) Comparison of aerobic exercise, diet or both on lipids and lipoproteins in adults: a meta-analysis of randomized controlled trials. *Clinical nutrition*, **31**, 156-167.

43.   Ornish, D., Lin, J., Daubenmier, J., Weidner, G., Epel, E., Kemp, C., Magbanua, M.J., Marlin, R., Yglecias, L., Carroll, P.R. *et al.* (2008) Increased telomerase activity and comprehensive lifestyle changes: a pilot study. *The lancet oncology*, **9**, 1048-1057.

44.   Franklin, S.S., Gustin, W.t., Wong, N.D., Larson, M.G., Weber, M.A., Kannel, W.B. and Levy, D. (1997) Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation*, **96**, 308-315.

45.   Benetos, A., Gardner, J.P., Kimura, M., Labat, C., Nzietchueng, R., Dousset, B., Zannad, F., Lacolley, P. and Aviv, A. (2005) Aldosterone and telomere length in white blood cells. *The journals of gerontology. Series A, Biological sciences and medical sciences*, **60**, 1593-1596.

46.   Graham, I.M. and O'Callaghan, P. (2002) Vitamins, homocysteine and cardiovascular risk. *Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy*, **16**, 383-389.

47.   Ulrich, C.M. and Potter, J.D. (2006) Folate supplementation: too much of a good thing? *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, **15**, 189-193.

48.   Sauer, J., Mason, J.B. and Choi, S.W. (2009) Too much folate: a risk factor for cancer and cardiovascular disease? *Current opinion in clinical nutrition and metabolic care*, **12**, 30-36.

49.   Dawson-Hughes, B., Harris, S.S., Krall, E.A. and Dallal, G.E. (1997) Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *The New England journal of medicine*, **337**, 670-676.

50.   Graham, L.S., Tintut, Y., Parhami, F., Kitchen, C.M., Ivanov, Y., Tetradis, S. and Effros, R.B. (2010) Bone density and hyperlipidemia: the T-lymphocyte connection. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, **25**, 2460-2469.

51.   Bernardes de Jesus, B., Schneeberger, K., Vera, E., Tejera, A., Harley, C.B. and Blasco, M.A. (2011) The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging cell*, **10**, 604-621.

25

Table 1.

Cross-Sectional Baseline Values for Metabolic and Cardiovascular Biomarkers and Relationship to Age

The mean, standard deviation, and linear regression statistics for test values vs age are provided for all baseline subjects (N=number). [1]Slope (m) of the linear regression line of test value versus age of subjects. [2]P-value for significance of the regression line slope. P-values less than 0.05 are shaded.

	AGE (Year)	Glucose (mg/dL)	Serum Insulin (µIU/mL)	DHEA Sulfate (mcg/dL)	Cholesterol (Total) (mg/dL)	Cholesterol (LDL) (mg/dL)
Mean	62.5	97	7.1	148	188	106
S.D.	12.5	15	7.0	131	38	34
N	114	107	107	106	105	104
m[1]	NA	0.33	0.025	-2.6	-0.29	-0.37
R-square	NA	0.08	0.002	0.06	0.01	0.02
p-value	NA	0.0046[2]	0.64	0.011	0.32	0.16
	SBP (mmHg)	DBP (mmHg)	Homo-cysteine (µmol/L)	Serum Folate (ng/mL)	Vit.D- 25-OH total (ng/mL)	Vit. B12 (pg/mL)
Mean	128	77	11	17	40	786
S.D.	19	10	3.6	5.8	16	409
N	97	97	107	105	107	105
m	0.51	-0.018	0.066	0.12	0.11	6.1
R-square	0.108	0.000	0.05	0.065	0.007	0.036
p-value	0.0010	0.83	0.016	0.0086	0.38	0.053

Table 2

Multi-Level ANOVA Analysis

Shown are the primary outputs from the model as described in the Methods Section.

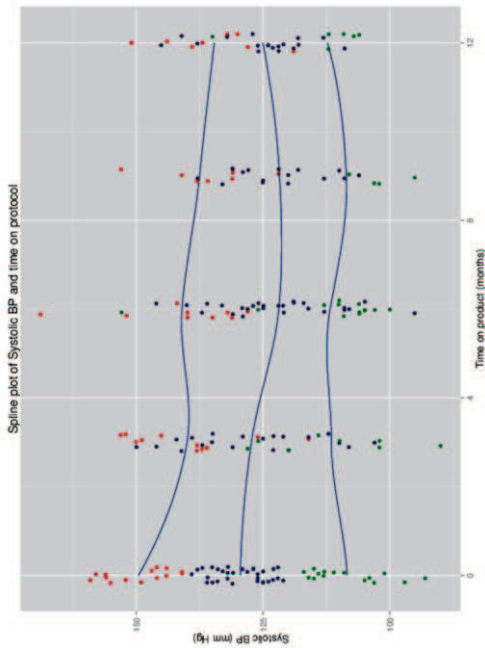| Dependent Variable	Intercept	Time on program (year)	Baseline Age (year)	Female
Glucose	84.32 (< 0.001)	-3.72 (0.02)	0.18 (0.01)	-3.03 (0.15)
Insulin	3.94 (0.07)	-1.32 (0.01)	0.04 (0.18)	-1.68 (0.07)
Total cholesterol	196.89 (<0.001)	-13.2 (0.002)	-0.23 (0.49)	7.59 (0.44)
LDL Cholesterol	123.49 (<0.001)	-11.8 (0.002)	-0.32 (0.27)	1.52 (0.86)
SBP	114.62 (< 0.001)	-17.3 (0.007)	0.23 (0.06)	-10.05 (0.005)
DBP	85.59 (< 0.001	-4.2 (0.001)	-0.11 (0.26)	-4.84 (0.08)
Homocysteine	8.97 (<0.001)	-3.6 (<0.001)	0.04 (0.10)	-0.94 (0.21)
Vitamin B12	382.56 (0.09)	72.4 (0.27)	6.88 (0.04)	-35.90 (0.71)
Vitamin D	27.2 (<0.001)	17.6 (0.008)	0.21 (0.06)	-4.83 (0.13)

Table 3.

Bone Mineral Density (L1-L4)

BMD (g/cm^3) data is reported for 26 evaluable subjects (individuals who changed hormone therapy during the 12-month period were excluded). P-values less than 0.05 are shaded. Average age of males at baseline was 64.9 ± 12.0 and that of females was 58.3 ± 5.1.

	All Subjects (n=26)	Males (n=21)	Females (n=5)
Baseline	1.239 ± 0.186	1.2720 ± 0.171	1.101 ± 0.202
12 Months	1.263 ± 0.202	1.298 ± 0.192	1.118 ± 0.198
Abs. Change	$+0.025 \pm 0.275$	$+0.026 \pm 0.257$	$+0.017 \pm 0.283$
Relative Change	2.0%	2.1%	1.5%
P-value	0.0034	0.0070	0.3271

Spline plot of Systolic BP and time on protocol

A natural product telomerase activator as part of a health maintenance program

B. Metabolic and cardiovascular response

Calvin B. Harley[1], Weimin Liu[2], Peter L. Flom[3] and Joseph M. Raffaele[4]

[1]Consultant to TA Sciences.

[2]TA Sciences, Inc., New York, NY.

[3]515 West End Ave., New York, NY, 10024

[4]PhysioAge Systems, LLC, New York, NY.

Word Count: 3300 excluding references/legends/abstract)

Address for correspondence and reprints:

Calvin B. Harley, Ph.D.

1177 Sandalwood Drive,

Murphys, CA, USA 95247

cbharley99@gmail.com

Phone #: 209-728-2821

Key Words: telomerase, telomere, health maintenance, TA-65

Abbreviated Title: A Telomerase Activator in Health Maintenance

ABSTRACT

Short average telomere length is associated with low telomerase activity and certain degenerative diseases. Studies in animals and with human cells confirm a causal mechanism for cell or tissue dysfunction triggered by critically short telomeres, suggesting telomerase activation may be an approach to health maintenance. We previously reported on positive immune remodeling in humans taking a commercial health maintenance program, PattonProtocol-1, composed of TA-65® (a natural product-derived telomerase activator) and other dietary supplements (1). In over a 5 year period and an estimated 7000 person-years of use, no adverse events or effects have been attributed to TA-65 by physicians licensed to sell the product. Here we report on changes in metabolic markers measured at baseline (n=97-107 subjects) and every 3-6 months (n=27-59 subjects) during the first 12 months of study. Rates of change per year from baseline determined by mixed effects ANOVA were -3.72 mg/dL for fasting glucose (p=.02), -1.32 mIU/mL for insulin (p=0.01), -13.2 and -11.8 mg/dL for total- and LDL-cholesterol (p=0.002, p=0.002, respectively), -17.3 and -4.2 mm Hg for systolic and diastolic blood pressure (p=0.007 and 0.001, respectively), and -3.6 umole/L homocysteine (p=0.001). In a subset of individuals with bone mineral density (BMD) measured at baseline and 12 months, density increased 2.0% in the spine (p=0.003). We conclude that in addition to apparent positive immune remodeling, PattonProtocol-1 may improve markers of metabolic, bone and cardiovascular health.

228 words

Introduction

Telomeres, the structures that protect the ends of our chromosomes, are fascinating components of our genome. They are complex, dynamic structures composed of DNA, RNA, and protein that play a key role in aging and age-related disease, including cancer (2-4). At a biochemical level, telomeres protect chromosome ends from degradation or recombination events, and facilitate faithful segregation of chromosomes during cell division. Early work over 20 years ago showed that average length of human telomeres in normal somatic (non-tumor, non-germline) cells is a measure of the remaining replicative capacity of those cells (5,6) and that short telomeres are associated with disease, including cardiovascular disease, cancer, HIV/AIDS and Downs Syndrome (6-11). Telomere length in general becomes shorter with each cell division, until one or more telomeres become critically short, triggering cell senescence, loss of normal cell function, genomic instability, and possibly cell death or tumor initiation (12-16).

Telomerase, the enzyme that synthesizes telomeric DNA (17), can slow or even reverse telomere shortening in normal human cells in culture (18,19), and contribute to slowing or reversal of degenerative, age-related disease in animals (14,20-22). In almost all large scale cross-sectional studies in humans, telomere length is inversely correlated with chronological age, but with the recent interest in longitudinal tracking of telomere length in humans, evidence for telomere lengthening within an individual is emerging (23-27). Because telomere length can vary within different cell lineages, average TL is a function of the distribution of different cell types and their telomere lengths at any given time. Thus, for complex tissues, one cannot easily distinguish between true telomere length changes versus a shift over time in

subsets of cells in the sampled tissue(s). However, given the fact that telomerase appears to be influenced by many environmental factors, including stress, exercise, active disease, diet, and supplements (28-31), it would be surprising if telomeres only monotonically declined with time.

In the late 1990s we (Harley and colleagues at Geron Corporation and the Hong Kong University of Science and Technology) conducted a screening program for telomerase activators, which led to the discovery that certain small molecule components of Huang Xi, a traditional Chinese medicine reputed to maintain health, were activators of telomerase (1,22,32-34). Definitive pharmacodynamic studies and randomized, placebo controlled studies with these molecules in humans have not been completed, but the majority of studies to date have suggested that they modestly activate telomerase and/or help maintain telomere length *in vitro* and *in vivo,* and that they have positive functional effects on human cells. In addition, a number of recent studies have suggested that telomerase activation in mice with shortened telomeres and signs of engrained age-related disease can be rescued in part through telomerase activation without signs of increased global cancer risk (14,20-22). Thus, telomerase activation is gaining support as a potential treatment or prevention of degenerative diseases using drugs, and for health maintenance using dietary supplements.

In our first analysis of Patton-Protocol-1, a commercial health maintenance program composed of TA-65® (a natural product-derived telomerase activator), other dietary supplements, and physician counseling), we reported positive immune remodeling over a one-year period relative to baseline values (1). These changes included significant declines in senescent cytotoxic (CD8$^+$/CD28$^-$) T cells, particularly in CMV seropositive subjects. In addition, we found that TA-65 alone increased telomerase in human cells in culture, and in aged mice

33

with shortened telomeres, TA-65 reduced the percentage of cells with short telomeres and improved the structure of multiple tissues (22). A recent study on the association between telomere length and experimentally induced upper respiratory track viral infection in healthy adults (35) underscored the predictive value of short telomeres for viral infections and clinical illness, especially when telomere length was measured in CD8+/CD28- cells.

Here we report on observed changes from baseline in metabolic and cardiovascular markers, including bone mineral density, from the same set of subjects consuming TA-65 for a one-year period.

Methods

PattonProtocol-1

PattonProtocol-1 was launched in January 2007 by TA Sciences (New York) as a commercial age-management product composed of a natural product-derived telomerase activator (TA-65®, described below), a dietary supplement pack, laboratory testing, and physician counseling. Further details, including the dietary supplement pack components, are found in Harley et al(1). A small number of subjects declared that they were taking DHEA (10-25mg) daily before or during the time on PattonProtocol-1 as part of their routine supplement use. All subjects signed a comprehensive Customer Acknowledgement Form. Baseline assays (Table 1) indicated that most individuals were within the normal ranges for the majority of tests (data not shown). The consulting physician prescribed new medications for only 3 subjects based upon clinical tests. There was no qualitative change in the overall conclusions whether these subjects were included or excluded from the analyses. The number of subjects at 3, 6, 9,

and 12 months for most tests was 43, 59, 27, and 37, respectively. The age and gender frequencies of the subset at each time-point were similar to those of the total baseline population (n=114; 63±12 years, 72% male).

TA-65®

As previously reported (1), TA-65® was exclusively licensed from Geron Corporation (Menlo Park) to TA Sciences (NY), and the manufacturing methodology at the time produced the single molecule entity at about 95% purity by HPLC from a proprietary extract of the dried root of Astragalus *membranaceus.* The molecule was then formulated into 5 or 10 mg capsules with inert excipients. All data in this report are derived from the original TA-65 product. Post 2011, the purity of the manufactured product has improved from roughly 95% to >98%.

Clinical Laboratory Assays

At baseline and each time-point after initiation of the product blood samples were drawn from each subject (for plasma or serum assays) and shipped the same day at ambient temperature to appropriate commercial, academic, or contract laboratories. Assays for standard blood counts, blood chemistry (including glucose, insulin, hemoglobin A1C, and serum vitamins), specialized immune subsets (CD8$^+$, CD28$^-$ and CD95, both gated on CD8$^+$), CMV antibody titer, and inflammation markers (homocysteine and cardio C-reactive protein) were all conducted at a standard clinical laboratory (either Quest Diagnostics, Bio-Reference Laboratory, or UCLA Clinical Laboratories and Pathology Services, as specified in Results). Data related to the immune markers have been reported previously (1).

35

Bone Mineral Density

Bone mineral density (BMD) was analyzed at baseline and at 12 months on the first available 31 subjects at PhysioAge Medical Group (New York, NY) to reach the 12 month time-point. Full AP spine L1-L4 and dual proximal femur (neck, trochanter, and dual mean) bone density data was generated using a DEXA (dual-energy x-ray absorptiometry) instrument (GE Healthcare).

Statistics

Since data were collected primarily as a hypothesis-generating exercise and the clients were not participating in a controlled clinical study, statistical analysis was not formally defined *a priori*. Baseline data was primarily analyzed for cross-sectional donor age effects to compare results with the "PattonProtocol-1" population. Where significance of linear regression lines is reported for cross-sectional analysis, the F-distribution was used to calculate the probability that the observed correlation occurred by chance and p-values are reported. ANOVA modeling of the 1 year change in values used a multilevel or mixed model with random intercepts and slopes. Each person has an individual starting point and an individual slope for change over time on treatment.   For each dependent variable, we first graphed the data, looking for outliers and for points that indicated the presence of disease. Erroneous data entries were corrected, as were entries indicating either a failure to follow protocol (e.g., glucose over 125 or insulin over 20 indicating non-fasting). SBP over 160 or DBP over 100 was taken to indicate hypertension. These relatively high cutoffs for SBP and DBP were chosen so as not to exclude those individuals with possible metabolic syndrome but not currently carrying a diagnosis of hypertension or on

anti-hypertensive medication. All single entries (that is, people who had only one measurement on a given dependent variable) were also deleted.

Next, the graphs were examined for nonlinear trends. After data cleaning and inspection, multilevel models were used to account for the dependence in the data. For each dependent variable (except folate, see below), we first tested a relatively full model that included fixed effects of time, time squared (where time is months on treatment), baseline age, sex, telomere length and CD8+CD28- T-cells. These were examined with unstructured covariance. It was decided that time, age and sex should remain in all models due to biological importance. Other variables were eliminated if the parameter estimate was close to 0 and the p value above 0.10. The initial model included random effects for intercept, slope and slope squared. The random effect of slope squared was deleted if the fixed effect for time squared was deleted. This step may be regarded as finding the mean structure of the model.

Because folate values were given an artificial ceiling by the lab (values over 24 were reported as 24) using a linear multilevel model was inappropriate. We attempted to model folate using a nonlinear multilevel model with beta distribution.

The resultant model was tested with three different covariance structures: Unstructured, compound symmetry, and auto-regressive. Briefly, the covariance structure models the relations among the residuals. An unstructured covariance matrix lets the covariances be set entirely by the data; a compound symmetric structure forces all the covariances to be identical. An autoregressive structure forces points that are closer in time to have higher covariance (that is, be more related). The choice was made based on the Akaike Information Criterion (other information criteria made identical choices).

In addition to the numerical analysis, four variables: Glucose, insulin, LDL cholesterol, and systolic blood pressure are presented visually as spline graphs. In these graphs, we divided the data for each variable into three groups based on the initial value, then tracked how people in each group changed over time. These graphs are presented with spline curves, which are nonparametric curves designed to fit data with various patterns.

Results and Discussion

Key Baseline Observations

Most of the subjects for this study were relatively affluent individuals motivated to maintain personal health, and thus may not reflect the general population in terms of age-specific health status.

The relationships between age and various biomarkers including telomere length have been reported in a number of cross-sectional studies. Table 1 shows mean values, standard deviations, count, slope and R^2 from linear regression on subject age, and the statistical significance of the slope for the baseline tests investigated in this report. As expected, this population showed a highly statistically significant increase as a function of client age in fasting blood glucose (0.33 mg/dL/year), systolic blood pressure (0.51 mmHg/year), and homocysteine (0.066 μmol/L/year), and a significant decrease in DHEA sulfate (-2.6 mcg/dL/y) and. The increase in serum folate (0.12 ng/ml/y) was unexpected but may reflect the relatively greater use of folic acid containing multivitamins in older compared to younger prior to starting the Protocol.

As described previously (1), telomere length declined in a characteristic manner in this population as a function of client age in both lymphocytes and granulocytes (55 and 34 bp/year; $p=10^{-15}$ and 10^{-8}, respectively).

Changes from Baseline While on PattonProtocol-1

The baseline analysis confirmed that test data from TA Sciences subjects were similar to those reported in other elderly groups and provided a benchmark(36) against which we could plot relative changes with time on the PattonProtocol-1. As discussed earlier, it is important to note that we cannot determine the contribution of any single component of the PattonProtocol-1 to the observed effects. Changes from baseline that are unusual in comparison to other dietary supplement studies could be due to TA-65® and its effects on telomeres, but it is also possible that placebo effects or the unique combination of ingredients in the TA Sciences dietary supplement pack also play a role. Medical/lifestyle questionnaires at baseline and post the 12 month period for each client were used to record changes in medications, diet or exercise while on the PattonProtocol-1. No clients reported significant changes in diet or exercise, but where clients reported a change in drug therapy, the test results which the physician felt could be significantly impacted by the medication change were excluded from the analysis. Since this study did not have a control group, seasonal effects could also be a factor, but since the 114 clients studied here initiated the program at a consistent rate of slightly under 1/week over a 2.5 year period, the baseline and follow-up data are relatively evenly spread across multiple years and it is unlikely that seasonal effects influenced the observed changes from baseline. Population trends over a multi-year timeframe however

39

cannot be ruled out. With these caveats in mind, we summarize the most interesting findings below.

Metabolic Improvements in Clients on PattonProtocol-1: Blood sugar, Cholesterol, Blood Pressure, Inflammatory markers, Vitamin levels, and Bone Mineral Density

Fasting glucose and insulin levels both declined with time in clients on the PattonProtocol-1 (at a rate of -3.72 mg/dL and -1.32 mIU/mLper year, respectively; Table 2). Decreases of this magnitude for fasting glucose are significant relative to the mean baseline values and the donor-age dependent change per year in the baseline population (98 mg/dL and +0.34 mg/dL/year, respectively, from Table 2). In the general population, Sehl et al. (36) reports 0.5-1.5 mg/dL//year increase in fasting glucose. On a relative basis, PattonProtocol-1 apparently reversed ≈11 years of mean increase in fasting glucose. The concomitant reduction in fasting glucose and insulin is indicative of an improvement in insulin sensitivity. Shorter leukocyte telomere length has been associated with insulin resistance, oxidative stress, and metabolic syndrome. (37-39) It is possible that the combination of antioxidants and increased telomerase activation improved insulin sensitivity in our subjects, though the exact mechanism is unknown.

Total and LDL cholesterol also decreased significantly with time on product . At 12 months, the 13.2 mg/dLand 11.8 mg/dL decreases in total and LDL cholesterol, respectively, were statistically significant (p=0.002 for both,) and large compared to the small decrease associated with client age in this population (-0.29mg/dL/y and -0.37 mg/dL/y, respectively).

Cholesterol generally increases with age (40), possibly due to reduced clearance of LDL from circulation and reduction in conversion of cholesterol to bile acids, but some studies have suggested a flat profile with aging or even a reduction in total cholesterol with age attributed to poor health(41). Since the baseline average total cholesterol in the PattonProtocol-1 clients was borderline high (mean 188mg/dL, Table 2), we consider the declines of ≈10mg/dL in total and LDL cholesterol to be beneficial as it is comparable to what has been achieved in successful diet and exercise programs (42). The mechanism of the reduction in cholesterol is unclear, but given no subjects reported changes in their exercise or dietary habits, it is possible that increased telomerase activation from the PattonProtocol1 contributed. A small study demonstrated that increased telomerase activity in PBMCs is significantly associated with a decrease in LDL cholesterol(43).

Systolic and diastolic blood pressure declined 17.8 mm/Hg and 4.2 mm/Hg, respectively over the 1 year period that clients were on the PattonProtocol-1 (Table 2). In the baseline population, DBP was relatively flat but SBP increased 0.51 mm Hg/year (Table 2), comparable to that seen in other studies (44) suggesting that the PattonProtocol-1 may have reversed >30 years of increase in SBP. The decrease in SBP is comparable to first line therapy with diuretics which confer a significant reduction in cardiovascular events. Using the Framingham 10-year CVD event calculator, these reductions in blood pressure and cholesterol would cause a 25% reduction in 10 year risk for males (from 8% to 6%). The mechanism of this reduction in blood pressure is difficult to determine given the observational nature of the study, but telomere attrition has been associated with higher blood pressure and increased aldosterone production(45) which in turn can increase oxidative stress and blood pressure by reducing nitric oxide production. If the reduction in percent short telomeres and senescent cytotoxic t-cells

41

we reported previously also resulted in decreased inflammatory cytokines (such as TNF-α and IL-6), then this process could have been reversed in our cohort and explain the reduction in blood pressure. This reversal of inflammation along with increased telomerase activation could rescue presenescent endothelial cells and increase the production of nitric oxide which could reduce SBP. Of course, the supplement packs could also have contributed to this reduction in oxidative stress.

Obviously, if the improvements calculated by our models continued year after year, they would no longer be beneficial as they would result in hypoglycemia, hypotension, and inadequate cholesterol production for steroid production and cell membrane fluidity. To explore whether the slope of these observed trends is constant regardless of the starting level of the dependent variable we generated sub-group spline graphs.

Figure 1 panel A shows a graph of fasting glucose levels over time. Each dot is a person at a given time point (baseline, 3 months, 6 months, 9 months and 1 year). To avoid overplotting symbols, a small amount of random noise (jitter) was added to each plot. Levels of glucose at baseline were divided into three groups: Low (below 88, the lowest group, colored green), high (above 102, the highest, colored red) and middle (the middle 50%, colored blue). Then splines were fit to each group. The overall trend was for those in the highest group to have relatively large declines in glucose, those in the mid-range to have smaller declines, and those in the lowest group to have a slight increase. Figure 1 panel B is similar for insulin, except that here the groups were set based on medical expertise: High was above 10, low was below 5 and middle was in between. Again, the highest group showed steep declines. At 12 months, the highest and middle groups were almost identical. This pattern of a simultaneous decrease

in glucose and insulin represents an improvement in insulin sensitivity, and it is beneficial that it occurs only in those subjects who have some degree of insulin resistance. Insulin resistance is a cardinal element of the metabolic syndrome and thought to be a result of increased inflammation which the PattonProtocol1 might have reduced. From a clinical standpoint, this pattern of effectiveness is similar to metformin, a first-line therapy for pre-diabetes which improves insulin sensitivity without causing hypoglycemia as can occur with the older oral hypoglycemic therapies.

The same pattern is demonstrated by the spline graph for LDL cholesterol (Figure 1 panel C). The groups were divided by the treatment categories defined by the National Cholesterol Education Program: Low was below 100, middle was 100 to 130 and high was above 130.) Although the trends are less pronounced, it should be noted that the highest group declined by about 20, the middle group by about 10, while the low group increased slightly. This moderating trend decreases the likelihood of any long-term adverse effect of over-reduction in cholesterol.

Figure 1 panel D shows similar effects for systolic BP; here the division was by thirds: High = above 138, low = below 120 and middle = 120-138, and again, there is a moderating trend which results in an improvement in SBP in those with high SBP, and no lowering in those who are in the optimal range for blood pressure. This moderating trend fits with the mechanisms of SBP reduction discussed above in which a dysfunctional endothelium (in those with higher baseline SBP) is repaired rather than a direct antihypertensive effect which could result in hypotension. One might see these moderating trends as simply a result of regression

43

to the mean phenomenon, but the decreasing variance over time that is evident from all four of

the spline graphs argues against this.

Homocysteine and C-reactive protein (CRP) are significant markers of inflammation and

are associated with arterial dysfunction and cardiovascular disease risk (16,46). We observed

significant reductions in both homocysteine (Table 2) and CRP (not shown) while clients were

on the PattonProtocol-1, but for CRP, a number of individuals had acute spikes in CRP, possibly

related to infections, which confounded the analysis. The 3.6 μmol/mL decrease in

homocysteine in clients on the Protocol (p=<0.001) is dramatic compared to the general

increase with age (0.066 μmol/mL/y) in this population. It is likely that folate and vitamins B12

and B6 in the supplement packs played a significant role in the decline in homocysteine, but a

number of individuals who were taking potent supplement packs before initiation of the

protocol also showed reductions in homocysteine, including some subjects whose folate levels

actually decreased (data not shown). It is also possible that other components of the Protocol,

including TA-65[®], reduced the inflammatory response and improved health through elongation

of short telomeres and remodeling of the immune system. If indeed it was shown that

reduction in inflammation and cardiovascular risk could be reduced through the

PattonProtocol-1 without the high levels of folate found in some aggressive dietary supplement

programs, this would be beneficial since recent reports warn of adverse effects from supra-

physiological levels of folate (47,48).

Table 2 shows the significant increases in levels of serum folate, vitamin B12, and 25-hydroxy vitamin D over the year on PattonProtocol-1. These increases likely reflect the relatively high amounts of these components in the supplement packs (1), but given the fact that an apparent plateau was not reached until ≈9-12 months (data not shown), other factors such as a gradual improvement in general health may also have played a role, for example by improving gastrointestinal epithelial function and thereby absorption of these nutrients.

Finally, bone mineral density was measured at baseline and 12 months in an essentially random subset of individuals (n=31). Of the 31 subjects, 26 were either not on hormone therapy or made no change in hormone therapy during the one year period. The remaining 5 subjects who changed therapy were excluded from the analysis. Of the 26 analyzed subjects, one had osteoporosis and 4 others had varying degrees of osteopenia. In the overall group, there was a 2.0% increase in AP spine L1-L4 BMD (p=0.003), Table 3. This is as large an increase in BMD as seen in a three year study of calcium and vitamin D therapy in comparable subjects without osteoporosis(49) and certainly contrasts with the expected ≈ 0.5% decrease per year. There were no significant changes in hip BMD (data not shown). Moreover, because the average baseline vitamin D levels were well into the normal range, it is less likely that the approximately 20% increase in vitamin D contributed significantly to the BMD increase. A plausible alternative explanation for this improvement in BMD could be related to the emerging understanding of the relationship among hyperlipidemia, T-cells, and bone density (50). The reduction in senescent suppressor cells we previously reported(1) combined with the reduction in LDL cholesterol could decrease the number of activated T-cells in the bone marrow and thereby reduce activated RankL and other inflammatory cytokines. This would reduce osteoclast activity and potentially explain the improved bone density.

Discussion and Conclusions

This study adds to the observations from 2011 that PattonProtocol-1, containing conventional supplements plus TA-65, a small molecule telomerase activator, has a positive impact on biomarkers of aging or age-related disease. The initial observational study of roughly 50 subjects on product(1) focused on results from independent diagnostic laboratory testing of immunological markers over a one-year period, while here we focus on similar laboratory testing of metabolic biomarkers and bone mineral density in the same cohort over the same 1 year period. We found reductions in fasting blood sugar, insulin, cholesterol, blood pressure, and homocysteine, and increases in bone mineral density, all considered positive health changes. These data suggest that PattonProtocol-1, and possibly TA-65 in combination with other supplements and physician counseling improves health and may reduce risk of morbidity and mortality.

The major limitation of these observational studies is that they are not randomized, placebo-controlled trials with defined dosing of TA-65. Such a study is in progress, but the observation that TA-65 was primarily beneficial to CMV+ humans who were "blind" to their CMV status(1), and that TA-65 had positive effects when given to aged telomerase-positive but not telomerase negative mice (51), suggests that TA-65 is an active molecule that should be further investigated.

Because telomerase may extend the lifespan of relatively rare pre-malignant cells which otherwise might die due to critical shortening of telomeres, it is possible that a telomerase activator could increase cancer risk in some individuals. However, prevention of critical shortening of telomeres in multiple tissues throughout an aging human by telomerase

activation could be a net tumor suppressive mechanism by reducing genomic instability and maintaining health of normal tissues. Much larger controlled studies will be needed to assess the potential risk and benefits of TA-65 in humans. Subjects taking TA-65 or any dietary supplement should consult their doctor and carefully consider the product's potential risks and benefits, including the data to date from the growing number of individuals on TA-65. There had been approximately 260 person-years of TA-65 dosing, mainly in the 5-50 mg/day range through June 2010, with no reports of new diagnoses of cardiovascular disease or cancer. This represented age-adjusted incidence rates significantly below the average US rates for CVD and cancer. As of June 2013, there are now an estimated 7000 person-years of TA-65 exposure at an average 50mg TA-65 dose equivalence. Disease and mortality was not formally tracked, but TA Sciences reports few if any adverse events, and no cases of adverse events being attributed to TA-65 or TA-65$_{MD}$ by the subjects' doctors. In the overall (all ages) US population, 7000 person years of life would entail about 30 new diagnoses each for cancer and CVD, and about 60 deaths, based on data from the Centers for Disease Control and Prevention. Because of lack of matching demographics for the study population, as well as lack of exact disease and mortality data, we cannot make a reliable comparison of incidence rates in the study population versus the general population, but the data to date do not point to increased risk of morbidity or mortality in subjects taking TA-65. In conclusion, TA-65, a moderate telomerase activator, is a novel dietary supplement which may enhance one's healthspan.

Acknowledgements

We thank Dr. Russ Kerschmann and Dr. Lennart Olsson for critical review of the manuscript.

Figure Legends

Figure 1. Spline graphs based on the multi-level ANOVA model, representing the change over time (baseline to 12 months) for (A) fasting glucose, (B) serum insulin, (C) LDL cholesterol, and (D) systolic blood pressure. In each panel, dots represent subjects on PattonProtocol-1. To avoid overplotting of symbols, a small amount of random noise (jitter) was added to each plot. Subjects with low, mid, and high baseline values are colored green, blue, and red, respectively. The cut-off values for these subgroups are given in the text. The shaded regions around each spline represents the 95% confidence intervals.

REFERENCES

1. Harley, C.B., Liu, W., Blasco, M., Vera, E., Andrews, W.H., Briggs, L.A. and Raffaele, J.M. (2011) A natural product telomerase activator as part of a health maintenance program. *Rejuvenation research*, **14**, 45-56.

2. Lin, J., Epel, E. and Blackburn, E. (2012) Telomeres and lifestyle factors: roles in cellular aging. *Mutation research*, **730**, 85-89.

3. Kropski, J.A., Lawson, W.E., Young, L.R. and Blackwell, T.S. (2013) Genetic studies provide clues on the pathogenesis of idiopathic pulmonary fibrosis. *Disease models & mechanisms*, **6**, 9-17.

4. Aubert, G., Baerlocher, G.M., Vulto, I., Poon, S.S. and Lansdorp, P.M. (2012) Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. *PLoS genetics*, **8**, e1002696.

5. Harley, C.B., Futcher, A.B. and Greider, C.W. (1990) Telomeres shorten during ageing of human fibroblasts. *Nature*, **345**, 458-460.

6. Allsopp, R.C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E.V., Futcher, A.B., Greider, C.W. and Harley, C.B. (1992) Telomere length predicts replicative capacity of human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 10114-10118.

7. Vaziri, H., Dragowska, W., Allsopp, R.C., Thomas, T.E., Harley, C.B. and Lansdorp, P.M. (1994) Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9857-9860.

8. Vaziri, H., Schachter, F., Uchida, I., Wei, L., Zhu, X., Effros, R., Cohen, D. and Harley, C.B. (1993) Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. *American journal of human genetics*, **52**, 661-667.

9. Counter, C.M., Avilion, A.A., LeFeuvre, C.E., Stewart, N.G., Greider, C.W., Harley, C.B. and Bacchetti, S. (1992) Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *The EMBO journal*, **11**, 1921-1929.

10. Counter, C.M., Hirte, H.W., Bacchetti, S. and Harley, C.B. (1994) Telomerase activity in human ovarian carcinoma. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 2900-2904.

11. Counter, C.M., Botelho, F.M., Wang, P., Harley, C.B. and Bacchetti, S. (1994) Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed human B lymphocytes. *Journal of virology*, **68**, 3410-3414.

12. Perera, S.A., Maser, R.S., Xia, H., McNamara, K., Protopopov, A., Chen, L., Hezel, A.F., Kim, C.F., Bronson, R.T., Castrillon, D.H. *et al.* (2008) Telomere dysfunction promotes genome instability and metastatic potential in a K-ras p53 mouse model of lung cancer. *Carcinogenesis*, **29**, 747-753.

13. Artandi, S.E. and DePinho, R.A. (2010) Telomeres and telomerase in cancer. *Carcinogenesis*, **31**, 9-18.

14. Sahin, E. and Depinho, R.A. (2010) Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*, **464**, 520-528.

15.   de Lange, T. (2009) How telomeres solve the end-protection problem. *Science*, **326**, 948-952.

16.   Ridker, P.M., MacFadyen, J., Cressman, M. and Glynn, R.J. (2010) Efficacy of rosuvastatin among men and women with moderate chronic kidney disease and elevated high-sensitivity C-reactive protein: a secondary analysis from the JUPITER (Justification for the Use of Statins in Prevention-an Intervention Trial Evaluating Rosuvastatin) trial. *Journal of the American College of Cardiology*, **55**, 1266-1273.

17.   Blackburn, E.H., Greider, C.W. and Szostak, J.W. (2006) Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nature medicine*, **12**, 1133-1138.

18.   Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S. and Wright, W.E. (1998) Extension of life-span by introduction of telomerase into normal human cells. *Science*, **279**, 349-352.

19.   Jiang, X.R., Jimenez, G., Chang, E., Frolkis, M., Kusler, B., Sage, M., Beeche, M., Bodnar, A.G., Wahl, G.M., Tlsty, T.D. *et al.* (1999) Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. *Nature genetics*, **21**, 111-114.

20.   Jaskelioff, M., Muller, F.L., Paik, J.H., Thomas, E., Jiang, S., Adams, A.C., Sahin, E., Kost-Alimova, M., Protopopov, A., Cadinanos, J. *et al.* (2011) Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*, **469**, 102-106.

21.   Bernardes de Jesus, B., Vera, E., Schneeberger, K., Tejera, A.M., Ayuso, E., Bosch, F. and Blasco, M.A. (2012) Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO molecular medicine*, **4**, 691-704.

22.   Bernardes de Jesus, B.B., Schneeberger, K., Vera, E., Tejera, A., Harley, C.B. and Blasco, M.A. (2011) The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging cell*, **10**, 604-621.

23.   Svenson, U., Nordfjall, K., Baird, D., Roger, L., Osterman, P., Hellenius, M.L. and Roos, G. (2011) Blood cell telomere length is a dynamic feature. *PloS one*, **6**, e21485.

24.   Epel, E.S., Merkin, S.S., Cawthon, R., Blackburn, E.H., Adler, N.E., Pletcher, M.J. and Seeman, T.E. (2009) The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging*, **1**, 81-88.

25.   Jacobs, T.L., Epel, E.S., Lin, J., Blackburn, E.H., Wolkowitz, O.M., Bridwell, D.A., Zanesco, A.P., Aichele, S.R., Sahdra, B.K., MacLean, K.A. *et al.* (2011) Intensive meditation training, immune cell telomerase activity, and psychological mediators. *Psychoneuroendocrinology*, **36**, 664-681.

26.   Farzaneh-Far, R., Lin, J., Epel, E.S., Harris, W.S., Blackburn, E.H. and Whooley, M.A. (2010) Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. *JAMA : the journal of the American Medical Association*, **303**, 250-257.

27.   Kiecolt-Glaser, J.K., Epel, E.S., Belury, M.A., Andridge, R., Lin, J., Glaser, R., Malarkey, W.B., Hwang, B.S. and Blackburn, E. (2013) Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: A randomized controlled trial. *Brain, behavior, and immunity*, **28**, 16-24.

28.   Ho, R.T., Chan, J.S., Wang, C.W., Lau, B.W., So, K.F., Yuen, L.P., Sham, J.S. and Chan, C.L. (2012) A randomized controlled trial of qigong exercise on fatigue symptoms, functioning, and telomerase activity in persons with chronic fatigue or chronic fatigue syndrome. *Annals of behavioral medicine : a publication of the Society of Behavioral Medicine*, **44**, 160-170.

29. Wolkowitz, O.M., Mellon, S.H., Epel, E.S., Lin, J., Reus, V.I., Rosser, R., Burke, H., Compagnone, M., Nelson, J.C., Dhabhar, F.S. *et al.* (2012) Resting leukocyte telomerase activity is elevated in major depression and predicts treatment response. *Molecular psychiatry*, **17**, 164-172.

30. Lavretsky, H., Epel, E.S., Siddarth, P., Nazarian, N., Cyr, N.S., Khalsa, D.S., Lin, J., Blackburn, E. and Irwin, M.R. (2013) A pilot study of yogic meditation for family dementia caregivers with depressive symptoms: effects on mental health, cognition, and telomerase activity. *International journal of geriatric psychiatry*, **28**, 57-65.

31. Epel, E. (2012) How "reversible" is telomeric aging? *Cancer prevention research*, **5**, 1163-1168.

32. Fauce, S.R., Jamieson, B.D., Chin, A.C., Mitsuyasu, R.T., Parish, S.T., Ng, H.L., Kitchen, C.M., Yang, O.O., Harley, C.B. and Effros, R.B. (2008) Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. *Journal of immunology*, **181**, 7400-7406.

33. Yung, L.Y., Lam, W.S., Ho, M.K., Hu, Y., Ip, F.C., Pang, H., Chin, A.C., Harley, C.B., Ip, N.Y. and Wong, Y.H. (2012) Astragaloside IV and cycloastragenol stimulate the phosphorylation of extracellular signal-regulated protein kinase in multiple cell types. *Planta medica*, **78**, 115-121.

34. Zhu, J., Lee, S., Ho, M.K., Hu, Y., Pang, H., Ip, F.C., Chin, A.C., Harley, C.B., Ip, N.Y. and Wong, Y.H. (2010) In vitro intestinal absorption and first-pass intestinal and hepatic metabolism of cycloastragenol, a potent small molecule telomerase activator. *Drug metabolism and pharmacokinetics*, **25**, 477-486.

35. Cohen, S., Janicki-Deverts, D., Turner, R.B., Casselbrant, M.L., Li-Korotky, H.S., Epel, E.S. and Doyle, W.J. (2013) Association between telomere length and experimentally induced upper respiratory viral infection in healthy adults. *JAMA : the journal of the American Medical Association*, **309**, 699-705.

36. Sehl, M.E. and Yates, F.E. (2001) Kinetics of human aging: I. Rates of senescence between ages 30 and 70 years in healthy people. *The journals of gerontology. Series A, Biological sciences and medical sciences*, **56**, B198-208.

37. Sahin, E., Colla, S., Liesa, M., Moslehi, J., Muller, F.L., Guo, M., Cooper, M., Kotton, D., Fabian, A.J., Walkey, C. *et al.* (2011) Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature*, **470**, 359-365.

38. Haendeler, J., Drose, S., Buchner, N., Jakob, S., Altschmied, J., Goy, C., Spyridopoulos, I., Zeiher, A.M., Brandt, U. and Dimmeler, S. (2009) Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. *Arteriosclerosis, thrombosis, and vascular biology*, **29**, 929-935.

39. Rentoukas, E., Tsarouhas, K., Kaplanis, I., Korou, E., Nikolaou, M., Marathonitis, G., Kokkinou, S., Haliassos, A., Mamalaki, A., Kouretas, D. *et al.* (2012) Connection between telomerase activity in PBMC and markers of inflammation and endothelial dysfunction in patients with metabolic syndrome. *PloS one*, **7**, e35739.

40. Berns, M.A., de Vries, J.H. and Katan, M.B. (1988) Determinants of the increase of serum cholesterol with age: a longitudinal study. *International journal of epidemiology*, **17**, 789-796.

41. Volpato, S., Zuliani, G., Guralnik, J.M., Palmieri, E. and Fellin, R. (2001) The inverse association between age and cholesterol level among older patients: the role of poor health status. *Gerontology*, **47**, 36-45.

42. Kelley, G.A., Kelley, K.S., Roberts, S. and Haskell, W. (2012) Comparison of aerobic exercise, diet or both on lipids and lipoproteins in adults: a meta-analysis of randomized controlled trials. *Clinical nutrition*, **31**, 156-167.

43. Ornish, D., Lin, J., Daubenmier, J., Weidner, G., Epel, E., Kemp, C., Magbanua, M.J., Marlin, R., Yglecias, L., Carroll, P.R. *et al.* (2008) Increased telomerase activity and comprehensive lifestyle changes: a pilot study. *The lancet oncology*, **9**, 1048-1057.

44. Franklin, S.S., Gustin, W.t., Wong, N.D., Larson, M.G., Weber, M.A., Kannel, W.B. and Levy, D. (1997) Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation*, **96**, 308-315.

45. Benetos, A., Gardner, J.P., Kimura, M., Labat, C., Nzietchueng, R., Dousset, B., Zannad, F., Lacolley, P. and Aviv, A. (2005) Aldosterone and telomere length in white blood cells. *The journals of gerontology. Series A, Biological sciences and medical sciences*, **60**, 1593-1596.

46. Graham, I.M. and O'Callaghan, P. (2002) Vitamins, homocysteine and cardiovascular risk. *Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy*, **16**, 383-389.

47. Ulrich, C.M. and Potter, J.D. (2006) Folate supplementation: too much of a good thing? *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, **15**, 189-193.

48. Sauer, J., Mason, J.B. and Choi, S.W. (2009) Too much folate: a risk factor for cancer and cardiovascular disease? *Current opinion in clinical nutrition and metabolic care*, **12**, 30-36.

49. Dawson-Hughes, B., Harris, S.S., Krall, E.A. and Dallal, G.E. (1997) Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *The New England journal of medicine*, **337**, 670-676.

50. Graham, L.S., Tintut, Y., Parhami, F., Kitchen, C.M., Ivanov, Y., Tetradis, S. and Effros, R.B. (2010) Bone density and hyperlipidemia: the T-lymphocyte connection. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, **25**, 2460-2469.

51. Bernardes de Jesus, B., Schneeberger, K., Vera, E., Tejera, A., Harley, C.B. and Blasco, M.A. (2011) The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging cell*, **10**, 604-621.