ABSRACT

Title of Thesis Defense:	RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND TELOMERE MAINTENANCE IN PERIPHERAL BLOOD MONONUCLEOCYTES
Degree Candidate:	Andrew Todd Ludlow
Degree and year:	Master of Arts, 2007
Thesis directed by:	Professor Stephen M. Roth, PhD Department of Kinesiology

Shortened telomere length and decreased telomerase activity have recently been associated with perceived psychological stress and cardiovascular disease risk factors. The purpose of this study was to examine the relationship between exercise energy expenditure (EEE) and telomere length, telomerase activity, and hTERT genotype. Sixty-nine male (n = 34) and female (n = 35) participants 50-70 yr. were assessed for weekly EEE level using the exercise component of the Yale Physical Activity Survey. Lifetime consistency of EEE was also determined. Subjects were recruited across a range of EEE levels and grouped according to EEE (0-1000, 1001-3500, and >3500 Kcal/wk). Relative telomere length and telomerase activity were measured in peripheral blood mononuclear cells (PBMCs). Consistency of physical activity was the only covariate in the final model and relative telomere length was significantly higher in the Middle EEE group compared to both the low and high EEE groups ($p<0.05$; means ± SE relative T/S ratio; low = 0.93 ± 0.06, n=17; Middle = 1.10 ± 0.05, n=30; high = 0.94 ± 0.06, n=19). Telomerase activity was not different among the three EEE groups. An association was observed between telomerase enzyme activity and hTERT genotype with the TT genotype ($1.02 \times 10^{-2} \pm 8.31 \times 10^{-3}$; n = 17) having significantly greater telomerase enzyme activity than both the

CT ($2.10 \times 10^{-3} \pm 6.47 \times 10^{-4}$; N = 29) and CC ($6.07 \times 10^{-4} \pm 2.01 \times 10^{-4}$; N = 17) (P = 0.013). In conclusion, the results indicate that moderate exercise energy expenditure levels may provide a protective effect on PBMC telomere length compared to both low and high exercise energy expenditure levels. hTERT genotype, but not EEE, was associated with telomerase activity.

RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND TELOMERE MAINTENANCE IN PERIPHERAL BLOOD MONONUCLEOCYTES

By

Andrew Todd Ludlow

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Arts
2007

Advisory Committee:
Assistant Professor Stephen M. Roth, Chair
Professor Bradley Hatfield
Assistant Professor Espen Spangenburg
Professor James M. Hagberg

Acknowledgements

There are many people who I would like to thank for all their support and guidance during my graduate career. I would first like to thank my family: my Mother and Father for their unconditional love, support, guidance and providing me the opportunity to pursue a life-long goal of mine. I would also like to thank them for what they have done for me over the past two and half years. I also would like to thank my brothers: Tom, Jim and Bob for their support and advice. Without my family, none of this would have been possible.

I would also like to acknowledge my advisor, Dr. Stephen Roth. I cannot thank Dr. Roth enough for his patience, knowledge, guidance, availability and advice and for setting high standards and a great example to follow. I will be grateful to him for taking me on as a student and providing me the opportunity to pursue a goal of mine to earn a degree in higher education.

Finally, I would like to extend my gratitude to my committee members for their willingness to serve on my thesis committee, for their time, and for their guidance. I

would like to thank Dr. Hatfield for allowing me to use part of his research data and for his advice through the writing process. I would like to thank Dr. Spangenburg for his laboratory knowledge and for his willingness to help solve difficult situations that arose through this research project. I would like to thank Dr. Hagberg for his advice and ability to see other solutions to problems that I may have overlooked. I would like to thank all the committee members for helping me develop as a graduate student thus far at the University of Maryland. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A – Adenine

Amoles - attomoles

ANCOVA – analysis of covariance

BMI – Body mass index

C – Cytosine

Ct – Critical threshold

CVD – Cardiovascular disease

DNA – Deoxyribonucleic acid

EDTA - ethylenediamine tetraacetic acid

EEE – exercise energy expenditure

exKcal/wk – exercise kilocalorie per week

G – Guanine

hTERT – Human telomere reverse transcriptase

Kcal/wk – kilocalorie per week

PAR – Stanford 7 day physical activity recall

PBMC – Peripheral blood mononuclear cells

PBS – phosphate buffered saline

PSS – Perceived Stress Survey

RFLP – restriction fragment length protocol

RT-PCR – Real – time polymerase chain reaction

SES – Socio-economic status

T – Thiamine

T/S ratio – Telomere copy number to single copy gene number ratio

TRAP – Telomere repeat amplification protocol

TRF – telomere restriction fragment length

VO_2max – maximum volume of oxygen consumed per unit time during a given activity

WBC – White blood cells

Ypas – Yale Physical Activity survey

Yr. – years

INTRODUCTION

Telomeres are found on the ends of linear chromosomes and act as a mitotic clock (58), in that as mitotic cells divide over time, DNA polymerase is unable to replicate the 3' end of linear DNA to its end and telomeres shorten (63). Thus with every cell division, telomere length progressively shortens until the cell reaches a senescent state (i.e., telomeres become too short to allow DNA replication). The number of cell divisions is limited by the replication of telomeres, which have been proposed as both a mechanism and a potential biomarker of the aging process (10, 17, 22, 37, 38). Telomere length has been associated with CVD disease risk and morbidity and mortality (4, 8-10, 27, 38).

Telomeres and their length are not, however, static entities, but rather should be considered a dynamic system that switches between a capped and uncapped state (15). As the telomeres shorten, a "critical length" or threshold is crossed resulting in "uncapping" to occur, at which time the cell becomes senescent. In certain cells, the ribonucleoprotein, telomerase, maintains and lengthens telomeres, allowing continued mitotic activity without progression to senescence (16). Human cells with telomerase activity include germ line cells, embryonic and stem cells, and adult proliferating cells including cells within the immune system, endothelium, skin, intestinal lining, and hair follicles (60). Thus, in cells with telomerase activity, telomere length can be maintained, thus delaying senescence and tissue aging (38).

Recently, both telomere length and telomerase activity have been shown to be influenced by various environmental factors such as oxidative stress and psychological stress (38, 43) and socioeconomic status (29). Collins et al. (32) found that athletes

diagnosed with 'fatigued athlete myopathic syndrome' (FAMS) had shorter muscle homogenate telomere length than age- and training-matched counterparts. These studies provide evidence that these cell traits are malleable, including the possibility that physical activity may play an influential role.

In addition to environmental factors, the gene that encodes for the catalytically active subunit of telomerase (hTERT) is known to have a functional single nucleotide polymorphism (SNP) in the promoter region (C–1327T; rs 2735940). The TT genotype has been associated with greater telomere length and greater telomerase activity compared to the CC genotype, apparently due to greater promoter activity of the hTERT gene with the TT genotype (72, 73). Thus, hTERT genotype may play a moderating role in how environmental factors may influence telomere length.

Telomere shortening occurs with aging in certain cells and accelerated telomere shortening occurs due to psychological stress and FAMS. Physical activity decreases the likelihood of developing CVD and reduces psychological stress, insulin resistance, and hypertension, all of which have been associated with telomere biology and aging (35, 37, 38, 40). Thus, the purpose of this study was to explore the relationship of physical activity and the telomere system in an older population by assessing telomere length and telomerase activity in the PBMCs of older men and women of varying physical activity levels. The association of hTERT genotype with telomere length and telomerase enzyme activity was also investigated.

Research Questions: Does telomere length or telomerase activity differ in PBMCs depending on an individual's physical activity or exercise energy expenditure level?

Is hTERT genotype associated with telomere length and telomerase enzyme activity?

Hypotheses: We hypothesized that men and women with greater levels of physical activity would have both longer PBMC telomere length and greater telomerase enzyme activity compared to men and women with lower levels of physical activity. We also hypothesized that men and women with the CC genotype would have shorter telomere length and less telomerase enzyme activity compared to the TT genotype.

METHODS

Subjects:

Sixty-nine male and post-menopausal females 50-70 years of age were recruited by newspaper advertisements, flyers, and word of mouth. Participants who had competed in and finished various road-running races and other local endurance events were sent letters inviting them to participate in the study. Volunteers who were enrolled in the "Legacy College program" were also invited to participate. The Legacy College is a volunteer, peer-led, peer-taught lifelong learning and civic engagement program for adults age 50 yr. and beyond. Other organizations that were contacted to recruit subjects included local civic groups. All subjects were apparently healthy according their responses to a medical health questionnaire. Potential volunteers were excluded if they had any underlying medical conditions, fatigue, acute myopathy, current infections or treatment for cancer, as indicated on the medical health history questionnaire. If a subject had diagnosed heart disease, un-medicated hypertension, and or previous blood and or lymphocyte cancer, they were excluded due the possible confounding effects of these conditions upon the dependent variables. The study was approved by the University of Maryland Institutional Review Board and all subjects provided written informed consent prior to participation.

Procedures: In order to assess each subject's individual physical activity experience and ability to participate, subjects completed both a medical health history status sheet and the Yale physical activity survey (36). The medical history status sheet was developed at the University of Maryland. The information extracted from the

medical health history includes date of birth, self-reported race and ethnicity, current medications, past and present medical disorders, self-reported physical activity consistency throughout past decades, memory function and family history of age related mental decline. This medical history sheet has not been validated, but is designed to assess basic information relevant to the exclusion criteria. Anthropometric measures, including height, weight, and body mass index (BMI) where collected using standardized procedures.

Physical Activity: Participants completed the Yale physical activity survey (Ypas) developed at Yale School of Medicine in 1993 (36). The Ypas is an "interviewer-administered survey that asks the individual to estimate time spent in a list of twenty-five activities in a typical week during the last month" (29). The list of activities has five sub-headings titled: Work, yard work, care- taking, exercise and recreational activities. Two indices are gleaned from the information: 1) time spent in each activity is multiplied by an intensity code and reported in kilocalories per minute and then 2) summed across all activities to create an index of weekly energy expenditure reported in kilocalories per week. The two measures are accompanied by the amount of time spent in each activity summed to provide a total time index reported in hours per week.

The Ypas was initially analyzed for repeatability and assessed using paired t-test and Pearson product moment correlation coefficient. Validity was assessed by the Spearman ranked correlation coefficient. The estimated maximal oxygen consumption was found to be significantly correlated to the summary index and vigorous index (0.58, P=0.004; and 0.60, P=0.003, respectively). To further validate the Ypas, Young et al, (108) compared it to another previously validated self-reported physical activity survey

called the Stanford 7-day Physical Activity Recall survey (PAR). Energy expenditure, total time, summary index and the vigorous index were found to be significantly correlated with daily energy expenditure (0.37, P = 0.0004; 0.30, P= 0.02; 0.33, P= 0.01; 0.45, P<0.001, respectively) (108). The validity of the Ypas for light-intensity activity was less clear and no significant correlations were found. More stringent validation qualities need be established for low intensity activity.

Based on the results of the Ypas the subjects had his/her physical activity level assessed. Another consideration that was made was the subject's self-reported past five-year physical activity consistency as reported on the medical screening instrument. To do this a summary question was asked, " Over the past 5 years how consistent has your physical activity remained? A) Consistent B) Somewhat consistent C) Inconsistent". The subjects were then asked to rate their physical activity through his/her life at ages 30-39, 40-49, 50-59 and 60-69 years. To do this subjects used a scale on the health history status sheet, where; 1 Very physically active (regular aerobic exercise and sports), 2 Fairly physically active (sports and active leisure), 3 Moderately physically active (hobbies, active leisure activities), 4 Fairly physically inactive (very few sports, light physical leisure activities) and 5 Very physically inactive (no sports, non-physical leisure activities). These responses were coded and used as possible covariates in the statistical analyses. To code the consistency and decade-to-decade level responses the following procedures were followed. First the summary questions were coded, if a subject responded Consistent of Somewhat consistent the response received a score of '1', otherwise the response received a score of '0'. Next we coded the decade-to-decade physical activity question, which simply consisted of adding and averaging the responses

to get a mean physical activity level for each subject. The two coded values were then summed to give a 'consistency score'.Since it is unknown how acute or recent changes in physical activity affect the telomere maintenance system, it is of importance to consider lifetime physical activity exposure.

The Ypas measure of exercise energy expenditure in kilocalories (exKcal) was used as the primary independent variable; since lifetime physical activity is a major concern, the decade-to-decade physical activity consistency was considered for the previous ten years of life for each subject. If the consistency score failed to match the exKcal measure, then the subject was excluded from the analyses. Subject whose responses on the Ypas and the health history questionnaire where inconsistent were excluded from the analysis. For example, a person who indicates a high-exercise energy expenditure (Ypas exKcal), compared to others in the study, and a low coded consistency score (< 3) was excluded. The other measures of physical activity were used as validation criteria for the Ypas exKcal score.

In an analysis using three groups, subjects were separated according to high exercise energy expenditure (Ypas exkcal/wk score > 3500kcal/wk), middle exercise energy expenditure (Ypas exkcal/wk score between1001 – 3500) and low exercise energy expenditure (Ypas exkcal/wk score < 1000kcal/wk). These cut off values were based on the relationship between physical activity energy expenditure and mortality rates (90). In the Paffenbarger et al. (90) study it was observed that individuals in the above kilocalorie exercise energy expenditure ranges had similar relative risks of death, respectively.

Perceived stress: In addition to the physical activity information a perceived stress survey was administered. The Perceived Stress Survey (PSS) is designed to measure the degree to which situations in one's life are appraised as stressful (31). The

questions in the PSS gather informational about the level of one's perceived life stress over the past month of time. The survey consists of ten questions, with a range of scores from 0- to 40. The reliability of the PSS was assessed and found to be satisfactory (31). The test-retest reliability was 0.85 at two days and 0.55 after six days after initial test (31). Since perceived psychological stress is known to affect the relationship of age and telomere length, the results of the survey were assessed as a possible covariate in the analyses (30).

Telomere Length: All subjects had blood drawn from the antecubital vein of the dominant arm using standard sterile phlebotomy techniques. The blood was collected in standard 10mL EDTA-treated vacutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation from each sample (Ficoll-Paque plus, Amersham Pharmacia Biotech, NJ) (69). The cells were placed into two separate 1.5 mL tubes, one for the telomere length assay and the other to be counted and frozen in 1 x PBS (Invitrogen) at -80°C. Once the cells were isolated, they were counted using a Neubauer ruled hemacytometer (Hausser Scientific, Horsham, PA) and a light microscope. In order to count the cells they were stained with Trypan Blue (Sigma-Aldrich). The counting was done to determine the amount of sample needed to ensure 1-2 million cells were aliquoted out for the telomerase assay. The cells did not need to remain alive, only the telomerase protein needed to remain active and hence freezing for up to one year with minimal freeze-thaw cycles was an appropriate storage method. The following equation was used to estimate the total number of PBMCs in the sample:

$$\text{Number of PBMCs}/\mu L = \text{number cells per } 1/5\ mm^3 \times 5 \times \text{dilution factor} \times 2000$$

The dilution factor was 10 for this cell isolation procedure.

The sample that was not frozen had the DNA extracted using a standardized and quality-controlled PureGene DNA isolation system (Gentra Systems, Minneapolis). The quantity and quality of the genomic DNA isolate was assessed by Quant-iT PicoGreen dsDNA kit (Invitrogen). The integrity of random isolates was evaluated by agarose gel electrophoresis.

Measurement of relative telomere lengths (T/S ratios) was determined by quantitative real time polymerase chain reaction (RT-PCR) as described by Cawthon et al. (26) with the following modifications. The forward primer for the telomere PCR was tel1b [5'-CGG TTT $(GTTTGG)_5$ GTT-3'] used at a final concentration of 125 nM. The reverse primer was tel2b [5'- GGC TTG $(CCTTAC)_5$ CCT-3'] used at a final concentration of 312.5 nM. The forward primer for the single-copy gene (acidic ribosomal phosphoprotein PO, 36B4) was RPLPO-F [5'- CCC ATT CTA TCA TCA ACG GGT ACA A –3'] used at a final concentration of 125 nM. The reverse primer was RPLPO-R [5'- AGG TAG AAG GCC ACA TCA CC –3'] used at a final concentration of 312.5 nM. SYBR Green Master mix (Applied Biosystems) was used and added to 8.75μL of sample and reference DNA. Tubes containing 50, 25, 12.5, 6.25 and 3.125 ng/μL of reference DNA were included in each PCR assay so that the quantity of research sample DNA could be determined relative to the reference DNA sample by the standard curve method. The same reference DNA was used in all PCR runs. The average intra-plate coefficient of variation was 0.5% and the inter-plate coefficient of variation was also 0.5% for both the T and S PCR. A plot of Ct versus log (amount of input reference DNA) is linear and used to determine the integrity of the assay. The Ct value is the fractional cycle number at which the fluorescence emitted by the sample

crosses a set threshold that is above the baseline fluorescence (26). The Telomere PCR (T-PCR) to Single copy gene PCR (S-PCR) ratio was derived from the Ct values of each unknown sample in each assay. The ratio of telomere repeat copy number to a single copy gene copy number allowed the relative quantification of PBMC telomere length (26). This ratio is proportional to the average telomere length (26). The assay was performed using an Applied Biosystems 7300 Real Time PCR System (Applied Biosystems, Foster City, CA). The telomere (T PCR) thermal cycling profile consisted of 10 min at 95°C followed by 30 cycles of 95°C for 15 sec., 67°C for 1 min, 95°C for 15 sec., followed by a dissociation stage of 95°C for 15 sec., 60°C for 30 sec., 95°C for 15 sec. The single-copy gene (S PCR) thermal cycling profile consisted of 10 min at 95°C followed by 40 cycles of 95°C for 15 sec., 57°C for 1 min., 95°C for 15 sec., followed by a dissociation stage of 95°C for 15sec., 60°C for 30 sec., 95°C for 15 sec.

Telomerase:

Telomerase enzyme activity was determined by a commercially available kit, which relies on the telomere repeat amplification protocol (TRAP) (Chemicon, TRAPeze) (37). PBMCs isolated and stored at -80°C will be thawed at 37°C, washed 3 times with 10mL of 4°C PBS, and resuspended in 1mL of 4°C PBS. Approximately $1x10^5$ - $1x10^6$ cells were suspended in 500μL of CHAPS lysis buffer. The assay procedures followed the recommendations of the manufacturer. Briefly, after the cells were prepared as stated above, a reaction mixture was made containing Titanium taq buffer, Trapeze master mix, Clone Tech Taq (hot start), and PCR grade water. The reaction mixture and each sample's cell preparation were combined into a 96-well ABI optical plate. The plate was then loaded into the ABI 7300 RT-PCR machine and PCR

performed with the following thermocycling profile 30°C for 20 min, 95°C for 2 min followed by 40 cycles of 94°C for 15s, 61°C for 40s, 45°C for 32s. The telomerase PCR extension and fluorescence took place in the same reaction vessel. The fluorescence was detected by the ABI 7300 and quantified by the software provided by ABI to determine a critical threshold (Ct) value. The enzyme activity was determined following the standard curve method. From a standard sample (TSR8) provided in the kit, five standards were diluted with a concentration ranging from 0.2 amoles/μL to 0.00002 amole/μL. By using a conversion factor provided by the kit, total product generated (TPG) units can be derived (1 amole of TSR8 = 1,000 TPG units). A plot of log (TSR8 concentration in TPG units) versus Ct value is linear. By inserting each unknown's Ct value into the X value of the linear standard curve and algebraically solving, each unknown's enzyme activity in 'telomerase units' was determined (107). The average intra-plate coefficient of variation was 1.59% and the inter-plate coefficient of variation was 1.58%.

Human Telomere Reverse Transcriptase (hTERT) Genotype–

A sub-sample (64) of subjects were genotyped by restriction fragment length genotyping (RFLP) for the C-1327T variant (rs22735940) in hTERT. The following primer sequences were designed for PCR: Forward hTERT-F1- 5'- ACA GAC GCC CAG GAC CGC TCT- 3'; Reverse hTERT-R1-5'- CAG CGC TGC CTG AAA CTC-3'. A base manipulation in the forward primer was necessary in order to generate a restriction enzyme cut site. A guanine in the gene primer was replaced with a thiamine in the forward primer (underlined in forward primer sequence) to generate a novel restriction site. This base change allowed for the use of RFLP methods and the ability to distinguish between the C and T alleles. The PCR was assembled with the following

parameters: 2.5 μL of 10 x PCR Buffer with $(NH_4)_2SO_4$ (Fermentas), 4.0 μL of 1.25 mM dNTPs, 1.0 μL of 25 mM $MgCl_2$, 0.4 μL of 20 mM hTERT- F1, 0.4 μL of 20 mM hTERT-R1, 0.2 μL of Taq Polymerase (Fermentas) and 14.5 μL of dH2O to make a 25 μL reaction mixture. The thermal cycling conditions were as follows; 95°C for 5min followed by a denaturation step (95°C for 30 sec), annealing step (62°C for 30 sec) and a extension step (72°C for 30 sec) for 40 cycles followed by extension at 72°C for 5min and then held as 4°C until ready for further analyses. The amplicon was ~225 base pairs in length and was verified on a 1% agarose gel. The restriction enzyme EAR I with T allele specificity was purchased from New England Biolabs®. After a 24 hours digestion at 37°C the products were visualized on 3% agarose gels. The fragment/band sizes were as follows for each genotype; TT = 225, CT 202 and 225, and CC = 202. To verify the RFLP results, controls were generated via direct sequencing using the following Primer: Forward hTERT –F-seq2- 5'- CAG AGC CTA GGC CGA TTC – 3'.

Statistical methods:

All statistical procedures were performed using SAS version 9.1. All data were tested to ensure conformity with the assumptions of regression analysis and analysis of covariance (ANCOVA) testing. The residuals for both T/S ratio and relative telomerase enzyme activity were checked for normality and homoscedasity. The residuals for T/S ratio were observed to be normally distributed and to have homogeneous variances. Relative telomerase enzyme activity was not normally distributed nor did it have homogeneous variances, so a logarithm base 10 transformation was performed for statistical analysis purposes. All statistical analyses were performed on the transformed telomerase data; however, non-transformed data are shown as these data are more

biologically relevant. ANCOVA was used to test the association of hTERT genotype with telomere length, telomerase activity, as well as with the interaction with EEE. The assumptions of ANCOVA and regression analysis were tested and observed to be satisfactory.

Correlation matrices were constructed to determine possible covariates according to a p value of 0.3. The variables included in the matrices were age, BMI, gender, T/S ratio, relative telomerase enzyme activity, Yale total energy expenditure, Yale exercise energy expenditure, Yale summary index, Yale vigorous index and physical activity consistency. Regression analysis was performed to explore the relationship between telomere length and telomerase enzyme activity and exercise energy expenditure (EEE). Similar analyses were done using Yale total energy expenditure, Yale summary index and Yale vigorous indices of physical activity. Subjects were grouped into three groups for EEE as follows for ANCOVA: low EEE, 0-1000Kcal/wk; middle EEE, 1001-3500 Kcal/wk; and high EEE, >3500Kcal/wk. ANCOVA was used to investigate the differences in telomere length and telomerase activity among EEE groups. If an ANCOVA model was statistically significant, specific contrasts were written to determine if significant differences existed in telomere length and telomerase enzyme activity between groups of exercise energy expenditure. Significance was accepted at $P \leq 0.05$.

RESULTS

Subject characteristics are shown in Table 1. Significant differences were observed among all groups for EEE, as expected (P<0.001), and in the overall group for PSS, specifically between the low and middle EEE groups (P = 0.014); no other significant differences were observed.

Table 1. Subject characteristics.

	Overall	Low EEE	Middle EEE	High EEE	P Value
N (M, F)	69 (34, 35)	18 (6, 12)	31 (13, 18)	20 (15, 5)	-
Age (yrs)	60.44 (4.09)	59.05 ± 1.14	60.16 ± 0.87	62.15 ± 1.08	0.1393
BMI (kg/m^2)	26.25 (4.49)	27.18 ± 1.13	25.88 ± 0.84	26.02 ± 1.06	0.6336
PSS	10.33 (4.87)	12.81 ± 1.16	9.00*± 0.90	10.18 ± 1.19	0.045
YPAS EEE (kcal/wk)	2697 (2309)	350 ± 285	2236* ± 217	5526*‡ ± 270	<0.001
Telomerase Enzyme activity (TPG-Amoles)	0.0039 (0.018)	0.0012 ± 0.0004	0.0021 ± 0.003	0.0087 ± 0.0004	0.3811
Yale Total EE (Kcal/wk)	8094 (4218)	5207 ± 818	7476* ± 623	11651*‡ ± 776	<0.001
Yale Summary	62.36 (24.46)	41.61 ± 4.80	63.77* ± 3.66	78.85*‡ ± 4.55	0.0001
Yale Vigorous	31.59 (20.51)	14.16 ± 4.1	35.48* ± 3.1	41.25* ± 3.97	0.0002

Descriptive statistics shown for the entire sample and by EEE groups, values in first column reported as means (SD) and all other values reported as least square (LS) means ± SE; * significantly different from the low EEE group (P < 0.05); ‡ significantly different from middle EEE group (P < 0.05) PSS = Perceived stress survey. TPG = Total product generated per 10,000 cells. Kcal/wk = Kilocalories per week. Amoles = attomoles. BMI = Body Mass index.

Table 2 Correlation Matrix.

	Age	BMI	Yale EEE	Ytotal	T/L	Tase	Log10 (TPG)	ConPA	PSS	Y Vigor	Y summary
Age	1	0.055 0.67	0.12 0.32	0.26 0.03	0.087 0.48	-0.054 0.66	-0.099 0.42	0.097 0.43	-0.065 0.61	0.012 0.91	0.008 0.94
BMI		1	-0.23 0.07	-0.058 0.65	-0.11 0.39	0.21 0.09	0.20 0.12	-0.083 0.52	-0.10 0.45	-0.174 0.17	-0.203 0.10
Yale EEE			1	0.58 0.001	-0.026 0.83	0.049 0.69	-0.081 0.51	0.22 0.06	-0.14 0.28	0.555 <.0001	0.628 <.0001
Ytotal				1	-0.003 0.97	0.093 0.45	-0.097 0.43	0.065 0.59	-0.19 0.12	0.191 0.11	0.386 0.001
T/L					1	-0.18 0.13	-0.11 0.35	0.23 0.06	-0.031 0.81	0.101 0.40	0.124 0.31
Tase						1	0.53 0.0001	0.036 0.77	-0.21 0.100	0.084 0.49	-0.014 0.90
Log10 (TPG)							1	-0.10 0.41	-0.17 0.17	0.013 0.91	-0.064 0.60
ConPA								1	-0.081 0.17	0.184 0.13	0.246 0.04
PSS									1	-0.115 0.37	-0.245 0.05
Y Vigor										1	0.791 <.0001
Y Summary											1

All values reported as Pearson's Correlation coefficient, P value. TPG = Total product generated per 10,000 cells. Kcal/wk = Kilocalories per week. Amoles = attomoles. BMI = Body Mass index. Ytotal = Yale total energy expenditure. T/L = T PCR to S PCR ratio. Tase = Telomerase enzyme activity (total product generated). Log (10)TPG = log base 10 transformation of total product generated in Amoles. ConPa = consistency of Physical activity. PSS = Perceived stress survey. Y vigor = Yale vigorous index. Y summary = Yale Summary index.

According to the correlation matrix (Table 2), only consistency of physical activity approached being significantly related to T/S ratio for inclusion as a covariate ($p = 0.063$); no other possible covariates were significant at the 0.3 level in any of the models. As such, only consistency of physical activity was included as a covariate in all-statistical models.

Hypothesis Ia: *Men and women with greater levels of physical activity will have longer PBMC telomere length.*

Telomere Length: In an analysis using three groups of exercise energy expenditure, ANCOVA was performed to test for differences between and among groups. The final ANCOVA model included consistency of physical activity as a covariate and a significant difference among the groups was observed ($P = 0.015$; Figure 1). The specific contrasts revealed a significantly higher T/S ratio in the middle compared to both the low EEE group ($P = 0.01$) and the high EEE group ($P = 0.03$). There were no differences between the low and high EEE groups for T/S ratio ($P = 0.60$).

Regression analysis revealed that there was not a significant relationship between EEE, total EE, Yale summary or Yale vigorous indices and relative telomere length. When we modeled the relationship using regression analysis with the inclusion of a quadratic term, EEE^2, the model was not significant ($P = 0.13$ $R^2 = 0.083$), indicating that

there was not a significant polynomial relationship between EEE and relative telomere length.

Figure 1.

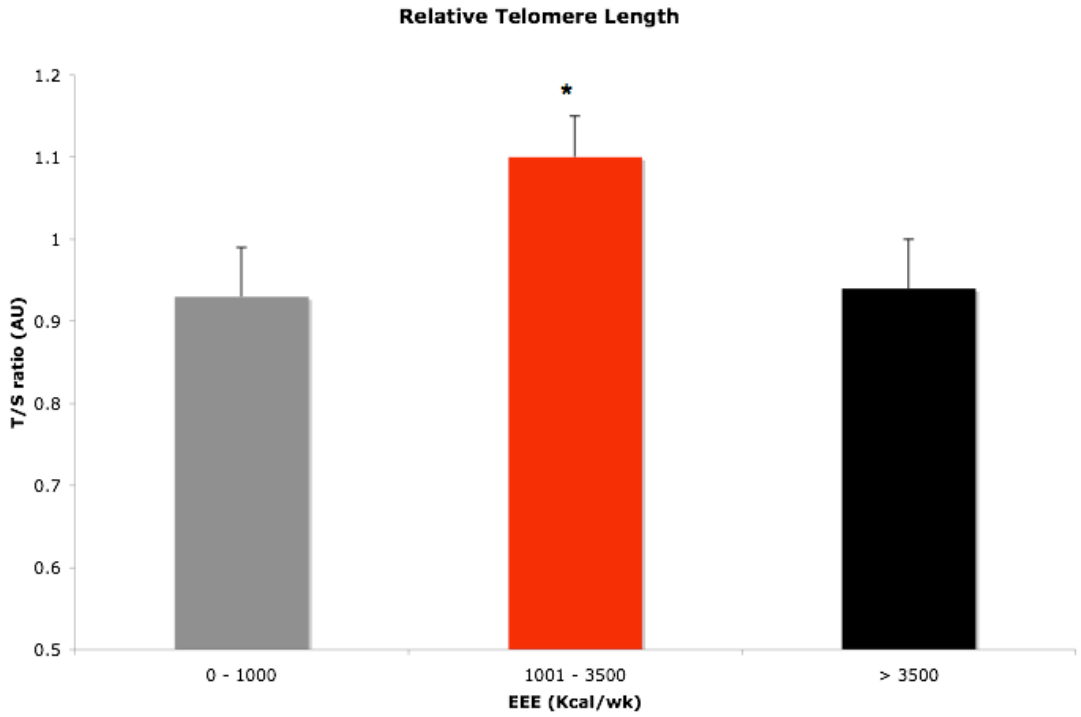

Figure 1.Telomere length by low, middle, and high EEE group. * Significantly different from the low and high EEE groups (P <0.05).

We also analyzed the data by grouping the subjects by quartiles of exercise energy expenditure (n = 16-17 per quartile). The quartile EEE values were low = < 780 kcal/wk, middle = 781 – 2250 kcal/wk, high = 2251 – 3539 kcal/wk and very high = < 3540 kcal/wk. Similar to the above analysis with three groups of EEE, the low EEE group (0.9423 ± 0.03860; Figure 2) had significantly shorter telomeres compared to the middle EEE group (1.1299 ± 0.04016; P = 0.0012). The low EEE group was similar to the upper middle EEE group (1.0313 ± 0.04029; P = 0.1216) and also similar to the high

EEE group (0.9750 ± 0.03503; P = 0.5353). The middle and high EEE groups were significantly different (P = 0.0061), the high and upper middle groups were not significantly different (P = 0.2971), and the middle and upper middle EEE groups approached being significantly different (P = 0.0916). These data indicate a similar relationship as observed with the three-group analysis.

Figure 2.

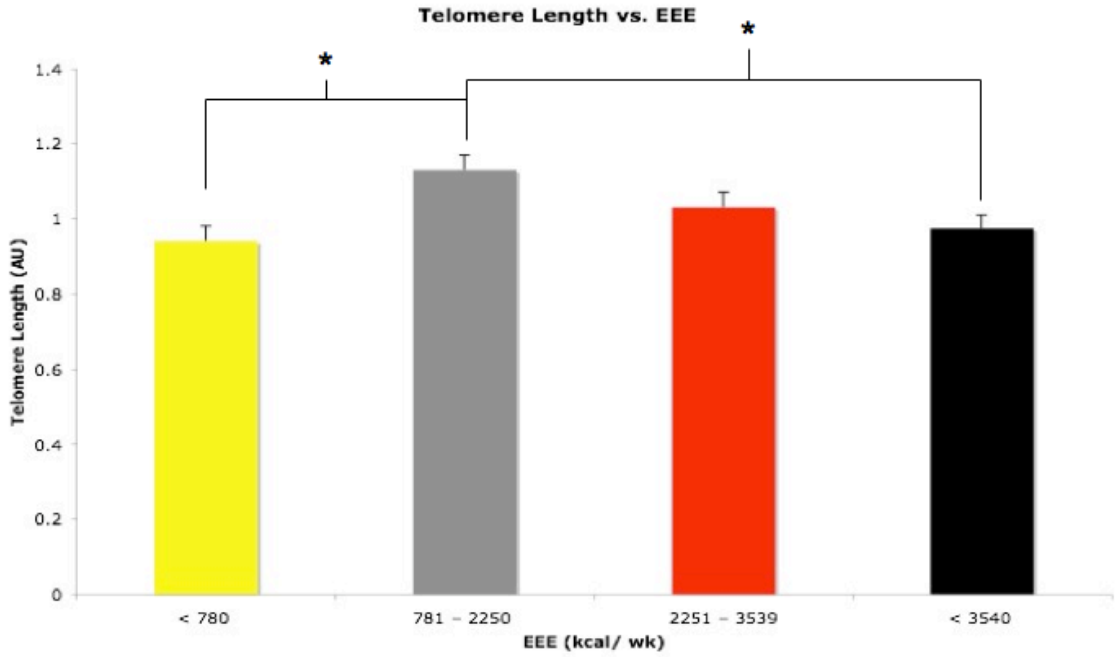

Figure 2. Telomere length by low, middle, upper middle, and high EEE group. *Significantly different from the low EEE group (P <0.05).

Hypothesis Ib: *Men and women with greater levels of physical activity will have greater PBMC telomerase enzyme activity.*

Telomerase Activity: Telomerase enzyme activity was analyzed using both regression and ANCOVA approaches. Regression analysis revealed no relationship between EEE or total EE and telomerase enzyme activity (p = 0.37). Similarly,

ANCOVA analyses revealed no differences among EEE groups for telomerase activity analyzed using either untransformed data (Table 1) or Log_{10} transformed data (not shown). Also the quartile ANCOVA analysis revealed no significant differences among groups (data not shown).

Hypothesis II: *Men and women with the CC genotype of the hTERT gene will have shorter telomere length and less telomerase enzyme activity compared to those with the TT genotype for hTERT.*

hTERT genotype:

Subject characteristics by hTERT genotype group are shown in Table 3.

Table 3 Descriptive statistics by genotype.

	CC	CT	TT	P value
N (M, F)	17 (6, 11)	29 (20, 9)	19 (9,10)	-
Age (yr)	60.2 ± 1.23	61.1 ± 0.94	60.2 ± 1.16	0.7763
BMI (kg/m^2)	25.7 ± 1.18	25.5 ± 0.81	27.7 ± 1.08	0.2696
PSS	10.7 ± 1.34	10.3 ± 0.93	9.8 ± 1.14	0.8655
YPAS (EEE) (Kcal/wk)	2647 ± 565	3184 ± 432	2402.53 ± 534	0.4974
Telomere length (arbitrary units)	1.01 ± 0.04	1.02 ± 0.03	0.99 ± 0.03	0.8468
Telomerase Enzyme activity (TPG- Amoles)	0.000607 ± 0.004	0.00210 ± 0.003	0.0102565 ± 0.004 *	0.0130_a
Yale Total EE (Kcal/wk)	9207 ± 1030	8135 ± 788	7471 ± 974	0.4718
Yale Summary	34.1 ± 5.92	33.8 ± 4.53	28.4 ± 5.60	0.2169
Yale Vigorous	62.9 ± 4.99	68.1 ± 3.82	55.3 ± 4.72	0.6214

All values reported as LS mean ± (SE), N. * CC and CT genotypes groups were significantly different from TT ($P < 0.05$). PSS = Perceived stress survey. TPG = Total product generated per 10,000 cells. Kcal/wk = Kilocalories per week. Amoles = attomoles. BMI = Body Mass index. Subscript a indicates the P value for the transformed data was used.

Across genotypes, subjects were statistically similar with respect to all variables except telomerase enzyme activity. Using ANCOVA it was observed that genotype was significantly associated with the transformed telomerase enzyme activity (data not shown) overall ($P = 0.0130$). The TT genotype had the greatest telomerase activity and was significantly different from both the CT and CC genotype groups, as shown in Figure 2).

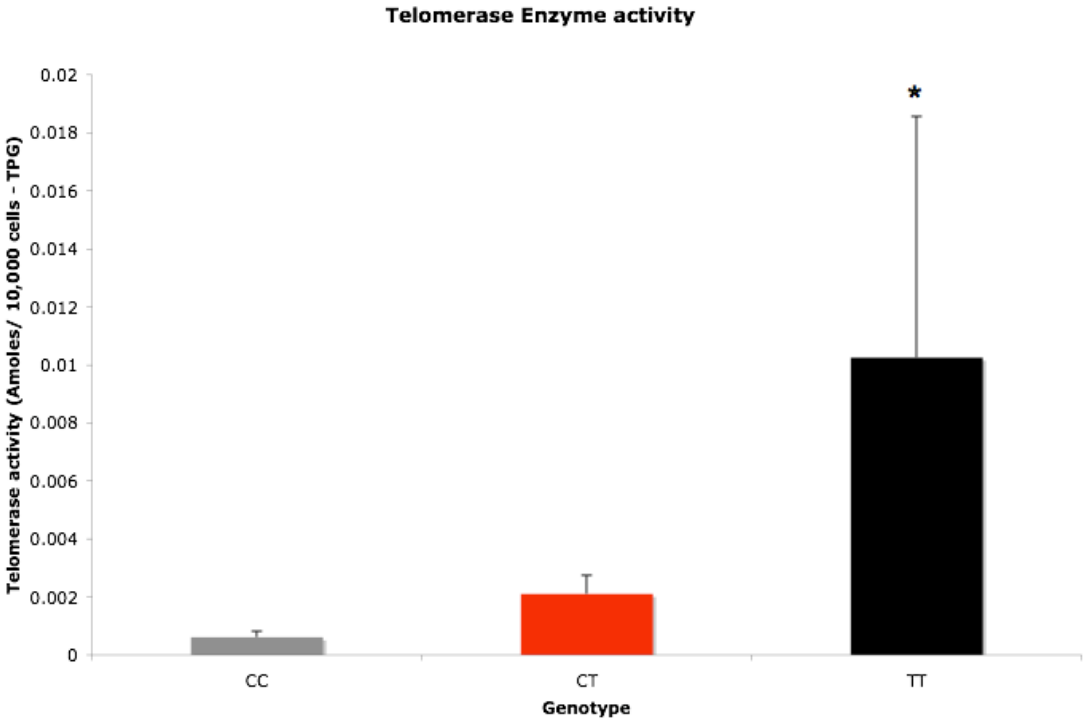

Figure 2 . Telomerase enzyme activity by hTERT genotype. *significantly different telomerase enzyme activity from both the CT and CC genotypes ($P < 0.05$).

Telomere length was not significantly different between genotype groups ($P = 0.8$; Table 3). When the interaction between genotype and exercise energy expenditure level was added to the ANOVA models a significant interaction was observed for telomerase enzyme activity ($P = 0.01$; Table 4), but not for relative telomere length ($P = 0.7$).

Table 4. Interaction between EEE, genotype and, telomerase.

	CC (n = 4)	CT (n = 6)	TT (n = 5)
Low EEE	$0.0016 \pm 0.00060_{a}$	$0.0012 \pm 0.00075_{a}$	$0.0008 \pm 0.00029_{a}$
	CC (n = 9)	CT (n = 12)	TT (n = 8)
Middle EEE	$0.00030 \pm 0.000094_{a,c}$	$0.0038 \pm 0.001_{a,d}$	$0.0016 \pm 0.0007_{a}$
	CC (n = 4)	CT (n = 11)	TT (n = 4)
High EEE	$0.00024 \pm 0.00013_{a,c}$	$0.00069 \pm 0.00023_{a}$	$0.039 \pm 0.034_{b}$

Table 4. Displays the LS means ± SE for the interaction of EEE, genotype and telomerase enzyme activity (TPG Amole/ 10,000 cells). Overall interaction between EEE group and hTERT genotype was significant ($P = 0.0195$). Subscripts a and b denote groups significantly different from high TT ($P = 0.01$- $P < 0.0001$). Subscripts c and d denote groups significantly different from Middle CT ($P = 0.03$ and 0.01).

DISCUSSION

The present study is the first to extensively explore the relationship between physical activity levels and telomere biology. The results of the present study provide evidence that a moderate level of physical activity may be beneficial to the replicative lifespan of immune cells (i.e., PBMCs) in an older population. To our knowledge, no studies to date have directly investigated the relationship between physical activity level and telomerase enzyme activity in an aged human sample, and the present study did not observe a relationship. The present study also observed an association between hTERT genotype and telomerase enzyme activity but did not confirm previous findings that telomere length was also associated with hTERT genotype.

The present results indicate that a middle level of EEE may provide a protective effect, maintaining telomere length in PBMCs. Both EEE and consistency of physical activity over the past five years were significantly associated with telomere length. Acute exercise or short-term physical activity alone may not be a strong enough stimulus to cause short term changes to telomere length but over five or more years of consistent exercise and physical activity, a protective effect may be manifested. The only other study that has addressed these questions was completed by Collins et al. (32), however, their work was related much more to athletic overtraining than to more typical physical activity. They observed that muscle cell homogenate telomere length from highly trained endurance athletes was significantly longer compared to age- and training-matched athletes with FAMS (32). Some of our subjects could have experienced 'overtraining' in their lifetime and hence lead to shorter PBMC telomere length, though none of the subjects reported unusual fatigue or any acute myopathy as part of the medical screening.

Both studies provide support for the hypothesis that physical activity can modify telomere length; with the present study providing evidence that commonly performed physical activity can have a beneficial effect.

We observed that the middle EEE group had the greatest telomere length compared to both the low EEE and high EEE, which were similar to each other. When an EEE quartile analysis was performed, a significant relationship was also observed, similar to the three EEE group analyses. Grouping of subjects by EEE (kcal/wk) has been done in numerous previous studies investigating the physiological outcomes of exercise on mortality and morbidity and cardiovascular disease. Our EEE groups, according to both the three-group analysis and quartile kcal/wk values, are similar to the kilocalorie group cutoffs used in two other studies. These cutoff values were used because subjects in the other studies in which our cutoff values for kilocalories of exercise energy expenditure are based upon have similar relative risks of morbidity and mortality (70) and relative risk of death (90). In Paffenbarger et al. (90), subjects that had exercise energy expenditures greater than 3500 kcal/wk actually had an increased risk of death compared to the 3000 – 34999 kcal/wk group.

Our PBMC telomere length data also fit well with the current literature on exercise training and immunology. A moderate level of endurance exercise training (3-5 days per week, < 90min per session, 55-75% VO_{2max}) elicits an enhanced immune response compared to both sedentary people and athletes who are either overtrained or have just completed an endurance event (77-85). Also there is a similar relationship between exercise workload and infection, again with sedentary and highly trained or overtrained individuals are at greater risk of infection (84, 86). Taken together these

results indicate that a 'J' shaped curve exists between exercise workload and infection rate (80). The telomere length data from this study indicate a similar relationship may be evident with exercise energy expenditure, with either low or high levels of physical activity associated with shorter telomere length compared to moderate levels of physical activity. PBMCs are immune cells that are influenced by both acute exercise and regular exercise training (84, 97). Acute endurance exercise of varying intensities is known to elicit a proliferative and functional response to the different subsets of PBMCs including T and B lymphocytes and natural killer cells (97). Acute exercise is also known to recruit a subset of PBMCs, CD4+ and CD8+ cells, with a greater replicative history and hence shortened telomeres compared to pre-exercise lengths (25). Consistent with our original hypothesis the low EEE group had shorter telomeres than the middle EEE group, while the middle EEE group had longer telomeres than the high EEE group, in contrast to our original hypothesis. A possible mechanism could be that because individuals with either low or high EEE may have compromised immune systems compared to the middle EEE, with the accumulative effect of infections and or exercise stress causing a greater proliferation of PBMCs, resulting in shorter telomeres over time. This is dependent on the fact that these cells' telomeres shorten with each replication and that telomerase levels are held constant across all groups. We did indeed find that telomerase enzyme activity was not significantly different among EEE groups, the first study to our knowledge that examined this issue.

The results of the present study support recent findings that moderate physical activity and exercise improve one's health as well if not better than more intense and frequent physical activity and exercise (52). It is important to note here that exercise

intensity was not directly assessed in the present study; however, the low, middle and high EEE groups did have significantly different vigorous scores from the Ypas. Low to moderate intensity exercise has been indicated to be more beneficial in the maintenance of antioxidant levels when compared to more intense exercise (13) and has been suggested to be better for the treatment of hypertension (52). High intensity exercise depletes most circulating antioxidants, thus increasing the likelihood that reactive oxygen species (ROS) could cause genomic damage and affect telomeres. This may point to a mechanism by which telomeres are damaged by higher intensity exercise. Hypertensive and normotensive subjects that were randomly assigned to three different intensities of cycling (low = 25% of VO_2 max, moderate = 50% of VO_2 max and high = 75% of VO_2 max) 3-5 days per week had markers of oxidative stress and endothelial-mediated vasodilation measured. After training, the high intensity group had greater levels of oxidative stress, as indicated by 8-hydroxy-2 –deoxyguanosine and malondialdehyde-modified low-density lipoprotein (46), providing further evidence that moderate exercise may be more physiologically beneficial. This is a complex issue, certainly not addressed in the present study, but one worth addressing by future investigations.

Figure 4.

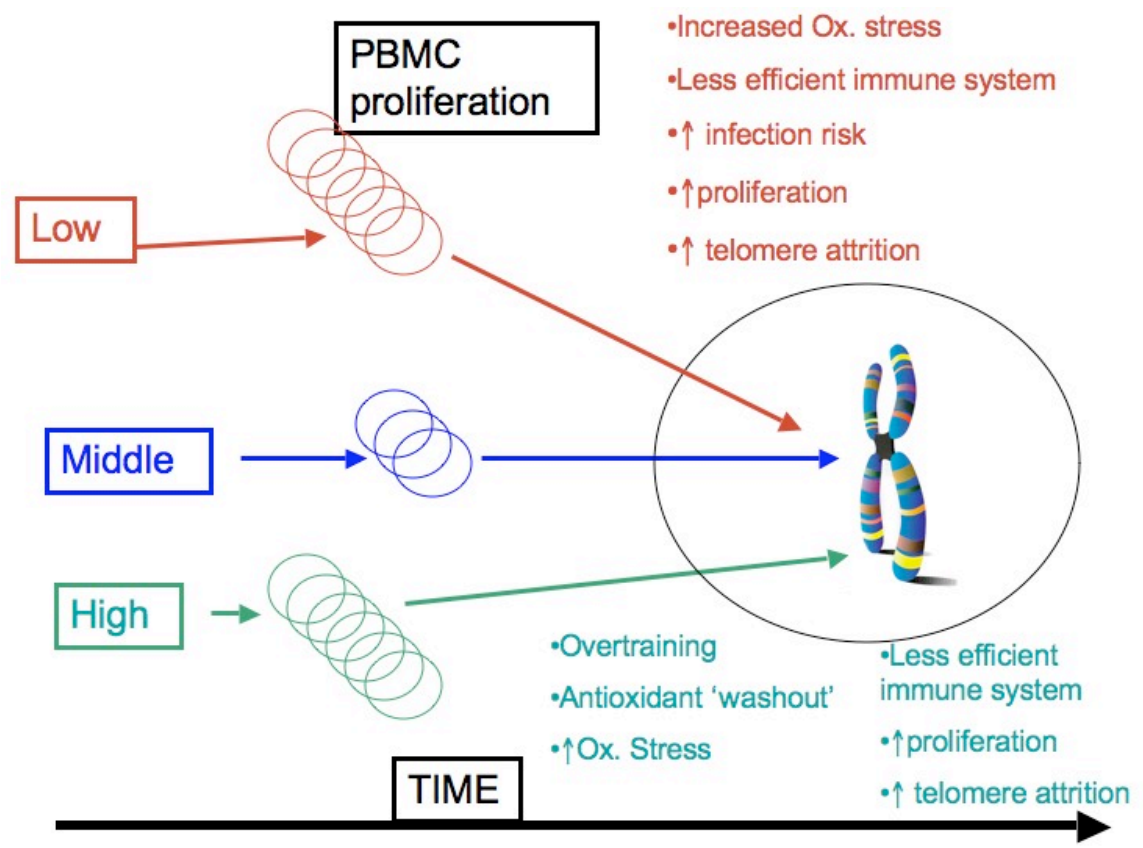

Figure 4. Shows the possible mechanisms that could be playing a role in the relationship between physical activity and telomere length.

There is a wide body of literature that indicates exercise and physical activity as being a way to reduce psychological stress (94). Epel et al. (37) separated subjects into quartiles based upon perceived stress scores. They observed that individuals in the extreme high stress quartile had shorter telomeres and decreased telomerase activity. In the present study, we did not observe a relationship between extreme high and low stress group and telomere length or telomerase enzyme activity in the present study (data not shown). That said, our subjects had much lower perceived stress scores and a much smaller range of values than did the subjects studied by Epel et al. (37) study, and the

present study was not designed to assess the relationship between stress and telomere variables.

In the present study, telomerase activity was not significantly associated with any of the exercise or physical activity measures, in contrast to our original hypothesis. The relationship between telomerase enzyme activity and physical activity and exercise deserves greater investigation. In an animal study of strenuous exercise training and telomerase enzyme activity in skeletal muscle, it was observed that mild or strenuous exercise training in rats did not significantly change telomerase enzyme activity (95). Although various environmental factors, such as oxidative stress, are known to up- and down-regulate telomerase activity (95), it seems from the present results and animal studies that exercise may not be such a stress. That said, in the animal study mentioned above, the authors also transplanted tumor cells into the rats and subjected the rats to exercise training. While no significant differences in telomerase activity were observed with the trained rats compared to the controls, the growth of the sarcoma was decreased in the trained animals compared to the controls (95). Telomerase activity within the tumor cells was not specifically measured, but it may be that exercise can alter telomerase activity in certain contexts, such as cancer.

Two papers by Matsubara and colleagues in 2006 showed a relationship between a functional polymorphism in the promoter region of the hTERT gene, which encodes the reverse transcriptase portion of the telomerase enzyme and transcriptional activity. Matsubara et al. (73) showed that the TT genotype had greater hTERT promoter activity, significantly longer telomeres and greater telomerase enzyme activity than the CC genotype. In a second study, Matsubara et al. (72) used a case control design to explore

the relationship between coronary artery disease and hTERT genotype and they observed a significant relationship between the hTERT genotype and coronary artery disease. Our findings are partly similar in that we observed greater telomerase enzyme activity in the TT genotype group compared both CT and CC groups. Our results indicate a lack of a relationship between TT genotype and telomere length in the present study. These results are consistent with recent findings of Nordfjall et al. (87) who reported that telomere length was not related to hTERT genotype. We also performed an exploratory analysis examining the potential interaction between EEE group and hTERT genotype in relation to telomere length and telomerase activity. A significant interaction was observed for EEE group by hTERT genotype for telomerase activity; however, the sample sizes of the resulting subgroups are so small as to warrant this finding uncertain. The results do raise an interesting future hypothesis about the possibility of a gene-environment interaction, which should be explored in future studies.

The present study is not without limitations. The physical activity information was obtained from self-report questionnaire. While the questionnaire used in the investigation has been widely used and validated, under- and over-reporting of physical activity occurs in studies of this nature, and recall bias may have occurred. Future studies should consider more sophisticated methods to assess physical activity such as doubly labeled water or accelerometry. Another limitation was that oxidative stress was not directly measured or statistically controlled, so possible-underlying mechanisms of the observed associations cannot be addressed in the current study. Oxidative stress is known to affect telomere biology, and vitamin supplementation, specifically antioxidant intake, was not controlled for and could have confounded the results. The goal of the

present study was to test the basic hypothesis that physical activity level is related to telomere biology, which provides a foundation for future, mechanistic studies. Finally the present study was under-powered to fully test for a genotype * EEE interaction in relation to the telomere variables, though a significant interaction was observed that will require further testing in future studies.

We have shown for the first time that long-term moderate exercise may be beneficial to telomere length in PBMCs. We also confirmed the results from two previous studies of an association between hTERT genotype and telomerase enzyme activity. Longitudinal studies looking at frequency, intensity and duration of physical activity are necessary in order to both confirm and gain insight into the mechanisms underlying these preliminary cross-sectional findings. Researching the possibility that telomere length maintenance may be occurring in a telomerase-independent manner when consistent physical activity is performed over a long period of time should also be explored. Finally, future studies should investigate how exercise and physical activity affect immunosenescence in the elderly, in that telomere length may be influencing the relationship between immunosenescence and physical activity.

CONCLUSIONS

Hypothesis Ia: *Men and women with greater levels of physical activity will have longer PBMC telomere length.*

In line with our hypothesis the low EEE groups had shorter telomere length compared to the middle EEE group. In contrast to our original hypothesis the middle EEE group had longer telomere length compared to the high EEE group. This relationship was observed in two separate analyses. Our results support the finding the hTERT TT genotype is associated with greater telomerase activity.

Hypothesis Ib: *Men and women with greater levels of physical activity will have greater PBMC telomerase enzyme activity.*

In contrast to our hypothesis no significant differences were observed between any of the EEE groups. Regression analysis also did not show any significant relationships. Further research is necessary to fully elucidate the relationship between physical activity and telomerase activity.

Hypothesis II: *Men and women with the CC genotype of the hTERT gene will have shorter telomere length and less telomerase enzyme activity compared to those with the TT genotype for hTERT.*

Partially in line with our original hypothesis we find individuals that have the hTERT TT genotype have significantly greater telomerase enzyme activity compared to both the CT and CC genotypes. However we did not observe a significant difference between any of the genotypes and telomere length.

REVIEW OF LITERATURE

The following review of literature is divided into four main parts and provides background information on aging, the telomere maintenance system, physical activity, and the group of survey instruments used to extract information from subjects for this thesis. Within each of the four main areas the review will focus on the following topics: 1) oxidative stress, senescence and how these affect tissue aging, telomere length and telomerase enzyme activity as biomarkers of aging 2) telomeres and telomerase 3) environmental factors associated with telomere maintenance 4) peripheral blood mononuclear cells 5) physical activity 6) perceived psychological stress 7) Yale physical activity survey 8) and telomere length and telomerase enzyme activity assays.

Aging

Aging is a very complex biological process. An enhanced life span has led to various conditions such as cardiovascular disease, cancer, decreased self-sufficiency, and sarcopenia. Aging is an inevitable process of human existence; it is a major risk factor for morbidity and mortality and is linked to oxygen metabolism and free radical production. Aging can be thought of as the turnover rate of our cells and when the loss of cells due to accumulated damage overcomes the replacement of cells leading to tissue failure. The next section will outline the basic cell biology, followed by the possible mechanisms and consequences of aging, and finally a section about the aging cell.

Basic cell biology: In mitotic cells the cell cycle consists of four defined phases known as G1 (G0), S G2 and M, growth phase 1, synthesis, growth phase 2 and mitosis and cytokinesis respectively. Each phase is characterized by specific cellular events. The

purpose of each phase is distinct and prepares the cell for division. Certain cells proliferate for a specified period of time before entering a quiescent state (G0), where the cell is said to be post-mitotic. This state is different from senescence, and is characterized by metabolic activity and the ability to repair DNA damage. Senescence has a main difference from quiescence, the inability to repair DNA damage and chromosome instability. Some cells enter a quiescent state (G0) for extended periods of time and only re-enter the cell cycle due to environmental or other stimulus. Other cells, once fully differentiated enter G0 and remain indefinitely, such as most neurons. However, other cells remain active in the cell cycle and can divide past the purposed Hayflick limit. Examples of cell lines capable of renewal are stem cells, progenitor cells, liver cells, immune cells and many cancer tumor cells.

Mechanisms of Aging: Biologists have long searched for the physiological mechanisms of aging and tried to understand the role of metabolism and oxidative stress in this process(56, 57, 59, 65). Oxidative stress, the production of reactive oxygen species (ROS) as a result of oxygen metabolism, leads to DNA damage and instability, protein denaturation and accumulation of lipid byproducts (Barouki, 2006). Oxidative stress also has implications in telomere biology and function that may point to mechanisms for organismal aging at the cellular level (7, 51, 102, 109).

Aerobic organisms must utilize oxygen in order to survive, but at the same time it is oxygen that is harmful to their existence. Oxygen metabolism produces reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical, singlet oxygen, and hydroxyl, that can cause oxidative damage to biomolecules, such as proteins, lipds, cardohydrates and nucleic acids, and are thus cytotoxic(28).

ROS have been associated with various pathologies such as cancer, heart and vascular diseases, hypertension, obesity, chronic inflammatory disease, diabetes, and aging. The body is equipped to handle oxidative stress with enzymatic and nonenzymatic defense or antioxidant systems. Typically in healthy young individuals, the rate of production of oxidatively modified cellular components is very similar to rate of removal or repair of such components (28). However these defense mechanisms are not without drawbacks, as even in the healthiest of people oxidized lipids, carbohydrates, nucleic acids and proteins accumulate adding to an aging phenotype. It also has been hypothesized that younger individuals can better or more efficiently respond to neutralize oxidative damage than older individuals (1). Therefore, older cells will have a high vulnerability to oxidative stress and tissue damage (1, 28). In essence, the accumulation of oxidative stress and the deleterious effects of this damage to tissues lead to aging. This theory was suggested by D. Harman nearly 60 years ago and is supported by many recent investigations (1, 28). When a cell's DNA and other biomolecules become damaged programmed cell death, apoptosis, and the cessation of cellular proliferation, cell senescence may occur adding to an aged phenotype.

In contrast to the ROS model, another model of investigating aging has been proposed by Kipling (66). In his model there are three components to aged tissues: aged extracellular environments, aging of cells that are non-mitotic, and the aging of mitotic cells. Each of these areas contributes to overall human aging. When cells that undergo division (i.e., mitosis) are removed from the body, they can only replicate a finite number of times before entering a senescent state (58). This senescent state should not be confused with cell death, necrosis, or apoptosis. In this mitosis arrested state,

(senescence) the cell is still metabolically active and can survive for many months or longer but is unable to replicate or repair damage (66).

Cellular senescence has been proposed as another important component of the aging process. The human body can be separated into major tissue or cell types; mitotic and non-mitotic tissues. Non-mitotic tissues do not under go cell division and hence lack the ability to replace damaged and dead cells. In mitotic tissues, cells must divide to maintain and replace normal physiological cell loss. The process known as mitosis or cell division is responsible for the replacement of lost or damaged cells. In vitro, this growth arrested state is apparently irreversible. Changes in the cells physiology, morphology and gene expression accompany this cessation of division (39). Senescent cells in culture take on an enlarged and flattened phenotype. Vascular cells also express a different set of genes, including negative cell cycle regulators p53 and p16 (75). It was hypothesized that this limited cellular division observed in vitro also occurred in vivo. A possible limiting factor to cell proliferation and division that has been proposed is a cells telomere length (17, 50, 54, 55, 58). It was observed that with each cell division a small section of telomere DNA was lost. As the telomeres shortened over time and with each division a critical length is reached and cell division ceases (58). Telomeres are found on the ends of linear chromosomes and function to protect the genomic DNA from damage. Telomeres will be discussed in greater detail later in this review.

The Aging Cell: During aging in vitro, telomeres shorten with each population doubling until a critical length is reached and replicative senescence occurs (34). Telomere length has been associated with aging in vivo as well (17, 27, 37, 38). Telomeres are said to act as a mitotic clock, keeping track of cell divisions until the

critical length is reached and the cell enters replicative senescence (34, 54). Thus, telomere length has been indicated as a novel biomarker of cellular aging.

Telomeres:

Structure: Telomeres are located on the ends of linear mammalian chromosomes. Telomeres consist of double stranded repeating $(TTAGGG)_n$ sequence and a single stranded Guanine-rich (G-rich) section of 150-300 nucleotides extending from the 3' hydroxyl group end of double stranded DNA, extending 10-14kb in humans (19). Telomere length in humans, specifically white blood cells, is highly variable and heritable (6, 9). Telomeres and telomere associated binding proteins function to protect the ends of chromosomes from damage that could result in recombination and degradation activities (14, 23, 66). Telomeres accomplish protecting chromosomes from damage and instability by forming a protective cap (14, 23, 66)(Blackburn, Blasco). When telomeres are sufficiently long, they will fold back and bind to telomere associated proteins, creating a T-loop or cap on the chromosome (14, 23, 66). Telomere binding proteins in mammals include TRF1, TRF2 and Pot1 has been associated with protection of the 3' G-rich strand (12, 14, 15, 18). Telomeres and the associated binding proteins may form a heterochromatin-like structure that has the ability to silence nearby genes when tightly folded (12). Telomeres, more specifically the progressive shortening of telomeres, have been implicated in replicative senescence and also aging.

Figure 1.

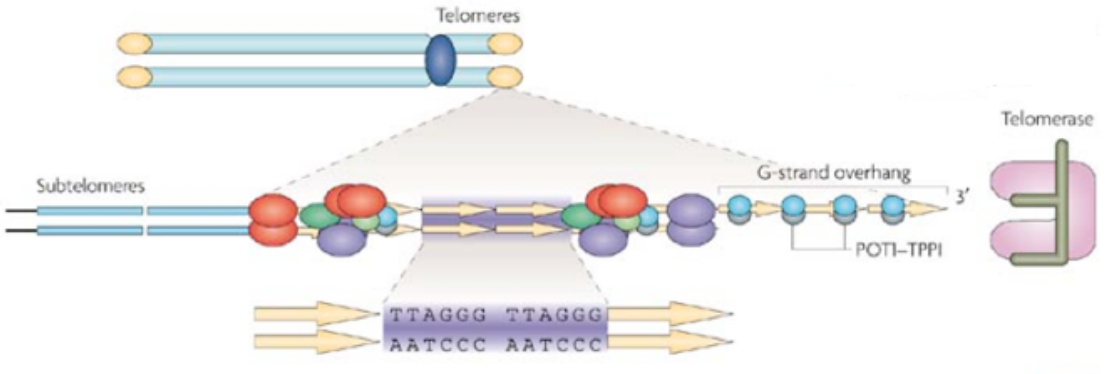

Figure 1. Telomere with subtelomeric and G-Strand overhang regions. Protection of telomeres (POT1 – a telomere associated binding protein) and telomerase also shown. Telomere displayed in elongated (not capped) conformation. Figure adapted from Blasco (24).

Telomere DNA shortens with each progressive cell division. The reason for this shortening is that DNA polymerase is unable to fully replicate telomere DNA in somatic cells. This is known as the end-replication problem (54). Each time a cell divides a small piece of telomere DNA is not fully replaced owing to the binding of the DNA polymerase. This leads to the progressive shortening of telomere with each division in vitro and is associated to telomere length being negatively related to aging (8, 9, 11, 16, 23, 27, 37, 54, 55). However, in certain cell types (stem cells immune cells etc.) the progressive shortening of telomeres does not occur (17, 23, 47-49).

Telomerease: It was first hypothesized that an enzyme to maintain telomeres existed in 1973 by a Russian, then USSR, scientist A.M Olovnikov. This hypothesis was later tested by E.H Blackburn and C. Grieder (49) when they discovered the enzyme that has become known as telomerase. Through further research it was found that only certain cell lines express the telomerase holoenzyme; stem cells, liver cells, progenitor cells, germ cells and certain immune cells such as peripheral blood mononuclear cells express the holoenzyme (16).

Telomerase is a specialized reverse transcriptase consisting of two components; a RNA component, encoded by the gene hTERC (human telomerase RNA component) and a protein component, encoded by the gene hTERT (human telomerase reverse transcriptase) (16). For telomerase to be functional and active, both components must be expressed and present in the cell. The hTERC gene is typically expressed ubiquitously by cells while the hTERT is only expressed in cells that have telomerase activity (24). Before this enzyme was discovered, telomere length was considered to be a static entity. It is now known that telomere length should be considered a dynamic system (14).

Telomeres are able to switch between a capped and uncapped state (Blackburn). Telomerase is only able to add telomere repeats from the RNA template, when the telomere is in an uncapped state and the telomere is accessible to telomerase (17, 23, 98-100, 106). When a telomere is in a capped state, the length is sufficiently long and able to fold back upon its self and bind to telomere binding proteins, forming a T-loop (17, 19, 24). Cells are only able to replicate when telomeres are sufficiently long enough to form a t-loop. Once a "critical length" of telomere DNA is reached the cell becomes senescent. In cells with telomerase activity telomere length is able to be maintained so that cell division and replication can occur as physiologically necessary, thus delaying tissue aging and senescence (38). However, cells with telomerase activity will still show shortened telomeres with aging. In cells with shortened telomeres and telomerase activity, cells will be able to maintain normal cell replication until part of the telomere maintenance system fails. The telomere maintenance system can stop functioning correctly for numerous reasons. One reason for this failure could be due to oxidative modification of the protein components of telomerase or modification in the expression

of the genes encoding for hTERT or hTERC. In certain cases, cells with shortened telomeres and lacking telomerase activity, can sustain an environmental stress that mutates these genes in a way that causes the over expression telomerase and leads to uncontrolled cell division and cancer (15, 17, 23, 66). There are certain environmental factors that have been associated to impaired telomere maintenance. Recently, a functional single nucleotide polymorphism (SNP) in the hTERT gene was found to be associated with increase risk of cardiovascular disease and lower telomerase enzyme activity (72, 73).

Functional hTERT single nucleotide polymorphism:

The gene that encodes for the catalytically active subunit of Telomerase (hTERT), is known to have a functional single nucleotide polymorphism. A C/T polymorphism is located in the promoter region at –1327 (rs 2853669); the T variant has been associated with greater telomere length and greater telomerase activity is known to be associated with greater telomere length and lower telomerase enzyme activity (Matsubara 2006). The –1327 T sequence is associated with greater promoter region activity than the –1327 C variant (72, 73). Recently Nordfjall et al. (87) confirmed that the T varant was associated with greater telomerase enzyme activity but did not confirm that the SNP was associated with longer telomeres.

Genetic and Gender influences on Telomere length: The following section will outline what is known about the heritability and gender differences within telomere length. We will explore the affect of gender and telomere length differences at birth and with in cell types. Telomere length has also been shown to be heritable (5, 6, 10, 61).

Telomere length has been investigated for birth and gender differences that may be used to explain the high variability observed in adult humans. Telomere length in newborns was assessed in three different tissues including white blood cells. This study found that within subject's tissues and between genders there was not a significant difference in telomere length (88, 89). They concluded that the reason for the gender difference observed in adult male and female telomere length is possibly due to a difference in telomere rate attrition between sexes (88, 89). However they could not account for the large differences observed between child and adult cell turnover rates.

One reason for the observed difference between adults and children could be that two sub-populations of PBMCs exist. One population of cells dividing about every twenty days and being more prevalent at birth having an annual telomere length decrease of 330bp/yr from birth to 1.5yrs; the second cell population divides about every six months, depending on infection, and has a telomere attrition rate of about 50bp/yr after the age of 23 (103). The second cell population is more prevalent in adults and may account for the observed differences between adult and child PBMC telomere length. Other environmental factors are also associated to observed differences in telomere length.

Environmental factors associated with Telomere length and telomerase activity:

The following section will review and outline some of the studies that have shown telomere length and telomerase enzyme activity to be malleable cellular traits. Environmental factors that have been associated with decreased telomerase enzyme activity and shortened telomere length include oxidative stress (2, 3, 61), perceived

psychological stress (37), cardiovascular disease risk factors (38), and morbidity and mortality (27, 71).

In a study of pre-menopausal mothers of healthy and chronically ill or disabled children, investigated the relationship of telomere length and telomerease enzyme activity and perceived psychological stress. The mothers were separated into groups of high and low perceived stress according to their responses to a questionnaire designed to assess perceived stress. Using ANOVA, and the covariates of age and body mass index, it was observed that mothers with higher perceived stress also had statistically significant shorter telomeres than mothers with less perceived stress. Telomerase enzyme activity was also analyzed in a similar manner, finding the association that higher perceived stress was associated to decreased telomerase enzyme activity. Telomere length and telomerase activity was measured in peripheral blood mononuclear cells (PBMCs), a density-specific type of immune cell with known telomerase enzyme activity.

In a continuation of this study, PBMC telomere length and telomerase enzyme activity was compared with cardiovascular disease risk factors, autonomic nervous system activation and stress arousal. The findings indicated that low telomerase activity was associated with exaggerated autonomic reactivity to acute mental stress, elevated nocturnal epinephrine and multiple risk factors of CVD and metabolic syndrome; including high fasting glucose, high systolic blood pressure, poor lipid profile, and elevated abdominal adiposity (38). Although this study was cross-sectional, the authors provided several possible mechanisms that this factors could be influencing these cellular factors. They also inferred that just the expression of telomerase can add to ‘healthly cell

life' (38). Other studies have also associated telomere length specifically to cardiovascular disease risk factors.

Two specific studies by Benetos and collegues associated telomere length with age, sex, and markers of cardiovascular disease. In a longitudinal study of French men and women Benetos et al. (9, 10) investigated the relationship between white blood cell telomere length and pulse wave velocity and pulse pressure. Pulse wave velocity and pulse pressure are both known to increase with age and are indicative of large artery stiffness (9, 10). Telomere length was measure by telomere restriction fragment (TRF) length analysis. Telomere length was found to be significantly longer in women than men after adjustment for age by 0.28 kb (8.67±0.09 versus 8.37±0.07kb for women and men, respectively; $P = 0.016$). They also found that telomere length was negatively correlated with age in both sexes (For men, r^2 -0.45, $P = 0.0001$; for women, r^2 -0.48; $P = 0.0001$). They found that the telomere attrition rate for men was 0.038 kb per year for men and 0.036 kb per year for women (9, 10). TRF length was significantly correlated to pulse wave velocity in men and women and to pulse pressure in men only. No physiological mechanism was discussed and they concluded that telomere length adds a new dimension to the study of cardiovascular disease (10).

In a second study Benetos el al. (9) explored the relationship between white blood cell telomere length and carotid artery increased carotid atherosclerosis in hypertensive subjects. After adjusting for age, hypertensive subjects with carotid artery plaques had significantly shortened telomeres compared to hypertensive subjects without plaques (9). Possible mechanisms for this relationship that were mentioned included oxidative stress and chronic inflammation. Cellular turnover rates are registered by a cell's telomeres,

this rate may be augmented by chronic inflammation and oxidative stress (9). There are many other studies that link cardiovascular disease and telomere biology but the studies mentioned above are examples of the recent findings in the field. Overall there is a relationship between white cell telomere length and risk factors for cardiovascular disease (9, 27, 38).

Another environmental factor that has been related to telomere length is socio-economic status (SES) (29). Female twins discordant for SES were evaluated for mean telomere white blood cell length. White blood cell telomere length was highly variable but significantly shorter in the lower SES groups (29). When manual versus non-manual employees were compared, manual workers were observed to have significantly shorter telomeres, mean difference 163.2 base pairs. Possible reasons for the observed differences were lack of exercise, smoking, and obesity in the low SES group (29). Another study has investigated the relationship between exercise and telomere length.

In this investigation telomere length was assessed in athletes (endurance) that had similar training volumes but one group had fatigued athlete myopathic syndrome (FAMS). Muscle biopsies were taken from both groups and the cell homogenate was assessed for telomere restriction fragment length. The symptomatic athletes had significantly shorter telomeres than the age, sex, and mileage matched healthy athletes (4.0 +/- 1.8 kb vs. 5.4 +/- 0.6 kb, $P < 0.05$). They concluded that a possible reason the symptomatic athletes may have shorter muscle cell telomere length is due to an increased rate of muscle satellite cell proliferation to repair extensive muscle damage due to constant training (32). However this claim may be unfounded because skeletal muscle

satellite cell telomere length was not measured directly, only a muscle cell homogenate that would contain many different cell lines.

Overall these studies have shown that telomere length and telomerase enzyme activity are modifiable factors. Although the mechanism is not be clearly understood, a moderate to strong relationship exists between oxidative stress, cardiovascular disease risk factors, psychological stress, SES, aging, and possibly exercise and telomere length. Telomerase enzyme activity has not been as extensively studied as telomere length but similar relationships do exist between cardiovascular disease risk factors, psychological stress and telomerase activity.

Peripheral Blood Mononuclear cells

Peripheral blood mononuclear cells (PBMCs) are a mixture of T (T cells) and B (B cells) lymphocytes, neutrophils, natural killer cells, and monocytes. These cells are isolated from EDTA treated whole blood by density gradient centrifugation using a Ficoll-paque method. T and B cells originate in the bone marrow and will eventually end up in the lymph nodes, the spleen and various other tissues and aid in immunity. Lymphocytes that will become T cells migrate to the thymus to mature. Neutrophils account for up to 60% of circulating PBMCs and aid in everyday immunity controlling bacteria that may enter the body. Natural killer cells originate from the bone marrow and travel to lymph nodes, specifically the spleen and tonsils, where they then enter one of two modes of immunity. In one mode of immunity, upon infection they secrete cytokines that direct T and B cells, in the second mode they become killers of tumor cells and foreign viruses (41, 76). PBMCs are among the essential cell mediators of stress and inflammation and produce cytokines, chemokines, and growth factors that produce

powerful effects that may be pathological of beneficial (33). Besides infection by foreign molecules another stimulus is known to increase circulating levels of PBMCs, exercise and physical activity (33).

Exercise is a powerful physiological stressor and is known to affect the gene expression profile in PBMCs (33). During relatively heavy bouts of exercise a large increase in number of circulating PBMCs occurs (33). In a study by Connolly et al. (33), the effect of acute strenuous exercise (30 min of cycling at 80% of the subjects peak oxygen uptake), and gene expression in PBMCs. The results indicated that 311 genes were differentially expressed between pre- and post exercise (33). Gene expression was also measured during recovery from exercise and they found that 552 genes were differentially regulated (33). In light of these factors we choose PBMCs as a cell source to study telomere length and telomerase enzyme activity.

Physical Activity and Exercise

Physical activity and exercise have been defined in various ways. In the Surgeon General's 1996 report *Physical Activity and Health* and also in the American College of Sports Medicine *Guidelines for Exercise Testing and Prescription 7th ed.* physical activity and exercise have different definitions. Physical activity is defined as any bodily movement caused by the contraction of skeletal muscle and substantially increases energy expenditure (ACSM 7th and *Physical Activity and Health* 1996). Exercise can be defined as a type of physical activity that is planned, structured, and repetitive bodily movement done to improve or maintain one of more components of physical fitness. Physical fitness is a set of attributes that people have or achieve relating to their ability to

perform physical activity. This review of literature will focus on physical activity and exercise energy expenditure and aging, and the various associated health outcomes.

Aging can be characterized by a loss of functional capacity. This loss of functional capacity can be broken down into mental and physical limitations and disabilities. It is a well-established paradigm that physical activity is related to physical and mental health. Factors such as cardiovascular disease, morbidity and mortality, sarcopenia, and oxygen transport have been studied in relation to aging. This section will focus on the physical decline associated with aging and how physical activity can alter the aging process.

In the Harvard Alumni study by Paffenbarger et al. (90), Harvard alumni age ranging from 35-74 yr. were followed and assessed by questionnaires. It was observed that those expending a greater amount of energy per week in exercise (walking, stair climbing, playing sports) had a 25-30% lower mortality rate than those with lower exercise energy expenditures (90). It has also been demonstrated that changing physical activity later in life is associated with a 23% decreased risk in all-cause mortality (91, 92). Another aspect of physical activity that has been associated with all-cause mortality is exercise intensity. Lee et al. (68) defined vigorous activity as intensity greater than 6 METs (1 MET = 3.5 ml/kg/min). They found a reduced risk of death for those that reported vigorous activity compared to those who reported no vigorous activity regardless of energy expenditure (68). The authors concluded that exercise or physical activity intensity might be responsible for the inverse relationship between physical activity and mortality, rather than the amount of exercise. In general it is well accepted that increased physical activity is associated with reduced all-cause mortality. However,

it is yet determined if the reduced risk of all-cause mortality with moderate levels and intensities of physical activity is affected by gender

Physical fitness levels have also been associated to all-cause mortality. In another very well known epidemiological study, Blair et al. (21) showed that those at the greatest risk of morbidity and mortality were in the lowest quintile of physical fitness. They also showed that improving physical fitness later in life reduced the risk of morbidity and mortality significantly (20). These studies were able to show a dose-response relationship to the reduction in risk with higher levels of physical fitness (20, 21). These data and others show that the protective effect of physical fitness is as strong or stronger in women as it is in men. Therefore a lack of physical fitness should be considered as serious a risk factor for mortality as smoking, high blood cholesterol, fasting blood glucose, blood pressure, high BMI, and genetics. Another physical parameter that decreases with aging is skeletal muscle strength.

In addition to the decreased levels of important circulating hormones physical inactivity adds to the age related loss of muscle mass and strength. Although there are only a few longitudinal animal studies directly investigating this, the evidence from the cross-sectional studies indicate that physical activity/ inactivity is important in the loss of muscle mass and strength. An extreme example of physical inactivity is bed rest, and/ or immobilization of a joint that leads to the loss of muscle mass and strength even in young individuals (42, 104). Also older individuals that are more active have greater lean mass and strength than age matched inactive individuals (67, 96). Physical activity, specifically resistance training, may provide the most effective non-drug intervention to help maintain muscle mass and strength (42, 104).

Change in fitness with Age:

Aging is also known to decrease one's aerobic capacity and maximal oxygen uptake (VO_2max). The loss in maximal oxygen transport is related to three factors, reduced physical activity, physiological aging, and an increased prevalence of pathological conditions. This loss of oxygen transport results in a loss of functional capacity that can contribute to a loss of independence, increased risk of disability, and a reduced quality of life with age (105). Also, maximal aerobic capacity is an independent risk factor for cardiovascular disease and morbidity and mortality. However there is large amount of variation in the rate of decline observed in individuals with a range of 5-15% per decade. The observed variation in the rate of decline is due to gender and exercise training differences, among other factors.

In a meta-analysis of trained and untrained men, the authors sought to describe the relationship between training status and decline in maximal aerobic capacity (105). The findings indicated the absolute rates of decline in VO_2max with age were not different (P = 0.05) for sedentary (-4.0 $ml*kg^{-1}*min^{-1}$) active (-4.0 $ml*kg^{-1}*min^{-1}$) and endurance trained (-4.6 $ml*kg^{-1}*min^{-1}$) (105). There were also no differences between groups in relative rates of decline in VO_2max with increasing age. In a meta-analysis of sedentary, active and endurance trained women, all groups were found to be significantly different from each other (P= 0.001). Endurance trained women had the greatest rate of decline per decade in VO_2max with age (-6.2 $ml*kg^{-1}*min^{-1}$), lower in active women (-4.4 $ml*kg^{-1}*min^{-1}$) and lowest in sedentary women (-3.5 $ml*kg^{-1}*min^{-1}$) (44). These results suggest, in contrast to the prevailing view, that endurance trained individuals may actually decline in maximal aerobic capacity at a greater rate. This may be due to the

enhanced starting values earlier in life, or a baseline effect (44). Although maximal aerobic capacity is not directly related to the study at hand, it is involved in aging and also is an important factor in physical activity.

Hormonal Changes, Aging, Disease, and Physical Activity:

Aging has many consequences, one being a change in the endocrine systems that regulate many of our physiological processes. The following section will outline the endocrine changes associated with aging and how physical can affect/ combat the negative effects. Three major hormonal systems will be discussed: hypothalamic-pituitary-gonadal axis, adrenal cortex, and growth hormone/ insuline- like growth factors axis.

Alterations in the Hypothalamic-Pituitary-Gonadal Axis:

Males and females differ in the amount of change that occurs with age in the hypothalamic-pituitary-gonadal axis. Females undergo menopause, starting at about at 50 and last up to five years. Menopause results from reductions stimulation from the hypothalamus and pituitary glands on the ovaries causing a reduction in esterdiol production. In men this reduction of stimulation from the hypothalamus and pituitary glands on the testes occurs more slowly and subtly. The decline in free and serum testosterone is gradual in males. Recently some sources have begun calling this andropause, due to the decline in adrogenic hormones(64). The reduction of these hormones maybe linked to an increased risk for the development of CVD, loss of muscle mass, increase risk of type II diabetes and increase risk of morbidity and mortality (64).

Adrenal Cortex:

The main hormone affected by aging that is produced in the adrenal cortex is dehydroepiandrosterone (DHEA). The other hormones produced by the adrenal cortex such as the glucocorticoid and mineralocorticoid adrenal steroids remain fairly stable throughout the aging process. Starting at age 30 yr. a slow decline of plasma levels of DHEA occurs until at age 75 yr. where only about 20-30% of the values at young age are present (53, 62). There are many aspects of DHEA functions that are unknown such as it role in health and aging, cellular or molecular functions, or it's receptor sites. However, research in animals suggests that DHEA may serve as a biomarker for aging and disease risk (93). Also research has shown that exogenous DHEA may protect aginst cancer, atherosclerosis, viral infections, obeisity, diabetes, enhances immune function and may even extend life.

Growth Hormone/ Insulin-Like Growth Factor Axis:

The amount and frequency of growth hormone released decrease gradually with aging, eventually resulting in 'somatopause' (45). A similar decrease in the amount of insulin-like growth factor- I (IGF-I). Both growth hormone and IGF-I stimulate tissue growth and protein synthesis. The interaction between the decrease in circulating sex hormones (females – estrogen; males – testosterone) and the decrease in growth hormone and IGF-I is not known. Research has shown that muscle size and strength, body composition, and bone mass alterations are related to the decreases in these hormones (101).

Physical activity and Exercise:

As reviewed in the above sections hormonal changes are related to an increased risk of various disease and morbidity and mortality. Also reviewed above it is noted that

physical activity and exercise are known to combat the age related increase in risk to many of the diseases associated with changes in endocrine levels. It has been estimated that a 'sedentary lifestyle produces losses in functional capacity at least as great as the effect of aging' (74). The greatest health benefits may be observed when a sedentary individual engages in a moderately physically activity lifestyle.

Perceived Psychological stress

Another factor that has implications in both aging and immunity is psychological stress. Exercise has been shown to combat the effects of stress, although the biological and cellular mechanisms are not well defined. The following will provide an overview of stress and the survey that was used in this project.

The impact of stress is agreed to be 'objective' and specific to the person experiencing the 'stressful' event, leading to the development of the term 'perceived stress' (lazarus 1966 1977). Perceived stress has been shown to impact one's behaviors and health through various avenues including immunity (31). In the past, both subjective and objective measures of stress have been used to quantify both number of stressful events and event specific stress (31). Using objective event specific measures of stress offer distinctive advantages over subjective measures of stress. First, because the event is identified and known, the risk of disease and rate of occurrence of disease follow such an event is more easily quantified (31). Second, the measurement is fairly simple and straightforward. Some researchers have tried to develop global scales of perceived stress with limited success. Cohen et al. (31) developed and validated a global measure of perceived stress using the objective rather than subjective criteria.

The Perceived stress survey (PSS) is a 14 question survey that measures the degree to which individuals appraise their life events as stressful (31). This survey is designed to measure the degree to which people found their life to be uncontrollable, unpredictable and overloading (31). The reason for using these three components is because they have been consistently associated with the experience of stress. The survey contains several questions that pertain to current stress levels. Within the survey there are seven positive and seven negatively slated questions that are answered on a scale of 1 – 4. The negative questions are reverse scored (ex. 1=4 for negatively slated questions) and then summed with the other seven questions. To assess the reliability and validity of the PSS, three samples were surveyed multiple times. A college aged group, an older group and a smoking cessation group were all assessed. The test-retest scores were very high at 0.84, 0.85, and 0.86 in the young, older, and smoking groups respectively (31). The PSS has been adequately correlated with life-event stress measures (0.18 to 0.36, P=0.05) and strongly correlated to symptomatological measures of depression (0.52 to 0.76, P=0.05). Sex and gender did not appear to have an effect on the outcome of the survey. Overall the survey has been proven to be valid and reliable and provides a simple means to investigate the role of perceived stress in the etiology of disease (31). The scale that was used for the present study was the 10 question PSS (PSS 10) which was found to be as good as the 14 question PSS (PSS 14) at quantifying perceived stress (31). The statistical parameters mentioned above as well as the reliability and validity were slightly better for the PSS 10 than the PSS 14 (31).

Yale Physical Activity Survey

The following section will outline the Yale Physical Activity Survey (Ypas). Reliability and validity of the survey will be discussed in terms of comparisons with similar physical activity surveys and objective physiological measures such as doubly-labeled water and maximal/peak oxygen consumption. This survey was developed at Yale School of Medicine in 1993 (36).

The Ypas is an "interviewer-administered survey that asks the individual to estimate time spent in a list of twenty-five activities in a typical week during the last month"(29). The list of activities has five sub-headings titled: Work, yard work, care-taking, exercise, and recreational activities. Two indices are gleaned from the information: 1) time spent in each activity is multiplied by an intensity code and reported in kilocalories per minute and then 2) summed across all activities to create an index of weekly energy expenditure reported in kilocalories per week. The two measures are accompanied by the amount of time spent in each activity summed to provide a total time index reported in hours per week. When the exercise portion of the survey is analyzed alone, exercise energy expenditure in kilocalories per week can be determined in addition to the other measures.

The Ypas was initially analyzed for repeatability and assessed using paired t-test and Pearson product moment correlation coefficient. Validity was assessed by the Spearman ranked correlation coefficient. The estimated maximal oxygen consumption was found to be significantly correlated to the summary index and vigorous index (0.58, P=0.004; and 0.60, P=0.003, respectively). To further validate the Ypas, Young et al, (108) compared it to another previously validated self-reported physical activity survey

called the Stanford 7-day Physical Activity Recall survey (PAR). Energy expenditure, total time, the summary index and the vigorous index were found to be significantly correlated with daily energy expenditure (0.37, P = 0.0004; 0.30, P= 0.02; 0.33, P= 0.01; 0.45, P<0.001, respectively)(29). The validity of the Ypas for light-intensity activity was less clear and no significant correlations were found. More stringent validation qualities need be established for low intensity activity. Overall the Ypas is a survey designed to assess an older individual's physical activity behavior. While the typical pitfalls of survey information are unavoidable, the survey is fairly reliable and valid.

Telomere length measurement

The following section will outline the technique used to determine relative PBMC telomere length for the present thesis project, known as T/S ratio. There are many methods that can be used to determine telomere length such as telomere restriction fragment length analysis and in situ hybridization and flow cytometry (FLOW-fish) and single telomere length assessment (STELA), but these methods will not be reviewed here. We used a modified Real-Time Polymerase chain reaction (RT-PCR) method that has been highly correlated to the telomere restriction fragment length analysis, the apparent 'gold standard' measure for telomere length measurment.

It was long presumed to be impossible to measure telomere length using PCR amplification because only primer dimer-derived products are expected. Cawthon et al. (26, 27) developed a pair of primers that eliminated this problem and was able to relatively quantify telomere length. The premise of the assay is based on the ratio of two quantitative real time PCR reactions. The factor by which the unknown sample differs from a reference DNA sample in its ratio of telomere repeat copy number (T) to single

copy gene number (S) should be proportional to the average telomere length (26). Thus if the T/S ratio = 1, then the unknown sample DNA is identical to the reference DNA in its ratio of telomere repeat copy number to single copy gene number (26). Peripheral blood mononuclear cells were used as the source of for the determination of relative telomere length in the present thesis project. The reasons for using these cells as the DNA source are stated above in the peripheral blood mononuclear cell section.

The detailed procedures are as follows: All subjects had blood drawn from the antecubital vein of the dominant arm using standard sterile phlebotomy techniques. The blood was collected in standard 10mL EDTA-treated vacutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMCs) were be isolated by density gradient centrifugation from each sample (Ficoll-Paque plus, Amersham Pharmacia Biotech, NJ) (69). The cells were placed into two separate 1.5 mL tubes, one for the telomere length assay and the other wascounted and frozen in 1 x PBS (Invitrogen) at -80°C. Once the cells were isolated, they were counted using a Neubauer ruled hemacytometer (Hausser Scientific, Horsham, PA) and Trypan Blue 0.04% staining solution (Invitrogen) and a light microscope. The following equation can be used to estimate the total number of PBMCs in the sample:

$$\text{Number of PBMCs}/\mu L = \text{number cells per } 1/5\ mm^3 \times 5 \times \text{correction factor} \times 10$$

The correction factor is 2000 for this cell isolation procedure.

The sample not frozen had the DNA extracted using a standardized and quality-controlled PureGene DNA isolation system (Gentra Systems, Minneapolis). The quantity and quality of the genomic DNA isolate was assessed by Quant-iT PicoGreen dsDNA kit

(Invitrogen). The integrity of random isolates was evaluated by agarose gel electrophoresis.

Measurement of relative telomere lengths (T/S ratios) was determined by quantitative real time polymerase chain reaction (RT-PCR) as described by Cawthon (9) with the following modifications. The forward primer for the telomere PCR was tel1b [5'-CGG TTT $(GTTTGG)_5$ GTT-3'] used at a final concentration of 125 nM. The reverse primer was tel2b [5'- GGC TTG $(CCTTAC)_5$ CCT-3'] used at a final concentration of 312.5 nM. The forward primer for the single-copy gene (acidic ribosomal phosphoprotein PO, 36B4) was RPLPO-F [5'- CCC ATT CTA TCA TCA ACG GGT ACA A –3'] used at a final concentration of 125 nM. The reverse primer was RPLPO-R [5'- AGG TAG AAG GCC ACA TCA CC –3'] used at a final concentration of 312.5 nM. SYBR Green Master mix (Applied Biosystems) was used and added to 8.75μL of sample and standard DNA. Tubes containing 50, 25, 12.5, 6.25 and 3.125 ng/μL of reference DNA were included in each PCR assay so that the quantity of research sample DNA could be determined relative to the reference DNA sample by the standard curve method. The same reference DNA was used in all PCR runs. A plot of Ct versus log (amount of input reference DNA) is linear and was used to determine the integrity of the assay. The Ct value is the fractional cycle number at which the fluorescence emitted by the sample crosses a set threshold that is above the baseline fluorescence (26). The Telomere PCR (T-PCR) to Single copy gene PCR (S-PCR) ratio was derived from the Ct values of each unknown sample in each assay. The ratio of telomere repeat copy number to a single copy gene copy number allowed the relative quantification of PBMC telomere length (26). This ratio was proportional to the average

telomere length (26). The assay was performed using an Applied Biosystems 7300 Real Time PCR System (Applied Biosystems, Foster City, CA). The telomere (T PCR) thermal cycling profile consisted of 10 min at 95°C followed by 30 cycles of 95°C for 15 sec., 67°C for 1 min, 95°C for 15 sec., followed by a dissociation stage of 95°C for 15 sec., 60°C for 30 sec., 95°C for 15 sec. The single-copy gene (S PCR) thermal cycling profile consisted of 10 min at 95°C followed by 40 cycles of 95°C for 15 sec., 57°C for 1 min., 95°C for 15 sec., followed by a dissociation stage of 95°C for 15sec., 60°C for 30 sec., 95°C for 15 sec.

Telomerase

The following section will outline the measurement of telomerase enzyme activity. Measuring telomerase can be done in a few different methods relying on the telomere repeat amplification protocol (TRAP) (Chemicon, TRAPeze)(37) or an enzyme linked immunsorbant assay (ELISA). For the present study we chose the TRAP that utilized real-time PCR amplification and emitted fluoresence as the means for quantifying telomerase enzyme activity. The assay uses two floumetrically labeled primers that when incorporated into the TRAP product emit fluorensence and allow for simple quantification of telomerase activity. This procedure eliminated isotopic, gel-based and ELISA complications and the risk of carryover contamination since the fluorescence are measured directly from the reaction vessel. Briefly, a reaction mixture containing a template stand and primers is added to the PBMCs and PCR is performed. During the PCR the telomerase, if active in the cell sample, will add the fluorescently labeled primers to the template strand. During PCR amplification the fluorescence

reaction will occur and accumulate as more primers are added by telomerase. Figure 1 below shows a schematic of this process.

Figure 1.

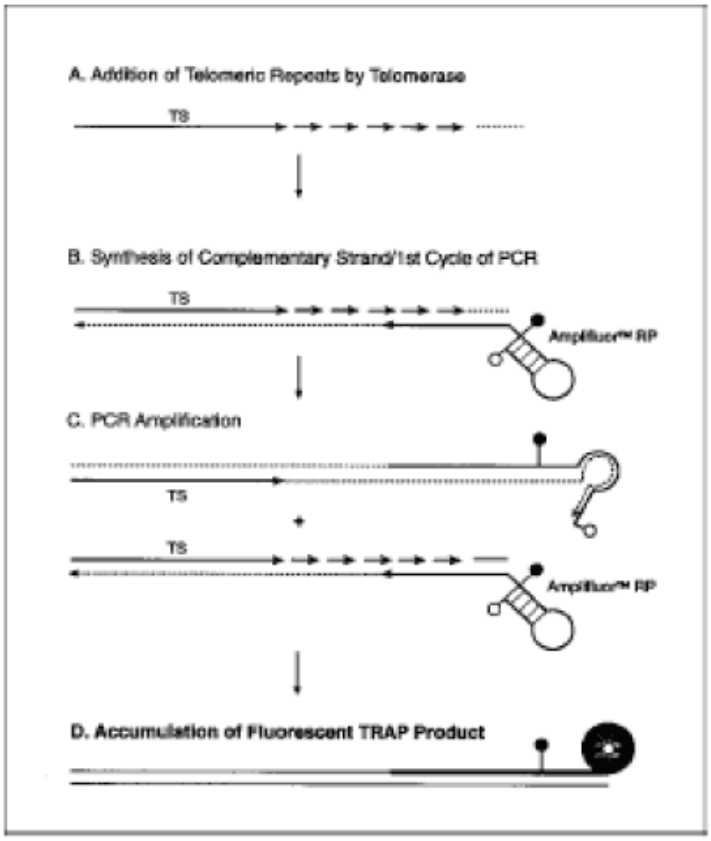

Figure 1. A. Showing the addtion of telomeris repeats by telomerase. B. The synthesis of complimentary strand during the first cycle of PCR. C. Amplification of the product and the activated fluorescence. D. Accumulation of the activated product allowing for visualiztion with the appropriate software. Figure adopted from the TRAPeze kit insert, Chemicon 2000.

PBMCs isolated and stored at -80°C were thawed at 37°C, washed 3 times with 10mL of 4°C PBS, and resuspended in 1mL of 4°C PBS. Approximately $1x10^5$ - $1x10^6$ cells were suspended in 500μL of CHAPS lysis buffer. The assay procedures followed the recommendations of the manufacturer. Briefly, after the cells are prepared as stated above, a reaction mixture was made containing Titanium taq buffer, Trapeze master mix,

Clone Tech Taq (hot start), and PCR grade water. The reaction mixture and each sample's cell preparation were combined into a 96-well ABI optical plate. The plate was then loaded into the ABI 7300 RT-PCR machine and PCR performed with the following thermocycling profile 30°C for 20 min, 95°C for 2 min followed by 40 cycles of 94°C for 15s, 61°C for 40s, 45°C for 32s. The telomerase PCR extension and fluorescence took place in the reaction vessel. The fluorescence was detected by the ABI 7300 and quantified by the software provided by ABI and a critical threshold (Ct) value determined. The enzyme activity will be determined following the standard curve method. From a standard sample (TSR8) provided in the kit, five standards will be diluted with a concentration ranging from 0.2 amoles/μL to 0.00002 amole/μL. By using a conversion factor provided by the kit, total product generated (TPG) units can be derived (1 amole of TSR8 = 1,000 TPG units). A plot of log(TSR8 concentration in TPG units) versus Ct value is linear. By inserting each unknown's Ct value into the X value of the linear standard curve and algebraically solving, one can determine each unknown's enzyme activity in 'telomerase units'.

Summary

Physical activity is an important component of a healthy lifestyle. As a society, we are living longer due to medical advances and better hygienic practices. This has lead to a surge in our geriatric population. Recent advances in molecular technologies have allowed us to study the molecular and cellular consequences of aging. Recent studies have shown that telomere length and telomerase enzyme activity may be malleable cellular traits that are associated with aging (8-10, 27, 37, 38). The purpose of this thesis

project is to determine if a relationship exists between physical activity levels and telomere length and telomerase enzyme activity in PBMCs.

APPENDIX A: Limitations of the Study

Delimitations

1. The scope of the study was delimited to 69 Caucasians males and females aged 50 – 70 yr. from the BRAIN study cohort. The results may not be generalizable beyond the characteristics of this sample.
2. Variables such as age, BMI, perceived stress and consistency of physical activity might affect telomere length and telomerase enzyme activity. Therefore, statistical control for these variables was applied where appropriate.
3. Telomere length was assessed by quantitative real- time PCR techniques.
4. Telomerase enzyme activity was assessed by telomere repeat amplification protocol.
5. The Yale Physical Activity Survey, a self-recall method, was used to assess physical activity.
6. Perceived stress was assessed by the 10-question Perceived Stress survey.
7. Pervious medical history was assessed by a general questionnaire, variables collected included height weight, present infection status, history of cancer incidence, and consistency of physical activity.

Limitations

1. The subjects in the study were generally healthy Caucasian volunteers from the BRAIN cohort and were not randomly selected from the general population.
2. Subjects self reported many variables, such as physical activity and perceived stress. Because these variables were not directly measured the accuracy of these reports cannot be verified. Inaccurate self-reporting could confound the results of this study.
3. Dietary intake and nutritional records or profiles of the subjects were not measured. Differences in these variables may have confounded the results of this study.
4. Other factors, such as oxidative stress, were not measured or controlled for in the present study, so no information on potential mechanisms of physical activity on telomere length or telomerase enzyme activity can be determined.

APPENDIX B: Statistical Power Analysis

Sample Size Estimates and Power Calculations:

Sample size estimates were determined for telomere length (T/S ratios) and telomerase enzyme activity. The effect size ratio used for telomere length was a difference of 0.2 units. A difference of 0.2 units has been significantly associated with psychological perceived stress (37) and has also been associated with poor survival rate and increased mortality from heart and infectious disease (27). To obtain appropriate statistical power (beta = 0.8; alpha 0.05), at least 50 subjects need to be analyzed (Table 1). Calculations were based on an expected average T/S ratio of 1.12 with a standard deviation of 0.3.

Table 1.

Power	0.9	0.8
Number of Subjects	66	50

Table 1. Power calculations for telomere length (TL) analysis.

The selected effect size for telomerase is 0.039 telomerase units, a difference of which has been associated with greater body mass index (BMI) and abdominal adiposity, higher resting heart rate, systolic blood pressure, fasting glucose, LDL and total cholesterol and total/HDL cholesterol ratio (38). These factors are related to metabolic syndrome and cardiovascular disease risk. To obtain appropriate power (beta = 0.8; alpha 0.05), at least 52 subjects need to be analyzed (Table 2). Calculations were made using an expected mean of 0.093 with a standard deviation of 0.01 telomerase units. All sample size calculations were performed using the statistical power calculators at

http://www.stat.uiowa.edu/~rlenth/Power/index.html. We anticipate a total of 70 total subjects to be included in the present study, which would provide approximately 90% power for both of the proposed dependent measures.

Power	0.9	0.8
Number of subjects	69	52

Table 2. Power calculations for telomerase activity analysis.

APPENDIX C: Human Subjects Approval

UNIVERSITY OF MARYLAND
INSTITUTIONAL REVIEW BOARD

2100 Blair Lee Building
College Park, Maryland 20742-5121
301.405.4212 TEL 301.314.1475 FAX
irb@deans.umd.edu
www.umresearch.umd.edu/IRB

November 10, 2006

MEMORANDUM

Renewal Application Approval Notification

To:	Dr. Brad Hatfield Dr. Stephen Roth Linzi Jones Michelle Costanzo Andy Ludlow Joanne Zimmerman Ming-Jung Woo Joe Hearn Ryan Conery Department of Kinesiology
From:	Roslyn Edson, M.S., CIP, IRB Manager University of Maryland, College Park
Re:	**IRB Application Number:** 05-0011 **Project Title:** "Age, Physical Activity, Genotype, and Cognitive Function"
Approval Date:	October 27, 2006
Expiration Date:	October 27, 2007
Type of Application:	Renewal
Type of Research:	Non-exempt
Type of Review For Application:	Expedited

The University of Maryland, College Park Institutional Review Board (IRB) approved your IRB application. The research was approved in accordance with 45 CFR 46, the Federal Policy for the Protection of Human Subjects, and the University's IRB policies and procedures. Please reference the above-cited IRB application number in any future communications with our office regarding this research.

Recruitment/Consent: For research requiring written informed consent, the IRB-approved and stamped informed consent document is enclosed. The IRB approval expiration date has been stamped on the informed consent document. Please keep copies of the consent forms used for this research for three years after the completion of the research.

Continuing Review: If you want to continue to collect data from human subjects or to analyze private, identifiable data collected from human subjects after the approval expiration date indicated above, you must submit a renewal application to the IRB Office · (Continued)

at least 30 days before the approval expiration date.

Modifications: Any changes to the approved protocol must be approved by the IRB before the change is implemented, except when a change is necessary to eliminate apparent immediate hazards to the subjects. If you would like to modify the approved protocol, please submit an addendum request to the IRB Office. The instructions for submitting an addendum request are posted on the IRB website at: **http://www.umresearch.umd.edu/IRB/irb_Addendum%20Protocol.htm.**

Unanticipated Problems Involving Risks: You must promptly report any unanticipated problems involving risks to subjects or others to the IRB Manager at 301-405-0678 or **redson@umresearch.umd.edu.**

Student Researchers: Unless otherwise requested, this IRB approval document was sent to the Principal Investigator (PI). The PI should pass on the approval document or a copy to the student researchers. This IRB approval document may be a requirement for student researchers applying for graduation. The IRB may not be able to provide copies of the approval documents if several years have passed since the date of the original approval.

Additional Information: Please contact the IRB Office at 301-405-4212 if you have any IRB-related questions or concerns.

APPENDIX D: Yale Physical Activity Survey

INTERVIEWER: PLEASE MARK TIME: HR__ MIN__ SEC__

INTERVIEWER: (Please hand the subject the list of activities while reading this statement.) Here is a list of common types of physical activities. Please tell me which of them you did during a typical week in the last month. Our interest is learning about the types of physical activities that are a part of your regular work and leisure routines.

For each activity you do, please tell me how much time (hours) you spent doing this activity during a typical week. (Hand subject card #1.)

Work	Intensity Time (hrs/wk) (Kcal/min)	Code
Shopping (e.g., grocery, clothes)	_____	3.5
Stair climbing while carrying a load	_____	8.5
Laundry (time loading, unloading, hanging, folding only)	_____	3.0
Light housework: tidying, dusting, sweeping, collecting trash in home, polishing, indoor gardening, ironing	_____	3.0
Heavy housework: vacuuming, mopping, scrubbing floors and walls, moving furniture, boxes, or garbage cans	_____	4.5
Food preparation (10+ minutes in duration): chopping, stirring, moving about to get food items, pans	_____	2.5
Food service (10+ minutes in duration: setting table, carrying food, serving food	_____	2.5
Dish washing (10+ minutes in duration): clearing table, washing/drying dishes, putting dishes away	_____	2.5
Light home repair: small appliance repair, light home maintenance/repair	_____	3.0
Heavy home repair: painting, carpentry,	_____	5.5

Yardwork	Time (hrs/wk)	Intensity Code (Kcal/min)
Gardening: planting, weeding, digging, hoeing	_____	4.5
Lawn mowing (walking only)	_____	4.5
Clearing walks/driveway: sweeping, shoveling, raking	_____	5.0
Other: _____	_____	___ #
Caretaking		
Older or disabled person (lifting, pushing wheelchair)	_____	5.5
Childcare (lifting, carrying, pushing stroller)	_____	4.0
Exercise		
Brisk walking (10+ minutes in duration)	_____	6.0
Pool exercises, stretching, yoga	_____	3.0
Vigorous calisthenics, acrobics	_____	6.0
Cycling, Exercycle	_____	6.0
Swimming (laps only)	_____	6.0
Other: _____	_____	___ #
Recreational Activities		
Leisurely walking (10+ minutes in duration)	_____	3.5
Needlework: knitting, sewing, needlepoint, etc.	_____	1.5
Dancing (mod/fast): line, ballroom, tap, square, etc.	_____	5.5
Bowling, bocci	_____	3.0
Golf (walking to each hole only)	_____	5.0
Racquet sports: tennis, racquet ball	_____	7.0
Billiards	_____	2.5
Other: _____	_____	___ #

INTERVIEWER: (Please read to subject.) I would now like to ask you about certain types of activities that you have done during the past month. I will ask you about how much vigorous activity, leisurely walking, sitting, standing, and some other things that you usually do.

1. About how many times during the month did you participate in vigorous activities that lasted at least 10 minutes and cause large increases in breathing, heart rate, or leg fatigue or caused you to perspire? (Hand subject card #2)

   Score:
   0 = Not at all (go to Q3)
   1 = 1-3 times per month
   2 = 1-2 times per week
   3 = 3-4 times per week
   4 = 5+ times per week
   7 = refused
   8 = don't know

   Frequency score = _____

2. About how long do you do this vigorous activity(ies) each time? (Hand subject card #3)

   Score:
   0 =Not applicable
   1 = 10-30 minutes
   2 = 31-60 minutes
   3 = 60+ minutes
   7 = refused
   8 = don't know

   Duration score = _____
   weight= 5

VIGOROUS ACTIVITY INDEX SCORE:
FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

3. Think about the walks you have taken during the past month. About how many times per month did you walk for at least 10 minutes or more without stopping which was not strenuous enough to cause large increases in breathing, heart rate, or leg fatigue or cause you to perspire'? (Hand subject card #2)

   Score:
   0 = Not at all (go to Q5)
   1 = 1-3 times per month
   2 = 1-2 times per week
   3 = 3-4 times per week
   4 = 5+ times per week
   7 = refused
   8 = don't know

   Frequency score = _____

4. When you did this walking, for how many minutes did you do it? (Hand subject card #3)

Score:
0 = Not applicable
1 = 10-30 minutes
2 = 31-60 minutes
3 = 60+ minutes
7 = refused
8 = don't know

Duration score = _____
weight = 4

LEISURELY WALKING INDEX SCORE:
FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

5. About how many hours a day do you spend moving around on your feet while doing things? Please report only the time that you are actually moving. (Hand subject card #4)

Score:
0 = Not at all
1 = less than 1 hr per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = refused
8 = don't know

Moving score = _____
weight = 3

MOVING INDEX SCORE:
FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

6. Think about how much time you spend standing or moving around on your feet on an average day during the past month. About how many hours per day do you stand? (Hand subject card #4)

Score:
0 = Not at all
1 = less than 1 hr per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = refused
8 = don't know

Standing score = _____
weight = 2

STANDING INDEX SCORE:
FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

7. About how many hours did you spend sitting on an average day during the past month? (Hand subject card #5)

Score:
0 = Not at all
1 = less than 3 hours
2 = 3 hrs to less than 6 hrs
3 = 6 hrs to less than 8 hrs
4 = 8+ hrs
7 = refused
8 = don't know

Sitting score = _____
weight = 1

SITTING INDEX SCORE:
FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing:)

8. About how many flights of stairs do you climb up each day? (Let 10 steps = 1 flight.)

_____

9. Please compare the amount of physical activity that you do during other seasons of the year with the amount you just reported for a typical week in the past month. For example, in the summer, do you do more or less activity than what you reported doing in the past month? (INTERVIEWER: PLEASE CIRCLE THE APPROPRIATE SCORE FOR EACH SEASON.)

	Lot More	Little More	Same	Little Less	Lot Less	Don't know
Spring	1.30	1.15	1.0	0.85	0.70	-
Summer	1.30	1.15	1.0	0.85	0.70	-
Fall	1.30	1.15	1.0	0.85	0.70	-
Winter	1.30	1.15	1.0	0.85	0.70	--

SEASONAL ADJUSTMENT SCORE = SUM OVER ALL SEASONS/ 4 _____

INTERVIEWER: PLEASE MARK TIME:

HR ___ MIN ___ SEC ___

APPENDIX E: Perceived Stress Survey

PSS

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please indicate how often you felt or thought a certain way by circling the appropriate item.

1. In the last month, how often have you been upset because of something that happened unexpectedly?

0 = Never 1 = Rarely 2 = Sometimes 3 Fairly often 4 = Very often

2. In the last month, how often have you felt that you were unable to control the important things in your life?

0 = Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 Very often

3. In the last month, how often have you felt nervous and "stressed"?

0 = Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 Very often

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

0 Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 = Very often

5. In the last month, how often have you felt that things were going your way?

0 = Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 = Very often

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

0 = Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 = Very often

7. In the last month, how often have you been able to control irritations in your life?

0 = Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 = Very often

8. In the last month, how often have you felt that you were on top of things?

0 = Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 = Very often

9. In the last month, how often have you been angered because of things that were outside of your control?

0 = Never 1 Rarely 2 Sometimes 3 = Fairly often 4 = Very often

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

0 = Never 1 = Rarely 2 = Sometimes 3 = Fairly often 4 = Very often

Rev 11/5/99

APPENDIX F: Health History Questionnaire

Medical History

Name _____ Telephone _____

Address _____

_____

Email address _____

Date of birth _____ Age _____ Gender M ___ F ___ Height: _____ Weight _____

Race/Ethnicity ___Caucasian ___African-American ___ Hispanic ___Asian _____Other

Color blind Yes ____ No _____

Years of education (high school = 12, plus any additional years of college) _____.

Please indicate your primary job/career during your lifetime. If you have had multiple careers, please list them.

_____

Medical History Are you presently taking or have taken any of the following medications within the past two months?

Aspirin, Bufferin, Anacin	Tranquilizers
Blood pressure pills	Weight reducing pills
Cortisone	Blood thinning pills
Cough medicine	Dilantin
Digitalis	Allergy shots
Hormones	Water pills
Insulin or diabetic pills	Antibiotics
Iron or blood medications	Barbiturates
Laxatives	Phenobarbital
Sleeping pills	Thyroid medicine

Other medications not listed _____

_____

Have you ever had any conditions or injuries which required brain surgery? Yes ___ No ___

If so, please explain _____

Do you currently or have you ever had any of the following medical disorders?

Heart attack	Yes ____	No ____	
Chest pain	Yes ____	No ____	
Hardening of the arteries	Yes ____	No ____	
Irregular heart beat	Yes ____	No ____	
Kidney disease	Yes ____	No ____	
Diabetes	Yes ____	No ____	
Cancer	Yes ____	No ____	
Gout	Yes ____	No ____	
Asthma	Yes ____	No ____	
Epilepsy or seizure disorder	Yes ____	No ____	
Migraine headaches	Yes ____	No ____	if yes, frequency/intensity _____
Psychiatric disorder	Yes ____	No ____	if yes, what diagnosis _____

Smoking History
Current smoker? Yes ___ No ___ If yes, how many years? _____
Previous smoker? Yes ___ No ___ If yes, how many years did you smoke? _____ When did you stop smoking? _____

Alcohol Consumption
Do you drink alcohol-containing beverages? Yes ___ No ___ If yes, how often? _____
How much do you drink in an average sitting? (A serving is 12 oz. of beer, 6 oz. of wine, or 1.5 oz. of distilled spirits.) ____ 1 serving _____ 2 to 4 servings _____ More than 4 servings

Physical Activity History

____ Has your physical activity level remained consistent during the previous 5 years?
a. very consistent b. somewhat consistent c. inconsistent

Have you had any injuries or medical conditions that caused you to be physically inactive for more than 3 months during the past 5 years? Yes ___ No ___
If yes, please explain _____
_____

We are interested in how stable your physical activity level has been over the course of your lifetime. Please use the scale below to rate your level of physical activity for the previous five decades.

1 **Fairly physically active,** sports and leisure activities.
2 **Moderately physically active,** hobbies, active leisure activities.
3 **Fairly physically inactive,** very few sports, light physical leisure activities.
4 **Very physically inactive,** no sports, non-physical leisure activities.

___ Between the ages of 20 and 29?
___ Between the ages of 30 and 39?
___ Between the ages of 40 and 49?
___ Between the ages of 50 and 59?
___ Between the ages of 60 and 69?

Memory, Family History

Do you have difficulty with your memory more than you used to? Yes ___ No ___
Do you forget where you have left things more than you used to? Yes ___ No ___
Do you forget the names of close friends or relatives? Yes ___ No ___
Have you ever been in your own neighborhood and forgotten your way? Yes ___ No ___
If you answered yes to any of these questions, please answer the following three questions.
When did this difficulty begin? (Duration in months) _____
Did it come on gradually or suddenly? _____
Has it become worse or better since it started? _____

Do you have any biological parents, siblings, or grandparents who have been clinically diagnosed with Alzheimer's disease? Yes __ No ___ If yes, please list how many. Do not give names.

Parents Yes ___ No ___ How many? _____
Siblings Yes ___ No ___ How many? _____
Grandparents Yes ___ No ___ How many? _____

APPENDIX G: Figures

Figure 4.

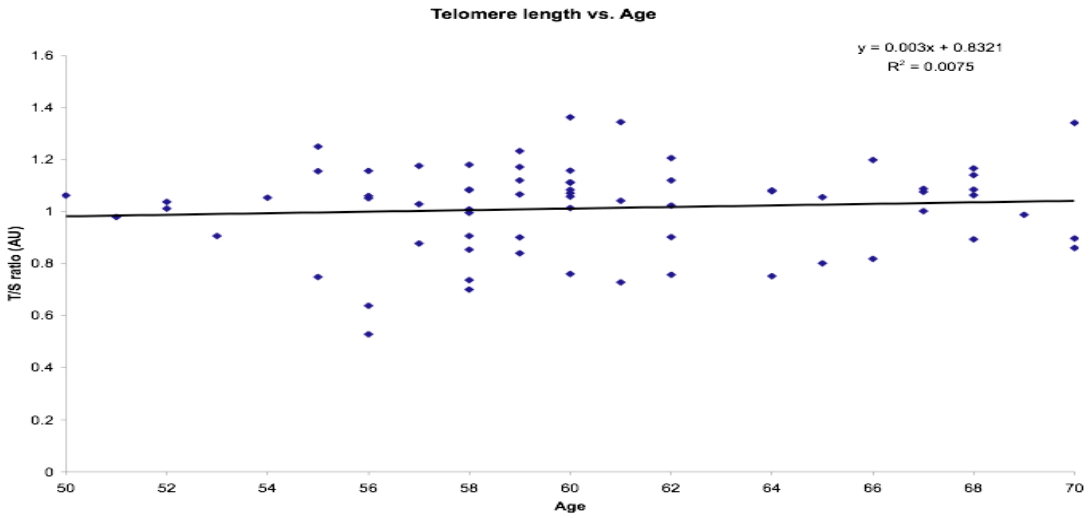

Figure 4. Non-significant relationship between telomere length and age.

Figure 5.

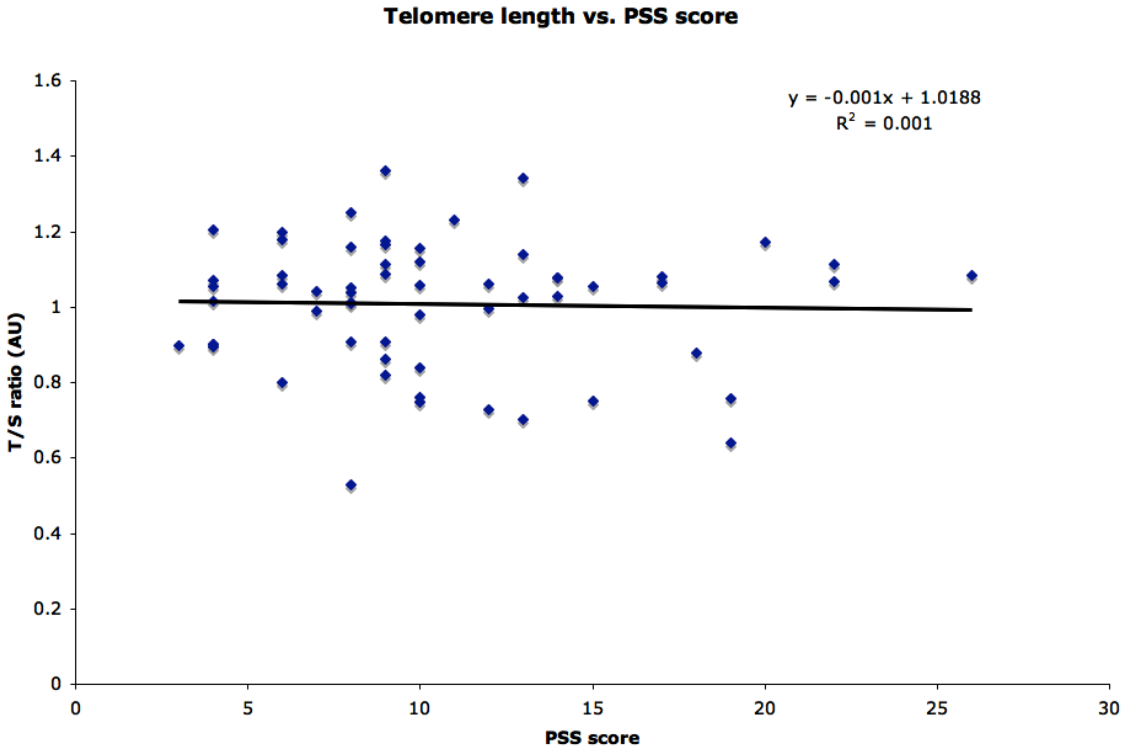

Figure 5. Non- significant relationship between telomere length and PSS score.

Figure 6.

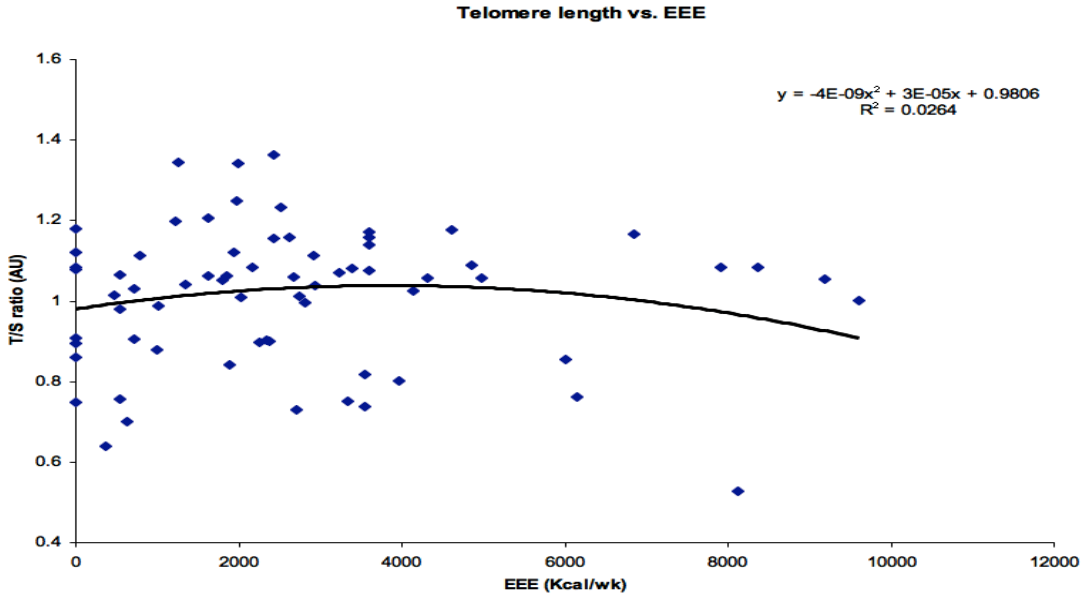

Figure 6. Non-significant relationship between telomere length and EEE with a polynomial term, EEE^2.

Reference List

1. **Agarwal S and Sohal RS.** Relationship between susceptibility to protein oxidation, aging, and maximum life span potential of different species. *Exp Gerontol* 31: 365-372, 1996.

2. **Aviv A.** Chronology versus biology: telomeres, essential hypertension, and vascular aging. *Hypertension* 40: 229-232, 2002.

3. **Aviv A.** Telomeres, sex, reactive oxygen species, and human cardiovascular aging. *J Mol Med* 80: 689-695, 2002.

4. **Aviv A and Aviv H.** Telomeres and essential hypertension. *Am J Hypertens* 12: 427-432, 1999.

5. **Aviv A and Aviv H.** Telomeres, hidden mosaicism, loss of heterozygosity, and complex genetic traits. *Hum Genet* 103: 2-4, 1998.

6. **Aviv A, Valdes AM, and Spector TD.** Human telomere biology: pitfalls of moving from the laboratory to epidemiology. *Int J Epidemiol* 35: 1424-1429, 2006.

7. **Barouki R.** [Ageing free radicals and cellular stress]. *MedSci(Paris)* 22: 266-272, 2006.

8. **Benetos A, Gardner JP, Kimura M, Labat C, Nzietchueng R, Dousset B, Zannad F, Lacolley P, and Aviv A.** Aldosterone and telomere length in white blood cells. *J Gerontol A Biol Sci Med Sci* 60: 1593-1596, 2005.

9. **Benetos A, Gardner JP, Zureik M, Labat C, Xiaobin L, Adamopoulos C, Temmar M, Bean KE, Thomas F, and Aviv A.** Short telomeres are associated with

increased carotid atherosclerosis in hypertensive subjects. *Hypertension* 43: 182-185, 2004.

10. **Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, and Aviv A.** Telomere length as an indicator of biological aging: The gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* 37: 381-385, 2001.

11. **Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, and Aviv A.** Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* 37: 381-385, 2001.

12. **Benetti R, Garcia-Cao M, and Blasco MA.** Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. *Nat Genet* 39: 243-250, 2007.

13. **Bergholm R, Makimattila S, Valkonen M, Liu ML, Lahdenpera S, Taskinen MR, Sovijarvi A, Malmberg P, and Yki-Jarvinen H.** Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilatation in vivo. *Atherosclerosis* 145: 341-349, 1999.

14. **Blackburn EH.** Switching and signaling at the telomere. *Cell* 106: 661-673, 2001.

15. **Blackburn EH.** Telomere states and cell fates. *Nature* 408: 53-56, 2000.

16. **Blackburn EH.** Telomeres and telomerase. *Keio JMed* 49: 59-65, 2000.

17. **Blackburn EH.** Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett* 579: 859-862, 2005.

18. **Blackburn EH.** The telomere and telomerase: nucleic acid-protein complexes acting in a telomere homeostasis system. A review. *Biochemistry (Mosc)* 62: 1196-1201, 1997.

19. **Blackburn EH, Chan S, Chang J, Fulton TB, Krauskopf A, McEachern M, Prescott J, Roy J, Smith C, and Wang H.** Molecular manifestations and molecular determinants of telomere capping. *Cold Spring HarbSympQuantBiol* 65: 253-263, 2000.

20. **Blair SN, Kohl HW, III, Barlow CE, Paffenbarger RS, Jr., Gibbons LW, and Macera CA.** Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *JAMA* 273: 1093-1098, 1995.

21. **Blair SN, Kohl HW, III, Paffenbarger RS, Jr., Clark DG, Cooper KH, and Gibbons LW.** Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA* 262: 2395-2401, 1989.

22. **Blasco MA.** Carcinogenesis Young Investigator Award. Telomere epigenetics: a higher-order control of telomere length in mammalian cells. *Carcinogenesis* 25: 1083-1087, 2004.

23. **Blasco MA.** Telomeres and human disease: ageing, cancer and beyond. *NatRevGenet* 6: 611-622, 2005.

24. **Blasco MA.** The epigenetic regulation of mammalian telomeres. *Nat Rev Genet* 8: 299-309, 2007.

25. **Bruunsgaard H, Jensen MS, Schjerling P, Halkjaer-Kristensen J, Ogawa K, Skinhoj P, and Pedersen BK.** Exercise induces recruitment of lymphocytes with an activated phenotype and short telomeres in young and elderly humans. *Life Sci* 65: 2623-2633, 1999.

26. **Cawthon RM.** Telomere measurement by quantitative PCR. *Nucleic Acids Res* 30: e47, 2002.

27. **Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, and Kerber RA.** Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361: 393-395, 2003.

28. **Chakravarti B and Chakravarti DN.** Oxidative modification of proteins: age-related changes. *Gerontology* 53: 128-139, 2007.

29. **Cherkas LF, Aviv A, Valdes AM, Hunkin JL, Gardner JP, Surdulescu GL, Kimura M, and Spector TD.** The effects of social status on biological aging as measured by white-blood-cell telomere length. *Aging Cell* 5: 361-365, 2006.

30. **Cohen LH, Towbes LC, and Flocco R.** Effects of induced mood on self-reported life events and perceived and received social support. *J Pers Soc Psychol* 55: 669-674, 1988.

31. **Cohen S, Kamarck T, and Mermelstein R.** A global measure of perceived stress. *J Health Soc Behav* 24: 385-396, 1983.

32. **Collins M, Renault V, Grobler LA, St Clair Gibson A, Lambert MI, Wayne Derman E, Butler-Browne GS, Noakes TD, and Mouly V.** Athletes with exercise-associated fatigue have abnormally short muscle DNA telomeres. *Med Sci Sports Exerc* 35: 1524-1528, 2003.

33. **Connolly PH, Caiozzo VJ, Zaldivar F, Nemet D, Larson J, Hung SP, Heck JD, Hatfield GW, and Cooper DM.** Effects of exercise on gene expression in human peripheral blood mononuclear cells. *J Appl Physiol* 97: 1461-1469, 2004.

34. **de Magalhaes JP and Toussaint O.** Telomeres and telomerase: a modern fountain of youth? *Rejuvenation Res* 7: 126-133, 2004.

35. **Demissie S, Levy D, Benjamin EJ, Cupples LA, Gardner JP, Herbert A, Kimura M, Larson MG, Meigs JB, Keaney JF, and Aviv A.** Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell* 5: 325-330, 2006.

36. **Dipietro L, Caspersen CJ, Ostfeld AM, and Nadel ER.** A survey for assessing physical activity among older adults. *MedSciSports Exerc* 25: 628-642, 1993.

37. **Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, and Cawthon RM.** Accelerated telomere shortening in response to life stress. *ProcNatlAcadSciUSA* 101: 17312-17315, 2004.

38. **Epel ES, Lin J, Wilhelm FH, Wolkowitz OM, Cawthon R, Adler NE, Dolbier C, Mendes WB, and Blackburn EH.** Cell aging in relation to stress arousal and cardiovascular disease risk factors. *Psychoneuroendocrinology* 31: 277-287, 2006.

39. **Faragher RG and Kipling D.** How might replicative senescence contribute to human ageing? *Bioessays* 20: 985-991, 1998.

40. **Farrell SW, Kampert JB, Kohl HW, III, Barlow CE, Macera CA, Paffenbarger RS, Jr., Gibbons LW, and Blair SN.** Influences of cardiorespiratory fitness levels and other predictors on cardiovascular disease mortality in men. *MedSciSports Exerc* 30: 899-905, 1998.

41. **Ferlazzo G.** Natural killer and dendritic cell liaison: recent insights and open questions. *Immunol Lett* 101: 12-17, 2005.

42. **Ferrando AA, Tipton KD, Bamman MM, and Wolfe RR.** Resistance exercise maintains skeletal muscle protein synthesis during bed rest. *J Appl Physiol* 82: 807-810, 1997.

43. **Finaud J, Lac G, and Filaire E.** Oxidative stress : relationship with exercise and training. *Sports Med* 36: 327-358, 2006.

44. **Fitzgerald MD, Tanaka H, Tran ZV, and Seals DR.** Age-related declines in maximal aerobic capacity in regularly exercising vs. sedentary women: a meta-analysis. *J Appl Physiol* 83: 160-165, 1997.

45. **Giordano R, Lanfranco F, Bo M, Pellegrino M, Picu A, Baldi M, Balbo M, Bonelli L, Grottoli S, Ghigo E, and Arvat E.** Somatopause reflects age-related changes in the neural control of GH/IGF-I axis. *J Endocrinol Invest* 28: 94-98, 2005.

46. **Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, Kawamura M, Chayama K, Yoshizumi M, and Nara I.** Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 108: 530-535, 2003.

47. **Greider CW.** Telomerase is processive. *Mol Cell Biol* 11: 4572-4580, 1991.

48. **Greider CW.** Telomeres, telomerase and senescence. *Bioessays* 12: 363-369, 1990.

49. **Greider CW and Blackburn EH.** Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* 43: 405-413, 1985.

50. **Greider CW and Blackburn EH.** Telomeres, telomerase and cancer. *SciAm* 274: 92-97, 1996.

51. **Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM, and Dimmeler S.** Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. *CircRes* 94: 768-775, 2004.

52. **Hagberg JM, Park JJ, and Brown MD.** The role of exercise training in the treatment of hypertension: an update. *Sports Med* 30: 193-206, 2000.

53. **Hahner S and Allolio B.** Do dehydroepiandrosterone supplements have anti-aging potential? *Nat Clin Pract Urol* 4: 302-303, 2007.

54. **Harley CB, Futcher AB, and Greider CW.** Telomeres shorten during ageing of human fibroblasts. *Nature* 345: 458-460, 1990.

55. **Harley CB, Kim NW, Prowse KR, Weinrich SL, Hirsch KS, West MD, Bacchetti S, Hirte HW, Counter CM, Greider CW, and et al.** Telomerase, cell immortality, and cancer. *Cold Spring Harb Symp Quant Biol* 59: 307-315, 1994.

56. **Harman D.** Aging and oxidative stress. *JIntFedClinChem* 10: 24-27, 1998.

57. **Harman D.** Aging: a theory based on free radical and radiation chemistry. *JGerontol* 11: 298-300, 1956.

58. **Hayflick L.** The limited in vitro lifetime of human diploid cell strains. *ExpCell Res* 37: 614-636, 1965.

59. **Hiramoto K, Yasuhara Y, Sako K, Aoki K, and Kikugawa K.** Suppression of free radical-induced DNA strand breaks by linoleic acid and low density lipoprotein in vitro. *Biol Pharm Bull* 26: 1129-1134, 2003.

60. **Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, Shay JW, Ishioka S, and Yamakido M.** Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol* 155: 3711-3715, 1995.

61. **Jeanclos E, Schork NJ, Kyvik KO, Kimura M, Skurnick JH, and Aviv A.** Telomere length inversely correlates with pulse pressure and is highly familial. *Hypertension* 36: 195-200, 2000.

62. **Kamel NS, Gammack J, Cepeda O, and Flaherty JH.** Antioxidants and hormones as antiaging therapies: high hopes, disappointing results. *Cleve Clin J Med* 73: 1049-1056, 1058, 2006.

63. **Katic M and Kahn CR.** The role of insulin and IGF-1 signaling in longevity. *Cell MolLife Sci* 62: 320-343, 2005.

64. **Kevorkian R.** Andropause. *Mo Med* 104: 68-71; quiz 71, 2007.

65. **Kikugawa K, Oikawa N, Miyazawa A, Shindo K, and Kato T.** Interaction of nitric oxide with glutathione or cysteine generates reactive oxygen species causing DNA single strand breaks. *BiolPharmBull* 28: 998-1003, 2005.

66. **Kipling D.** Telomeres, replicative senescence and human ageing. *Maturitas* 38: 25-37, 2001.

67. **Kuta I, Parizkova J, and Dycka J.** Muscle strength and lean body mass in old men of different physical activity. *J Appl Physiol* 29: 168-171, 1970.

68. **Lee IM, Hsieh CC, and Paffenbarger RS, Jr.** Exercise intensity and longevity in men. The Harvard Alumni Health Study. *Jama* 273: 1179-1184, 1995.

69. **Machalinski B, Honczarenko M, Gontarewicz A, and Ratajczak MZ.** [Isolation of human mononuclear cells from bone marrow, peripheral blood and cord blood using Ficoll-Paque (Pharmacia) and Gradisol L (Polfa). Comparative study]. *Pol Arch Med Wewn* 99: 15-23, 1998.

70. **Manini TM, Everhart JE, Patel KV, Schoeller DA, Colbert LH, Visser M, Tylavsky F, Bauer DC, Goodpaster BH, and Harris TB.** Daily activity energy expenditure and mortality among older adults. *Jama* 296: 171-179, 2006.

71. **Martin-Ruiz CM, Gussekloo J, van Heemst D, von Zglinicki T, and Westendorp RG.** Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. *Aging Cell* 4: 287-290, 2005.

72. **Matsubara Y, Murata M, Watanabe K, Saito I, Miyaki K, Omae K, Ishikawa M, Matsushita K, Iwanaga S, Ogawa S, and Ikeda Y.** Coronary artery disease and a functional polymorphism of hTERT. *Biochem Biophys Res Commun* 348: 669-672, 2006.

73. **Matsubara Y, Murata M, Yoshida T, Watanabe K, Saito I, Miyaki K, Omae K, and Ikeda Y.** Telomere length of normal leukocytes is affected by a functional polymorphism of hTERT. *Biochem Biophys Res Commun* 341: 128-131, 2006.

74. **McArdle W, D. Katch, Frank, I. Katch, Victor, L.** *Exercise Physiiology: Energy, Nutrition, & Human Performance*. Baltimore: Lippincott, Williams & wilkins, 2007.

75. **Minamino T and Komuro I.** Vascular cell senescence: contribution to atherosclerosis. *Circ Res* 100: 15-26, 2007.

76. **Munz C, Dao T, Ferlazzo G, de Cos MA, Goodman K, and Young JW.** Mature myeloid dendritic cell subsets have distinct roles for activation and viability of circulating human natural killer cells. *Blood* 105: 266-273, 2005.

77. **Nieman D, Henson D, Gojanovich G, Davis JM, Dumke C, Utter A, Murphy A, Pearce S, McAnulty S, and McAnulty L.** Immune Changes: 2 h of Continuous vs. Intermittent Cycling. *Int J Sports Med*, 2007.

78. **Nieman DC.** Exercise and resistance to infection. *Can J Physiol Pharmacol* 76: 573-580, 1998.

79. **Nieman DC.** Exercise, infection, and immunity. *Int J Sports Med* 15 Suppl 3: S131-141, 1994.

80. **Nieman DC.** Exercise, upper respiratory tract infection, and the immune system. *Med Sci Sports Exerc* 26: 128-139, 1994.

81. **Nieman DC.** Immune response to heavy exertion. *J Appl Physiol* 82: 1385-1394, 1997.

82. **Nieman DC.** Immunologic changes associated with strenuous exercise. *Clin J Sport Med* 6: 140, 1996.

83. **Nieman DC.** Influence of carbohydrate on the immune response to intensive, prolonged exercise. *Exerc Immunol Rev* 4: 64-76, 1998.

84. **Nieman DC.** Is infection risk linked to exercise workload? *Med Sci Sports Exerc* 32: S406-411, 2000.

85. **Nieman DC.** The effect of exercise on immune function. *Bull Rheum Dis* 43: 5-8, 1994.

86. **Nieman DC, Buckley KS, Henson DA, Warren BJ, Suttles J, Ahle JC, Simandle S, Fagoaga OR, and Nehlsen-Cannarella SL.** Immune function in marathon runners versus sedentary controls. *Med Sci Sports Exerc* 27: 986-992, 1995.

87. **Nordfjall K, Osterman P, Melander O, Nilsson P, and Roos G.** hTERT (-1327)T/C polymorphism is not associated with age-related telomere attrition in peripheral blood. *Biochem Biophys Res Commun* 358: 215-218, 2007.

88. **Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, and Aviv A.** Telomere length in the newborn. *Pediatr Res* 52: 377-381, 2002.

89. **Okuda K, Khan MY, Skurnick J, Kimura M, Aviv H, and Aviv A.** Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. *Atherosclerosis* 152: 391-398, 2000.

90. **Paffenbarger RS, Jr., Hyde RT, Wing AL, and Hsieh CC.** Physical activity, all-cause mortality, and longevity of college alumni. *N Engl J Med* 314: 605-613, 1986.

91. **Paffenbarger RS, Jr., Hyde RT, Wing AL, Lee IM, Jung DL, and Kampert JB.** The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. *N Engl J Med* 328: 538-545, 1993.

92. **Paffenbarger RS, Jr., Kampert JB, Lee IM, Hyde RT, Leung RW, and Wing AL.** Changes in physical activity and other lifeway patterns influencing longevity. *Med Sci Sports Exerc* 26: 857-865, 1994.

93. **Perret M and Aujard F.** Aging and season affect plasma dehydroepiandrosterone sulfate (DHEA-S) levels in a primate. *Exp Gerontol* 40: 582-587, 2005.

94. **Petruzzello SJ, Landers DM, Hatfield BD, Kubitz KA, and Salazar W.** A meta-analysis on the anxiety-reducing effects of acute and chronic exercise. Outcomes and mechanisms. *Sports Med* 11: 143-182, 1991.

95. **Radak Z, Taylor AW, Sasvari M, Ohno H, Horkay B, Furesz J, Gaal D, and Kanel T.** Telomerase activity is not altered by regular strenuous exercise in skeletal muscle or by sarcoma in liver of rats. *Redox Rep* 6: 99-103, 2001.

96. **Rantanen T, Era P, and Heikkinen E.** Physical activity and the changes in maximal isometric strength in men and women from the age of 75 to 80 years. *J Am Geriatr Soc* 45: 1439-1445, 1997.

97. **Rowbottom DG and Green KJ.** Acute exercise effects on the immune system. *Med Sci Sports Exerc* 32: S396-405, 2000.

98. **Shay JW and Wright WE.** Hallmarks of telomeres in ageing research. *J Pathol* 211: 114-123, 2007.

99. **Shay JW and Wright WE.** Telomerase therapeutics for cancer: challenges and new directions. *Nat Rev Drug Discov* 5: 577-584, 2006.

100. **Shay JW and Wright WE.** Telomeres are double-strand DNA breaks hidden from DNA damage responses. *Mol Cell* 14: 420-421, 2004.

101. **Sherlock M and Toogood AA.** Aging and the growth hormone/insulin like growth factor-I axis. *Pituitary* 10: 189-203, 2007.

102. **Sherr CJ and DePinho RA.** Cellular senescence: mitotic clock or culture shock? *Cell* 102: 407-410, 2000.

103. **Sidorov IA, Gee D, and Dimitrov DS.** A kinetic model of telomere shortening in infants and adults. *J Theor Biol* 226: 169-175, 2004.

104. **Volpi E, Nazemi R, and Fujita S.** Muscle tissue changes with aging. *Curr Opin Clin Nutr Metab Care* 7: 405-410, 2004.

105. **Wilson TM and Tanaka H.** Meta-analysis of the age-associated decline in maximal aerobic capacity in men: relation to training status. *Am J Physiol Heart Circ Physiol* 278: H829-834, 2000.

106. **Wright WE and Shay JW.** Telomere biology in aging and cancer. *J Am Geriatr Soc* 53: S292-294, 2005.

107. **Wright WE, Shay JW, and Piatyszek MA.** Modifications of a telomeric repeat amplification protocol (TRAP) result in increased reliability, linearity and sensitivity. *Nucleic Acids Res* 23: 3794-3795, 1995.

108. **Young DR, Jee SH, and Appel LJ.** A comparison of the Yale Physical Activity Survey with other physical activity measures. *MedSciSports Exerc* 33: 955-961, 2001.

109. **Zhang X, Mar V, Zhou W, Harrington L, and Robinson MO.** Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. *Genes Dev* 13: 2388-2399, 1999.