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Telomeres, Nutrition and Mortality: Risk Factors for the Rate of Telomere Length Decline and the Associations Between Telomere Length, Nutrition and Mortality

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TELOMERES, NUTRITION AND MORTALITY: RISK FACTORS FOR THE RATE OF TELOMERE LENGTH DECLINE AND THE ASSOCIATIONS BETWEEN TELOMERE LENGTH, NUTRITION AND MORTALITY

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Abstract

Introduction: Telomeres are nucleoprotein structures located at the ends of eukaryotic chromosomes, thought to protect the DNA from damage. As a person experiences stressors, harmful exposures, and other diseases throughout their life, telomeres are thought to become damaged and their length shortened, decreasing their ability to protect the DNA. Nutrition is an important aspect of healthy aging. Preservation of telomere length (TL) is thought to be one of the mechanisms by which good nutrition can delay or prevent the development of chronic disease and death. Recent evidence of preservation of TL with good nutrition is promising. Thus, the aim of this dissertation is to study the relationship of TL with nutrition and mortality, which will be accomplished through three distinct, but ancillary aims. The first aim of this research is to examine rates of age-related TL change and evaluate variability in the rate by gender, chronic stress, and chronic diseases. The second aim is to comprehensively examine the linear associations between different macro- and micro-nutrients and healthy eating and TL. The third aim is to assess the associations of TL with survival and lifespan.

Methods: For the first and second study aim, cross-sectional data on individuals, aged ≥ 20 years with a TL measurement available, were included from the National Health and Nutrition Examination Survey, years 1999-2002. Crude and adjusted linear regression models were used to estimate the rate of decline in TL across 10-year age categories, and any variations in the decline with respect to sex, measure of chronic stress, and presence of chronic diseases. To evaluate the associations between TL and a variety of macro- and micro-nutrients, and healthy eating, multivariate linear regression models using energy-adjusted nutrients were implemented. For the third aim of the study, data on 328 elderly (oldest-old) men from the Zutphen (The Netherlands) and Cretan (Greece) Elderly Study, with available TL measurement in 2000 were included.

Follow-up for vital status was available until September 2015. Kaplan-Meier survival estimation assessed differences in survival between men with short and long TL. Univariate and multivariable Cox proportional hazard models assessed the associations of TL (standardized continuous measure) on survival. Linear regression models assessed the relation of TL on age at death.

Results: In an adjusted model, the population rate of decline in TL with age was consistent and linear for only three age categories: 20-29 (β=-0.0196, 95% CI: -0.0360, -0.0032), 50-59 (β=-0.0200, 95% CI: -0.0326, -0.0074) and 70-79 (β=-0.0164, 95% CI: -0.0318, -0.0010) years. The population rate of decline in TL with age was significantly greater for males and those with high allostatic load (a measure of chronic stress) and a history of comorbidities. When the population rate of decline in TL was analyzed by gender in 10-year age bins, a fairly consistent yet statistically non-significant decline for males was observed; however, a trough in the rate was observed for females in the age categories 20-29 years (β=-0.0283, 95% CI: -0.0468, -0.0099) and 50-59 years (β=-0.0216, 95% CI: -0.0396, -0.0036). To further elucidate the gender difference observed in the primary analyses, secondary analyses were conducted with reproductive and hormonal status; a significant inverse association was found between TL and parity, menopause, and age at menopause. In a linear regression model to study associations between TL and nutrients, adjusted for demographics and health-related behaviors, of the macronutrients examined, total fat (β= -83.24, 95% CI: -153.31, -13.17), mono-unsaturated (β= -107.40, 95% CI: -175.39, -39.40) and poly-unsaturated fatty acids (β=-54.01, 95% CI: -99.81, -8.19) were inversely associated with TL. Of the micro-nutrients examined, potassium was the only element (β= 90.17, 95% CI: 7.54, 172.79) and riboflavin or Vitamin B2 (β= 85.04, 95% CI: 1.53, 168.54) was the only vitamin that was significantly associated with TL in fully adjusted

models. However, Vitamin A, B12, and E were inversely associated with TL in energy adjusted models. After adjustment for total calorie, demographic, and health-related behaviors, the overall healthy eating index (HEI) score was positively associated with TL (β= 3.42, 95% CI: 0.29, 6.53, p-value=0.033); every unit increase in HEI score increased the TL by 3.42 base pairs. In the mortality study, all but nine men were deceased by the end of the study period. TL was not related to socio-demographic factors, lifestyle factors and prevalent chronic diseases. Kaplan-Meier survival analysis did not show statistically significant differences in all-cause or cardiovascular mortality between long and short TL groups. In Cox proportional hazards models, long TL was not associated with all-cause (HR: 0.90, 95%CI: 0.68 - 1.17) and cardiovascular mortality (HR: 0.96, 95%CI 0.62 - 1.50). In linear regression models, TL was not a significant predictor of age at death. Findings were also not significant when analyzed separately for the Zutphen and Cretan cohort.

Conclusions: TL was shorter with increasing age and this decline was modified by gender, chronic stress and comorbidities; individuals with chronic morbidity and/or chronic stress and females in their twenties and fifties experienced greater decline. Female reproductive factors, i.e., parity and menopause, were associated with TL. Certain nutrients, as well as healthy eating in general, are associated with preservation of TL. Health gains associated with telomere preservation could potentially be achieved relatively easily through promotion of healthy and adequate diets. Finally, the lack of associations of TL with mortality and lifespan in the elderly men from Zutphen and Crete is consistent with previous literature.

Acknowledgements

Most women in my native country of Nepal are limited by our traditional culture, expected to be nothing more than obedient wives and daughters-in-law as well as caretakers of children and the extended family. As a first-generation high school graduate, I had to struggle, at every step, to define and frame my own path to success. In May 2019, when I undergo the hooding ceremony at UNLV's Commencement, I will be not only the first person in my family with a terminal degree but also one of few female PHDs from my country. Dreaming big, as I have always done, was rebellious, yet prerequisite to pursuing my dreams. Fortunately, I have been welcomed here at UNLV, where I am now a very proud UNLV REBEL.

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To

Dr. Mark P. Buttner

who always believed in me and inspired me to persevere in my PHD journey despite the obstacles

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List of Abbreviations

AFAR	American Federation for Aging Research
ANOVA	Analysis of Variance
BMI	Body Mass Index
CAPI	Computer-Assisted In-Person Interview
CCI	Charlson Comorbidity Index
CVD	Cardiovascular Disease
HDL	High-Density Lipoprotein
HEI	Healthy Eating Index
HR	Hazard Ratios
ICD-9	International Classification of Diseases, Ninth Revision
Kbp	Kilobase Pairs
MEC	Mobile Examination Center
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NH	Non-Hispanic
PCR	Polymerase Chain Reaction
PIR	Poverty Income Ratio
Qol	Quality of Life
SEM	Standard Error of Mean
SES	Socioeconomic Status
TL	Telomere Length
US	United States

Chapter 1. Introduction

Burgeoning geriatric populating

Aging is a relatively new area of research as the proportion of elderly in the population grows globally, demanding attention for elderly health. Annually, around 58 million people celebrate their 60th birthday throughout the globe, a number equivalent to two persons becoming elderly every second (McNicoll, 2002). In 2012, about 11.5% of the total global population was represented by the elderly population and the proportion is expected to double by 2050 (McNicoll, 2002). By 2050, estimates suggest that there will be more elderly (60 or over) than children under 15 years old in the world (McNicoll, 2002). This scenario of a growing geriatric population is also evident in the United States (US). The proportion of the US population (in 2014) that was 65 or older was 14.5% (46.3 million) and this is projected to reach to 23.5% (98 million) by 2060 (Colby & Ortman, 2015). The rapidly increasing growth rate of the proportion of geriatric individuals in the population has a direct and significant impact on the individual, family, society and the nation in social and economic constructs (Knickman & Snell, 2002; National Research Council Committee on Population, 2001).

Health and quality of life (QoL) are major concerns for the aging population. A greater demand for geriatric health care is expected than ever before, and the geriatric population's health management has become an increasing concern for both developing and developed countries in terms of funding and resources available (National Research Council Committee on Population, 2001). During the life transition, various unique physical, mental and psycho-social changes accompany the phenomenon of aging (World Health Organization, 2015). With increasing age, people are more likely to experience multiple chronic diseases, which has a significant impact on

QoL and demand for health care. The impact on an individual with multiple chronic diseases is greater than the additive effect of individual conditions (Marengoni et al., 2011). In response, current research attention is focused on promoting a healthy aging experience and enhancing QoL in older adults (Centers for Disease Control and Prevention, 2003).

Elderly definition in research

Although the global population is aging, a uniform definition of aging has not been established and, therefore, measuring aging can be difficult. Biologically, aging is inevitable in all living species, including human beings (Gilbert, 2000). Aging is defined by Gilbert et al as "the time-related deterioration of the physiological functions necessary for survival and fertility" (Gilbert, 2000). However, the definition is vague in itself and researchers have no clear cutoff for defining who is an elder.

The demographics of aging is interesting, as well as complex. The literature identifies three different constructs of measuring aging: chronological, social, and biological aging. Chronological aging is simply a calculation of a number of years lived after birth. A limitation of the chronological age construct is that it cannot accurately differentiate an individual as young or elderly because two people of the same age may have different physical and mental functioning and life span (Choi, DiNitto, & Kim, 2014).

Globally, the established criteria of aging come from a social construct and an individual is defined as elderly at the age they are eligible for retirement. In the US, it often coincides with eligibility for Social Security benefits. Based on the social definition (i.e., age eligible for retirement), developed and developing countries define elderly at different age cutoffs. In line with the United Nations, the developing countries use 60 years to refer to older people (United

Nations Population Fund, 2012; World Health Organization, 2002). In fact, most of the developed countries set the reference criteria at 60 years (United Nations Population Fund, 2012). In the US, elderly, also called as senior citizens, are defined as the population aged 65 years and above (Federal Interagency Forum on Aging-Related Statistics, 2016). But that number no longer holds the accepted status it once did. For example, the retirement age for Social Security benefit eligibility has already been increased from 65 to 66 and is scheduled to rise to 67 shortly (Moon, Guo, & McSorley, 2015).

Within the research literature and the health surveys on aging, differences in the defined age range are widespread. The survey on health, well-being, and aging in Latin America and the Caribbean includes persons aged 60 and older as elderly (Albala et al., 2005). The English longitudinal study of aging in United Kingdom (Steptoe, Breeze, Banks, & Nazroo, 2013) and the health and retirement study in the US (Sonnega et al., 2014) includes individuals over age 50 years as elderly. Similarly, World Health Organization's study on global aging and adult health collects data on adults aged 50 years and older (Chatterji, 2013). Such inconsistencies do not only limit the comparability of any research finding, but also obscure the translation of findings into clinical or public health practice.

Political, social and research entities clearly have inconsistencies in the definitions of aging. From a clinical and public health perspective, it is more important to study the biologically aged population. However, capturing biological aging is not as simple as counting numbers of years since birth. Telomere length (TL) has been proposed as a candidate biomarker of aging (Butler et al., 2004; Guralnik, 2008; von Zglinicki & Martin-Ruiz, 2005). Additionally, TL is also considered as a biomarker of health status, disease risk and early mortality.

Biomarkers of aging

Human aging is a complex process, and despite extensive efforts to develop the biomarkers of human aging, success so far has been limited (Sprott, 2010). The American Federation for Aging Research (AFAR) proposed that biomarkers of aging (Lara et al., 2015; Simm et al., 2008):

1. *must predict the rate of aging (it should tell exactly where a person is in their total lifespan and it must be a better predictor of lifespan than chronological age);*
2. *it must monitor a basic process that underlies the aging process, not the effects of disease;*
3. *it must be able to be tested repeatedly without harming the person (for example a blood test or an imaging technique);*
4. *it must be something that works in humans and in laboratory animals, such as mice (so that it can be tested in laboratory animals before being validated in humans).*

Biomarkers meeting all of the above criteria are practically implausible (Johnson, 2006). In a 2005 review of the in-vitro and in vivo evidence, von Zglinicki and Martin-Ruiz (von Zglinicki & Martin-Ruiz, 2005) stated that "*as TL changes with age, has high inter-individual variability, is linked to basic biology, and correlates with aging and aging-related disease*", thus it satisfies several criteria for a biomarker of aging. Therefore, many research studies since then have advocated TL as a candidate biomarker of aging (Butler, et al., 2004; Guralnik, 2008; von Zglinicki & Martin-Ruiz, 2005).

Telomeres, a nucleoprotein structures situated at the ends of eukaryotic chromosomes (Figure 1-1), are called as the chromosome's protective cap due to their role in protecting the end of the chromosome from degradation and end-to-end fusion (Blackburn, Greider, & Szostak, 2006). Additionally, telomeres play a vital role in preserving our genomic information by protecting the

genome from nucleolytic degradation, unnecessary recombination, and repair (Shammas, 2011). With each somatic cell division, there is a gradual attrition of the telomere, resulting in TL shortening with increasing age (Slagboom, Droog, & Boomsma, 1994). However, TL decreases are not directly proportional with age. TL decreases have also been associated with stressors, occurrence of chronic disease, obesity, etc. (Haycock et al., 2014; Sampson, Winterbone, Hughes, Dozio, & Hughes, 2006; van der Harst et al., 2007; Weischer et al., 2012; Wikgren et al., 2012). TL has been proposed as a candidate biomarker of aging (von Zglinicki & Martin-Ruiz, 2005) whereby longer TL is an indicator of healthy aging (Cherkas et al., 2008). Several human studies provide support for this proposed hypothesis by providing evidence to suggest that TL is inversely related to several age-sensitive measures (Aviv, 2006; Houben, Moonen, van Schooten, & Hageman, 2008), such as oxidative stress (Demissie et al., 2006; Houben, et al., 2008), diabetes (Sampson, et al., 2006), and heart disease (Brouilette et al., 2007; Haycock, et al., 2014; van der Harst, et al., 2007; Weischer, et al., 2012), as well as overall mortality (Needham et al., 2015; Rode, Nordestgaard, & Bojesen, 2015).

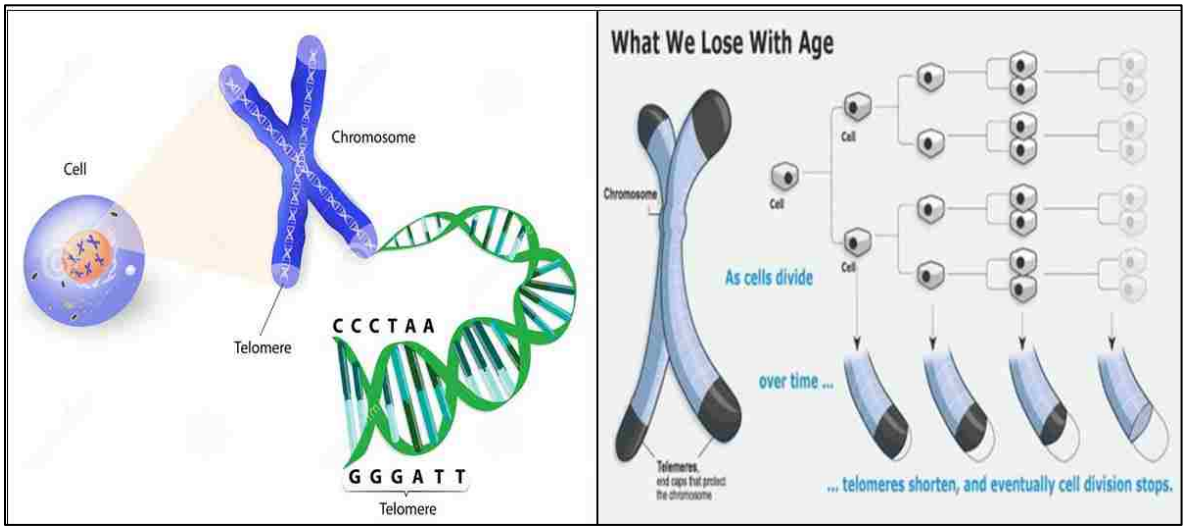

Figure 1-1. Telomere in cell

(Source: TA Science ***https://www.tasciences.com/what-is-a-telomere/***)

Study aims and hypotheses

Preservation of TL among healthy individuals is thought to be one of the several ways for the prevention of chronic diseases and mortality. This research examines one of the several theories linking TL with chronic diseases and mortality. The aims of the proposed dissertation are to describe telomere rate of decline with age; generate initial hypotheses about specific nutritional factors and TL; and to assess the associations of TL with survival and lifespan. The dissertation will be operationalized in terms of three distinct, but ancillary research aims and hypotheses, as defined below:

- Aim 1: To assess the cross-sectional rates of change in TL by age, and evaluate any variability in the rates by gender, measures of chronic stress and presence of chronic diseases.
- Aim 2: To estimate the association between TL and nutritional factors, and healthy eating index (Basiotis, Carlson, Gerrior, Juan, & Lino, 2002).
- Aim 3: To assess the association of TL with mortality and lifespan among elderly men.

This dissertation follows the three-paper dissertation format. Thus, the subsequent chapters (Chapters 2, 3, and 4) are three unique manuscripts written in peer-reviewed journal style, reflecting the above three study aims. Chapter 5 presents an overall conclusion.

Chapter 2. Manuscript 1

"Decline in Telomere Length by Age and Effect Modification by Gender, Allostatic Load and Comorbidities in National Health and Nutrition Examination Survey (1999-2002)"

Abstract

Background

This study aims to assess the decline in telomere length (TL) with age and evaluate effect modification by gender, chronic stress, and comorbidity in a representative sample of the US population.

Methods

Cross-sectional data on 7826 adults with a TL measurement were included from the National Health and Nutrition Examination Survey, years 1999-2002. The population rate of decline in TL across 10-year age categories was estimated using crude and adjusted regression.

Results

In an adjusted model, the population rate of decline in TL with age was consistent and linear for only three age categories: 20-29 (β=-0.0196, 95% CI: -0.0360, -0.0032), 50-59 (β=-0.0200, 95% CI: -0.0326, -0.0074) and 70-79 (β=-0.0164, 95% CI: -0.0318, -0.0010) years. The population rate of decline in TL with age was significantly greater for males and those with high allostatic load (a measure of chronic stress) and a history of comorbidities. When the population rate of decline in TL was analyzed by gender in 10-year age bins, a fairly consistent yet statistically

non-significant decline for males was observed; however, a trough in the rate was observed for females in the age categories 20-29 years (β=-0.0283, 95% CI: -0.0468, -0.0099) and 50-59 years (β=-0.0216, 95% CI: -0.0396, -0.0036). To further elucidate the gender difference observed in the primary analyses, secondary analyses were conducted with reproductive and hormonal status; a significant inverse association was found between TL and parity, menopause, and age at menopause.

Conclusions

TL was shorter with increasing age and this decline was modified by gender, chronic stress and comorbidities; individuals with chronic morbidity and/or chronic stress and females in their twenties and fifties experienced greater decline. Female reproductive factors, i.e., parity and menopause, were associated with TL.

Keywords

Telomere Length, NHANES, Allostatic Load, Comorbidities

Introduction

Telomeres, nucleoprotein structures located at the ends of eukaryotic chromosomes, protect the end of the chromosome from degradation and end-to-end fusion (Blackburn, et al., 2006). With each somatic cell division, there is a gradual attrition of the telomere, resulting in telomere length shortening with increasing age (Blackburn, et al., 2006). Telomere length (TL) has been proposed as a candidate biomarker of aging (von Zglinicki & Martin-Ruiz, 2005) whereby longer TL is an indicator of healthy aging. Preservation of TL among healthy individuals, in comparison to those with multiple morbidities, is thought to be one of the several pathways by which the development of chronic diseases and mortality can be explained. Although the association between health status and TL has been fairly well established, it is unclear how quickly TL declines with increasing age, or whether there is any effect modification by gender, chronic stress and morbidities, all of which influence telomere dynamics (Ahrens, Rossen, & Simon, 2016; Epel et al., 2004; Fuster & Andres, 2006; Gardner et al., 2014).

Although TL shortens with increasing age (Slagboom, et al., 1994), TL decreases are not directly proportional with age. Gender, chronic stress, and comorbidities may modify the relationship between age and TL. Females have longer telomeres than males (Gardner, et al., 2014). Chronic psychosocial stress, depression, anxiety, and childhood trauma was associated with shorter TL (Epel, et al., 2004; O'Donovan et al., 2011; Okereke et al., 2012; Simon et al., 2006; Tyrka et al., 2010). Perceived stress was associated with lower telomerase activity, and shorter TL among healthy premenopausal women (Epel, et al., 2004). Shorter TL in adulthood was associated with childhood trauma (Tyrka, et al., 2010). Stressful life events within the last five years was associated with associated with shorter telomeres in Netherlands Study of Depression and Anxiety (Verhoeven, van Oppen, Puterman, Elzinga, & Penninx, 2015). A systematic review

aimed to examine whether chronic social stress is associated with TL throughout the life course, concluded that chronic social stress was associated with shorter telomeres in both early and adult exposures (Oliveira et al., 2016). Further, evidence suggests that shorter telomeres is associated with greater cortisol reactivity to stress, central elements of the physiological stress response system (Gotlib et al., 2015; Tomiyama et al., 2012). "Allostatic load," also called the wear and tear in the body (McEwen & Seeman, 1999), has been proposed as a conceptualization of cumulative stress exacted on the body through attempts to adapt to life's demands (Seeman, McEwen, Rowe, & Singer, 2001). Stress, an inevitable condition of human existence, has been associated with poor health outcomes (McEwen & Seeman, 1999). Limited research has looked at the relationship between TL and allostatic load (Ahrens, et al., 2016).

TL has been linked to various morbidities, such as diabetes (Sampson, et al., 2006), heart disease (Brouilette, et al., 2007; Haycock, et al., 2014; van der Harst, et al., 2007; Weischer, et al., 2012), hypertension (Yang et al., 2009) cancer (Zhu et al., 2016), and depression (Ridout, Ridout, Price, Sen, & Tyrka, 2016), as well as overall mortality (Needham, et al., 2015; Rode, et al., 2015). A meta-analysis of 62 population based studies found a non-significant association between short telomeres and overall risk of cancer, but an increased risk for gastrointestinal tumor and head and neck cancer, indicating that telomeres may play diverse roles for risk in different cancers (Zhu, et al., 2016). In contrast, a Mendelian Randomization Study reported an increased risk for several cancers with longer telomeres but reduced risk for cardiovascular diseases (CVD) (Haycock et al., 2017). Another meta-analysis reported negative association between depression and TL (Ridout, et al., 2016). The Charlson comorbidity index (CCI) is commonly used to provide a cumulative weighted score of 17 comorbid conditions (Charlson, Pompei, Ales, & MacKenzie, 1987). The measure of decline in TL with age by allostatic load and comorbidities could be

useful, particularly in elucidating the biologic pathway by which chronic stress and comorbidities can affect TL and accelerate the rate of aging. Once this pathway is better understood, this information can be used to develop effective prevention measures in at-risk populations. Therefore, we aim to assess the decline in TL with age and evaluate any effect modification by gender, chronic stress and comorbidities in a representative sample of the US population. Guided by the results of the primary analyses and to further elucidate the observed gender differences, secondary analyses were conducted to examine the association between TL, parity and menopause.

Methods

Study design

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional nationally representative survey of the US civilian noninstitutionalized population, conducted using complex, multistage, stratified, clustered sampling (Curtin et al., 2012; Johnson et al., 2013b). Details of NHANES methodology have been reported elsewhere (Curtin, et al., 2012; Johnson, et al., 2013b). We used data from the cycles 1999-2000 and 2001-2002, combining them following the National Center for Health Statistics (NCHS) recommendations (Johnson, et al., 2013b).

Study participants

NHANES 1999-2000 and 2001-2002 included a total of 9965 and 11,039 participants, respectively. For our analyses, we included those aged ≥ 20 years who had a measure of TL, for a total of 7826 individuals from both the cycles, NHANES 1999-2002.

Ethical approval

NHANES was approved by the NCHS Research Ethics Review Board (https://www.cdc.gov/nchs/nhanes/irba98.htm). All participants provided written informed consent. The Institutional Review Board at the University of Nevada Las Vegas approved the current study.

Measurements

2.3.4.1 Telomere length

Telomere length in leukocytes was measured from whole blood using the quantitative polymerase chain reaction method, described in detail elsewhere (Cawthon, 2002; Needham et al., 2013). The Mean T/S ratio, which is the measure of TL relative to standard reference DNA, were provided in the NHANES dataset. During data analysis, the T/S ratio was converted to kilobase pairs (kbp) using the following formula: (3,274 + 2,413 * (T/S))/1000.

2.3.4.2 Allostatic load

Chronic stress, a hypothesized effect modifier, was measured in terms of allostatic load, quantified using nine biomarkers of cardiovascular, inflammatory, and metabolic system functioning. The nine biomarkers with corresponding cutoffs (Chen, Redline, Shields, Williams, & Williams, 2014; Parente, Hale, & Palermo, 2013) were: systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, heart rate ≥ 90 beats/minute, total cholesterol level ≥ 240 mg/dL, high-density lipoprotein (HDL) cholesterol < 40mg/dL, BMI ≥ 30 kg/m2, glycosylated hemoglobin ≥ 6.4%, C-reactive protein ≥ 0.3 mg/dL, and albumin < 3.8 g/dL. Each measure was coded as a dichotomous variable at the cutoff (1, if the respondent had indicated the condition; 0, if otherwise). The cumulative score of the nine indicators was then converted into a dichotomous

variable, with high allostatic load defined as an allostatic load score ≥ 3. The same cutoff values and measures have been used to quantify allostatic load with the NHANES dataset in previous studies (Chen, et al., 2014; Parente, et al., 2013).

2.3.4.3 Comorbidity

We calculated the Charlson Comorbidity Index (Charlson, et al., 1987), another hypothesized effect modifier, to account for the impact of any comorbid conditions on TL. Different health conditions included in calculating CCI, their definations and corresponding weights in the calculation, are provided in Appendix A. Because a score of ≥4 points is associated with an estimated 53% 10-year mortality, a weighted combined index score of ≥4 points was used to define a history of chronic comorbid conditions (Charlson, et al., 1987).

2.3.4.4 Other covariates

Covariates for this study, selected based on the literature, were race/ethnicity, educational attainment, socioeconomic status (SES), and body mass index (BMI) (Gardner, et al., 2014; Needham, et al., 2013; Rehkopf et al., 2016).

Age (in years and 10-year categories), gender, race/ethnicity (nominal: Hispanic, Non- Hispanic [NH] white, NH Black, and others including multi-racial); educational level (ordinal: <12th grade, high school graduate/some college, and college graduate or above); and marital status (nominal: married/living with partner, divorced/widowed/separated, and never married) were self-reported by the participants. SES was measured on a continuous scale, in terms of poverty income ratio (PIR). PIR, the ratio of family income to the poverty threshold, was calculated following the U.S. Department of Health and Human Services' poverty guidelines and described in detail elsewhere (Johnson, et al., 2013b). BMI, the ratio of weight/height^2 measured in kg/m^2,

was available as a continuous measure and was categorized as normal weight (<25 kg/m^2), overweight (25- <30 kg/m^2) and obese (≥30 kg/m^2). Following the definition for menopause proposed by McKinlay (McKinlay, 1994), menopause was defined as one or more of the given criteria: over 55 years, had a hysterectomy or both ovaries removed, and menopause as the reason for no periods in the past 12 months. Age at menopause as well as number of pregnancies resulting in live birth were self-reported by women.

Statistical analyses

To generate a nationally representative sample, we adjusted sample weights in the analyses following the NHANES guidelines to (Johnson, et al., 2013b). For nominal variables, percentages with 95% CIs are provided; for continuous variables, unadjusted means with standard error (SEM) are provided (Table 2-1). TL was divided into quartiles based on the weighted population distribution. Covariate characteristics, between the quartiles of TL, were compared using Rao-Scott Chi-Square tests and analysis of variance. Linear regression with TL (kbp) as the outcome was used to assess the association with socio-demographic factors, biomarkers of allostatic load, and reproductive factors among females. Initially, univariate models were evaluated, then a group of covariates (age, gender, race/ethnicity, education, PIR, and BMI) were added to the model.

The main outcome of this study is rate of decline in TL, which would best use a longitudinal design study. Since this is a cross-sectional study, we aim to examine the cross-sectional rates of TL decline as a proxy for rate of decline in TL by age. For this, we are assuming that an individual with a TL at a certain age will, later in life, have the same TL as another individual at an older age with similar covariates. Therefore, the rate we are measuring is a population rate of decline in TL, not an individual rate of decline. To measure the population rate of decline in TL

in different age categories, we used 10-year bins and calculated the slope of the rate of decline in TL as a function of age in each bin, using a linear regression with TL (kbp) as outcome and age (years) as predictor. All population rates of decline in TL are adjusted for gender, ethnicity, CCI, and allostatic load. A two-tailed p-value less than 0.05 was considered statistically significant. Data analyses were performed using the survey procedures that account for the weights and complex survey design of NHANES, in SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Participant's characteristics

The mean (±SEM) age and TL of the participants were 45.2±0.4 years and 5.8±0.04 kbp, respectively (Table 2-1). Participants with shorter TL, i.e., in lower quartiles, were older, NH White, married, and had higher BMI, allostatic load, and CCI. A gradual decreasing trend in mean age, BMI, allostatic load and CCI was noted from lowest to highest quartiles (Table 2-1).

Telomere length and associated factors

In analyses adjusted for age, gender, race/ethnicity, education, PIR, and BMI (Table 2-2), increasing age, having less than high school education, and higher BMI were associated with lower TL. Likewise, NH Black compared to NH White and unmarried compared to married/living with a partner had higher TL. A further stratified analysis was conducted to assess the role of age and gender in the association between marital status and TL. We found that the marital status and TL association was significant only for younger (20-29 years) females (β: 0.5194, 95%CI: 0.1129-0.9258, p-value: 0.0141) and older (80 years and above) females (β: 0.2770, 95%CI: 0.1217-0.4323, p-value: 0.001). Although female gender demonstrated a positive trend and family PIR demonstrated an inverse trend with TL, the findings did not reach the statistical significance of <0.05 (Table 2-2).

Table 2-1. Socio-demographic characteristics of the study participants by quartiles of leukocyte telomere length- NHANES 1999-2002

	Overall (N=7826)		Quartiles, % (95% CI)				
	N	**% (95% CI)**	**One (N=2415)**	**Two (N=2120)**	**Three (N=1633)**	**Four (N=1658)**	**p-Value**
Age, years, mean ±SEM	7826	45.2 ±0.4	54.6 ±0.7	46.2 ±0.8	41.5 ±0.7	37.4 ±0.8	**<0.001** [a]
TL, kbp, mean ±SEM[b]	7826	5.8 ± 0.04	5.1 ±0.0	5.6 ±0.0	6.0 ±0.0	6.7 ±0.0	**<0.001** [a]
Gender							
Male	3770	48.6 (47.6 - 49.5)	50.7 (48.3 - 53.2)	48.0 (45.1 - 50.9)	47.7 (45.5 - 49.9)	47.7 (45.2 - 50.3)	0.287
Female	4056	51.4 (50.5 - 52.4)	49.3 (46.8 - 51.7)	52.0 (49.1 - 54.9)	52.3 (50.1 - 54.5)	52.3 (49.7 - 54.8)	
Race/Ethnicity							
Hispanic	2292	13.8 (9.7 - 17.8)	11.8 (6.0 - 17.7)	13.4 (9.3 - 17.4)	14.2 (9.7 - 18.6)	15.9 (11.1 - 20.6)	**<0.001**
NH White	3965	72.8 (69.0 - 76.5)	77.4 (71.8 - 82.9)	74.7 (70.5 - 78.9)	71.1 (66.4 - 75.8)	67.2 (62.4 - 72.0)	
NH Black	1333	9.4 (7.2 - 11.6)	7.2 (5.2 - 9.2)	8.1 (5.6 - 10.6)	9.8 (7.6 - 12.1)	12.8 (9.4 - 16.3)	
Other	236	4.1 (2.7 - 5.4)	3.6 (1.8 - 5.5)	3.9 (2.7 - 5.0)	4.9 (3.1 - 6.7)	4.0 (1.8 - 6.3)	
Educational Status							
<12th Grade	2640	21.3 (19.4 - 23.2)	26.3 (23.4 - 29.2)	20.6 (18.3 - 22.9)	20.8 (17.4 - 24.2)	17.1 (15.3 - 18.9)	**<0.001**
High School/Some College	3733	54.6 (51.7 - 57.5)	53.0 (49.1 - 56.9)	55.2 (51.6 - 58.8)	52.1 (47.1 - 57.1)	57.9 (53.4 - 62.5)	
College Graduate	1441	24.1 (20.7 - 27.5)	20.7 (16.8 - 24.6)	24.2 (20.7 - 27.7)	27.1 (21.6 - 32.7)	24.9 (19.8 - 30.1)	
Marital Status							
Married/Living with Partner	4759	65.1 (63.0 - 67.2)	68.8 (66.4 - 71.1)	67.3 (64.2 - 70.3)	63.9 (60.5 - 67.3)	59.5 (55.7 - 63.3)	**<0.001**
Divorced/Widowed/Separated	1566	17.5 (16.2 - 18.9)	23.0 (21.0 - 24.9)	19.4 (17.3 - 21.6)	14.4 (11.9 - 16.8)	12.1 (9.8 - 14.4)	
Never Married	1123	17.4 (15.6 - 19.1)	8.3 (6.8 - 9.8)	13.3 (10.8 - 15.8)	21.8 (19.1 - 24.4)	28.4 (25.4 - 31.4)	
Family PIR, mean ±SEM	7128	3.0 ±0.1	3.0 ±0.1	3.1 ±0.1	3.1 ±0.1	2.8 ±0.1	**<.0001** [a]
BMI, kg/m^2, mean ±SEM	7577	28.0 ±0.1	28.7 ±0.2	28.2 ±0.2	27.8 ±0.2	27.4 ±0.3	**<.0001** [a]
Allostatic load, mean± SEM [b]	7826	2.4 ±0.0	2.7 ±0.0	2.5 ±0.0	2.4 ±0.0	2.2 ±0.1	**<0.001** [a]
CCI, mean ±SEM [b]	7826	1.5 ±0.0	2.2 ±0.1	1.5 ±0.1	1.2 ±0.1	1.0 ±0.1	**<0.001** [a]

[a]: p-values from one-way ANOVA; all others from a Chi-square test. [b] SEM values of 0 indicates a value <0.1. Abbreviations: BMI: body mass index, CCI: Charleston's comorbidity index, CI: confidence interval, Kbp: kilo base pairs, TL: telomere length, NH: Non-Hispanic, PIR: poverty income ratio, SEM: standard error of mean. Values expressed are % (95% CI) unless otherwise stated.

Table 2-2. Multivariable regression for factors associated with telomere length- NHANES 1999-2002

	Model 1 unadjusted			Model 2 adjusted [a]		
	β	**95%CI**	**p-value**	**β**	**95%CI**	**p-value**
Age	**-0.0146**	**-0.0162, -0.0130**	**<0.001**	**-0.0141**	**-0.0157, -0.0126**	**<0.001**
Gender (Reference= Male)						
Female	0.0252	-0.0265, 0.0769	0.328	0.0425	-0.0071, 0.0921	0.0903
Race/Ethnicity (Reference= NH White)						
Hispanic	0.0681	-0.0692, 0.2054	0.319	-0.0044	-0.1391, 0.1302	0.9467
NH Black	**0.1687**	**0.0802, 0.2572**	**<0.001**	**0.1257**	**0.0342, 0.2172**	**0.0088**
Other	0.0396	-0.1005, 0.1797	0.568	-0.0237	-0.1634, 0.1160	0.7312
Educational Status (Reference=High School)						
<12th Grade	**-0.1203**	**-0.1825, -0.0581**	**<0.001**	**-0.0775**	**-0.1417, -0.0132**	**0.0198**
College Graduate	-0.0023	-0.0689, 0.0642	0.943	0.0285	-0.0363,0.0933	0.3754
Marital Status (Reference= Married/with Partner)						
Divorced/Widowed/Separated	**-0.1111**	**-0.1655, -0.0566**	**<0.001**	-0.0177	-0.0767, 0.0413	0.5445
Never Married	**0.3162**	**0.2422, 0.3901**	**<0.001**	**0.0963**	**0.0257, 0.1669**	**0.009**
Family PIR	-0.0127	-0.0354, 0.0100	0.261	-0.0043	-0.0281, 0.0194	0.7116
Biomarkers of allostatic load						
Systolic blood pressure	**-0.0030**	**-0.0044, -0.0015**	**<0.001**	0.0012	-0.0003, 0.0027	0.1105
Diastolic blood pressure	**0.0041**	**0.0022, 0.0060**	**<0.001**	0.0007	-0.0001, 0.0023	0.4329
Heart rate	-0.0008	-0.0026, 0.0009	0.325	-0.0019	-0.0036, -0.0003	**0.0237**
Total cholesterol	-0.0002	-0.0007, 0.0004	0.516	0.0001	-0.0006, 0.0006	0.9649
High-density lipoprotein cholesterol	**0.0027**	**0.0009, 0.0044**	**0.004**	0.0024	0.0003, 0.0045	**0.0272**
BMI (kg/m2)	**-0.0093**	**-0.0133, -0.0052**	**<0.001**	**-0.0069**	**-0.0106, -0.0033**	**<0.001**
Glycosylated hemoglobin	**-0.0370**	**-0.0573, -0.0167**	**<0.001**	-0.0054	-0.0302, 0.0194	0.6571
C-reactive protein	**-0.0538**	**-0.0993, -0.0083**	**0.022**	-0.0380	-0.0791, 0.0032	0.0692
Albumin	0.1664	-0.3897, 0.7225	0.545	0.1664	-0.3897, 0.7225	0.5453
Allostatic load (Reference=Low)						
High	**-0.1735**	**-0.2226, -0.1244**	**<0.001**	-0.0351	-0.0927, 0.0225	0.2232
Charleston Comorbidity Index	**-0.0723**	**-0.0823, -0.0624**	**<0.001**	-0.0020	-0.0147, 0.0107	0.7501

DV: Telomere length in kbp; [a] Adjusted for age, gender, race/ethnicity, education, PIR, and BMI (when applicable, the variable for which coefficient is reported, adjustments were made for the remaining variables). Abbreviations: BMI: body mass index, CI: confidence interval, NH: Non-Hispanic, PIR: poverty income ratio. p-value less than 0.05 are bold.

Of the nine biomarkers of allostatic load, after controlling for covariates, only the regression coefficients of heart rate, HDL cholesterol, and BMI had the 95% CIs that did not include the null of no association (Table 2-2); while a higher heart rate and BMI was associated with shorter TL, a higher value of HDL cholesterol preserved it. In an unadjusted analysis, a one-unit difference in allostatic load and CCI were associated with 17.3% and 7.2% decrease in TL, respectively. However, the estimates did not remain statistically significant when controlled for covariates. Specifically, age had a strong confounding effect on our estimates because most of the covariates with a significant coefficient in unadjusted models retained their statistical significance when adjusted for other covariates except age (Appendix B).

Cross-sectional population rates of telomere length decline

The population rates of decline in TL stratified by 10-year age bins showed that decline in TL with age was initiated early in life (20-29 years) and was consistent and linear for only three age categories: 20-29 (β=-0.0196, 95% CI: -0.0360, -0.0032), 50-59 (β=-0.0200, 95% CI: -0.0326, -0.0074) and 70-79 (β=-0.0164, 95% CI: -0.0318, -0.0010) years. The population rate of decline in TL was sharp in the age category 70-79 years; whereas, in the oldest age category examined (80-89 years), the rate of decline (β=-0.0103, 95% CI: -0.0412, 0.0207) was highly variable and did not follow a linear pattern. Likewise, the population rate of decline in TL stratified by allostatic load and comorbid conditions showed a significantly greater rate of decline among those with high allostatic load (β=-0.0123, 95% CI: -0.0143, -0.0102), and history of comorbid conditions (β=-0.0137, 95% CI: -0.0167, 0.0106).

Gender stratified analyses, adjusted for ethnicity, CCI, and allostatic load, (Figure 2-1) showed a significantly greater rate of decline for males (β=-0.0153, 95% CI: -0.0170, -0.0135, p-value <0.001) compared to females (β=-0.0129, 95% CI: -0.0154, -0.0105, p-value <0.001). When the

population rate of decline in TL was further stratified by gender in 10-year age bin (Figure 2-2), we observed a fairly consistent population rate of decline in TL for males; none of the regression coefficients were statistically significant (Figure 2-2). Interestingly, for females, a peak in the population rate of decline in TL was observed in the age categories of 20-29 years (β=-0.0283, 95% CI: -0.0468, -0.0099) and 50-59 years (β=-0.0216, 95% CI: -0.0396, -0.0036). Although women in the age categories of 30-39 years and 60-69 years displayed a minimal reduction in TL, the estimates were not statistically significant (Figure 2-2).

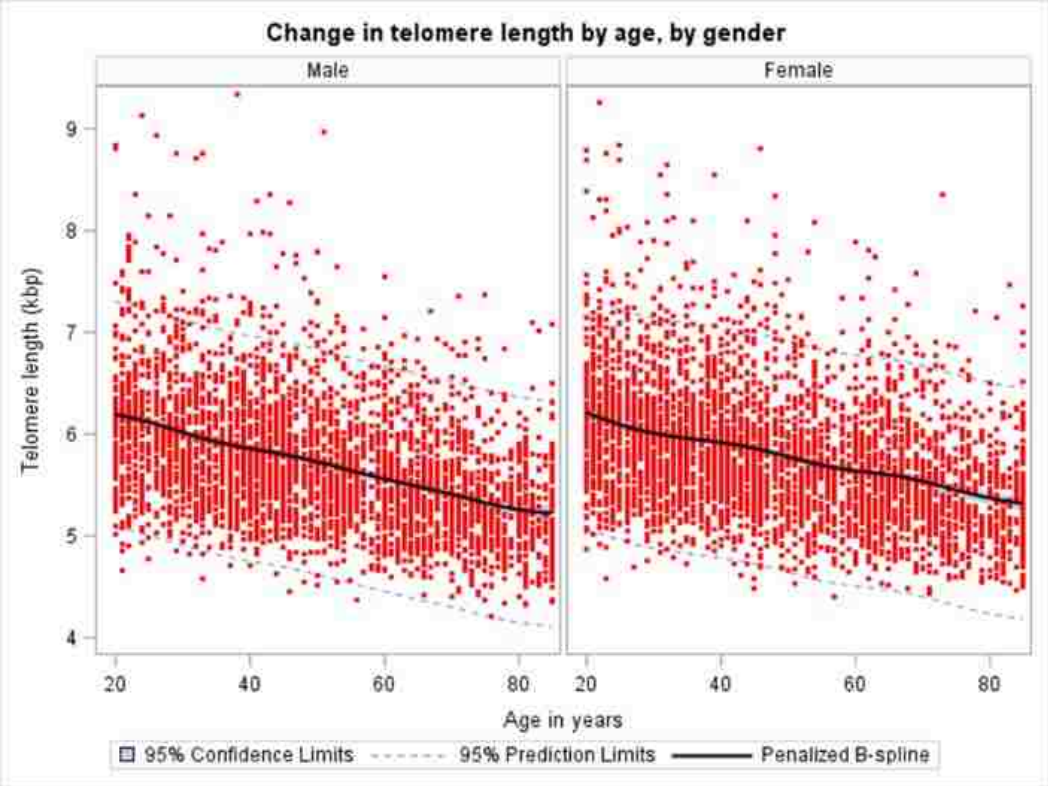

Figure 2-1. Telomere length by age and gender in NHANES 1999-2002:

(a) Male (β= -0.0153; p-value <0.001), **(b) Female** (β= -0.0129; p-value <0.001). Estimates, obtained from linear regression with telomere length (kbps) as outcome and age (years) as predictor, are adjusted for ethnicity, allostatic load and comorbidity index.

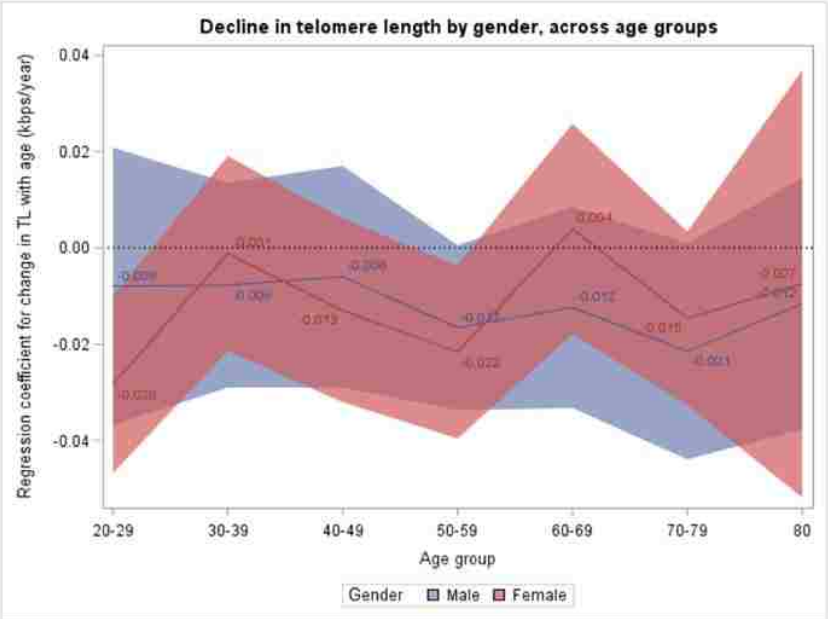

Figure 2-2. Decline in telomere length by gender, across age groups, NHANES 1999-2002.

Y-axis represents slope from a linear regression with telomere length (kbps) as outcome and age (years) as predictor. X-axis represents the age bins for the slope reported in Y-axis. Regression coefficients adjusted for ethnicity, allostatic load, and comorbidity index.

To explore the heterogeneity in the population rate of decline in TL among females, we conducted additional analyses for women's reproductive history, particularly parity and menopause, since ages 20-29 and 50-59 years are common ages for parity and menopause, respectively. After controlling for ethnicity, education, PIR, and BMI, the number of live children a woman had was negatively associated with TL (β=-0.0318, 95% CI: -0.0510, -0.0125). Further, compared to nulliparous women, decline in TL was noted for women with live births (Appendix C). Similarly, women's menopausal status (β=-0.2875, 95% CI: -0.3517, -0.2232), age at menopause (β=-0.0077, 95% CI: -0.0130, -0.0023) as well as years passed since menopause (β=-0.0095, 95% CI: -0.0127, -0.0063) were inversely associated with TL. Further,

variation in slope of decline in TL with years passed since menopause was noted by women's weight status (Appendix D).

Discussion

Overall, TL significantly reduced with age, but this decline was modified by gender, chronic stress and comorbidities; those with chronic morbidities and chronic stress experienced greater decline. Gender stratified analysis was more interesting, showing a fairly consistent and statistically non-significant rate for males but a substantial decline in TL for females in the age categories 20-29 and 50-59 years. Female reproductive factors, i.e., parity and menopause, were associated with TL.

Decline in TL with age is unanimously supported by the literature (Broer et al., 2014; Needham, et al., 2013). Heterogeneity in rate of decline in TL is suggested by a previous study from the Erasmus Rucphen Family data, which found a significant reduction in variance in TL from young adulthood to old age by using the TL of grandchildren as a proxy for participants' TL at childhood (Broer, et al., 2014). For females, a significant decline in the adjusted population rate of decline in TL was noted in early (20-29) and midlife (50-59) years. In general, for females the 20-29 and 50-59 age bands are periods of important hormonal changes, as these are the common ages for parity and menopause, respectively. Consistent with previous studies, we found a significant negative association between TL and parity, menopausal status, and age at menopause (Gray et al., 2014). The observed inverse relationship between TL and parity is supported by the life history theory, which postulates that the energy used during reproduction reduces the energy available for tissue maintenance because the total amount of energy available for mobilization by an organism is finite at any given time, and that poor tissue maintenance, in turn, leads to faster cellular degradation and aging (Jasienska, 2001). Biologically, ovarian tissue aging or

tumorigenesis may be caused by hyper- or hypo-activity of estrogen (Bayne et al., 2011). Therefore, telomere attrition rate should accelerate after menopause in response to a decrease in estrogen (Bayne, et al., 2011). In vitro, telomerase activity is upregulated by estrogen (Cha, Kwon, Seol, & Park, 2008; Misiti et al., 2000). Population-based studies also provide evidence that greater estrogen exposure, as measured by the use of hormone therapy (Lee, Im, Kim, Lee, & Shim, 2005), and longer duration of reproductive years (Lin et al., 2011), are related to significantly higher TL in postmenopausal women. Oxidative stress and proinflammatory cytokines have also been associated with telomere shortening (O'Donovan et al., 2009; Passos, Saretzki, & von Zglinicki, 2007); thus, antioxidants aid in the attenuation of telomere shortening (Serra, von Zglinicki, Lorenz, & Saretzki, 2003; von Zglinicki et al., 2000). Estrogen is known to have antioxidant properties (Aviv, 2002; Vasa, Breitschopf, Zeiher, & Dimmeler, 2000); thus, the conferred protective role may be due to its ability to lower oxidative stress and reduce inflammation (Stice, Lee, Pechenino, & Knowlton, 2009; Xing, Nozell, Chen, Hage, & Oparil, 2009).

This study found that one-unit increases in allostatic load were associated with 17.3% decreases in TL. However, the findings did not remain statistically significant after controlling for covariates. Regardless, there is biological plausibility linking allostatic load and comorbidities with TL. Further analyses stratified by allostatic load levels showed a greater decline in TL among those with higher allostatic load. The allostatic load model posits that repeated or inadequate physiological adaption to social and environmental stress over time results in dysregulation of cortisol (via dysfunction of the hypothalamic-pituitary-adrenal axis) and catecholamines (via the sympathetic nervous system), which may in turn result in dysfunction of the body's cardiovascular, immune, and metabolic systems (McEwen & Seeman, 1999).

Therefore, it is likely that TL may serve as an important cellular-based indicator of systemic allostatic load. Additionally, since TL is strongly correlated with chronological age, the latter had a powerful confounding effect on most of our estimates which lost statistical significance when adjusted for age.

The associations between TL and various morbidities such as diabetes (Sampson, et al., 2006), heart disease (Brouilette, et al., 2007; Haycock, et al., 2014; van der Harst, et al., 2007; Weischer, et al., 2012), hypertension (Yang, et al., 2009) cancer (Zhu, et al., 2016), and depression (Ridout, et al., 2016), as well as overall mortality (Needham, et al., 2015; Rode, et al., 2015) have been established. In this study, one-unit increases in comorbidities, as measured by the CCI, were associated with 7.2% decreases in TL, although findings lost significance after adjusting for age. Further, analyses stratified by comorbidity levels showed a greater decline in TL among those with four or more comorbidities. It has been suggested that telomere shortening might contribute to various morbidities through pathways involving cellular senescence, chronic inflammation and endothelial dysfunction (Minamino et al., 2002; Rufer et al., 1999).

Many of the covariates we selected were associated with TL. TL in our study was positively correlated with HDL cholesterol and inversely correlated with BMI and heart rate (Rehkopf, et al., 2016). The existing literature on race/ethnicity and TL is inconsistent (Needham, et al., 2013; Rewak et al., 2014). In our study, NH Black had higher allostatic load compared to NH White (OR=1.32, 95% CI= 1.13-1.53), after adjustment for age, gender, and PIR. Despite the higher allostatic load, in overall and stratified analysis by allostatic load status, NH Black had higher TL than NH White. Although unexpected given that African-Americans experience greater stress in various life domains, sociocultural factors, such as social support and religion/spirituality, may enhance resilience when dealing with psychological distress for this group (McCreary,

Cunningham, Ingram, & Fife, 2006). These factors may nurture coping efficacy, which fosters an ability to manage adversity (McCreary, et al., 2006). More research is needed to understand relationships between high effort coping styles and TL for racial and ethnic populations in the US. Another surprising finding in our study was being unmarried was associated with longer TL than being married. This finding conflicts with other studies that showed being married is associated with longer TL (Mainous et al., 2011; Yen & Lung, 2013). In general, being unmarried is associated with poor health outcomes, presence of systemic inflammation (Engstrom, Hedblad, Rosvall, Janzon, & Lindgarde, 2006; Sbarra, 2009) increased mortality risk, and a shorter lifespan (Engstrom, et al., 2006; Molloy, Stamatakis, Randall, & Hamer, 2009; Sbarra, 2009; Scafato et al., 2008). Although the protective role of marriage is not completely understood, it has been hypothesized that the social, emotional, and financial support provided by a spouse/partner acts as a buffer to life stressors (Michael, Berkman, Colditz, Holmes, & Kawachi, 2002). Even among laboratory animals, social isolation was related to increased oxidative stress (Zhuravliova et al., 2009), which is related to telomere attrition. In our stratified analyses, we found that age and gender played a role in the association between marital status and TL. Thus, different mediators and moderators may explain the inconsistency observed in the relationship between marital status and TL attrition, which should be explored in future research.

Strengths, limitations and implications

The strengths of the study include a large, nationally representative sample, rigorous methodology and the comprehensive quality control procedures of NHANES. During data analyses, adjustments were made for sampling weight and design to reduce errors in estimation. Given the cross-sectional design of NHANES, no causal inferences should be made from this study. Limitations were also observed for the quantification of allostatic load and CCI. Currently

no gold standard measure exists to quantify allostatic load, and while our approach was similar to others (Chen, et al., 2014; Parente, et al., 2013), the extent to which the measures of allostatic load actually reflect the complex concept of "wear and tear" is uncertain, which may impact the accuracy of the measurement, and consequently, the quality of the evidence generated. Of the 17 comorbidities used in the original CCI, we were unable to include the measures of hemiplegia and metastatic cancer due to unavailability of data on these conditions in NHANES 1999-2002. Some of the disease statuses used in calculating CCI were self-reported. Lastly, the possibility of residual confounding due to unmeasured covariates cannot be ruled out.

If the findings from this current study could be replicated with longitudinal data, there may be several important implications. First, our findings imply that interventions aimed at preserving TL should be targeted at younger ages, not just at old age. The heterogeneity in decline in TL and absence of a linear pattern in the oldest age category (≥80 years) may also partially explain the lack of statistical association between TL and survival, as seen in some studies conducted among the elderly. For women, a trough in decline in TL with age was noted in early and midlife years. Although 20-29 and 50-59 years are common ages for first childbirth and menopause, respectively, the underlying cause of the decline in these age categories is still not clear and should be addressed by future research.

Conclusions

We found shorter TL with increasing age; this decline was modified by gender, chronic stress and comorbidities. Females in their twenties and fifties and those with chronic morbidities and/or chronic stress experienced greater TL decline. Female reproductive factors, i.e., parity and menopause, were associated with TL. Given the cross-sectional design of our study, future research should attempt to replicate our findings, specifically those related to parity and

menopause, in a longitudinal design. Females in their twenties and fifties are potential subgroups of interest for any interventions or programs aimed at preserving TL.

Chapter 3. Manuscript 2

"Can Healthy Eating Preserve Telomere Length? Association of Telomere Length with Nutrients and Healthy Eating Index"

Abstract

Background

Preservation of telomere length (TL), a biomarker of aging, is hypothesized as one pathway by which proper nutrition can delay or prevent the development of chronic disease. Recent evidence that nutrition may preserve TL is promising. Here we examined the potential of healthy eating patterns or individual macro- and micro-nutrients as potential factors related to the preservation or attenuation of TL in a large, nationally representative US sample.

Methods

Cross-sectional data from 6645 non-pregnant individuals, aged ≥ 20 years with a TL measurement available, were included from the National Health and Nutrition Examination Survey, years 1999-2002. Multivariate linear regression models using energy-adjusted nutrients were used to quantify the associations between TL and individual macro- and micronutrients, and a Healthy Eating Index (HEI).

Results

Of the macronutrients examined, total fat (β= -83.24, 95% CI: -153.31, -13.17), mono-unsaturated (β= -107.40, 95% CI: -175.39, -39.40) and poly-unsaturated fatty acids (β=-54.01, 95% CI: -99.81, -8.19) were inversely associated with TL. Of the micronutrients examined,

potassium was the only element (β= 90.17, 95% CI: 7.54, 172.79) and riboflavin, or Vitamin B2, (β= 85.04, 95% CI: 1.53, 168.54) was the only vitamin that was significantly associated with TL in fully adjusted models, although Vitamins A, B12, and E were inversely associated with TL in energy-adjusted models. After adjustment for total calorie, demographic factors, and health-related behaviors, the overall HEI score was positively associated with TL (β= 3.42, 95% CI: 0.29, 6.53, p-value=0.033); every unit increase in HEI score increased the TL by 3.42 base pairs.

Conclusions

Our findings suggest that certain nutrients and healthy eating in general, easily achievable through adequate and healthy diets, are associated with preservation of TL.

Keywords

Telomere Length, NHANES, Nutrients, Healthy Eating

Introduction

Telomere length (TL) attrition has been proposed as a primary hallmark of biological aging (Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013) (Butler, et al., 2004; Guralnik, 2008; von Zglinicki & Martin-Ruiz, 2005). Telomeres, nucleoprotein structures located at the ends of eukaryotic chromosomes, protect the ends of the chromosome from degradation and end-to-end fusion (Blackburn, et al., 2006); therefore, they are also known as the chromosome's protective cap. With each somatic cell division, there is a gradual attrition of the telomere, resulting in TL shortening with increasing age (Slagboom, et al., 1994). Hence, longer TL is an indicator of healthy aging (Cherkas, et al., 2008). Furthermore, a shorter TL is associated with disease (Haycock, et al., 2014; Sampson, et al., 2006) and death (Needham, et al., 2015). Independent of chronological age, there is substantial variation in the rate of telomere shortening (Ghimire, Hill, Sy, & Rodriguez, 2019), which suggests that telomere attrition may be a modifiable factor (Aviv, 2006). Indeed, many population-based studies have provided evidence supporting the link between various lifestyle and behavioral risk factors and TL, suggesting that TL can be preserved (Aviv et al., 2009; Shammas, 2011).

Eating a suboptimal diet is related to many health problems such as obesity, diabetes, cardiovascular diseases (CVD), and diet-related cancers (Danaei et al., 2009; Murray et al., 2013). In the US, 650,000 deaths per year and 14% of all disability-adjusted life-years are attributed to dietary factors (Murray, et al., 2013). Preservation of TL may be one of the several pathways by which good nutrition can delay or prevent the development of chronic disease. Studies have provided evidence of preservation of TL with good nutrition (Freitas-Simoes, Ros, & Sala-Vila, 2016). Dietary antioxidants (Sen et al., 2014), Mediterranean diet (Boccardi et al., 2013; Marin et al., 2012), Omega-3 fatty acids (Farzaneh-Far, Lin, Epel, Harris, et al., 2010),

seeds or derived products such as legumes and nuts (Lee, Jun, Yoon, Shin, & Baik, 2015) may help to preserve TL. In contrast, total and saturated fat intake (Cassidy et al., 2010; Tiainen et al., 2012) and consumption of refined flour cereals (Cassidy, et al., 2010), meat and meat products (Nettleton, Diez-Roux, Jenny, Fitzpatrick, & Jacobs, 2008), and sugar-sweetened beverages (Leung et al., 2014) may shorten TL. A recent review revealed that evidence on the relationships between TL and fruits, vegetables, alcohol and dairy products is less robust (Freitas-Simoes, et al., 2016). Despite some evidence, the research on nutrition and TL is still in its infancy. Given that improved nutrition is an achievable and relatively inexpensive public health intervention compared to intensive medical interventions, it is important to confirm the counteracting role of improved nutrition in telomere epidemiology and the biological aging process. Prior investigations have focused on the link between TL and a limited number of nutrient components, but a comprehensive assessment of a wide range of micronutrients and macronutrients, including both total intake and intake within the context of dietary recommendations, has not yet been undertaken.

The primary aim of our study is to examine the cross-sectional associations between micronutrients and macronutrients that may attenuate or preserve TL attrition in a large, US nationally representative sample. Additionally, we also examined the association of TL with each of the nutrients' recommended daily intake and with the overall Healthy Eating Index (HEI).

Methods

Study design

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional survey of nationally representative U.S. civilian noninstitutionalized population, conducted using a

complex, multistage, stratified, clustered sampling (Johnson, et al., 2013b). The details of NHANES data have been reported elsewhere (Johnson, et al., 2013b). We used data from the cycles 1999–2000 and 2001-2002 and combined them following the National Center for Health Statistics (NCHS) recommendations (Johnson, et al., 2013b). NHANES 1999-2001 oversamples Mexican Americans, non-Hispanic blacks, pregnant females, and adolescents 12-19 years of age, and adults > 60 years.

Study participants

NHANES 1999-2000 and 2001-2002 included a total of 9,282 and 10,477 participants, respectively, in the mobile examination. Of these, 18,613 participated in the dietary interview but only 7,504, aged 20 and above, were eligible to provide DNA. Given that pregnant women have different nutritional requirements and survival bias is a potential issue among the extremely old, we excluded pregnant women (n= 446) and older adults 85 years and older (n= 199) and those with extreme total energy intake values (<600 or >5000 kcal/d) (n=214). For our analyses, we used data from a total of 6645 individuals, aged 20 to 84 years, from both NHANES 1999–2002 cycles.

Ethical approval

The study procedure of NHANES was approved by the NCHS Research Ethics Review Board. All participants provided written informed consent. Institutional Review Board at University of Nevada Las Vegas determined exempt status for this study.

Data collection

Data collection in NHANES 1999–2002 was accomplished by NCHS-trained professionals through a computer-assisted in-person interview (CAPI), followed by assessments at a mobile

examination center (MEC) for a subset of participants. Data on participants' demographics, self-reported medical conditions, and behavioral factors were collect by CAPI; extensive physical examinations, blood and urine collection for laboratory measures were performed in the MEC (Johnson, Dohrmann, Burt, & Mohadjer, 2014).

Measurements

3.3.5.1 Telomere length

Leucocyte TL was measured from whole blood using the quantitative polymerase chain reaction method, described in detail elsewhere (Cawthon, 2002; Needham, et al., 2013). The mean and standard deviation of the T/S ratio (i.e., TL about standard reference DNA) are provided in the public dataset of NHANES. The lab conducting the DNA assay was blinded to all other measurements in the NHANES. Before releasing the public data of TL, a quality control review was conducted (CDC, 2010). During data analysis, T/S ratio was converted to kilobase-pairs (kbp) using the formula: (3,274 + 2,413 * (T/S))/1000 (Farzaneh-Far, Lin, Epel, Lapham, et al., 2010).

3.3.5.2 Dietary assessment in NHANES

Participant's total nutrient intakes was assessed by trained interviewers via in-person 24-hour dietary recalls (Blanton, Moshfegh, Baer, & Kretsch, 2006; Moshfegh et al., 2008) whereby detailed descriptions of all food and quantities eaten by an individual were recorded; description of the dietary interview methods is provided in the NHANES Dietary Interviewer's Training Manual (Blanton, et al., 2006; CDC, 2014). The details of all the macro- and micro-nutrients assessed in this study, with their measurement unit, are listed in Appendix E. Briefly, seven macronutrients (carbohydrate, protein, dietary fiber, total fat, total saturated fatty acids, total

mono- and poly-unsaturated fatty acids), nine elements (calcium, copper, iron, magnesium, phosphorus, selenium, zinc, potassium, sodium) and ten vitamins (Vitamins A, B1, B2, B3, B6, B12, C, E, K and folate) were assessed. Furthermore, to calculate nutrient intake within the context of dietary recommendations, the proportion of participants meeting the recommended age- and sex-specific daily intake were calculated, using the 2005 Dietary Guidelines for Americans and Dietary Reference Intakes recommendations (Appendix E).

3.3.5.3 Healthy Eating Index

The HEI is a diet quality index that measures conformance to federal Dietary Guidelines for Americans (Guenther et al., 2013). Used to monitor the quality of American diets, it is the basis of nutrition policy for the US government and the foundation of all federal nutrition guidelines. The components of the HEI were calculated using NHANES dietary data and dietary weights from day 1, as described elsewhere (Basiotis, et al., 2002). Briefly, the HEI score is the sum of 10 components (listed in Appendix F), each representing different aspects of a healthful diet. Components 1-5 measure the degree to which a person's diet conforms to serving size recommendations for the five major food groups of the Food Guide Pyramid: grains, vegetables, fruits, milk, and meat. Components 6 and 7 measure total fat and saturated fat consumption, respectively, as a percentage of total food energy (calorie) intake. Components 8 and 9 measure total cholesterol intake and total sodium, and component 10 examines variety in a person's diet. Each of these 10 components have a score ranging from 0-10; thus the HEI index ranges from 0-100. High component scores indicate intakes close to the recommendation; low component scores indicate less compliance with recommended ranges or amounts. The mean HEI score for the US population was 63.8 in 1999-2000 (Basiotis, et al., 2002) and 53.5 in 2007–2008 based on NHANES data (Guenther, et al., 2013).

3.3.5.4 Other covariates

Covariates for this study, selected based on findings from previous literature, were age (Mirabello et al., 2009), gender (Gardner, et al., 2014), race/ethnicity (Diez Roux et al., 2009; Hamad, Tuljapurkar, & Rehkopf, 2016; Hunt et al., 2008; Rewak, et al., 2014), educational attainment (Needham, et al., 2013), socioeconomic status (SES) (Cherkas, et al., 2008; Robertson et al., 2013), smoking (Cherkas, et al., 2008; Needham, et al., 2013), alcohol consumption (Pavanello et al., 2011), physical activity levels (Ludlow et al., 2008; Savela et al., 2013), sedentary lifestyle (Cherkas, et al., 2008; Du et al., 2012), and body mass index (BMI) (Kim et al., 2009; Valdes et al., 2005).

Age, sex, race/ethnicity (Hispanic, non-Hispanic [NH] white, NH Black, and others including multi-racial); educational level (<12th grade, high school graduate/some college and college graduate or above), and marital status (married/living with partner, divorced/widowed/separated, and never married) were self-reported by the participants. SES was measured in terms of poverty income ratio (PIR), the ratio of family income to the poverty threshold, calculated following the U.S. Department of Health and Human Services' poverty guidelines (HHS, 2013) and described in detail elsewhere (Johnson et al., 2013a). In short, PIR was calculated by dividing family income by the poverty guidelines specific to family size, as well as the appropriate year and state (Johnson, et al., 2013a). BMI, the ratio of weight/height^2 measured in kg/m^2, was available as a continuous measure.

We categorized smoking into every day, some days, not at all. Alcohol intake was assessed by lifetime and current alcohol use and classified into three categories: abstainers, occasional/moderate drinkers, and heavy drinkers, based on the Dietary Guidelines for Americans, 2005 (HHS, 2005) that recommend a sex-specific cut point of one drink or fewer per

day for women and two drinks or fewer per day for men. Respondents who consumed alcohol more than the recommended levels were considered heavy drinkers; otherwise, they were classified as occasional/moderate drinkers. Those reporting no alcohol consumption ever were considered abstainers. Physical activity was categorized into inactive (no physical activity), moderate (self-reported participation, over the past 30 days, in moderate activities such as brisk walking, bicycling for pleasure, golf and dancing that cause only light sweating or a slight to moderate increase in breathing or heart rate) and vigorous (self-reported participation, over the past 30 days, in vigorous activities such as brisk walking, bicycling for pleasure, golf and dancing that caused heavy sweating or large increases in breathing or heart rate). Participants were also classified in terms of their health outcomes. Participants were considered morbid if they had at least one of the following chronic conditions: diabetes, CVD, cancer, kidney disease, or liver disease (Detailed definitions provided in Appendix G).

Statistical analyses

Sample weights, the four-year dietary weight, were used to adjust data according to NHANES guidelines to generate a nationally representative sample (Johnson, et al., 2013b). Percentages with 95% CIs and unadjusted means with standard error (SEM) are provided for nominal and continuous variables, respectively (Table 3-1). Differences in covariate characteristics between the quartiles of TL were compared using Rao-Scott Chi-Square test and analysis of variance (ANOVA). The normality of continuous variables was assessed; non-normal variables were subsequently log–transformed. The associations between TL and different nutrients and HEI were quantified in multivariable linear regression models. For all regression analyses, TL was expressed in the unit of base pairs. Intake of all dietary components was energy-adjusted using the residual method whereby energy-adjusted nutrient intakes are computed as the residuals from

a regression model with total energy intake as the independent variable and absolute nutrient intake as the dependent variable; this adjustment minimizes measurement error in dietary estimates (Willett, 2013). Model 1 provides the energy-adjusted coefficient. Model 2 is adjusted for demographic variables (i.e., age, gender, race/ethnicity, marital status, education, socio-economic status, BMI, smoking, alcohol use, and physical activity), shown to be predictors of TL in this study population by a previous study (Needham, et al., 2013). Since TL is strongly correlated with chronological age, the latter had a potential for nonlinearity and a powerful confounding effect on the estimates in previous studies (Ghimire, et al., 2019; Rehkopf et al., 2013). Thus, we used age both as a continuous linear and as an age-squared measure. Additionally, in subgroup analyses, we examined the effect measure modification of our observed estimates by age groups (20–44, 45-64, and 65–85) and by gender.

We also performed a sensitivity analysis, restricting the population to individuals who did not use a multi-vitamin supplement and had chronic disease conditions. In the sensitivity analysis, we replaced the continuous level of nutrient intake with meeting the daily recommended threshold measures (binary, yes/no). A 2-tailed p-value less than 0.05 was considered statistically significant. The survey procedures in SAS 9.4 (SAS Institute Inc., Cary, NC) was used for data analysis.

Results

Characteristics of participants

Table 3-1 shows the demographic characteristics of the study participants by quartiles of TL. The mean (±SEM) TL of the participants was 5.8±0.04 kbp (Table 3-1). In bivariate analyses, participants with shorter TL, i.e., in lower quartiles, were older, male, NH White, with less than

high school education, married, and had higher BMI and at least one morbid condition (Table 3-1).

The mean intakes of total energy, various macro- and micro-nutrients are presented in Appendix H. Although the mean intakes of energy, various macro- nutrients, elements and vitamins, except Vitamin A, E, and K, significantly differed between the quartiles of TL, no clear trend was noted (Appendix H).

Table 3-1. Socio-demographic characteristics of the study participants by quartiles of telomere length- NHANES 1999-2002

	Overall (N= 6645)		Quartiles 1 (N=1917)	Quartiles 2 (N=1703)	Quartiles 3 (N=1625)	Quartiles 4 (N=1400)	p-Value
	N	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	
Age, Mean ± SEM	6645	46.4 ± 0.3	55.6 ± 0.7	48.1 ± 0.8	43.1 ± 0.7	38.5 ± 0.8	<0.001 [a]
Telomere length (Kbp), Mean ± SEM	6645	5.8 ± 0.0	5.1 ± 0.0	5.6 ± 0.0	5.9 ± 0.0	6.7 ± 0.0	<0.001 [a]
Gender							
Male	3393	49.5 (48.1-50.9)	51.4 (48.0-54.9)	48.9 (45.9-51.8)	47.8 (45.1-50.5)	49.9 (47.3-52.5)	0.3111
Female	3252	50.5 (49.1-51.9)	48.6 (45.1-52.0)	51.1 (48.2-54.1)	52.2 (49.5-54.9)	50.1 (47.5-52.7)	
Race/Ethnicity							
Hispanic	1954	13.0 (9.4-16.6)	11.1 (6.1-16.2)	12.8 (8.9-16.7)	14.0 (9.5-18.4)	14.3 (10.1-18.5)	0.0185
NH White	3378	74.1 (70.6-77.5)	78.1 (73.4-82.9)	75.9 (71.9-79.9)	72.0 (67.1-77.0)	70.2 (65.1-75.2)	
NH Black	1128	9.0 (6.9-11.1)	7.0 (5.0-8.9)	8.3 (5.8-10.7)	8.8 (6.8-10.9)	12.0 (8.4-15.5)	
Other	185	3.9 (2.7-5.1)	3.8 (1.7-5.9)	3.0 (1.8-4.2)	5.2 (3.4-7.0)	3.6 (1.5-5.8)	
Educational Status							
<12th Grade	2198	20.4 (18.5-22.3)	25.1 (22.2-28.1)	20.2 (17.6-22.8)	19.7 (16.5-22.9)	16.5 (14.0-19.1)	0.005
High School/Some College	3206	54.6 (51.8-57.4)	53.7 (49.5-57.8)	55.5 (51.4-59.5)	53.4 (49.2-57.6)	55.9 (50.3-61.5)	
College Graduate	1234	25.0 (21.8-28.2)	21.2 (17.0-25.4)	24.4 (20.5-28.2)	26.9 (21.9-32.0)	27.6 (21.9-33.4)	
Marital Status							
Married or Living with Partner	4079	65.3 (63.2-67.4)	70.0 (67.4-72.6)	66.5 (63.1-69.9)	63.0 (59.4-66.6)	61.4 (57.4-65.5)	<0.001
Divorced/Widowed/Separated	1300	18.0 (16.6-19.4)	22.0 (19.7-24.3)	20.5 (18.1-22.9)	16.6 (13.5-19.7)	12.5 (9.8-15.1)	
Never Married	949	16.7 (14.8-18.6)	8.0 (6.3-9.7)	13.0 (10.1-15.9)	20.4 (17.6-23.3)	26.1 (22.7-29.6)	
Socio-economic Status							
Below official poverty threshold	1035	13.5 (11.9-15.2)	12.6 (10.3-14.9)	12.1 (9.6-14.6)	13.8 (11.3-16.4)	15.6 (12.3-18.9)	0.1545
Above official poverty threshold	5030	86.5 (84.8-88.1)	87.4 (85.1-89.7)	87.9 (85.4-90.4)	86.2 (83.6-88.7)	84.4 (81.1-87.7)	
Body Mass Index, Mean ± SEM	6508	28.1 ± 0.2	28.7 ± 0.2	28.3 ± 0.3	27.9 ± 0.3	27.3 ± 0.2	<0.001 [a]
Smoking							
Every day	1231	40.6 (37.6-43.6)	36.9 (32.0-41.8)	40.2 (33.4-47.1)	43.3 (38.2-48.4)	42.8 (37.3-48.2)	<0.001
Some days	264	8.0 (6.4-9.5)	4.6 (2.8-6.4)	5.1 (3.3-6.9)	7.5 (5.2-9.8)	15.7 (11.2-20.2)	
Not at all	1805	51.4 (48.4-54.4)	58.5 (53.9-63.2)	54.7 (47.6-61.8)	49.2 (44.2-54.2)	41.6 (35.3-47.8)	
Alcohol use							
Nondrinker/abstainers	896	14.3 (10.0-18.6)	15.1 (11.7-18.5)	13.3 (9.2-17.5)	15.4 (9.3-21.5)	13.3 (7.8-18.9)	<0.001
Moderate/occasional drinker	2741	47.2 (44.2-50.1)	54.9 (51.2-58.5)	49.9 (46.0-53.8)	42.9 (37.4-48.4)	41.4 (37.2-45.6)	
Heavy drinker	2072	38.5 (35.5-41.6)	30.1 (26.7-33.4)	36.7 (32.2-41.3)	41.7 (37.1-46.3)	45.2 (40.1-50.4)	
Physical Activity							
Physically active	3981	68.4 (66.0-70.8)	61.4 (58.1-64.6)	67.1 (63.5-70.6)	70.9 (67.5-74.4)	74.3 (70.4-78.2)	<0.001
Physically inactive	2662	31.6 (29.2-34.0)	38.6 (35.4-41.9)	32.9 (29.4-36.5)	29.1 (25.6-32.5)	25.7 (21.8-29.6)	

At least one morbid condition	2231	28.8 (26.9-30.7)	41.3 (38.0-44.6)	29.6 (26.4-32.8)	25.6 (21.9-29.3)	18.6 (15.9-21.3)	<0.001
Cardiovascular disease	697	8.5 (7.6-9.5)	15.3 (13.1-17.4)	9.6 (7.2-12.1)	5.7 (4.1-7.2)	3.5 (2.3-4.7)	<0.001
Diabetes	882	9.4 (8.4-10.4)	14.1 (12.5-15.7)	10.5 (8.4-12.5)	7.4 (5.3-9.5)	5.6 (4.3-6.9)	<0.001
Cancer	552	8.3 (7.2-9.4)	13.1 (10.5-15.7)	7.8 (5.9-9.8)	7.2 (5.4-8.9)	5.0 (3.4-6.6)	<0.001
Kidney disease	826	9.7 (8.6-10.8)	12.7 (10.4-14.9)	9.4 (7.7-11.0)	9.0 (6.7-11.2)	7.6 (5.2-10.1)	0.0046
Liver disease	214	3.3 (2.5-4.1)	3.8 (2.7-4.9)	3.9 (2.3-5.5)	2.4 (1.2-3.6)	3.0 (1.8-4.1)	0.2266

[a]: p-values from one-way ANOVA; all others from a Chi-square test. Abbreviation: CI: confidence interval, Kbp: kilo base pairs, NH: Non-Hispanic, SEM: standard error of mean. Values expressed are % (95% CI) unless otherwise stated.

Association between telomere length and nutrients

3.4.2.1 Macronutrients

Among the macronutrients examined (listed in Table 3-2), total fat, saturated and unsaturated fatty acids were inversely associated with TL. In the model adjusted for total energy intake, demographic and health-related behaviors, an increase in one gram of total fat intake was associated with a decrease in 83.24 base pairs of TL (Table 3-2). However, in subgroup analyses by gender (Appendix I), the inverse association between fat intake and TL was significant only for females. Further, statistical significance was lost in sensitivity analyses limited to healthy participants and those not taking any dietary supplements (Appendix J).

3.4.2.2 Micronutrients

We evaluated the relationship between TL and nine different elements and ten different vitamins (listed in Table 3-2). Of the elements, only potassium was significantly associated with TL, in both energy-adjusted and fully-adjusted models. In the model adjusted for total energy intake, demographic and health-related behaviors, an increase in one gram of potassium increased the TL by 90.17 base pairs (Table 3-2). No effect modification by age and sex was observed in subgroup analyses (Appendix I). Moreover, of the vitamins examined, Vitamins A, B2 (riboflavin), B12, and E were inversely associated with TL in energy adjusted models (Table 3-2). However, statistical significance was lost for all vitamins except for riboflavin, in the fully adjusted models (Table 3-2). In sub-group analyses, riboflavin was significantly positively associated with TL among males (Appendix I).

Table 3-2. Multivariable regression for macro- and micro-nutrients associated with telomere length (base pairs) - NHANES 1999-2002

Total nutrients intake	Model 1– energy adjusted			Model 2– energy + demographic + health-related behaviors adjusted		
	β	95% CI	p-value	β	95% CI	p-value
Macronutrients						
Carbohydrate (gm)	103.83	-6.64, 214.29	0.064	**105.99**	**13.42, 198.55**	**0.026**
Protein (gm)	-27.38	-81.53, 26.77	0.310	27.87	-57.56, 113.30	0.510
Dietary fiber (gm)	**-63.96**	**-116.51, -11.40**	**0.019**	-3.17	-61.02, 54.69	0.912
Total fat (gm)	**-144.64**	**-218.03, -71.24**	**0.000**	**-83.24**	**-153.31, -13.17**	**0.022**
Total saturated fatty acids (gm)	**-70.34**	**-116.28, -24.39**	**0.004**	-53.39	-122.58, 15.80	0.125
Total monounsaturated fatty acids (gm)	**-121.21**	**-181.77, -60.65**	**0.000**	**-107.40**	**-175.39, -39.40**	**0.003**
Total polyunsaturated fatty acids (gm)	**-89.41**	**-139.09, -39.73**	**0.001**	**-54.01**	**-99.81, -8.19**	**0.023**
Elements						
Calcium (mg)	-12.63	-55.90, 30.64	0.555	15.50	-56.12, 87.12	0.661
Copper (µg)	-38.48	-98.95, 21.98	0.203	54.09	-29.32, 137.50	0.195
Iron (mg)	-41.38	-115.96, 33.19	0.266	31.23	-55.31, 117.77	0.466
Magnesium (mg)	-73.66	-157.36, 10.05	0.082	59.55	-47.99, 167.09	0.267
Phosphorus (mg)	-55.68	-121.10, 9.73	0.092	13.31	-93.96, 120.58	0.801
Selenium (µg)	-42.11	-98.74, 14.51	0.139	-11.53	-87.83, 64.77	0.760
Zinc (mg)	-30.05	-73.78, 13.68	0.171	49.73	-20.72, 120.17	0.160
Potassium (g)	**-129.00**	**-202.07, -55.92**	**0.001**	**90.17**	**7.54, 172.79**	**0.034**
Sodium (g)	-53.20	-122.14, 15.73	0.125	-18.18	-117.47, 81.12	0.711
Vitamins						
Vitamin A (µg)	**-54.01**	**-100.24, -7.77**	**0.024**	-9.84	-64.95, 45.27	0.718
Thiamin (Vitamin B1) (mg)	-11.76	-58.86, 35.33	0.613	58.35	-12.57, 129.27	0.103
Riboflavin (Vitamin B2) (mg)	**-64.49**	**-126.81, -2.17**	**0.043**	**85.04**	**1.53, 168.54**	**0.046**
Niacin (Vitamin B3) (mg)	-32.09	-90.91, 26.72	0.274	-19.40	-96.78, 57.98	0.612
Vitamin B6 (mg)	-17.57	-71.17, 36.03	0.508	56.29	-14.91, 127.50	0.117
Vitamin B12 (µg)	**-30.34**	**-56.40, -4.27**	**0.024**	17.80	-28.96, 64.55	0.443
Folate (µg)	2.31	-57.45, 62.08	0.938	35.86	-46.22, 117.94	0.379
Vitamin C (mg)	-2.91	-34.83, 29.00	0.853	18.51	-13.83, 50.85	0.251
Vitamin E (mg)	**-78.93**	**-139.29, -18.55**	**0.012**	-44.37	-101.22, 12.47	0.121
Vitamin K (µg)	-20.22	-49.68, 9.24	0.171	19.80	-25.55, 65.15	0.379

Model 1– energy adjusted, Model 2– energy, age, age-squared, sex, race/ethnicity, marital status, education, socio-economic status, BMI, smoking, alcohol use, and physical activity adjusted.

Association between telomere length and recommended daily intakes of nutrients

In an unadjusted model (Table 3-3), compared to participants who met the recommended daily intakes for a given element, participants not meeting the recommended daily intakes of calcium, magnesium, phosphorus, and zinc were more likely to have shorter telomeres. However, the findings were not statistically significant when adjusted for covariates. Likewise, for vitamins, participants who did not meet the daily intake of Vitamin B3 and Vitamin B6 had shorter TL by 83 and 134 base pairs, respectively, compared to those who met the recommendation for each given vitamin. However, statistical significance was again lost in the adjusted model (Table 3-3).

Association between telomere length and Healthy Eating Index

Appendix K shows the association between telomere length and each of the ten components of the HEI. After adjustment for total calorie, demographic, and health-related behaviors, the overall HEI score was positively associated with TL (β= 3.42, 95% CI: 0.29, 6.53, p-value=0.033); every unit increase in HEI score increased TL by 3.42 base pairs.

Table 3-3. Multivariable regression for recommended daily intakes of nutrients associated with telomere length- NHANES 1999-2002

	Model 1–unadjusted			[1]Model 2– demographic + health-related behaviors adjusted		
	β	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
Macronutrients	**Reference = Meeting the recommended daily intakes for respective nutrients**					
Carbohydrate (gm)	-92.91	-209.35, 23.54	0.114	-41.61	-198.93, 115.70	0.593
Protein (gm)	-67.64	-137.56, 2.28	0.057	-10.56	-93.26, 72.14	0.796
Dietary fiber (gm)	-29.5	-165.51, 106.52	0.661	**136.86**	**5.08, 268.65**	**0.042**
Elements						
Calcium (mg)	**-153.07**	**-226.90, -79.24**	**<0.001**	-35.31	-175.66, 105.04	0.611
Copper (µg)	-54.85	-133.83, 24.12	0.166	-64.28	-174.82, 46.27	0.244
Iron (mg)	**152.05**	**60.32, 243.78**	**0.002**	51.39	-67.16, 169.94	0.383
Magnesium (mg)	**-120.71**	**-204.40, -37.01**	**0.006**	24.24	-72.26, 120.74	0.611
Phosphorus (mg)	**-84.86**	**-168.59, -1.14**	**0.047**	-18	-108.41, 72.42	0.687
Selenium (µg)	-71.31	-161.95, 19.34	0.119	25.93	-96.71, 148.57	0.669
Zinc (mg)	**-84.67**	**-142.39, -26.95**	**0.006**	-1.39	-99.38, 96.59	0.977
Potassium (g)	-114.94	-264.99, 35.11	0.128	63.09	-166.91, 293.09	0.579
Sodium (g)	-25.82	-113.15, 61.50	0.550	93.32	-29.25, 215.89	0.130
Vitamins						
Vitamin A (µg)	-77.66	-164.48, 9.16	0.078	25.82	-105.37, 157.01	0.690
Thiamin (Vitamin B1) (mg)	-58.8	-125.70, 8.09	0.083	-26.52	-112.34, 59.31	0.532
Riboflavin (Vitamin B2) (mg)	-38.78	-131.82, 54.26	0.401	-106.08	-212.80, 0.63	0.051
Niacin (Vitamin B3) (mg)	**-82.52**	**-151.04, -14.00**	**0.020**	-21.47	-121.71, 78.78	0.665
Vitamin B6 (mg)	**-134.57**	**-197.92, -71.23**	**<0.001**	-82.06	-167.22, 3.10	0.058
Vitamin B12 (µg)	-28.7	-97.19, 39.80	0.399	31.01	-66.44, 128.45	0.520
Folate (µg)	-58.88	-143.98, 26.23	0.168	-34.74	-163.06, 93.58	0.584
Vitamin C (mg)	-50.67	-117.96, 16.62	0.134	-14.3	-80.18, 51.58	0.660
Vitamin E (mg)	-11.81	-137.09, 113.47	0.848	69.71	-109.00, 248.42	0.431
Vitamin K (µg)	-26.58	-87.47, 34.31	0.379	-10.96	-110.84, 88.92	0.824

[1]Model 1– unadjusted, Model 2– Model 2– energy, age, age-squared, sex, race/ethnicity, marital status, education, socio-economic status, BMI, smoking, alcohol use, and physical activity adjusted.

Discussion

In a large, nationally representative sample of Americans, this study aimed to comprehensively examine the cross-sectional associations between micronutrients and macronutrients that may attenuate or preserve TL attrition. An inverse association between TL and fat intake was found,

as well as a positive association between TL and two micronutrients: the mineral potassium and Vitamin riboflavin. Additionally, the overall HEI score was positively associated with TL.

Among the various macro- and micro-nutrients examined in the current study, fat (total fat and unsaturated fatty acids) was the only macronutrient significantly associated with TL with an inverse relationship. This finding is consistent with previous studies (Cassidy, et al., 2010; Tiainen, et al., 2012), which suggests that reduction in fat intake may increase longevity. Given that the cardioprotective benefits offered by polyunsaturated fatty acids are well-established (Harris et al., 2009), the observed inverse association between unsaturated fatty acids and TL was particularly concerning. Our finding is consistent with a few previous studies (Cassidy, et al., 2010; Kiecolt-Glaser et al., 2013). However, others found positive or non-significant associations between TL and polyunsaturated fatty acids (Farzaneh-Far, Lin, Epel, Harris, et al., 2010; Song et al., 2013). A randomized controlled trial, assessing the impact of n-3 polyunsaturated fatty acid supplementation on leukocyte TL and telomerase, suggests that decreasing n-6:n-3 polyunsaturated fatty acids ratios increases TL (Kiecolt-Glaser, et al., 2013). The relationship between TL and polyunsaturated fatty acids warrants further investigation and clarification. Overall, previous findings regarding the association between TL and different nutrients, including those statistically non-significant in this study, are inconsistent and complex to summarize. Appendix L tabulates the literature on the association between TL and various nutrients.

Among the micronutrients, only potassium and riboflavin were significantly positively associated with TL. (Sen, et al., 2014). Specific nutrients, such as folate (Moores, Fenech, & O'Callaghan, 2011), vitamins B, D, E, and C (Sen, et al., 2014) and zinc (Sharif, Thomas, Zalewski, & Fenech, 2012), provide the necessary building blocks to support telomere health;

thus, they are anticipated to preserve TL. However, previous research has shown mixed results. One cross-sectional study among 586 U.S. women who did not use multivitamin supplements reported a significant direct association of TL with vitamin A, vitamin C, vitamin E, folic acid, calcium, and magnesium but no association with vitamin B6, vitamin B12, vitamin D, selenium, iron, and zinc (Xu et al., 2009). In contrast, a cross-sectional study among 786 Austrian males found a significant direct associations with vitamin C but no association with vitamin A, vitamin E, and total antioxidant status. Other studies show similarly inconsistent results (Appendix L).

Our finding that higher HEI scores were associated with longer TL is important. Previously, some dietary patterns, especially the Mediterranean and calorie-reduced diets, were positively linked to TL (Boccardi, et al., 2013; Freitas-Simoes, et al., 2016; Marin, et al., 2012). Moreover, previous studies using NHANES data also linked Mediterranean Diet scores and Dietary Approaches to Stop Hypertension (DASH) scores with longer telomere length in women (Leung, Fung, McEvoy, Lin, & Epel, 2018); the pro-inflammatory diet was significantly associated with shorter TL (Shivappa, Wirth, Hurley, & Hebert, 2017). It is possible that the synergistic effects of nutrients, usually consumed together, may explain the observed non-significance at individual nutrient level but positive associations with overall HEI score. Higher HEI scores, a snapshot of higher overall diet quality or intakes close to the recommended amounts, have been associated with lower risk of chronic diseases such as type 2 diabetes, CVD, cancer (Chiuve et al., 2012), and all-cause, CVD, and cancer mortality (Reedy et al., 2014). Thus, emphasis should probably be placed not on individual nutrients but rather the importance of overall healthy eating. The mechanism linking higher diet quality and preservation of TL has not yet been delineated, but may be similar to the mechanism by which it reduces the risk of chronic disease and mortality:

by reducing oxidative stress, inflammation, and metabolic dysregulation (Sanders & Newman, 2013; von Zglinicki & Martin-Ruiz, 2005).

This study has some strengths and limitations. The strengths of the study include a large, nationally representative sample, rigorous methodology and the comprehensive quality control procedures of NHANES. During data analyses, adjustments were made for sampling weight and design to reduce errors in estimation. Given the cross-sectional design of NHANES, no causal inferences should be made from this study regarding the relationship between TL, nutrients and HEI. Limitations can also be seen in terms of participants' self-reported dietary data. In general, self-reported dietary data are attenuated compared to their respective standard biomarker (Day, McKeown, Wong, Welch, & Bingham, 2001; Schatzkin et al., 2003). Nevertheless, self-reported 24-hour recall is a convenient, inexpensive and widely-used method for estimating an individual's dietary intake (Schatzkin, et al., 2003). Additionally, the 24-hour recall used in NHANES is a highly standardized version developed by the US Department of Agriculture for use in national dietary surveillance (Moshfegh, et al., 2008). Finally, our data are not recent; they date back to 1999–2002. However, the TL assays were done in 2011, following recent methodological approaches. Since the measure of TL varies with different cell types (Lin et al., 2010), our findings may only be comparable to other studies assessing TL from leucocytes. Given that the majority of the literature on the relationship between nutrition and TL comes from cross-sectional designs, longitudinal studies are needed in the future to make valid causal inferences.

Conclusions

In conclusion, this study suggests that certain micro-nutrients and overall healthy eating in general may be associated with preservation of TL whereas fat consumption may be associated

with attenuation of TL. Given that malnutrition is indisputably a prominent problem among the geriatric population (Lee & Frongillo, 2001; Pillsbury, Miller, Boon, & Pray, 2010) and that optimum nutrition is an easier to achieve and relatively inexpensive public health intervention compared to intensive medical interventions, the importance of healthy eating for overall health benefits, healthy aging, and preserving telomeres should be emphasized. Specifically, improving overall diet quality rather than emphasizing individual nutrients should be encouraged.

Chapter 4. Manuscript 3

"Association between Telomere Length, Survival, and Lifespan among Elderly Men from the Zutphen and Crete Elderly Study"

Abstract

Background

This study assesses the associations of telomere length (TL), a biomarker of aging, with risk factors, survival, and lifespan among the oldest old men from the Zutphen (The Netherlands) and Cretan (Greece) Elderly Study.

Methods

In Zutphen and Crete, 328 elderly men with available TL measurement, aged 79-98 years, were enrolled in 2000; follow-up for vital status was available until September 2015. TL measurements and risk factors were available for 144 men in Zutphen and 122 on Crete and included in the current analyses. Kaplan-Meier survival estimation assessed differences in survival between men with short and long TL. Univariate and multivariable Cox proportional hazard models assessed the associations of TL (standardized continuous measure) on survival. Linear regression models assessed the relation of TL on age at death.

Results

All but nine men were deceased by the end of the study period. TL was not related to socio-demographic factors, lifestyle factors, and prevalent chronic diseases. Kaplan-Meier survival analysis did not show statistically significant differences in all-cause or cardiovascular mortality between long and short TL groups. In Cox proportional hazards models, long TL was not

associated with all-cause (HR: 0.90, 95%CI: 0.68 - 1.17) and cardiovascular mortality (HR: 0.96, 95%CI 0.62 - 1.50). In linear regression models, TL was not a significant predictor of age at death. Findings were also not significant when analyzed separately for the Zutphen and Cretan cohort.

Conclusions

In this homogeneous, healthy and long surviving older adult population, TL was not associated with lifespan or mortality, which is consistent with previous literature suggesting survival effect; the known predictors of mortality do not demonstrate an association among long-surviving individuals.

Keywords

Telomere length, Mortality, Lifespan, Zutphen, Crete, Elderly Men.

Introduction

Telomeres are nucleoprotein structures located at the ends of eukaryotic chromosomes, protecting the end of the chromosome from degradation and end-to-end fusion (Blackburn, et al., 2006), and are thus also known as the chromosome's protective cap. With each somatic cell division, there is a gradual attrition of the telomere, and thus their length shortens with increasing age (Muezzinler, Zaineddin, & Brenner, 2013). When telomeres reach a critical minimal length, the cell may enter apoptosis (Blackburn, 2000; Blasco, 2007). Telomere length (TL) has been proposed as a candidate biomarker of human aging (von Zglinicki & Martin-Ruiz, 2005), whereby longer TL is an indicator of healthy aging (Cherkas, et al., 2008). Telomere shortening has been linked to oxidative stress, inflammation, and metabolic dysregulation (Sanders & Newman, 2013; von Zglinicki & Martin-Ruiz, 2005).

Over a decade has passed since the early reports of an inverse association between TL and mortality (all-cause, heart, and infectious disease mortality) (Cawthon, Smith, O'Brien, Sivatchenko, & Kerber, 2003). Since then, many studies looking at the association between TL and mortality have been conducted, but findings are inconsistent over the lifespan. Most studies have provided evidence that short TL is associated with increased mortality (Astrup et al., 2010; Bakaysa et al., 2007; Cawthon, et al., 2003; Epel et al., 2008; Farzaneh-Far et al., 2008; Fitzpatrick et al., 2007; Kimura et al., 2008; Martin-Ruiz et al., 2006; Needham, et al., 2015), whereas other studies did not show an association between TL and all-cause mortality (Bendix et al., 2014; Bischoff et al., 2006; Fitzpatrick, et al., 2007; Harris et al., 2006; Houben, Giltay, Rius-Ottenheim, Hageman, & Kromhout, 2011; Martin-Ruiz, Gussekloo, van Heemst, von Zglinicki, & Westendorp, 2005; Njajou et al., 2009; Strandberg et al., 2011; Zekry et al., 2012). The link between TL and mortality is an ongoing area of research.

Previously, using data from the Zutphen Elderly Study, it was reported that TL was not predictive of all-cause mortality, cardiovascular mortality, or cancer mortality among elderly men in Zutphen (Houben, et al., 2011). The current study is an update to the previously published study (Houben, et al., 2011). However, the relevance of the current study is multi-fold.

First, the mortality data in the Zutphen Elderly Study (the Netherlands) has been updated through 2015; the former analyses had a follow-up of 7 years from 1993 until 2000. So, it would be worth updating the previous study in old men (Houben, et al., 2011). Second, in the current study, data were included from the Cretan Elderly Study (Greece). Since elderly men from Crete are thought to have, on average, healthier lifestyles than the Northern European Zutphen men due to the Mediterranean diet, physical activity and longer survival, it would be interesting to investigate the differences in survival between the elderly men from two different European countries, in terms of TL (Keys et al., 1980; Kromhout et al., 1989; Menotti et al., 2001). Finally, in addition to linking TL to all-cause mortality, we also assessed TL in relation to age at death or the lifespan. Therefore, this study assessed the association of TL with mortality and lifespan among elderly men from Zutphen and Crete.

Methods

Study population

The Zutphen Elderly Study (the Netherlands) and the Cretan Elderly Study (Greece) are components of the Seven Countries Study (Buijsse et al., 2007), a longitudinal study to investigate dietary and other risk factors for chronic diseases. The details of the Zutphen Elderly Study and the Cretan Elderly Study are provided elsewhere (Buijsse, et al., 2007; Houben, et al., 2011). Briefly, The Zutphen Elderly Study consisted of 887 men, born between 1900 and 1920, in 1985 were followed over time, every five years between 1985 and 2000, as well as in 1993. In

the follow-up examination in 2000, 240 men aged 80–98 were still alive, of whom 176 men were enrolled in the study and 144 men had TL measurements. The Cretan sample in 2000 consisted of 165 men, aged 79–96 years, of whom 152 consented to participate, and 122 men had TL measurements. Thus, information on 144 men from Zutphen cohort and 122 from Cretan cohort were used for the present study. The survey took place in Crete between May and August 2000, and in Zutphen between March and June 2000.

Although TL in the Zutphen Elderly Study was available for 203 men in 1993, of whom 75 men had TL assessed both in 1993 and in 2000, we limited our analysis to the 'baseline' year 2000 because TL was measured for the Cretan sample only in 2000, and not in 1993. Furthermore, a previous study on 7 year mortality has already been published that included the Zutphen participants from 1993 (Houben, et al., 2011). Therefore, we define 2000 as our baseline measurement for the present analyses, for which the relationship with mortality had not yet been analyzed.

Ethics

All participants, in the Zutphen Elderly Study and the Cretan Elderly Study, provided written informed consent. The Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research (TNO) approved The Zutphen Elderly Study, and a local medical ethics committee approved The Cretan Elderly Study. The Institutional Review Board at the University of Nevada Las Vegas approved the current study.

Measurements

4.3.3.1 Blood samples

Non-fasting blood samples were collected from the participants. In Zutphen, the samples were collected in the morning, kept in a box with cooling elements, and in the afternoon plasma and

serum were obtained. EDTA tubes (Becton Dickinson Vacutainer Systems, Plymouth, UK) were used to draw blood and prepare the plasma. In Crete, plasma and serum were obtained after allowing the blood samples to stand for two hours at room temperature. Samples were stored at −30°C in Zutphen and at −80°C in Crete and usually transported within a few days of collection. From both Zutphen and Crete, samples were transported, for analyses, to the National Institute for Public Health and Environment (RIVM), Bilthoven, The Netherlands, where they were stored at −80 °C until analyses. Samples from Crete were transported to the Netherlands on dry ice by plane.

4.3.3.2 Leukocyte telomere length measurement

An aliquot of 200 µl of buffy coat was used to extract genomic DNA with the QIAamp DNA Mini Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol. The DNA was quantified using a Nanodrop instrument (Isogen Life Science, Belgium). Leukocyte TL was determined by quantitative polymerase chain reaction (PCR) as described previously (Cawthon, 2002; Houben et al., 2009). The PCR was performed using a BioRad MyiQ iCycler Single Color real-time PCR detection system using iQ SYBR Green Supermix, containing iTaq Polymerase, dNTPs, SYBRGreen I, and buffers (BioRad). Cycle threshold or *C*t values from the real-time PCR technique were converted into kilobase-pairs by using the standard curve of the reference cell line. By adding reference DNA to each PCR reaction, a standard curve was created, and the absolute TL of the samples was calculated.

4.3.3.3 Mortality

The vital status of the participants was verified approximately every five years during 15 years of follow-up until September 1, 2015. No one was lost to follow-up both in Zutphen and on Crete. The causes of death were coded by an experienced clinical epidemiologist, according to the

World Health Organization's International Classification of Diseases, Ninth Revision (ICD-9 codes) (Slee, 1978). The underlying cause of death was classified as a cardiovascular disease (CVD) if the primary cause of death was ICD-9 Codes 390–459. The clinical epidemiologist was blinded to the outcome of TL measurement.

4.3.3.4 Life span

Life span or the age-at-death was calculated as the difference between birth date and death date. For the 9 (3.4%) participants [Zutphen: 4 (2.8%) and Crete: 5 (4.1%)] who were still surviving until September 1, 2015, we imputed their lifespan.

4.3.3.5 Other variables

Protocolized structured questionnaires were used to collect data on sociodemographic and lifestyle variables (Buijsse, et al., 2007). Covariates for this study, selected based on the literature, were age, marital status, smoking, alcohol consumption, physical activity, body mass index (BMI), self-rated health, and prevalent chronic diseases (CVD, diabetes and cancer) at baseline.

Information was collected on age (in years and dichotomised into <85 and ≥85 years), marital status (binary, unmarried and married), smoking (binary: never, and former/current), and alcohol intake obtained from the cross-check dietary history method (binary, yes/no). Physical activity was assessed with a validated questionnaire designed for retired men to calculate the total minutes per week spent in different physical activities (i.e., walking, cycling, gardening, odd jobs, sports, and hobbies) (Caspersen, Bloemberg, Saris, Merritt, & Kromhout, 1991), which was further categorized into two levels as: less than 150 min/week, and 150 min/week and above. BMI, the ratio of weight/height^2 measured in kg/m^2, was dichotomized into less than 25 kg/m^2, and 25 kg/m^2 and above. Participants were asked to report their general health status in terms of

a 5-point Likert scale (excellent, very good, good, poor, and bad) which was dichotomized into healthy (excellent, very good, good), and unhealthy (poor, and bad). Prevalence of chronic diseases (binary, yes/no) was defined as a diagnosis of one or more of these conditions: CVD (i.e., stroke and myocardial infarction), diabetes mellitus, and cancer. The disease status was determined by a survey questionnaire and verified with hospital discharge data or by information from general practitioners.

Statistical analyses

Descriptive data are present as frequency (percentage), mean (±SD) and (range), as applicable. TL was dichotomized as short (defined as TL<5 kbp) and long (defined as TL ≥ 5 kbp), based on the average TL in our population. Kaplan–Meier curves were used to present crude all-cause and CVD mortality risk with reference to short and long TL. Cox proportional hazards regressions, with TL treated as a binary (short vs. long) variable, was used to compute the Hazard Ratios (HR) with 95% confidence intervals for all-cause and CVD mortality in crude and adjusted models. Two multivariable models were developed: Model 1 adjusted for age, self-rated health, prevalence of chronic diseases and Model 2 adjusted for age, marital status, smoking, alcohol and BMI. Since TL differs by cell type and measurement methods (Blackburn, 2000), which may impair the comparability of findings, we used standardized TL in all linear regressions and Cox proportional hazards models (per 1-standard-deviation increment in TL). Sensitivity analyses examined the hazards models for the men who were free of CVD, diabetes and cancer at baseline. Linear regression was used to evaluate the association between continuous measure of TL and life span. A 2-tailed p-value less than 0.05 was considered statistically significant. Data analyses were performed in SAS 14.1 (SAS Institute Inc., Cary, NC).

Results

Participant's characteristics

The general characteristics of the study participants at baseline is provided in Table 4-1. In the Zutphen cohort, the mean age of the participants was 84 years (range: 79.5-98.2; SD: 3.6), and mean TL was 4.8 kbp. Likewise, in the Cretan cohort, the mean age of the participants was 85 years (range: 79.2-96.1; SD: 4.2), and mean TL was 5.0 kbp. About 33% of the participants from Zutphen and 47% from Crete were 85 years or older. The Cretan cohort was slightly older, had longer telomeres, and had a lower prevalence of chronic diseases (Table 4-1). There was no statistically significant difference in mean TL (adjusted and unadjusted) for sociodemographic factors, lifestyle factors and prevalence of chronic diseases, in both Zutphen and Crete (Table 4-2).

Table 4-1. Baseline sociodemographic, lifestyle factors, and mortality characteristics of participants in Zutphen and Crete

Characteristics	Zutphen (N=144)	Crete (N=122)
Age (years):		
Mean ± SD (range)	84.0 ± 3.6 (79.5-98.2)	85.0 ± 4.2 (79.2-96.1)
<85 years, n (%)	97 (67.4)	65 (53.3)
≥85 years, n (%)	47 (32.6)	57 (46.7)
Telomere length (kbp):		
Mean ± SD (range)	4.8 ± 0.4 (3.7-6.1)	5.0 ± 0.5 (3.7-7.1)
Short telomere (<5 kbp, n (%))	111 (77.1)	64 (52.5)
Long telomere (≥ 5 kbp, n (%))	33 (22.9)	58 (47.5)
Marital status, n (%):		
Unmarried	56 (38.9)	37 (31.4)
Married	88 (61.1)	81 (68.6)
Smoking status, n (%):		
Never	39 (27.1)	37 (30.3)
Former/current	105 (72.9)	85 (69.7)
Alcohol intake, n (%):		
Yes	110 (76.4)	65 (55.1)
No	34 (23.6)	53 (44.9)
Physical activity, n (%):		
< 150 min/week	62 (43.1)	50 (42.4)
≥ 150 min/week	82 (56.9)	68 (57.6)
Body mass index (BMI, kg/m^2):		
Mean ± SD	25.8 ± 3.7	24.5 ± 4.1
≥ 25, n (%)	79 (55.2)	46 (42.2)
< 25, n (%)	64 (44.8)	63 (57.8)
Self-rated health, n (%)		
Healthy	112 (78.3)	55 (47.0)
Not healthy	31 (21.7)	62 (53.0)
Chronic diseases[1], n (%):		
Present	70 (48.6)	29 (23.8)
Absent	74 (51.4)	93 (76.2)
Cardiovascular disease, n (%):		
Present	41 (28.5)	20 (16.4)
Absent	103 (71.5)	102 (83.6)
Diabetes, n (%):		
Present	18 (12.5)	10 (8.2)
Absent	126 (87.5)	112 (91.8)
Cancer, n (%):		
Present	29 (20.1)	6 (4.9)
Absent	115 (79.9)	116 (95.1)

[1]: Chronic disease was defined as having prevalent cardiovascular disease (i.e., myocardial infarction, stroke, and heart failure), cancer, or diabetes mellitus.

Table 4-2. Telomere length according to sociodemographic and lifestyle factors in Zutphen and Crete, at baseline

Characteristics	Zutphen (N=144)				Crete (N=122)			
	Mean (SEM)	P value	*Adjusted Mean (SEM)	P value	Mean (SEM)	P value	*Adjusted Mean (SEM)	P value
Age (years)								
< 85	4.80 (0.04)	0.25	4.75 (0.05)	0.18	5.06 (0.06)	0.09	5.06 (0.09)	0.09
≥ 85	4.73 (0.04)		4.66 (0.06)		4.91 (0.06)		4.86 (0.09)	
Marital status								
Unmarried	4.79 (0.05)	0.67	4.72 (0.06)	0.73	5.04 (0.09)	0.43	5.02 (0.10)	0.33
Married	4.77 (0.04)		4.69 (0.05)		4.95 (0.05)		4.91 (0.07)	
Smoking status								
Never	4.69 (0.06)	0.07	4.64 (0.06)	0.07	5.06 (0.09)	0.29	5.03 (0.10)	0.23
Former/current	4.81 (0.04)		4.77 (0.05)		4.96 (0.05)		4.89 (0.07)	
Alcohol intake								
Yes	4.80 (0.04)	0.13	4.74 (0.05)	0.37	4.99 (0.05)	0.87	4.98 (0.08)	0.65
No	4.70 (0.05)		4.67 (0.07)		4.97 (0.07)		4.94 (0.09)	
Physical activity								
< 150 min/week	4.81 (0.05)	0.36	4.77 (0.05)	0.08	4.90 (0.08)	0.14	4.93 (0.10)	0.62
≥ 150 min/week	4.75 (0.04)		4.64 (0.06)		5.04 (0.05)		4.99 (0.08)	
Body mass index								
≥ 25 kg/m^2	4.78 (0.04)	0.91	4.68 (0.06)	0.54	4.93 (0.06)	0.36	4.91 (0.09)	0.29
< 25 kg/m^2	4.77 (0.05)		4.72 (0.05)		5.02 (0.07)		5.02 (0.08)	
Self-rated health								
Healthy	4.79 (0.04)	0.41	4.74 (0.05)	0.35	5.03 (0.06)	0.25	4.97 (0.10)	0.82
Not healthy	4.73 (0.06)		4.67 (0.07)		4.93 (0.06)		4.95 (0.08)	
Chronic diseases[1]								
Present	4.74 (0.04)	0.25	4.66 (0.05)	0.16	4.89 (0.07)	0.15	4.91 (0.11)	0.40
Absent	4.81 (0.04)		4.75 (0.06)		5.02 (0.05)		5.01 (0.07)	
Cardiovascular disease								
Present	4.75 (0.05)	0.55	4.64 (0.07)	0.42	4.94 (0.09)	0.58	4.83 (0.16)	0.80
Absent	4.79 (0.04)		4.69 (0.07)		5.00 (0.05)		4.86 (0.14)	
Diabetes								
Present	4.75 (0.10)	0.77	4.65 (0.09)	0.70	4.79 (0.12)	0.11	4.75 (0.2)	0.35
Absent	4.78 (0.03)		4.68 (0.05)		5.01 (0.05)		4.94 (0.12)	

Cancer								
Present	4.74 (0.06)	0.55	4.64 (0.08)	0.56	4.80 (0.14)	0.23	4.79 (0.22)	0.66
Absent	4.78 (0.03)		4.69 (0.06)		5.00 (0.05)		4.9 (0.12)	

[1]: Chronic disease was defined as having prevalent cardiovascular disease (i.e., myocardial infarction, stroke, and heart failure), cancer, or diabetes mellitus.

[*]Adjusted for age, marital status, smoking, alcohol intake, physical activity, body mass index, self-rated health, chronic diseases.

Telomere length and mortality

In the 15 years of follow-up, there was a total of 257 deaths (Table 4-3); 140 (97.2%) from the Zutphen cohort and 117 (95.9%) from Crete. The mean lifespan of men from Crete (91.3 years) was slightly higher than men from Zutphen (89.9 years) but they were not statistically significant. Results from the Kaplan-Meier analysis of survival, according to dichotomized TL groups, showed statistically non-significant differences in all-cause and CVD mortality (Figure 4-1) between long and short TL groups in both cohorts. In sub-group analysis by age groups, there were no statistically significant differences in all-cause mortality between older adults less than 85 years) and those 85 years or older in both cohorts (Appendix M). Again, the results from Cox proportional hazards regressions (Table 4-3) suggested that TL, as a standardized continuous measure, was not significantly associated with all-cause and CVD mortality in the combined cohorts. Findings were similarly non-significant when analyzed separately for the Zutphen and Cretan cohort. When analyses were limited to men who were free of prevalence of chronic diseases at baseline, the null findings persisted for Zutphen and Crete when analyzed separately. For the combined cohorts, in the model adjusted for age, marital status, smoking, alcohol and BMI, there were statistically significant difference for CVD mortality, with a lower HR of 0.46 (95% confidence interval [CI]: 0.22-0.98; P=0.04) (Appendix N).

Table 4-3. Hazard ratios of overall and cardiovascular mortality according to telomere length

	Short TL	**Long TL**	***P* value**
Zutphen:			
No. of men	111	33	
Cases of all–cause mortality	109 (77.9%)	31 (22.1%)	
Crude	1.0 (ref.)	0.83 (0.55 - 1.24)	0.35
Model 1	1.0 (ref.)	0.89 (0.59 - 1.34)	0.58
Model 2	1.0 (ref.)	0.64 (0.41 - 1.00)	0.05
Cases of cardiovascular mortality	38 (79.2%)	10 (20.8%)	
Crude	1.0 (ref.)	1.09 (0.53 - 2.21)	0.82
Model 1	1.0 (ref.)	1.00 (0.46 - 2.18)	0.99
Model 2	1.0 (ref.)	0.96 (0.42 - 2.23)	0.93
Crete:			
No. of men	64	58	
Cases of all–cause mortality	61 (52.1%)	56 (47.9%)	
Crude	1.0 (ref.)	0.96 (0.66 - 1.39)	0.84
Model 1	1.0 (ref.)	1.28 (0.87 - 1.89)	0.22
Model 2	1.0 (ref.)	0.97 (0.64 - 1.46)	0.88
Cases of cardiovascular mortality	27 (49.1%)	28 (50.9%)	
Crude	1.0 (ref.)	0.90 (0.52 - 1.57)	0.71
Model 1	1.0 (ref.)	0.86 (0.46 - 1.58)	0.62
Model 2	1.0 (ref.)	0.73 (0.38 - 1.40)	0.34
All participants combined[†]:			
No. of men	175	91	
Cases of all–cause mortality	170 (66.2%)	87 (33.9%)	
Crude	1.0 (ref.)	0.90 (0.68 - 1.17)	0.42
Model 1	1.0 (ref.)	1.03 (0.79 - 1.35)	0.83
Model 2	1.0 (ref.)	0.87 (0.65 - 1.16)	0.34
Cases of cardiovascular mortality	65 (63.1%)	38 (36.9%)	
Crude	1.0 (ref.)	0.96 (0.62 - 1.50)	0.86
Model 1	1.0 (ref.)	0.95 (0.60 - 1.51)	0.83
Model 2	1.0 (ref.)	0.84 (0.51 - 1.37	0.48

[a]Model 1: adjusted for age, self-rated health, prevalence of CVD, diabetes and cancer.
[b]Model 2: adjusted for age, marital status, smoking, alcohol and BMI.
†: additionally adjusted for country.

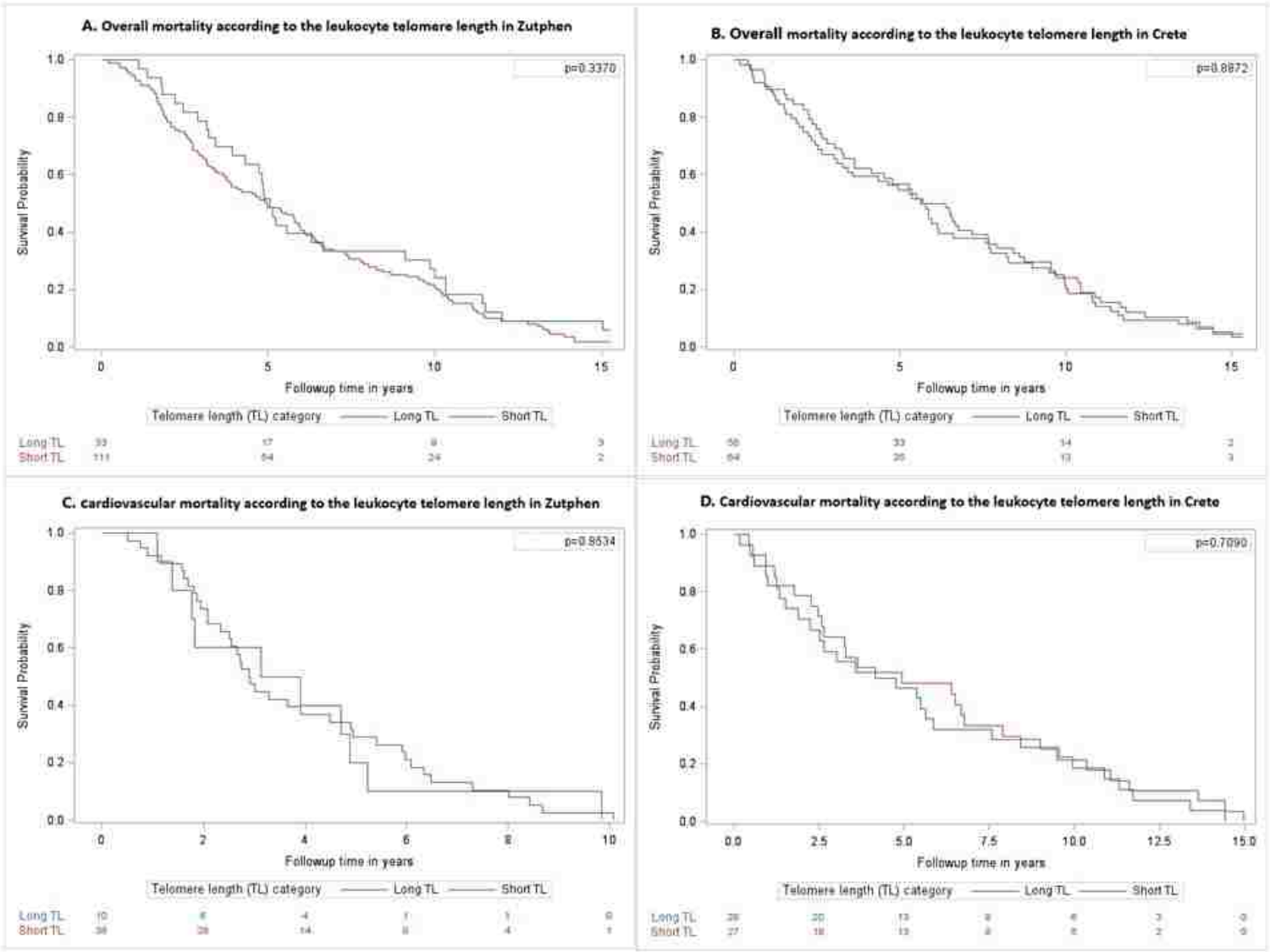

Figure 4-1. Figure 1. Kaplan–Meier survival plot for overall (A, B) and cardiovascular mortality (C, D) according to the leukocyte telomere length in elderly men from (A) Zutphen and (B) Crete.

Pearson's correlation coefficient showed a non-significant relationship between TL and age-at-death in both the Zutphen (r = -0.11, p =0.17) and Crete cohorts (r = 0.01, p =0.89) (Figure 4-2). In the regression analysis, we explored the relationship of TL with risk factors, mortality and lifespan, but did not find statistically significant associations in either cohort (Appendix O).

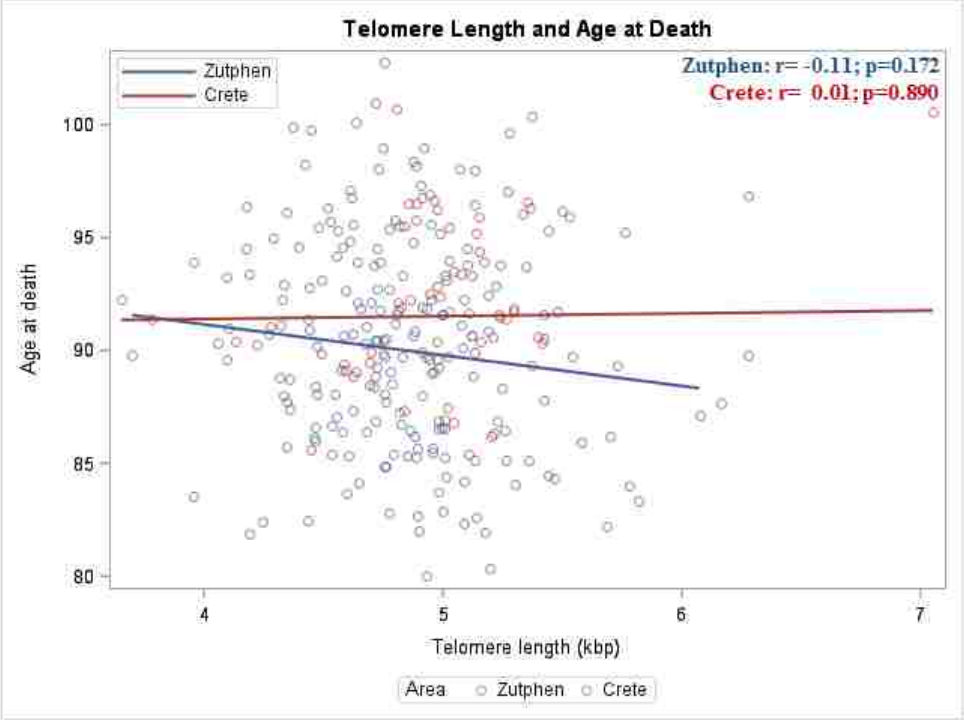

Figure 4-2. Relationship between leukocyte telomere length and age at death in Zutphen and Crete, 2000.

Discussion

Our study showed that TL was not related to demographic factors, lifestyle factors and prevalence of chronic diseases among elderly men in Zutphen and on Crete. TL was also not related to lifespan, all-cause and CVD mortality. The findings may be explained by the survival effect, since our participants were long surviving healthy and homogeneous older adults.

It is hypothesized that lifestyle and environmental stressors upsurge oxidative stress that will increase the telomere attrition rates, resulting into a shortened TL, which would impair survival in the long run (von Zglinicki & Martin-Ruiz, 2005). The alleged associations are quoted repeatedly, but strong evidence to support these hypotheses is difficult to find. So far, the literature on the relationship between TL and mortality has been inconsistent, possibly related to the age at the time of TL assessment. The first published literature on the association between TL and mortality, by Cawthon et al. (Cawthon, et al., 2003), reported a significant increased risk of overall death (HR: 1.86, 95%CI: 1.22-2.83) associated with short TL among individuals aged ≥60 years. Since then studies have provided evidence in support (Bakaysa, et al., 2007; Epel, et al., 2008; Fitzpatrick, et al., 2007; Kimura, et al., 2008) as well as against this observation (Bischoff, et al., 2006; Fitzpatrick, et al., 2007; Harris, et al., 2006; Martin-Ruiz, et al., 2005; Njajou, et al., 2009). In the largest study conducted so far, a Danish study with 64,637 participants and 7,607 deaths during 22 years of follow-up, reported modestly increased hazards for all-cause (HR:1.40, 95%CI: 1.25-1.57), CVD (HR:1.36, 95%CI: 1.12-1.66), and cancer mortality (HR:1.35, 95%CI: 1.11-1.65) among participants in the shortest TL deciles compared to those in the longest deciles (Rode, et al., 2015). Similarly, the most recent meta-analysis, using data from two large prospective cohort studies, on 12,199 participants aged 43–75 years from Germany and the US, corroborated the previous evidence suggesting that TL predicts all-cause mortality beyond its association with age (Mons et al., 2017). Another recent prospective study, with multiple measure of TL, with 10 years follow-up, found no association between change in TL and risk of all-cause mortality, cancer, chronic obstructive lung disease, diabetes mellitus, ischemic cerebrovascular disease, or ischemic heart disease (Weischer, Bojesen, & Nordestgaard, 2014). Thus, our findings supports some of the previously published reports

(Bendix, et al., 2014; Bischoff, et al., 2006; Fitzpatrick, et al., 2007; Harris, et al., 2006; Martin-Ruiz, et al., 2005; Njajou, et al., 2009; Strandberg, et al., 2011; Zekry, et al., 2012) but does not support others (Bakaysa, et al., 2007; Cawthon, et al., 2003; Fitzpatrick, et al., 2007; Kimura, et al., 2008; Mons, et al., 2017; Rode, et al., 2015).

A meta-analysis of previous studies showed that human leucocyte TL predicts mortality, but the mortality association diminished with increasing age (Boonekamp, Simons, Hemerik, & Verhulst, 2013). Consequently in the studies with elderly participants, TL did not significantly predict survival (Bischoff, et al., 2006; Martin-Ruiz, et al., 2005; Strandberg, et al., 2011; Zekry, et al., 2012). In a recent, well-powered, population-based prospective study (MrOS-Sweden), among 2744 older Swedish men with a mean age of 75.5 years, no significant association between TL and all-cause or cause-specific mortality was observed (Svensson et al., 2014). Our study, included men from The Netherlands and Greece, had older participants than the Swedish study, had almost complete mortality follow-up and found similarly non-significant associations.

The findings of these epidemiological studies are consistent with the idea that the variability in TL in a population decreases with increasing age (Bischoff, et al., 2006; Martin-Ruiz, et al., 2005; Sanders & Newman, 2013). In a previous study (Ghimire, et al., 2019), heterogeneity in decline in TL and absence of a linear pattern in the oldest age category (≥80 years) was noted, which partially explains the lack of statistical association between TL and survival, as seen in ours and other studies conducted among the elderly. Another possible explanation for our null findings could be that long TL and low mortality were both causes of continued participation in our study of older men, which may have served as a 'collider' (in a graphical model with inverted forks). Potential bias from the underrepresentation of men with both short TL and a high risk of mortality may have weakened the association between long TL and low mortality (i.e.,

Berkson's paradox). Factors such as frailty, telomerase insufficiency, and oxidative stress which has been implicated in telomere attrition in older people, may explain our non-significant findings. Several biomarkers associated with health outcomes and the known predictors of mortality no longer demonstrate an association in the older elderly (Ben-Ezra & Shmotkin, 2006; Forette, 1999; Nybo et al., 2003). In a large population-based study of 85 year olds, limited evidence was found for the role of immune-senescence in frailty, and no evidence for TL, markers of oxidative stress or DNA damage and repair (Collerton et al., 2012). Furthermore, two recent studies, a study from California (Lapham et al., 2015), and another from Costa Rica (Rehkopf, et al., 2013), provide some evidence of a reversed trend in telomere shortening with increasing age among participants with extreme longevity, defined as above 75 years in California study and 95 years and above in Costa Rica study. The observed reversed trends in the oldest-old suggest selection for survival and the survival bias might explain non-significant associations of TL with mortality and lifespan.

Notably, our participants were males and previous studies suggest a gender difference in survival with reference to TL (Epel, et al., 2008). In previous studies, shorter TL and telomere-associated single nucleotide polymorphisms were associated with CVD mortality only in women (Burnett-Hartman et al., 2012; Epel, et al., 2008). In another study, TL declined gradually throughout life span only in women, but in men, TL declined marginally only (Bischoff et al., 2005). Although the specific reason for gender difference in variability in TL and survival is unknown, sex steroids have been shown to affect telomerase activity and estrogen has been indicated to increasing telomerase activity (Liu & Li, 2010) whereas androgens may either increase or decrease the activity of telomerase (Calado et al., 2009)(Moehren et al., 2008).

Strengths and limitations

The generalizability of our findings may be limited due to homogeneous participants, i.e., long-surviving white elderly males. Thus, findings may not be generalized to younger age groups, females and different ethnic groups. Our sample size in both cohorts was small. However, the study also has strengths, such as high reliability of TL analysis in one laboratory, and a complete mortality follow-up. Furthermore, it is one of the few studies assessing the relationship between TL and life span at the end of life. Future studies should evaluate whether TL differentiates survival among an elderly population, surviving to an advanced age, including women and other racial and ethnic groups.

Conclusions

In conclusion, in our population of elderly men from Zutphen and Crete, TL was not related to lifespan, all-cause and cardiovascular mortality.

Chapter 5. Conclusions

With an overall aim to study the relationships between TL, a biomarker of aging, with nutrition and mortality, this dissertation included three distinct but ancillary studies, following the multiple paper dissertation format. The first and second study used data from NHANES (1999-2002) and the third study used data from the Zutphen and Cretan Elderly Study (2000).

The first study examined cross-sectional rates of age-related TL change and evaluated variability in the rate by gender, chronic stress, and chronic diseases, finding that the population rate of decline in TL with age was significantly greater for certain age groups, for males and for those with high allostatic load and a history of comorbidities. Analyses by gender was interesting and showed a fairly consistent, yet statistically non-significant decline for males; however, a decline in the rate was observed for females in the age categories 20-29 years and 50-59 years. Further, among women, a significant inverse association was found between TL and parity, menopause, and age at menopause. Particularly, the effect modification by gender was useful in elucidating the at-risk populations, i.e., females in their twenties and fifties, who could benefit from targeted interventions aimed at preserving telomeres.

The second study comprehensively examined the role of specific nutrients and a HEI in attenuating or preserving TL and found that dietary intake of potassium, Vitamin B2 (riboflavin), and the overall HEI score were positively associated with TL. Nutrition is an important factor contributing to longevity and healthy aging (Bengmark, 2006; Kiefte-de Jong, Mathers, & Franco, 2014). Although discovery of the "elixir" for long life is ever elusive, the importance of healthy eating for overall health benefits, healthy aging, and preserving telomeres should be emphasized, given that optimum nutrition is an easier to achieve and relatively inexpensive

public health intervention compared to intensive medical interventions. Further, healthy dietary habits are proactive rather than reactive, preventing disease rather than treating it after often irreversible damage has been done. Nonetheless, malnutrition is indisputably a prominent problem among the geriatric population (Lee & Frongillo, 2001; Pillsbury, et al., 2010).

The third study assessed the association of TL with survival and lifespan and did not show statistically significant differences in all-cause or cardiovascular mortality between long and short TL groups. Also, TL was not a significant predictor of age at death for all men combined or analyzed separately for the Zutphen and Cretan cohort. The findings are consistent with the literature, which posits that among the highly selective sample of long-surviving individuals, TL does not determine survival.

Aging is a relatively new area of research. As the proportion of elderly in the population grows globally, the demand for attention for elderly health increases. Current research is focused on promoting a healthy aging experience among "baby boomers" and the burgeoning elderly populations (World Health Organization, 2011). Therefore, in line with current recommendations, the findings in this dissertation align with the health promotion and prevention goals of public health. Healthy aging is not just an effect of fate, but the consequence of a complex interweaving between environmental and genetic factors (Sharpless & DePinho, 2007). Findings from these three studies provide a better understanding of the relationship between TL and health outcomes, nutrition, and mortality. With TL serving as a potential biomarker of aging, understanding telomere dynamics and epidemiology may provide new insights to promoting healthy aging.

Appendix A. Comorbid conditions included in Charlson's Comorbidity Index

SN	Condition	Definition of the condition	Weight
1.	Myocardial infarction	Participant's self-report of ever being diagnosed with a heart attack or myocardial infarction.	1
2.	Congestive heart failure	Participant's self-report of ever being diagnosed with congestive heart failure.	1
3.	Peripheral vascular disease	Ankle Brachial Blood Pressure Index of <0.90 in either leg [1].	1
4.	Cerebrovascular disease	Participant's self-report of ever being diagnosed with stroke or cerebrovascular disease.	1
5.	Dementia	Cognitive functioning was assessed by the Wechsler Adult Intelligence Scale (WAIS), third edition [2]. Dementia was defined as a score of more than 1.5 SD from mean WAIS or use of dementia medication (galantamine, rivastigmine, memantine, donepezil, or tacrine) [3].	1
6.	Pulmonary disease	Participant's self-report of ever being diagnosed with asthma or chronic bronchitis or wheezing in the chest.	1
7.	Connective tissue disorder	Connective tissue disorder was defined as participant's self-report of ever being diagnosed with arthritis, or rheumatoid arthritis or osteoporosis; currently taking prescription antirheumatic drugs or glucocorticoid medications or prednisone or cortisone.	1
8.	Liver disease	Participant's self-report of ever being diagnosed with a liver condition.	1
9.	Diabetes	Diabetes was defined as: a previous diagnosis of diabetes, or current use of diabetic pills or insulin, or a hemoglobin A1c level of 6.5% or greater, or a fasting plasma glucose level of ≥126 mg/dL, or 2-hour plasma glucose level of ≥200 mg/dL [4].	1
10.	Diabetes complications	Diabetes complications were considered if the participant reported retinopathy, unhealed ulcer/sore within four weeks, numbness in hands-feet, pain/tingling in hands feet and pain in either leg while walking due to diabetes.	2
11.	Renal disease	Renal disease was defined by ever diagnosis of weak/failing kidneys or received dialysis in past 12 months, or a glomerular filtration rate (GFR)< 60 [5], or presence of microalbuminuria (urine albumin-to-creatinine ratio of ≥30 mg/g) [6]. GFR was estimated using the equation developed by Modification of Diet in Renal Disease Study Group [5].	2
12.	Cancer	Participant's self-report of ever being diagnosed with cancer or malignancy of any type.	2

13.	Leukemia	Participant's self-report of ever being diagnosed with leukemia.	2
14.	Lymphoma	Participant's self-report of ever being diagnosed with lymphoma.	2
15.	Moderate or severe liver disease	Participant's self-report of ever being diagnosed with a liver condition.	3
16.	HIV	Laboratory confirmed cases with HIV antibody.	6

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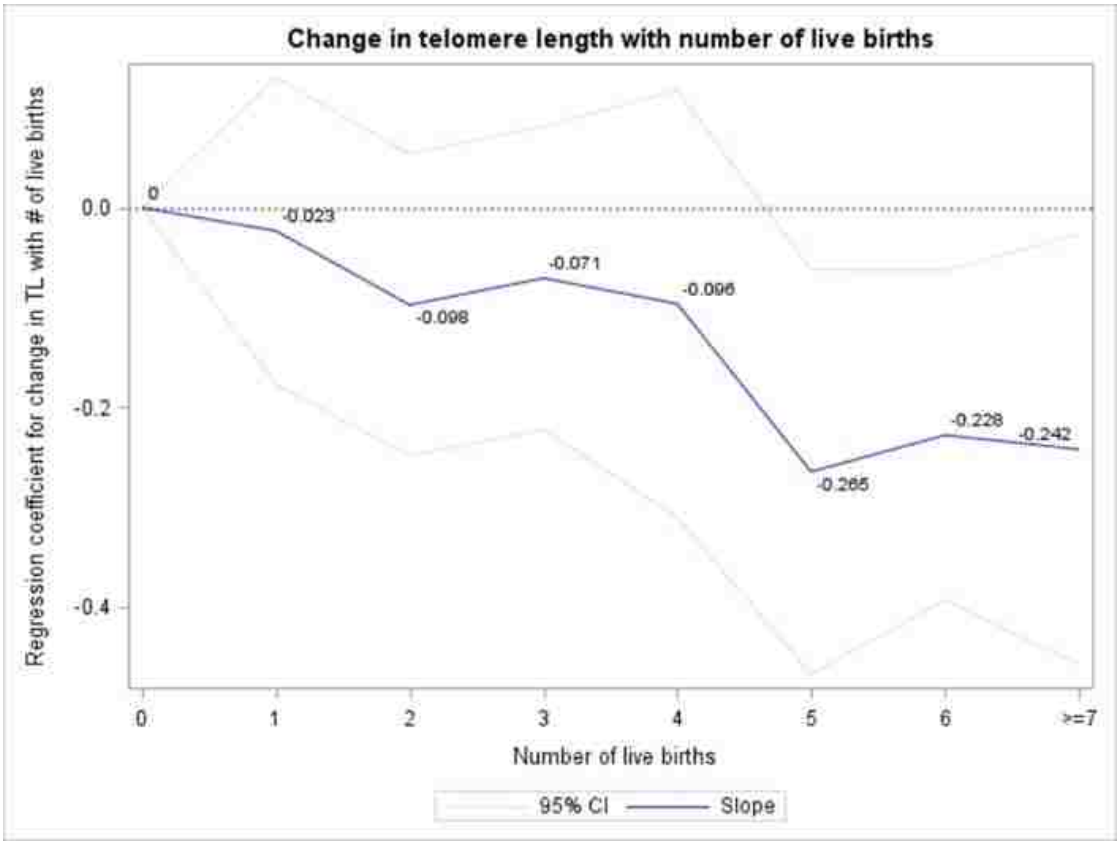
Appendix B. Multivariable regression for factors associated with telomere length- NHANES 1999-2002

	Adjusted Model [a]		
	β	**95%CI**	**p-value**
Age	**-0.0141**	**-0.0157, -0.0126**	**<0.001**
Gender (Reference= Male)			
Female	0.0165	-0.0354, 0.0684	0.5204
Race/Ethnicity (Reference= NH White)			
Hispanic	0.0948	-0.0477, 0.2372	0.184
NH Black	**0.1948**	**0.0987, 0.2909**	**0.0003**
Other	0.0269	-0.1179, 0.1718	0.7065
Educational Status (Reference=High School)			
<12th Grade	**-0.1717**	**-0.2357, -0.1078**	**<0.001**
College Graduate	0.0237	-0.0421, 0.0895	0.4677
Marital Status (Reference= Married/with Partner)			
Divorced/Widowed/Separated	**-0.1283**	**-0.1895, -0.0672**	**0.0002**
Never Married	**0.2860**	**0.2148, 0.3572**	**<0.001**
Family PIR	-0.0224	-0.0495, 0.0047	0.1016
Smoking (Reference=Smokers)			
Non-smokers	0.0433	-0.016, 0.1026	0.146
Alcohol use (Reference= Abstainers)			
Moderate drinker	**-0.0995**	**-0.1942, -0.0048**	**0.0401**
Heavy drinker	0.0385	-0.0819, 0.1589	0.518
Physical Activity (Reference=Physically active)			
Physically inactive	**-0.0985**	**-0.1436, -0.0534**	**<0.001**
Biomarkers of allostatic load			
Systolic blood pressure	-0.0024	-0.0039, -0.0009	**0.0027**
Diastolic blood pressure	0.0037	0.0019, 0.0054	**0.0002**
Heart rate	-0.0004	-0.0024, 0.0016	0.6809
Total cholesterol	-0.0001	-0.0006, 0.0005	0.8304
High-density lipoprotein cholesterol	0.0013	-0.0008, 0.0034	0.2252
BMI (kg/m2)	**-0.0100**	**-0.0138, -0.0062**	**<0.001**
Glycosylated hemoglobin	**-0.0237**	**-0.0443, -0.0032**	**0.0253**
C-reactive protein	-0.0412	-0.0864, 0.004	0.0726
Albumin	0.2563	-0.4608, 0.9735	0.4706
Allostatic load (Reference=Low)			
High	**-0.1487**	**-0.2053, -0.0921**	**<0.001**
Charleston Comorbidity Index	**-0.0649**	**-0.0744, -0.0555**	**<0.001**

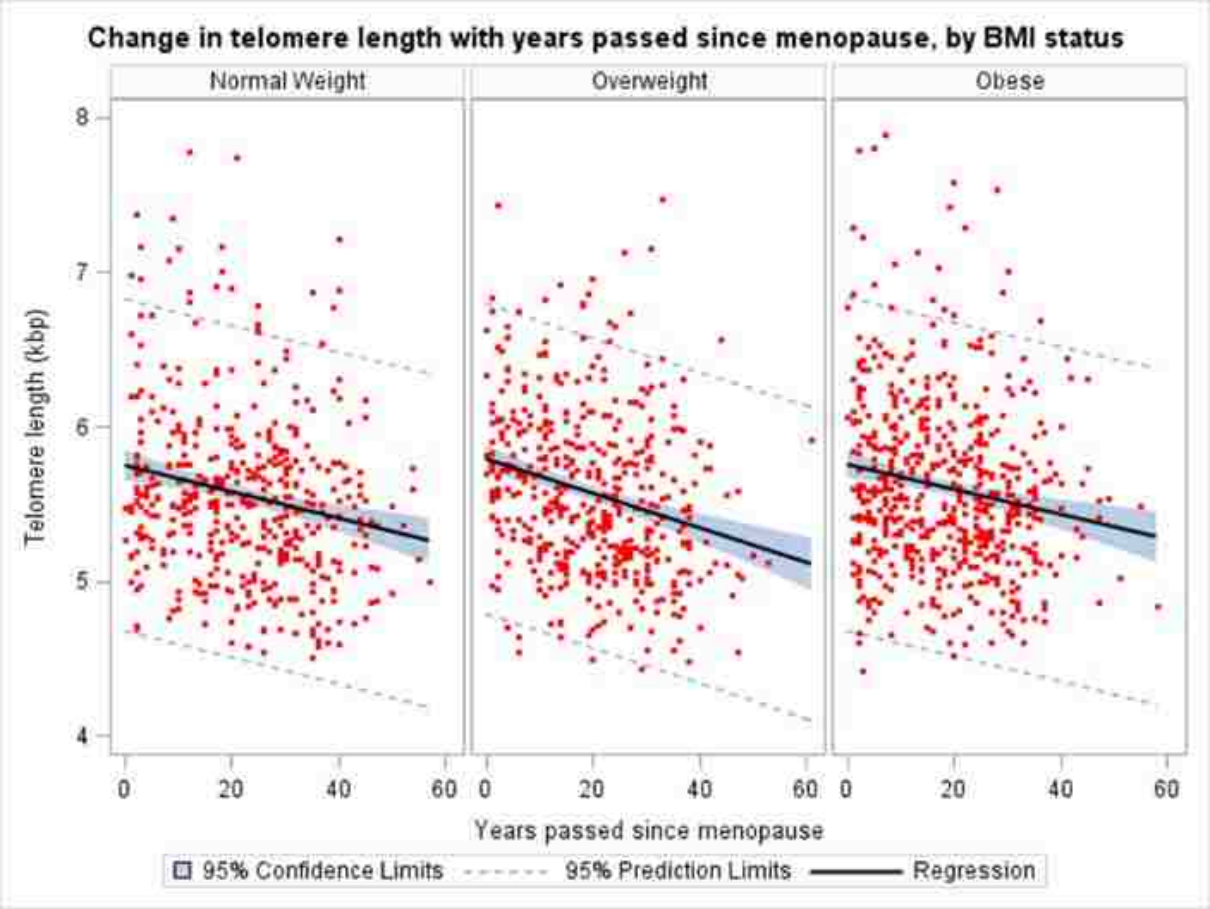
DV: Telomere length in kbp; [a] Adjusted for gender, race/ethnicity, education, PIR, and BMI. Abbreviations: BMI: body mass index, CI: confidence interval, NH: Non-Hispanic, PIR: poverty income ratio. p-value less than 0.05 are bold.

Appendix C. Association between telomere length and parity in NHANES 1999-2002



Appendix C footnote: Y-axis represents the slope of change in telomere length with the number of live births. Regression coefficients adjusted for ethnicity, education, poverty income ratio, and body mass index.

Appendix D. Association between telomere length and years passed since menopause, by BMI status of women in NHANES 1999-2002



Appendix D footnote: The slope for each weight category is: Normal weight (β= -0.0101; p =0.001), Overweight (β= -0.0111; p =0.005) and Obese (β= -0.0076; p= 0.007). Estimates adjusted for ethnicity, education, poverty income ratio, and body mass index.

Appendix E. The nutrients assessed in manuscript 2 and their age and sex specific recommended daily intake

Nutrients (Unit)	Recommended daily intake (Unit/day)							
	Males				Females			
	19–30 y	31–50 y	51–70 y	> 70 y	19–30 y	31–50 y	51–70 y	> 70 y
Macronutrients								
Carbohydrate (gm)	130	130	130	130	130	130	130	130
Protein (gm)	56	56	56	56	46	46	46	46
Dietary fiber (gm)	38	38	30	30	25	25	21	21
Total fat (gm)	Not determined							
Total saturated fatty acids (gm)	Not determined							
Total monounsaturated fatty acids (gm)	Not determined							
Total polyunsaturated fatty acids (gm)	Not determined							
Cholesterol (mg)	Not determined							
Elements								
Calcium (mg)	1,000	1,000	1,000	1,200	1,000	1,000	1,200	1,200
Copper (µg)	900	900	900	900	900	900	900	900
Iron (mg)	8	8	8	8	18	18	8	8
Magnesium (mg)	400	420	420	420	310	320	320	320
Phosphorus (mg)	700	700	700	700	700	700	700	700
Selenium (µg)	55	55	55	55	55	55	55	55
Zinc (mg)	11	11	11	11	8	8	8	8
Potassium (g)	4700	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Sodium (g)	1.5	1.5	1.3	1.2	1.5	1.5	1.3	1.2
Vitamins								
Vitamin A (µg)	900	900	900	900	700	700	700	700
Thiamin (Vitamin B1) (mg)	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1
Riboflavin (Vitamin B2) (mg)	1.3	1.3	1.3	1.3	1.1	1.1	1.1	1.1
Niacin (Vitamin B3) (mg)	16	16	16	16	14	14	14	14
Vitamin B6 (mg)	1.3	1.3	1.7	1.7	1.3	1.3	1.5	1.5
Vitamin B12 (µg)	2.4	2.4	2.4h	2.4h	2.4	2.4	2.4h	2.4h
Folate (µg)	400	400	400	400	400	400	400	400
Vitamin C (mg)	90	90	90	90	75	75	75	75
Vitamin E (mg)	15	15	15	15	15	15	15	15
Vitamin K (µg)	120	120	120	120	90	90	90	90

Appendix F. Healthy Eating Index: components and standards for scoring

SN	Component	Score ranges	Criteria for minimum score of 0	Criteria for maximum score of 10
1.	Grain consumption	0 to 10	0 servings	6 - 11 servings
2.	Vegetable consumption	0 to 10	0 servings	3 - 5 servings
3.	Fruit consumption	0 to 10	0 servings	2 - 4 servings
4.	Milk consumption	0 to 10	0 servings	2 - 3 servings
5.	Meat consumption	0 to 10	0 servings	2 - 3 servings
6.	Total fat intake	0 to 10	45% or more energy from fat	30% or less energy from fat
7.	Saturated fat intake	0 to 10	15% or more energy from saturated fat	Less than 10% energy from saturated fat
8.	Cholesterol intake	0 to 10	450 mg or more	300 mg or less
9.	Sodium intake	0 to 10	4800 mg or more	2400 mg or less
10.	Variety	0 to 10	3 or fewer different items in a day	8 or more different items in a day
	Total HEI score	0-100		

Appendix G. Definition of chronic conditions

SN	Condition	Definition of the condition
1.	Diabetes	Diabetes was defined as: a previous diagnosis of diabetes, or current use of diabetic pills or insulin, or a hemoglobin A1c level of 6.5% or greater, or a fasting plasma glucose level of ≥126 mg/dL, or 2-hour plasma glucose level of ≥200 mg/dL [1].
2.	Cardioascular disease	Participant's self-report of ever being diagnosed with congestive heart failure, coronary heart disease, angina pectoris, heart attack, and stroke.
3.	Cancer	Participant's self-report of ever being diagnosed with cancer or malignancy of any type.
4.	Kidney disease	Renal disease was defined by ever diagnosis of weak/failing kidneys or received dialysis in past 12 months, or a glomerular filtration rate (GFR)< 60 [2], or presence of microalbuminuria (urine albumin-to-creatinine ratio of ≥30 mg/g) [3]. GFR was estimated using the equation developed by Modification of Diet in Renal Disease Study Group [2].
5.	Liver disease	Participant's self-report of ever being diagnosed with a liver condition.

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Appendix H. Nutrients intake of the study participants by quartiles of leukocyte telomere length- NHANES 1999-2002

	Overall (N= 6645)		Quartiles, Mean ± SEM				
Total nutrients intake	**N**	**Mean ± SEM**	**One (N=1917)**	**Two (N=1703)**	**Three (N=1625)**	**Four (N=1400)**	**p-value**
Energy (kcal)	6645	2172.2 ±18.2	2038.6 ±29.0	2155.6 ±32.3	2208.0 ±33.7	2288.2 ±33.7	<0.001
Macronutrients							
Carbohydrate (gm)	6645	271.1 ± 3.0	253.1 ± 3.4	267.7 ± 4.8	274.3 ± 4.8	289.6 ± 4.3	<0.001
Protein (gm)	6645	81.3 ± 0.8	76.4 ± 1.3	80.6 ± 1.4	84.1 ± 1.6	84.2 ± 1.5	<0.001
Dietary fiber (gm)	6645	15.9 ± 0.3	16.0 ± 0.5	15.7 ± 0.4	15.6 ± 0.4	16.4 ± 0.5	0.0003
Total fat (gm)	6645	81.2 ± 0.8	78.2 ± 1.6	81.9 ± 1.4	81.5 ± 1.7	83.2 ± 2.0	<0.001
Total saturated fatty acids (gm)	6645	26.5 ± 0.3	25.1 ± 0.5	26.6 ± 0.5	26.9 ± 0.7	27.5 ± 0.7	<0.001
Total monounsaturated fatty acids (gm)	6645	30.3 ± 0.3	29.1 ± 0.7	30.5 ± 0.6	30.2 ± 0.6	31.2 ± 0.8	<0.001
Total polyunsaturated fatty acids (gm)	6645	16.8 ± 0.2	16.4 ± 0.4	17.0 ± 0.4	16.7 ± 0.4	16.9 ± 0.5	0.0193
Elements							
Calcium (mg)	6645	852.8 ± 13.0	797.4 ± 20.4	846.2 ± 18.8	878.3 ± 27.6	889.8 ± 21.2	<0.001
Copper (µg)	6645	1329.4 ± 29.1	1274.8 ± 29.0	1341.5 ± 69.5	1321.8 ± 32.1	1380.0 ± 42.4	0.0001
Iron (mg)	6645	15.5 ± 0.3	15.2 ± 0.4	15.2 ± 0.4	15.4 ± 0.4	16.2 ± 0.4	<0.001
Magnesium (mg)	6645	284.4 ± 4.3	276.1 ± 6.1	281.1 ± 5.1	285.6 ± 5.8	295.1 ± 7.3	<0.001
Phosphorus (mg)	6645	1322.1 ± 13.6	1250.2 ± 22.2	1308.2 ± 20.2	1360.3 ± 28.7	1370.3 ± 26.2	<0.001
Selenium (µg)	6645	108.2 ± 1.3	103.1 ± 1.9	108.0 ± 1.9	111.1 ± 2.4	110.7 ± 2.3	<0.001
Zinc (mg)	6645	11.9 ± 0.2	11.4 ± 0.3	11.9 ± 0.3	12.1 ± 0.3	12.2 ± 0.3	<0.001
Potassium (g)	6645	2.8 ± 0.0	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.0	2.8 ± 0.1	0.0029
Sodium (g)	6645	3.4 ± 0.0	3.3 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	<0.001
Vitamins							
Vitamin A (µg)	6645	787.2 ±24.9	791.0 ±31.6	793.5 ±54.9	793.4 ±39.0	770.8 ±47.2	0.7225
Thiamin (Vitamin B1) (mg)	6645	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.1	1.7 ± 0.1	<0.001
Riboflavin (Vitamin B2) (mg)	6645	2.1 ± 0.0	2.0 ± 0.0	2.1 ± 0.0	2.1 ± 0.1	2.1 ± 0.1	<0.001
Niacin (Vitamin B3) (mg)	6645	23.0 ± 0.3	22.0 ± 0.4	22.8 ± 0.5	23.3 ± 0.5	23.9 ± 0.6	<0.001
Vitamin B6 (mg)	6645	1.9 ± 0.0	1.8 ± 0.0	1.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1	<0.001
Vitamin B12 (µg)	6645	5.2 ± 0.2	5.0 ± 0.2	5.2 ± 0.4	5.6 ± 0.4	5.0 ± 0.2	0.0045
Folate (µg)	6645	398.6 ± 8.3	383.3 ±10.5	394.0 ± 9.6	396.0 ±10.7	421.3 ±13.4	<0.001
Vitamin C (mg)	6645	95.7 ± 3.4	92.0 ± 3.5	91.7 ± 3.5	96.6 ± 5.1	102.6 ± 6.5	<0.001
Vitamin E (mg)	6645	8.1 ± 0.1	8.3 ± 0.3	8.1 ± 0.3	8.0 ± 0.2	8.2 ± 0.3	0.1326
Vitamin K (µg)	3594	91.6 ± 4.7	91.4 ± 6.9	88.8 ± 6.7	91.9 ± 3.8	94.3 ±10.5	0.3071

Appendix I. Subgroup analyses: regression of telomere length (base pairs) on different nutrients by age and gender- NHANES 1999-2002

Adjusted models[1]										
Nutrients	**20-44**		**45-64**		**65-85**		**Male**		**Female**	
	ß	**95% CI**	**ß**	**95% CI**	**ß**	**95% CI**	**ß**	**95% CI**	**ß**	**95% CI**
Macronutrients										
Carbohydrate (gm)	74.71	-80.71, 230.13	136.93	-70.24, 344.10	114.03	-62.61, 290.66	67.24	-71.39, 205.87	**172.19**	**19.83, 324.55**
Protein (gm)	20.43	-113.56, 154.42	20.54	-126.68, 167.77	46.93	-157.74, 251.59	-25.58	-184.29, 133.13	76.89	-78.50, 232.29
Dietary fiber (gm)	-12.54	-110.04, 84.97	-1.99	-97.58, 93.60	22.67	-97.88, 143.23	11.86	-36.93, 60.65	-18.93	-126.22, 88.35
Total fat (gm)	-48.79	-158.58, 61.00	-165.94	-339.20, 7.32	-85.41	-313.56, 142.75	-72.32	-168.85, 24.22	**-98.5**	**-192.03, -4.97**
Total saturated fatty acids (gm)	-18.17	-103.32, 66.98	-95.85	-287.49, 95.79	-79.01	-249.68, 91.67	-22.84	-121.30, 75.62	**-84.01**	**-162.90, -5.11**
Total monounsaturated fatty acids (gm)	-63.16	-166.08, 39.75	**-193.3**	**-335.79, -50.80**	**-150.62**	**-346.95, 45.71**	**-100.44**	**-198.23, -2.64**	**-111.5**	**-218.69, -4.31**
Total polyunsaturated fatty acids (gm)	-56.36	-133.75, 21.03	-67.14	-182.27, 47.99	-17.37	-135.55, 100.80	**-74.67**	**-140.68, -8.65**	-41.8	-106.86, 23.26
Elements										
Calcium (mg)	29.76	-54.84, 114.37	47.34	-82.93, 177.60	-71.68	-171.18, 27.82	12.47	-58.96, 83.90	20.84	-102.03, 143.71
Copper (µg)	69.84	-56.91, 196.60	12.74	-103.94, 129.41	117.59	-28.14, 263.32	100.75	-25.33, 226.83	-3.43	-134.81, 127.95
Iron (mg)	43.63	-53.87, 141.13	28.11	-102.13, 158.35	-31.06	-169.68, 107.56	9.57	-86.52, 105.66	52.94	-54.29, 160.16
Magnesium (mg)	54.55	-93.94, 203.05	92.82	-82.17, 267.80	70.4	-101.36, 242.15	90.86	-35.06, 216.78	22.66	-139.20, 184.53
Phosphorus (mg)	-26.93	-212.21, 158.35	123.79	-98.85, 346.42	17.12	-160.53, 194.77	-20.43	-183.07, 142.21	57.73	-100.75, 216.21
Selenium (µg)	-34.99	-138.40, 68.43	-52.73	-186.30, 80.84	124.35	-26.02, 274.72	-36.57	-135.84, 62.70	8.87	-123.07, 140.81
Zinc (mg)	50.53	-53.45, 154.50	65.98	-87.96, 219.92	0.36	-95.55, 96.28	47.45	-35.86, 130.75	55.09	-34.65, 144.83
Potassium (g)	80.09	-42.52, 202.71	99.77	-37.86, 237.40	94.76	-89.64, 279.16	117.71	-5.42, 240.85	50.84	-132.13, 233.81
Sodium (g)	52.86	-108.20, 213.92	-70.28	-186.76, 46.19	-158.32	-374.69, 58.06	28.85	-112.93, 170.63	-92.41	-203.45, 18.62
Vitamins										
Vitamin A (µg)	-29.51	-106.91, 47.88	21.2	-38.96, 81.37	-18.96	-95.38, 57.46	1.7	-49.11, 52.51	-20.01	-96.40, 56.39
Thiamin (Vitamin B1) (mg)	49.27	-53.78, 152.33	101.48	-37.28, 240.24	-32.11	-151.81, 87.58	75.03	-14.16, 164.22	34.55	-69.27, 138.36
Riboflavin (Vitamin B2) (mg)	91.98	-6.95, 190.92	84.29	-69.00, 237.57	34.91	-105.02, 174.84	**119.24**	**19.58, 218.90**	54.77	-49.35, 158.89
Niacin (Vitamin B3) (mg)	4.55	-97.00, 106.10	-54.35	-212.50, 103.81	-63.06	-206.47, 80.35	-98.79	-250.76, 53.19	59.83	-47.09, 166.75
Vitamin B6 (mg)	14.98	-84.58, 114.55	**161.87**	**58.39, 265.35**	-23.71	-134.42, 87.01	3.18	-92.96, 99.32	106.84	-12.75, 226.42
Vitamin B12 (µg)	11.7	-58.79, 82.19	33.71	-52.21, 119.63	-19.49	-65.19, 26.22	13.54	-41.94, 69.02	18.14	-41.58, 77.87
Folate (µg)	31.44	-66.30, 129.19	75.49	-59.30, 210.28	-42.51	-167.92, 82.90	47.65	-59.41, 154.70	25.81	-92.70, 144.32
Vitamin C (mg)	13.11	-41.65, 67.88	30.48	-2.71, 63.68	-14.67	-60.87, 31.53	23.78	-7.81, 55.38	10.53	-52.85, 73.91

Vitamin E (mg)	-55.68	-138.60, 27.24	-48.97	-153.24, 55.29	21.05	-90.33, 132.42	-43.5	-119.79, 32.80	-54.37	-123.09, 14.35
Vitamin K (µg)	17.74	-58.37, 93.85	11.63	-59.11, 82.37	-1.89	-98.96, 95.18	45.7	-18.88, 110.28	-7.74	-77.08, 61.60

[1]Adjusted for energy, age, age-squared, sex, race/ethnicity, marital status, education, socio-economic status, BMI, smoking, alcohol use, and physical activity.

Appendix J. Sensitivity analyses: regression of telomere length (base pairs) on different nutrients among dietary supplement non-users, and participants without chronic diseases, NHANES 1999-2002

Nutrients	Healthy participants		Participants without dietary supplement use	
	ß[1]	95% CI	ß[1]	95% CI
Macronutrients				
Carbohydrate (gm)	63.92	-74.13, 201.97	**146.21**	**40.64, 251.78**
Protein (gm)	11.02	-108.17, 130.20	49.71	-42.45, 141.88
Dietary fiber (gm)	-29.29	-109.26, 50.69	19.97	-45.34, 85.27
Total fat (gm)	-69.05	-152.55, 14.45	-49.09	-181.46, 83.29
Total saturated fatty acids (gm)	-28.37	-114.58, 57.85	-16.01	-132.52, 100.50
Total monounsaturated fatty acids (gm)	**-104.62**	**-184.44, -24.80**	-81.05	-178.97, 16.86
Total polyunsaturated fatty acids (gm)	-55.01	-117.19, 7.18	-65.23	-154.63, 24.16
Elements				
Calcium (mg)	6.91	-64.20, 78.02	37.5	-43.34, 118.33
Copper (µg)	44.45	-51.27, 140.16	57.14	-72.41, 186.70
Iron (mg)	23.6	-89.39, 136.59	66.31	-49.17, 181.80
Magnesium (mg)	65.72	-46.91, 178.36	49.81	-90.25, 189.87
Phosphorus (mg)	-13.93	-125.13, 97.26	-3.65	-141.91, 134.60
Selenium (µg)	-70.86	-196.98, 55.26	12.68	-99.92, 125.28
Zinc (mg)	84.8	-8.43, 178.03	61.39	-27.85, 150.63
Potassium (g)	93.04	-0.92, 187.00	**153.16**	**54.61, 251.71**
Sodium (g)	-43.48	-162.57, 75.62	76.27	-74.96, 227.51
Vitamins				
Vitamin A (µg)	-1.35	-68.31, 65.61	-0.18	-65.97, 65.60
Thiamin (Vitamin B1) (mg)	33.76	-69.03, 136.54	104.06	-10.24, 218.36
Riboflavin (Vitamin B2) (mg)	71.69	-23.71, 167.08	**117.55**	**27.12, 207.98**
Niacin (Vitamin B3) (mg)	-18.87	-123.58, 85.84	0.03	-114.82, 114.88
Vitamin B6 (mg)	77.93	-8.01, 163.86	54.55	-33.12, 142.23
Vitamin B12 (µg)	33.89	-20.58, 88.36	21.65	-28.40, 71.70
Folate (µg)	20.3	-81.51, 122.11	19.92	-75.87, 115.72
Vitamin C (mg)	14.17	-27.47, 55.80	27.12	-17.88, 72.12
Vitamin E (mg)	**-62.54**	**-120.21, -4.87**	-21.31	-117.42, 74.80
Vitamin K (µg)	29.37	-25.70, 84.43	55.04	-17.71, 127.79

[1]Adjusted for energy, age, age-squared, sex, race/ethnicity, marital status, education, socio-economic status, BMI, smoking, alcohol use, and physical activity.

Appendix K. Multivariable regression of telomere length (base pairs) on different components of Healthy Eating Index- NHANES 1999-2002

Healthy Eating Index components	Model 1– energy adjusted			[1]Model 2– energy + demographic + health-related behaviors adjusted		
	β	95% CI	p-value	β	95% CI	p-value
Grain intake score	-2.50	-11.03, 6.03	0.553	-3.67	-15.35, 8.02	0.526
Vegetable intake score	0.18	-5.86, 6.22	0.952	3.04	-5.02, 11.10	0.447
Fruit intake score	-2.93	-11.57, 5.71	0.494	**13.56**	**4.11, 23.01**	**0.007**
Dairy intake score	4.33	-2.44, 11.11	0.201	2.04	-7.29, 11.38	0.658
Meat/meat alternatives intake score	2.99	-3.66, 9.63	0.365	-1.71	-9.02, 5.59	0.635
Total fat intake score	**10.18**	**3.18, 17.17**	**0.006**	8.33	-2.50, 19.15	0.127
Saturated fat intake score	4.19	-0.99, 9.37	0.109	6.47	-3.99, 16.93	0.216
Cholesterol intake score	-2.37	-8.01, 3.26	0.396	-0.22	-10.15, 9.72	0.965
Sodium intake score	**-7.35**	**-14.34, -0.35**	**0.040**	0.05	-9.03, 9.13	0.991
Variety score	3.15	-7.53, 13.83	0.551	10.63	-1.55, 22.81	0.085

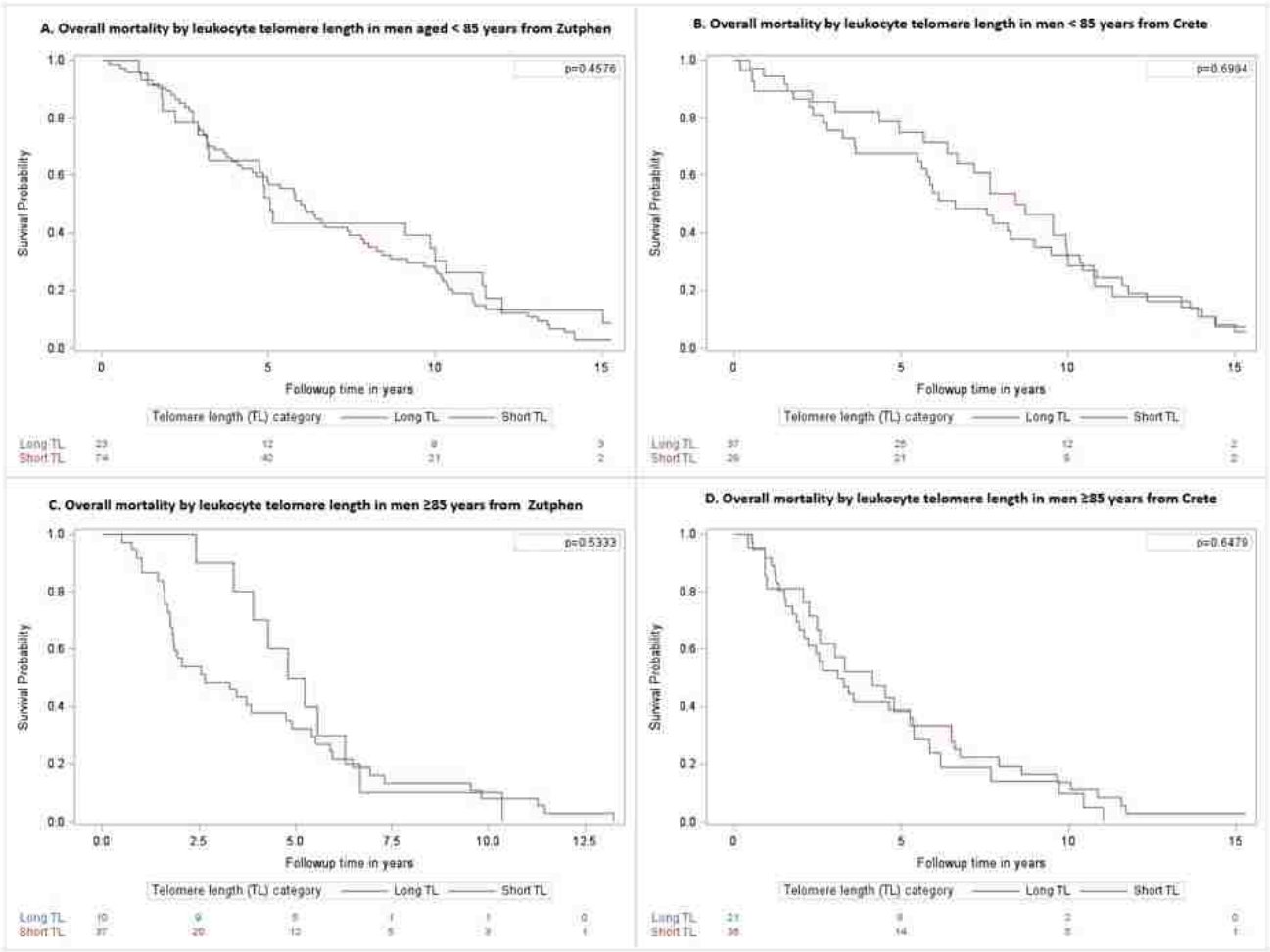
[1]Adjusted for energy, age, age-squared, sex, race/ethnicity, marital status, education, socio-economic status, BMI, smoking, alcohol use, and physical activity.

Appendix L. Literature review on nutrition and telomere length

Nutrient	Epidemiological evidence	Possible biological mechanism
Folate	Plasma folate concentration above the median was positively associated with telomere length but plasma folate concentration below the median was inversely associated with telomere length (Paul et al., 2009).	Methylene-tetrahydrofolate (THF) is used for the synthesis of the pyrimidine thymidylate and of purines, thus providing precursors for DNA synthesis. Thus, folate plays an important role in maintenance of DNA integrity and DNA methylation, both of which influence telomere length (Paul, et al., 2009).
Vitamin B12	No association between telomere length and vitamin B12, intake and plasma concentration, (Paul, et al., 2009; Xu, et al., 2009) but women who use vitamin B12 supplements have longer telomeres than nonusers (Xu, et al., 2009).	Vitamin B12 plays a role in generation of methyl groups for methylation reactions (Paul, 2011). Additionally, the strong antioxidant and anti- inflammatory properties of Vitamin B12 may help in preservation of telomeres (Paul, 2011).
Vitamin A	Telomere length is positively associated with dietary intake of vitamin A and β-carotene in women who do not take multivitamins (Xu, et al., 2009).	Vitamin A plays an important role in immune response (Mora, Iwata, & von Andrian, 2008)). Deficiency of vitamin A predisposes individuals to infections (Aukrust et al., 2000) that can lead to telomere attrition.
Vitamin D	A positive association between serum concentration of vitamin D and telomere length in women (Richards et al., 2007).	The biologically active form of vitamin D, 1α,25 dihydroxyvitamin D3, possesses immunosuppressive properties (Mora, et al., 2008) and is inversely related to the inflammatory marker CRP (Oelzner et al., 1998). In addition, vitamin D also reduces the expression of inflammation mediators interleukin-2 (Lemire et al., 1985) and interferon gamma (Reichel, Koeffler, Tobler, & Norman, 1987). These anti-inflammatory and antiproliferative properties of vitamins D limit the turnover of cells, thus potentially reducing their telomere length attrition.
Vitamins C and E	Intake of vitamin C and E is positively associated with longer telomeres in a dose-dependent manner in women (Xu, et al., 2009).	Antioxidant properties of vitamin C and E are widely known (Honarbakhsh & Schachter, 2009). They limit shortening of telomere length by preventing oxidative damage to telomeric DNA (Furumoto, Inoue, Nagao, Hiyama, & Miwa, 1998).
Iron	Use of iron supplements is associated with shorter telomeres (Aviv, 2009; Xu, et al., 2009). But iron intake from diet or multivitamins (low dose of iron than iron supplements) is not negatively associated with telomere length (Xu, et al., 2009).	The shorter telomeres observed in iron supplement users could be due the free radical generating capacity of iron and resultant oxidative stress which is one of the factors that result in telomere attrition (Aviv, 2009).
Magnesium	Dietary magnesium intake is positively related to telomere length in women (Xu, et al., 2009).	Magnesium is required for catalytic activity of enzymes involved in DNA replication, DNA repair and RNA synthesis (Hartwig, 2001). In addition to its potential role in DNA integrity and repair, magnesium may also affect telomere length by oxidative stress and inflammation (Martin et al., 2008).

Zinc		DNA polymerases, RNA polymerases and reverse transcriptases are Zinc-dependent enzymes in the cell (Poiesz, Seal, & Loeb, 1974; Terhune & Sandstead, 1972). Thus, Zince deficiency cause DNA damage (Song et al., 2009). In addition, zinc deficiency may also affect telomere length through increased oxidative stress and susceptibility to infection (Paul, 2011).
Omega-3 fatty acids	Plasma concentration of omega-3 fatty acids is positively associated with reduced attrition of telomere length (Farzaneh-Far, Lin, Epel, Harris, et al., 2010).	The anti-inflammatory and antioxidant properties of omega-3 fatty acids decrease the cell turnover and oxidative DNA damage and thus may reduce telomere shortening (Ferrucci et al., 2006).

Appendix M. Kaplan–Meier survival plot for overall mortality according to the leukocyte telomere length in elderly men



Appendix M footnote: Age group < 85 years from (A) Zutphen and (B) Crete and aged ≥85 from (C) Zutphen and (D) Crete.

Appendix N. Hazard ratios of overall and cardiovascular mortality according to telomere length for men who were free of cardiovascular disease, diabetes and cancer

	Short TL	**Long TL**	***P* value**
Zutphen:			
Cases of all–cause mortality			
Crude	1.0 (ref.)	0.75 (0.42 - 1.34)	0.33
Model 1	1.0 (ref.)	0.84 (0.47 - 1.52)	0.57
Model 2	1.0 (ref.)	0.62 (0.32 - 1.19)	0.15
Cases of cardiovascular mortality			
Crude	1.0 (ref.)	0.75 (0.22 - 2.62)	0.65
Model 1	1.0 (ref.)	1.14 (0.29 - 4.45)	0.85
Model 2	1.0 (ref.)	0.23 (0.03 - 1.78)	0.16
Crete:			
Cases of all–cause mortality			
Crude	1.0 (ref.)	1.00 (0.65 - 1.53)	0.99
Model 1	1.0 (ref.)	1.23 (0.79 - 1.91)	0.36
Model 2	1.0 (ref.)	0.93 (0.57 - 1.52)	0.77
Cases of cardiovascular mortality			
Crude	1.0 (ref.)	0.81 (0.41 - 1.60)	0.54
Model 1	1.0 (ref.)	0.69 (0.32 - 1.48)	0.34
Model 2	1.0 (ref.)	0.46 (0.18 - 1.13)	0.09
All participants combined[†]:			
Cases of all–cause mortality			
Crude	1.0 (ref.)	0.90 (0.64 - 1.27)	0.56
Model 1	1.0 (ref.)	1.07 (0.76 - 1.51)	0.70
Model 2	1.0 (ref.)	0.89 (0.62 - 1.29)	0.55
Cases of cardiovascular mortality			
Crude	1.0 (ref.)	0.79 (0.44 - 1.44)	0.45
Model 1	1.0 (ref.)	0.81 (0.42 - 1.54)	0.52
Model 2	1.0 (ref.)	0.46 (0.22 - 0.98)	**0.04**

Model 1: adjusted for age, self-rated health, prevalence of CVD, diabetes and cancer.
Model 2: adjusted for age, marital status, smoking, alcohol and BMI.
†: additionally adjusted for country.

Appendix O. Associations between leukocyte telomere length at baseline and age at death in Zutphen and Crete

	β (95% CI)	P-value
Zutphen:		
Crude	-0.11 (-3.33, 0.60)	0.17
Model 1	-0.03 (-2.18, 1.36)	0.65
Model 2	0.04 (-1.27, 2.22)	0.59
Crete:		
Crude	0.01 (-1.63, 1.88)	0.89
Model 1	0.04 (-1.09, 1.88)	0.60
Model 2	0.07 (-0.94, 2.16)	0.44

Data are standardized beta-coefficients (β, with the 95% confidence interval [CI]).
Model 1: adjusted for age, self-rated health, prevalence of CVD, diabetes and cancer.
Model 2: adjusted for age, marital status, smoking, alcohol and BMI.

Appendix P. UNLV IRB approval for study 1 and 2

UNLV

UNLV Biomedical IRB - Administrative Review
Notice of Excluded Activity

DATE:	July 6, 2017
TO:	Rachelle Rodriguez, Ph.D.
FROM:	UNLV Biomedical IRB
PROTOCOL TITLE:	[1085277-1] Investigate the evidence of a biological pathway between poor nutrition, telomere length decline and morbidity or mortality.
SUBMISSION TYPE:	New Project
ACTION:	EXCLUDED - NOT HUMAN SUBJECTS RESEARCH
REVIEW DATE:	July 6, 2017
REVIEW TYPE:	Administrative Review

Thank you for your submission of New Project materials for this protocol. This memorandum is notification that the protocol referenced above has been reviewed as indicated in Federal regulatory statutes 45CFR46.

The UNLV Biomedical IRB has determined this protocol does not meet the definition of human subjects research under the purview of the IRB according to federal regulations. It is not in need of further review or approval by the IRB.

We will retain a copy of this correspondence with our records.

Any changes to the excluded activity may cause this protocol to require a different level of IRB review. Should any changes need to be made, please submit a Modification Form.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

Office of Research Integrity - Human Subjects
4505 Maryland Parkway . Box 451047 . Las Vegas, Nevada 89154-1047
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Appendix Q. UNLV IRB approval for study 3

UNLV

UNLV Biomedical IRB - Administrative Review
Notice of Excluded Activity

DATE:	July 6, 2017
TO:	Rachelle Rodriguez, Ph.D.
FROM:	UNLV Biomedical IRB
PROTOCOL TITLE:	[1085261-1] Telomere Length and Mortality by Age among the Elderly Men in the Zutphen Elderly Study.
SUBMISSION TYPE:	New Project
ACTION:	EXCLUDED - NOT HUMAN SUBJECTS RESEARCH
REVIEW DATE:	July 6, 2017
REVIEW TYPE:	Administrative Review

Thank you for your submission of New Project materials for this protocol. This memorandum is notification that the protocol referenced above has been reviewed as indicated in Federal regulatory statutes 45CFR46.

The UNLV Biomedical IRB has determined this protocol does not meet the definition of human subjects research under the purview of the IRB according to federal regulations. It is not in need of further review or approval by the IRB.

We will retain a copy of this correspondence with our records.

Any changes to the excluded activity may cause this protocol to require a different level of IRB review. Should any changes need to be made, please submit a Modification Form.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

Office of Research Integrity - Human Subjects
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Curriculum Vitae

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Publications

Published/Accepted Peer-Reviewed Articles

1. Mishra SR, **Ghimire S**, Joshi C, Gyawali B, Shrestha A, Neupane D, Sharma SR, Pokharel Y, Virani SS. Cardio-metabolic disease risk factors among South Asian labour migrants to the Middle East: A scoping review. Accepted for publication by *Globalization and Health*.
2. **Ghimire S**, Singh DR, Shrestha N, Nath D, Baral BK. Nursing Students' Knowledge of Aging, Attitudes toward and Perceptions of Working with Older Adults in Kathmandu Nepal. Accepted for publication by International Journal of Nursing Sciences.
3. **Ghimire S**, Mishra SR, Sharma A, Siweya A, Shrestha N, Adhikari B. Geographic and socio-economic variation in markers of indoor air pollution in Nepal: evidence from a nationally-representative data. *BMC Public Health*. 2019 19:195.
4. Acharya S, **Ghimire S**, Jeffers E, Shrestha N. Health Care Utilization and Health Care Expenditure of Nepali Older Adults in Pokhara Lekhnath Metropolitan. February 2019 *Frontiers in Public Health.* DOI: https://doi.org/10.3389/fpubh.2019.00024.
5. **Ghimire S**, Baral BK, Feng D, Sy FS, Rodriguez R. Is Selenium Intake Associated with the Presence of Depressive Symptoms among U.S. adults?: Findings from National Health and Nutrition Examination Survey (NHANES) 2011-2014. December 2018. *Nutrition*. DOI: 10.1016/j.nut.2018.12.007
6. Amatya P, **Ghimire S**, Callahan KE, Baral BK, Poudel KC. Practice and Lived Experience of Menstrual Exiles (Chhaupadi) among Adolescent Girls in Far Western Nepal. December 2018. *PLoS ONE* 13(12): e0208260. DOI: 10.1371/journal.pone.0208260

7. **Ghimire S**, Cheong P, Sagadraca L, Chien LC, Sy FS. A Health Needs Assessment of the Filipino American Community in the Greater Las Vegas Area. November 2018. *Health Equity*. DOI: 10.1089/heq.2018.0042

8. **Ghimire S**, Baral BK, Pokhrel BR, Pokhrel A, Acharya A, Amatya D, Amatya P, Mishra SR. Depression, malnutrition, and health-related quality of life among Nepali older patients. BMC geriatrics. 2018 Dec; 18(1):191. PMID: 30143004.

9. **Ghimire S**, Baral BK, Karmacharya I, Callahan KE, Mishra SR. Life satisfaction among elderly patients in Nepal: associations with nutritional and mental well-being. Health and quality of life outcomes. 2018 Dec; 16(1):118. PMID: 29880002.

10. **Ghimire S**, Singh DR, Nath D, Jeffers EM, Kaphle M. Adult children's migration and well-being of left behind Nepalese elderly parents. Journal of Epidemiology and Global Health. 2018 Jul 31. https://doi.org/10.1016/j.jegh.2018.07.004.

11. Baral BK, Pokhrel A, Pokhrel BR, Rana D, Pandey R, Bhandari P, **Ghimire S.** Type 2 Diabetes Mellitus Risk Assessment Survey among Medical Students. Nepal Med Coll J 2018; 20(1-3): 19-24.

12. **Ghimire S**, Shrestha N, Callahan KE. Barriers to Dietary Salt Reduction among Hypertensive Patients. Journal of Nepal Health Research Council. 2018 Jul 3; 16(2):124-30. PMID: 29983423.

13. **Ghimire S**, Pradhananga P, Baral BK, Shrestha N. Factors associated With health-related Quality of life among hypertensive Patients in Kathmandu, Nepal. Frontiers in cardiovascular medicine. 2017 Nov 6; 4:69. PMID: 29164136.

14. **Ghimire S**. Barriers to Diet and Exercise among Nepalese Type 2 Diabetic Patients. International scholarly research notices. 2017. PMID: 29349287.

15. **Ghimire S**, Baral BK, Callahan KE. Nutritional assessment of community-dwelling older adults in rural Nepal. PloS one. 2017 Feb 14; 12(2):e0172052. PMID: 28196115.

16. Pokhrel D, Banstola R, Basnet S, Baral B, Shrestha N, **Ghimire S**. Patient's Medicine Cabinet Composition Study in Tilahar VDC, Parbat. Nepal Med Coll J 2016; 18 (1-2): 16-19.

17. Khatiwada B, **Ghimire S**, Shrestha N, Shrestha KB, Dahal PK. Willingness to Pay for Health Insurance in Mangalbare Village Development Committee of Illam District. MOJ Public Health. 2017 5(2): 00120. DOI: 10.15406/mojph.2017.05.00120.

18. Wu Q, Wang Y, Demaerschalk BM, **Ghimire S**, Wellik KE, Qu W. Bone marrow stromal cell therapy for ischemic stroke: A meta-analysis of randomized control animal trials. International Journal of Stroke. 2017 Apr; 12(3):273-84. PMID: 27794139.

19. Shrestha R, Shrestha KB, **Ghimire S**, Shrestha N. Knowledge and Preventive Practices related to Avian Influenza among Poultry Workers of Kamalamai Municipality, Sindhuli, Nepal. Journal of Nepal Health Research Council. 2016 Jan; 14(32):7-12. PMID: 27426705.
20. Shrestha M, Maharjan R, Prajapati A, **Ghimire S**, Shrestha N, Banstola A. Assessment of knowledge and practice of community pharmacy personnel on diabetes mellitus management in Kathmandu district: A cross sectional descriptive study. Journal of Diabetes & Metabolic Disorders. 2015 Dec; 14(1):71. PMID: 26396963.
21. **Ghimire S**, Sikharam HK, Baral BK. Maternal knowledge and management practice towards childhood diarrhea in Bhaktapur, Nepal. International Journal of Recent Scientific Research, 2015; 6(5).
22. **Ghimire S**, Shrestha N, Baral BK. Oral Contraceptives as a Risk Factor for Developing Breast Cancer in Breast Cancer (BRCA) Gene Carrier Female in-The 30-60 Years Age Group; A Meta-Analysis. International Journal of Medical Research and Health Sciences. 2015 Jan 1; 4(1):135-43.

Invited Papers

23. **Ghimire S**, Mishra SR, Baral BK, Dhimal M, Callahan KE, Bista B, Aryal KK. Non-communicable disease risk factors among older adults aged 60-69 years in Nepal: findings from The STEPS Survey 2013. Journal of Human Hypertension, 2019. DOI: https://doi.org/10.1038/s41371-019-0161-7.
24. Mishra SR, **Ghimire S**, Shrestha N, Shrestha A, Virani S. Socio-economic inequalities in hypertension burden and cascade of services: nationwide cross-sectional study in Nepal. Journal of Human Hypertension, 2019. DOI: https://doi.org/10.1038/s41371-019-0165-3.

Manuscripts Submitted/Under Review

1. **Ghimire S**, Hill CV, Sy FS, Rodriguez R. Decline in Telomere Length by Age, Gender, Allostatic Load and Comorbidities in National Health and Nutrition Examination Survey (1999-2002). Submitted to *PLoS ONE*; manuscript under revision.
2. Singh DR, **Ghimire S**, Jeffers E, Szabo S, Nath D. Food Insecurity among Senior Citizens in High Out-migration Areas: Evidence from Western Nepal. Submitted to Agriculture & Food Security; manuscript under revision.

3. Mishra SR, Shrestha N, **Ghimire S,** Gyawali B, Pradhan PMS, Schwarz D. A multi-level analysis exploring geographic and socioeconomic variation in underweight and overweight/obesity in Nepal. Submitted to Nutrition and Diabetes; manuscript under review.
4. Singh DR, Singh S, Bista B, Karki K, **Ghimire S.** Awareness of Thyroid Disorders among Women: A Cross-Sectional Study in Nepal. Submitted to International Journal of Women's Health; manuscript under review.
5. Ghimire S, Shrestha N, **Ghimire S.** Practice related to pesticide use and health hazards amongst pesticides user farmers in Gotikhel, Lalitpur. Submitted to Journal of Nepal Health Research Council; manuscript under review.
6. Baral SR, Parajuli DR, Shrestha S, Acharya SR, Dahal P, Poudel P, **Ghimire S**, Palaian S, Shrestha N. Undergraduate pharmacy students' attitudes and perceived barriers toward provision of pharmaceutical care: A multi-institutional study in Nepal. Submitted to Integrated Pharmacy Research and Practice; manuscript under review.

Manuscripts in Preparation

1. **Ghimire S**, Rodriguez R, Sy FS, Giltay EJ, Waterham E, Geleijnse M, Hageman G, Kromhout D. Association between Telomere Length, and Mortality, and Lifespan in Elderly Men: The Zutphen and Crete Elderly Study.
2. Karmacharya I, **Ghimire S**, Bhujel K, Shrestha-Dhauvadel A, Adhikari S, Baral S, Shrestha N. Health Services Utilization among Elderly Population in Pokhara Lekhnath Metropolitan City.
3. **Ghimire S**, Cross CL, Sy FS, Rodriguez R. The Association between Telomere Length, Nutrients and a Healthy Eating Index in the National Health and Nutrition Examination Survey (NHANES), 1999-2002.
4. Cheong P, Coughenour C, Shegog M, **Ghimire S**, Sagadraca L, Sy FS. An Evaluation of Food Insecurity and Its Correlates in a Filipino American Population Sample Residing in Clark County, Nevada.

Technical Reports

1. Shrestha N, Gurung SC, **Ghimire S**, Devkota BM. Study on Effectiveness of Integration of Family Planning into Agriculture and Economic Empowerment Program for Access and Coverage. Funded by USAID. January 2016.
2. Pandey BD, Gurung JK, Shrestha N, **Ghimire S**, Shakya S. Facility based assessment for reproductive health commodities and services. Funded by UNFPA. March 2015.

Books

1. Giri RK, **Ghimire S.** Community Health I. *Vidharyathi Pustak Bhandar*, Bhotahiti, Kathmandu, First edition 2015 (ISBN: 9789994618590).
2. **Ghimire S**, Dahal S. A Textbook of Applied Epidemiology in Nepalese Context. *Navodit Hamro Pustak Bhandar*, Bagbazar, Kathmandu, First edition 2014 (ISBN: 9789937885232).

Presentations

Oral Presentations at Scientific Meetings

1. Siweya A, **Ghimire S**, Sagadraca L, Cheong P, Sy FS. Access and Barriers to Health Care among Filipino Immigrants in the Greater Las Vegas Area. American Public Health Association Conference, San Diego, CA, November 2018.
2. **Ghimire S**. Decline in Telomere Length by Age, Gender, Allostatic Load and Comorbidities in National Health and Nutrition Examination Survey (1999 - 2002). 2018 Southern Gerontological Society and Georgia Gerontological Society Joint Conference, Buford, GA, April 14, 2018.
3. **Ghimire S**. Depression, Malnutrition, and Health-Related Quality of Life among Nepalese Elderly. 2018 Southern Gerontological Society and Georgia Gerontological Society Joint Conference, Buford, GA, April 14, 2018.
4. **Ghimire S**. Decline in Telomere Length by Age, Gender, Allostatic Load and Comorbidities in National Health and Nutrition Examination Survey (1999 - 2002). 20th Annual Graduate & Professional Student Research Forum, University of Nevada Las Vegas. February 3, 2018.
5. **Ghimire S**, Baral BK, Callahan KE. Nutritional Assessment of Older Adults in Nepal. 2016 Annual Meeting of Nevada Public Health Association. Las Vegas, NV. September 2016.
6. Ghimire S, Sharma DR, Shrestha N, **Ghimire S.** Practice related to pesticide use and health hazards amongst pesticides user farmers in Gotikhel, Lalitpur. First National Summit of Health and Population Scientists in Nepal, Nepal Health Research Council (NHRC), Kathmandu, Nepal. 11-12 April 2015.
7. Shrestha R, Shrestha KB, **Ghimire S**, Shrestha N. Knowledge and preventive practices related to avian influenza among poultry workers of Kamalamai Municipality, Sindhuli.

First National Summit of Health and Population Scientists in Nepal. Nepal Health Research Council (NHRC), Kathmandu, Nepal. 11-12 April 2015.

8. **Ghimire S**. Conducting a Facility Based Assessment for Reproductive Health Commodities and Service. Logistics Regional Review of Central Region. Kathmandu, Nepal. March 11, 2015.

Poster Presentations at Scientific Meetings

1. **Ghimire S,** Sy FS, Rodriguez R. Association between Telomere Length, 31 Nutrients and a Healthy Eating Index in the National Health and Nutrition Examination Survey (NHANES), 1999-2002. GSA 2018 Annual Scientific Meeting, Boston, MA, November 2018.
2. Cheong P, Coughenour C, Shegog M, **Ghimire S**, Sagadraca L, Sy FS. An Evaluation of Food Insecurity and Its Correlates in a Filipino American Population Sample Residing in Clark County, Nevada. American Public Health Association Conference, San Diego, CA, November 2018.
3. **Ghimire S,** Sy FS, Rodriguez R. Association between Telomere Length, 31 Nutrients and a Healthy Eating Index in the National Health and Nutrition Examination Survey (NHANES), 1999-2002. American Public Health Association Conference, San Diego, CA, November 2018.
4. **Ghimire S.** Can Low Serum Selenium Concentrations Increase Risk for Depression in U.S. adults?: National Health and Nutrition Examination Survey (NHANES) 2011-2014. American Public Health Association Conference, Atlanta, GA, November 2017.
5. Cheong P, **Ghimire S**, Sagadraca L, Sy FS. An Assessment of Filipino American Health in the Greater Las Vegas Area: A Pilot Study. American Public Health Association Conference, Atlanta, GA, November 2017.
6. **Ghimire S**, Shrestha N, Callahan KE. Factors Associated with Health-Related Quality of Life among Hypertensive Patients in Kathmandu, Nepal. 2017 Annual Meeting Society for Epidemiological Research. Seattle, WA. June 21, 2017.
7. **Ghimire S**, Baral BK, Callahan KE. Nutritional Assessment of Older Adults in Nepal. 18th Annual Graduate & Professional Student Research Forum, University of Nevada Las Vegas. March 12, 2016.
8. **Ghimire S**, Maheshwari S, Callahan KE, Wu Q. The Risk of Incident Asthma among Overweight and Obese Children: A Meta-Analysis of Prospective Studies. Nevada Institute of Personalized Medicine, 2016 Retreat. Las Vegas, NV. February 12, 2016.

9. Wu Q, Wang Y, Demaerschalk BM, **Ghimire S**, Wellik KE, Qu W. Bone Marrow Stromal Cell Therapy for Ischemic Stroke: A Meta-Analysis of Randomized Control Animal Trials. Nevada Institute of Personalized Medicine, 2016 Retreat. Las Vegas, NV. February 12, 2016.
10. Palisoc B, **Ghimire S**, Wu Q. Depression Onset as an Adverse Event of Tetrabenazine on Individuals with Huntington's Disease: Preliminary Results of Systematic Review and Meta-Analysis. Nevada Institute of Personalized Medicine, 2016 Retreat. Las Vegas, NV. February 12, 2016.

Presentations in Trainings and Workshops

1. **Research Evaluator**: UNLV Office of Undergraduate Research /CSUN Undergrad Research Conference, University of Nevada Las Vegas. April 27, 2018.
2. **Presenter**: SPSS Fundamentals Workshop. Graduate College and the Graduate & Professional Student Association (GPSA), University of Nevada Las Vegas. March 4, 2016.
3. **Presenter**: Operational Research and Report Writing. National Health Training Center Teku, Kathmandu. July 8, 2015.
4. **Workshop Leader**: Research Proposal Development - Training Workshop. Purbanchal University College of Medical and Allied Sciences (PUCMAS), Morang Nepal. June 17-19, 2015.
5. **Presenter**: Data Entry by using Epidata - Training Workshop. AHEAD-Nepal, Kathmandu. November 2, 2014.
6. **Workshop Leader**: Literature Review, Citation and Referencing by Using EndNote - Training Workshop. AHEAD-Nepal, Kathmandu. November 5, 2014.
7. **Workshop Leader:** Data Management and Analysis by using SPSS - Training Workshop. AHEAD-Nepal, Kathmandu. November 8-10, 2014.

Trainings and Workshops Attended

1. UNLV Graduate College Communication Certification Program, August 2018-May 2019 (ongoing).
2. UNLV Graduate College Research Certification Program, August 2018-May 2019 (ongoing).
3. Grad Rebel Writing Boot Camp, August 13-17, 2018, UNLV Graduate College.
4. Three-day workshop on Meta-Analysis by Dr. Michael Borenstein, April 18-20, 2016 New York.

5. Collaborative Institutional Training Initiative (CITI) Training, Biomedical Responsible Conduct of Research, Expires: August 2020.
6. Research Ethics organized by The Global Health Network, June 2015; Certificate Number 59144.
7. Training Workshop on "Data Management and Analysis", organized by the National Health Research Council (NHRC), 08 - 13 March 2015, Kathmandu, Nepal.
8. National workshop on "Neglected Tropical Disease (NTDs) in Nepal: Identifying the Gaps and Mapping the Way Forward", organized by the National Health Research Council (NHRC) and World Health Organization, Country office Nepal, 26-27 March 2015; Kathmandu, Nepal.
9. Advanced course "Ethics of Public Health and Biomedicine in a Nutshell" (3 higher education credits), Sahlgrenska Academy, University of Gothenburg, Sweden, April 8-12, 2013; Kathmandu, Nepal.

Service

Professional Service as Journal Peer Reviewer

1. **Editor and Reviewer**: Health Prospect, Journal of Public Health (ISSN: 2091-2021) https://www.nepjol.info/index.php/HPROSPECT.
2. **Reviewer**: Journal of Human Hypertension (ISSN 1476-5527) https://www.nature.com/jhh/ .
3. **Reviewer**: Center for Disease Control and Preventions' Preventing Chronic Disease, (https://www.cdc.gov/pcd/about_the_journal/index.htm).
4. **Reviewer**: Journal of Geriatric Medicine and Gerontology (https://www.clinmedjournals.org/Journal-of-Geriatric-Medicine-and-Gerontology.php).
5. **Reviewer**: World Research Journal of Cardiology (ISSN: 2321-4422 & E-ISSN) 2015-2018.
6. **Reviewer**: AIDS Education and Prevention https://guilfordjournals.com/loi/aeap.
7. **Reviewer**: Current Research in Nutrition and Food Science www.foodandnutritionjournal.org
8. **Reviewer:** Sexual and Relationship Therapy https://www.tandfonline.com/toc/csmt20/current
9. **Reviewer:** Journal of International Medical Research https://journals.sagepub.com/home/imr

10. **Abstract Reviewer:** 50th Anniversary Meeting of the Society for Epidemiologic Research.

Community Service

1. **Graduate Liaison Officer**: Public Health Student Association, University of Nevada Las Vegas, 2017-2018.
2. **School of Community Health Sciences Representative**: Graduate and Professional Student Association (GPSA), Summer 2016 and 2017.
3. **Treasurer**: Nepalese Students Association, University of Nevada Las Vegas, 2015-2017.

Honors and Awards

Awards

1. UNLV Graduate College Spring 2019 Medallion Recipient, Las Vegas, May 2019.
2. 2019 GPSA Merit Award, GPSA and Graduate College Annual Research Forum, Las Vegas, February 2019.
3. Delta Omega Best Student Poster Award, Delta Omega National Meeting, San Diego, November 2018.
4. Outstanding Graduate Student 2018, Department of Environment and Occupational Health, School of Community Health Sciences Honors Reception, UNLV, May 2018.
5. Southern Gerontological Society Student Paper Award, 2018 Southern Gerontological Society and Georgia Gerontological Society Joint Conference, Buford, GA, April 14, 2018.
6. Selected for membership. Delta Omega, The Honor Society of Public Health, Delta Theta Chapter at UNLV, December 2017.
7. Laurence G. Branch Doctoral Student Research Award, American Public Health Association Conference, Atlanta, GA, November 2017.
8. Delta Omega Best Student Poster Award, Delta Omega National Meeting, Atlanta, GA, November 2017.
9. Selected for Membership, The Honor Society of Phi Kappa Phi Chapter 100, April 2017.
10. Outstanding Graduate Student 2017, Department of Environment and Occupational Health, School of Community Health Sciences Honors Reception, UNLV, May 2017.
11. Honorable Mention: Outstanding Poster, “Nutritional Assessment of Older Adults in Nepal”; 18th Annual Graduate & Professional Student Research Forum, University of Nevada Las Vegas, March 12, 2016.

12. Mini Research Award 2071/2072, University Grants Commission, Sanothimi, Bhaktapur, Nepal, July 2015.
13. First position with Distinction in Intermediate of Science (I.Sc.) (2001-2003), Siddhartha Vanasthali Institute, Tribhuvan University, Kathmandu, Nepal, May 2003.
14. Girls Topper in Eastern Development Region- SLC 2000, Rumpum Excellency Awards, Kathmandu, Nepal, May 2000.

Scholarships, Fellowships and Travel Awards

1. 2019 Nevada Regents' Scholar Awards - Graduate Students.
2. UNLV Graduate and Professional Student Association (GPSA) Merit Award 2019.
3. 2018-2019 President's UNLV Foundation Graduate Research Fellowship, University of Nevada, Las Vegas.
4. 2018 Summer Doctoral Research Fellowships, University of Nevada, Las Vegas.
5. UNLV Graduate and Professional Student Association (GPSA) Travel Sponsorship, November 2018, Boston, MA.
6. UNLV School of Community Health Sciences - Conference Travel award for APHA meeting, November 2018. San Diego, CA.
7. Southern Gerontological Society Student Scholarship Award, 2018 Southern Gerontological Society and Georgia Gerontological Society Joint Conference, Buford, GA, April 2018.
8. UNLV School of Community Health Sciences - Conference Travel award for Southern Gerontological Society and Georgia Gerontological Society Joint Conference, April 2018. Buford, GA.
9. Society for Epidemiologic Research (SER) "SPC Travel Scholarship" Seattle, WA, Jun 2017.
10. UNLV School of Community Health Sciences - Conference Travel award for SER meeting, June 2017. Seattle, WA.

Professional Affiliations

1. **Member**: American Public Health Association, 2017, 2018.
2. **Member:** The Gerontological Society of America, 2018.
3. **Member**: Society for Epidemiologic Research, 2016.
4. **Member**: Nevada Public Health Association, 2016.
5. **Member**: Nepal Public Health Association, 2012-Present.

6. **Member**: Nepal Health Professional Council (Council Registration No: 28 Jan PH), 2012-Present.
7. **Member**: University of Wolverhampton Alumni Association, 2011-Present.
8. **Member**: Tribhuvan University Alumni Association, 2008 –Present.

Students mentored

1. Saroj Rimal, Bachelor's in Public Health (BPH) Thesis Mentee (2014), currently employed by Ministry of Health and Population, Nepal Government.
2. Ramlal Thaguna, BPH Thesis Mentee (2014), currently employed by Ministry of Health and Population, Nepal Government.
3. Kiran Budathoki, BPH Thesis Mentee (2014), currently employed by Ministry of Health and Population, Nepal Government.
4. Sudarsan Dhungana, BPH Thesis Mentee (2013), currently Field Coordinator for Action Nepal, Non-governmental organization.

Technical/Software Skills

Proficient in Microsoft Office, MS Access, Endnote, Epidata, Epi Info, SPSS, SAS, STATA, familiar with R.