

Stress and cellular aging – Associations between stress-related factors and leukocyte telomere length

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Abstract

It is well established that stress poses an elevated risk for non-communicable diseases and early mortality. However, the underlying mechanisms are not fully understood. There is strong, yet not conclusive, evidence showing that telomeres, non-coding DNA-protein complexes (TTAGGG) located at the end of chromosomes, are associated with aging-related diseases including cardiovascular diseases, stroke and type 2 diabetes. The aim of this thesis is to expand the relatively scant literature on stress and telomere length and to study whether stress-related factors are associated with leukocyte telomere length (LTL), a possible biomarker of cellular aging.

The participants were from the Helsinki Birth Cohort Study, which comprises 13,345 individuals born from 1934 to 1944 in Finland. Between the years 2001 and 2004 a randomly selected sample of subjects participated in a detailed clinical examination including blood sampling for LTL measurement. LTL data, measured by the quantitative Polymerase Chain Reaction (qPCR) method, was available for 1964 participants (men $n = 912$ and women $n = 1052$) at a mean age of 61.5 (SD = 2.9, Range = 56.7 - 69.8) years. In conjunction with the clinical examination, the participants reported on the following stress-related factors: the Beck Depression Inventory ($n = 1954$), the Mental Health Index ($n = 1950$) and the Vitality Scale ($n = 1950$) from the SF36/RAND to capture depressive symptoms. After clinical examination they completed the NEO Personality Inventory ($n = 1671$) to capture personality dimensions that either render individuals vulnerable to or buffer them from stress and the Traumatic events checklist ($n = 1486$) to capture traumatic experiences across the lifespan. Objective stress-related factors were derived from Finnish registries. These included mental disorders ($n = 116$ vs. 1840), sleep apnea ($n = 44$ vs. 1875) and snoring ($n = 29$ vs. 1875) that were identified from the Finnish Hospital Discharge Register, psychotropic medication use ($n = 665$ vs. 1291) that was identified from the Finnish National Social Insurance Institution Register, and early life stress ($n = 215$ vs. 1271), namely temporary separation from both parent(s) due to child evacuations from Finland during World War II, that was identified from the National Archives' register. In addition, a subsample of the participants with LTL

measurement underwent the Trier Social Stress Test (n = 287) during which HPA axis stress reactivity was measured.

The results showed no associations between personality dimensions, mental disorders, depressive symptoms, objectively measured early life stress, self-reported traumatic experiences across the lifespan, or HPA axis stress reactivity and LTL. However, a combination of objectively measured early life stress and self-reported traumatic experiences, and a history of sleep apnea, were associated with shorter LTL. Two unpredicted findings were also made. Individuals hospitalized for mental disorders who also used psychotropic medication had longer LTL than non-hospitalized controls; also an agreeable personality dimension was associated with LTL in a sex-specific manner such that more agreeable men and less agreeable women had shorter LTL.

As a majority of the findings did not support the study hypotheses, and the effect sizes in the few existing associations were relatively small, the results in this PhD thesis suggest that stress related factors are not strongly associated LTL, at least when measured with qPCR in an elderly sample showing little variation in age.

Tiivistelmä

Aikaisemmat tutkimukset ovat osoittaneet että stressi lisää riskiä sairastua ikääntymiseen liittyviin sairauksiin sekä todennäköisyyttä ennenaikaiseen kuolemaan. Välittävää mekanismia ei kuitenkaan tunneta vielä täysin. On olemassa vahvaa, ei kuitenkaan täysin yhdenmukaista, tutkimusnäyttöä siitä, että ikääntymiseen liittyvät sairaudet ovat yhteydessä telomeereihin. Telomeerit ovat kromosomien päissä olevia toistuvia DNA jaksoja (TTAGGG), jotka eivät sisällä informaatiota proteiinien koodaamiseen. Lyhemmät telomeerit on yhdistetty ikääntymiseen liittyviin sairauksiin, kuten sydän- ja verisuonitauteihin, aivohalvaukseen ja 2 tyypin diabetekseen. Tämän väitöskirjan tarkoitus on laajentaa olemassa olevaa suhteellisen suppeaa kirjallisuutta stressin ja telomeeripituuden välisistä yhteyksistä, ja tutkia ovatko stressiin liittyvät tekijät yhteydessä lyhempään veren leukosyyttien telomeeripituuteen; mahdolliseen fyysisen ikääntymisen biomarkkeriin.

Tutkimukseen osallistuneet olivat osa Helsinki Syntymäkohorttia, johon kuuluu kokonaisuudessaan 13,345 vuosina 1934 - 1944 syntynyttä suomalaista. Vuosien 2001 - 2004 aikana sattumanvaraisesti valittu otos osallistui tarkkoihin kliinisiin tutkimuksiin, joiden aikana kerättiin myös verinäyte leukosyyttien telomeeripituuden määrittystä varten. Leukosyyttien telomeeripituus määritettiin Kvantitatiivista polymeerasiketjureaktio (qPCR) mittausmenetelmää käyttäen 1964 osallistujalta (miehiä $n = 912$ ja naisia $n = 1052$) keskimäärin 61.5 (keskihajonta = 2.9, vaihteluväli = 56.7 - 69.8) vuoden iässä. Kliinisen tutkimuksen yhteydessä osallistujat täyttivät seuraavat stressiin liittyvät kyselyt; depressiivisiä oireita kuvastavat Beckin depressiokyselyn ($n = 1954$) sekä mielenterveysindeksin ($n = 1950$) ja vitaliteetti-indeksin ($n = 1950$) SF-36/RAND kyselystä. Kliinisen tutkimuksen jälkeen tutkittavat täyttivät heille lähetetyt Viiden faktorin mallin persoonallisuuskyselyn ($n = 1671$) stressille altistavien ja stressiltä suojaavien persoonallisuuspiirteiden kartoittamiseksi ja Traumaattisten tapahtumien kyselyn ($n = 1486$) elämän aikana koettujen traumaattisten tapahtumien selvittämiseksi. Objektiivisesti mitatut stressiin liittyvät tekijät koottiin suomalaisista rekistereistä. Tiedot sairaalahoitoa vaatineista mielenterveyshäiriöistä ($n = 116$ vs. 1840), uniapneasta ($n = 44$ vs.

1875) ja kuorsauksesta (n = 29 vs. 1875) kerättiin sairaalapoistorekisteristä, psykieläkkeiden käyttö (n = 665 vs. 1291) Kansaneläkelaitoksen lääkekorvausrekisteristä ja varhainen stressikokemus, jollaiseksi määriteltiin väliaikainen erokokemus molemmista vanhemmista toiseen maailmansotaan liittyneiden väliaikaisten sotaevakoiden vuoksi (n = 215 vs. 1271), Suomen Kansallisarkistosta. Tämä lisäksi osa tutkittavista (n = 287) osallistui Trierin sosiaaliseen stressitestiin, jossa tutkittiin HPA akselin stressireagoivuutta.

Tulokset osoittivat, että persoonallisuuspiirteet, mielenterveyshäiriöt, depressiiviset oireet, objektiivisesti mitattu varhainen stressikokemus, itseraportoidut traumakokemukset ja stressireagoivuus eivät olleet yhteydessä leukosyyttien telomeeripituuteen. Havaitimme kuitenkin, että yhdistelmänä varhainen stressikokemus ja myöhemmät traumakokemukset olivat yhteydessä lyhempiin leukosyyttien telomeereihin, kuten myös uniapnea. Tämän lisäksi teimme kaksi yllättävää löydöstä. Havaitimme, että tutkittavat joilla oli mielenterveyshäiriö ja jotka käyttivät psykieläkkeitä, oli pidemmät telomeerit kuin terveillä verrokeilla; myös sovinnollinen persoonallisuus oli yhteydessä leukosyyttien telomeereihin sukupuolispesifisti niin, että enemmän sovinnollisilla miehillä ja vähemmän sovinnollisilla naisilla oli lyhemmät leukosyyttien telomeerit.

Koska suurin osa löydöksistä ei tukenut alkuperäistä tutkimushypoteesia, eivätkä muutamat löydetty yhteydet olleet vaikutukseltaan suuria, viittaavat tämän väitöskirjan tulokset siihen, ettei leukosyyttien telomeeripituus, ainakaan mitattuna qPCR:llä ikäjakaumaltaan kapeasta iäkkäiden henkilöiden otoksesta, ole vahvassa yhteydessä stressiin liittyviin tekijöihin.

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List of original publications

- I Savolainen, K., Eriksson, J.G., Kajantie, E., Pesonen, A.K., Räikkönen, K. (2015). Associations between the five-factor model of personality and leukocyte telomere length in elderly men and women: The Helsinki Birth Cohort Study (HBCS). *Journal of Psychosomatic Research*, 79, 233–238.
- II Savolainen, K., Räikkönen, K., Kananen, K., Kajantie, E., Hovatta, I., Pesonen, A.K., Heinonen, K., Eriksson, J.G. (2012). History of mental disorders and leukocyte telomere length in late adulthood: The Helsinki Birth Cohort Study (HBCS). *Journal of Psychiatric Research*, 10, 1346–1353.
- III Savolainen, K., Eriksson, J.G., Kajantie, E., Pesonen, A.K., Heinonen, K., Räikkönen, K. (2014). Associations between early life stress, self-reported traumatic experiences across the lifespan and leukocyte telomere length in elderly adults. *Biological Psychology*, 97, 35–42.
- IV Savolainen, K., Eriksson, J.G., Kajantie, E., Lahti, M., Räikkönen, K. (2014). The history of sleep apnea is associated with shorter leukocyte telomere length: the Helsinki Birth Cohort Study. *Sleep Medicine*, 15, 209–212.
- V Savolainen, K., Eriksson, J.G., Kajantie, E., Lahti, J., Räikkönen, K. (2015). Telomere length and hypothalamic-pituitary-adrenal axis response to stress in elderly adults. *Psychoneuroendocrinology*, 53, 179–84.

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Abbreviations

| | |
|-----------|---|
| ACTH | adrenocorticotrophic hormone |
| ALT | Alternative lengthening of telomeres |
| AUC | Area under curve |
| AVP | Arginine vasopressin hormone |
| BDI | Beck depression inventory |
| BMI | Body mass index |
| bp | base pair |
| CHD | Coronary heart disease |
| CHR | Corticotropin-releasing hormone |
| CRP | C-reactive protein |
| CV | Coefficient of variation |
| DNA | Deoxyribonucleic acid |
| DSM | Diagnostic and statistical manual of mental disorders |
| ELS | Early life stress |
| FFM | Five factor model |
| Flow-FISH | Fluorescence in situ hybridization |
| GCs | Glucocorticoids |
| GR | Glucocorticoid receptor |
| GWAS | Genome wide association study |
| HBCS | Helsinki birth cohort study |
| HDR | Hospital discharge register |
| HPA | Hypothalamic-pituitary-adrenal |
| ICD | International classification of diseases |
| IL | Interleukin |
| LTL | Leukocyte telomere length |
| MHI | Mental health index |
| MR | Mineralocorticoid receptors |
| n | Number of cases |

| | |
|--------|---|
| NEO-PI | Neuroticism-Extraversion-Openness Personality Inventory |
| QC | Quality control |
| Q-FISH | Quantitative fluorescence in situ hybridization |
| qPCR | Quantitative polymerase chain reaction |
| RNA | Ribonucleic acid |
| ROS | Reactive oxygen species |
| SAM | Sympathetic-adrenal-medullary |
| SASP | Senescence associated secretory phenotype |
| SD | Standard deviation |
| SES | Socio economic status |
| SNP | Small nucleotide polymorphism |
| STELA | Single telomere length analyses |
| T/S | Ratio of telomere- / the β -hemoglobin reactions |
| TEC | Traumatic events checklist |
| TL | Telomere length |
| TNF | Tumor necrosis factor |
| TRF | Telomere restriction fragment |
| TSST | Trier social stress test |
| VS | Vitality scale |

1 Introduction

The number of individuals aged 80 years and above is growing throughout the world. As more and more people are surviving into old and very old age, at the same time as fertility rates are declining, the age structure of populations are shifting towards aging societies (Jin, Simpkins, Ji, Leis, & Stambler, 2015). Also in Finland, it is estimated that there will be more than 730 000 individuals over 80 years of age in year 2065 compared to the 280 000 in year 2015 (Figure 1) and the number of persons who reach the age of 100 years will rise from 700 to 7000 respectively (Official Statistics of Finland, 2015). This, however, does not mean that the extra years are healthier, rather the contrary, old age is still usually associated with biological degeneration and thus increasing risk for non-communicable diseases (Jin et al., 2015). Gaining better understanding on the mechanisms that render individuals vulnerable to aging-related diseases is important for both economical and individual reasons.

It is well established that stress and stress-related factors pose an elevated risk for aging-related diseases, including infections, autoimmune diseases, coronary heart diseases, hypertension and type 2 diabetes (Cohen & Williamson, 1991; Cohen, Janicki-Deverts, & Miller, 2007; Pouwer, Kupper, & Adriaanse, 2010; Rozanski, Blumenthal, & Kaplan, 1999; Spruill, 2010; Stojanovich & Marisavljevich, 2008; Wright, Rodriguez, & Cohen, 1998). However, the mechanisms between stress, stress-related factors and aging-related diseases are not fully understood (Charles et al., 2008; Cohen et al., 2007; Gozal & Kheirandish-Gozal, 2008; McEwen, 2008; Pouwer et al., 2010; Spruill, 2010). Telomere biology may offer an additional window to study the mechanisms that link stress with disease risks. The overarching aim of this thesis is to expand the scant literature, and to study whether stress and stress-related factors are associated with leukocyte telomere length (LTL).

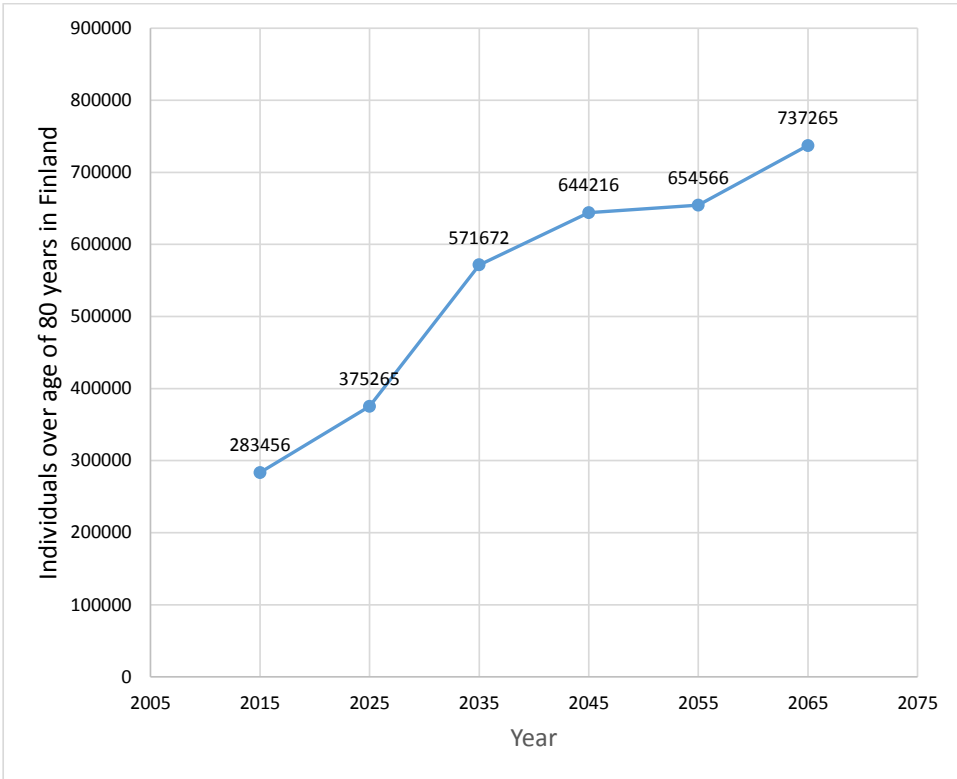


Figure 1. Estimated number of individuals aged over 80 years in Finland between years 2015-2065 (Official Statistics of Finland, 2015).

1.1 Stress

1.1.1 Definition and basic functions

First definitions for stress come from Selye, who defined stress as a nonspecific general response of the body to variety of demands characterized by the secretion of glucocorticoids (Selye, 1936). He also developed a concept of general adaptation syndrome which includes alarm reaction, stage of resistance and finally exhaustion caused by stressful stimulus (Selye, 1936). Later stress has been defined as state of threatened homeostasis (complex of dynamic and harmonious equilibrium of a body) (Chrousos & Gold, 1992). Homeostasis can be threatened by internal or external stimulus and the steady homeostatic state

gained back by successful counteracting responses that can be physical or mental and stressor specific or more general (Chrousos & Gold, 1992). Behavioural adaptation to stress includes increased arousal, alertness, anxiety, and attention as well as suppression of reproductive behaviours and alteration in appetite (Chrousos & Gold, 1992; Sanford, Suchecki, & Meerlo, 2014; Yau & Potenza, 2013). Physical adaptation to stress include sympathetic nervous system, immune system and hypothalamus pituitary axis activation causing direction of energy to central nervous system and stressed body sites, altered cardiovascular tone, increased blood pressure, heart rate, respiratory rate, glycogenesis and lipolysis as well as inhibition of growth and reproductive systems (Chrousos & Gold, 1992). Coping theories of stress have highlighted the individual differences in stress responses and stress itself is defined as relationship between person and environment and stress reaction is seen to be dependent on its personal significance, controllability and current coping resources available (Lazarus & Folkman, 1984).

In a short term, stress can be beneficial as it can improve the performance and fitness of the individual, however severe chronic stress in turn can decrease the overall fitness and affect brain (changes in hippocampal, frontal cortex and amygdala volume), cognition (poorer learning and memory) and physical and mental health as it heightens the risks for metabolic and chronic inflammation related diseases (depression, cardiovascular disease, type 2 diabetes) (Chrousos & Gold, 1992; Lupien, McEwen, Gunnar, & Heim, 2009; Möstl & Palme, 2002).

1.1.2 Stress at cellular level

Key physiological responses to stress include sympathetic-adrenal-medullary system (SAM), Hypothalamic-pituitary-adrenal (HPA) axis, and immune system (Chrousos & Gold, 1992; Gunnar & Quevedo, 2007; Lupien et al., 2009). SAM, sympathetic division of autonomic nervous system, releases adrenaline and noradrenaline hormones and promote the instant “fight or flight” response to stress while HPA axis controls more for longer term stress response through glucocorticoids (Wetherell et al., 2006). Both of these SAM and HPA systems are innervated by amygdala and hippocampus and are partially modulated by

prefrontal cortex (Compas, 2006). Amygdala can activate the systems in a rapid and automatic manner, where prefrontal cortex activation and modulation is more slow and conscious process (Compas, 2006).

The SAM axis is activated rapidly in response to stress or a threat to prepare the body for physical reaction of either approach or escape (Compas, 2006). Sympathetic nervous system activation through sympathetic preganglionic neurons in the intermediolateral grey matter of spinal cord, triggers epinephrine and norepinephrine production by the medulla of an adrenal glands located above kidneys (Compas, 2006; Gunnar & Quevedo, 2007). Epinephrine and norepinephrine can bind to several adrenergic and noradrenergic receptors in the body (not crossing the brain barrier in a significant degree) and thus the effect depends on receptor and tissue type (Gunnar & Quevedo, 2007; Kudielka & Wüst, 2010). These activations of receptors lead to rapid increase in heart rate, respiration, blood flow, blood pressure, glycolysis, inhibition of insulin secretion, and decreased activity in digestive system (Chrousos & Gold, 1992; Compas, 2006).

Coordinated response of HPA axis gets activated somewhat more slowly after detected stress. The process is started by activation of neurons in paraventricular nucleus of the hypothalamus, which release corticotropin-releasing hormone (CHR) and arginine vasopressin (AVP) (Lupien et al., 2009). These in turn activate pituitary gland, attached to the bottom of the hypothalamus, that releases adrenocorticotrophic hormone (ACTH) that travel through blood to adrenal cortex, attached above kidneys, to trigger production of glucocorticoids (GCs); steroids including cortisol (Lupien et al., 2009). ACTH also activates adrenal medulla, attached below adrenal cortex, triggering production of adrenalin and noradrenalin (Lupien et al., 2009). GCs have wide range of effects in the body for example in foetal development and programming, upregulation of anti-inflammatory proteins, stimulation of glycogenesis, inhibition of glucose uptake, \cap -shape effects on vigilance, memory, learning as well as brain structure (Chrousos & Gold, 1992; Lupien et al., 2009). Once GCs are released from adrenal cortex, they also have the ability to regulate the HPA axis stress reaction by regulating further ACTH and CHR release (Lupien et al., 2009). This regulation is often referred as negative feedback loop (Lupien et al., 2009). Briefly, GCs bind

to the glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) that are located at almost all cells, also cells in the various levels in HPA axis including frontal cortex, hippocampus, hypothalamus and pituitary gland, thus activated GRs and MRs can regulate gene expression of the cell, for example downregulate CHR and ACTH and pro-inflammatory cytokine secretion (Lupien et al., 2009).

Immune system, SAM and HPA axis are tightly associated. Both SAM and HPA axis activation can modulate immune system functioning and immune system also takes part in SAM and HPA axis regulation (Glaser & Kiecolt-Glaser, 2005). For example, noradrenalin, adrenalin, epinephrine and norepinephrine produced by activation of stress systems cause increase in circulating levels of proinflammatory cytokines IL-6 and IL1-beta (Cattaneo et al., 2015; Padgett & Glaser, 2003; Steptoe, Hamer, & Chida, 2007) and inflammatory cytokines including tumour necrosis factor- α , IL1 and IL6 can further activate HPA axis (Flier, Underhill, & Chrousos, 1995). However glucocorticoids, including cortisol produced also by HPA activation, can also inhibit inflammation in various levels including leukocyte traffic, function and production of cytokines and mediators of inflammation by activating glucocorticoid receptors and the negative feedback loop of stress system (Flier et al., 1995).

Long term unbalance in immune system between the accumulating and inhibiting systems can cause elevated inflammatory state that have several harming effects for the body (Padgett & Glaser, 2003). Chronic stress has shown to cause mild inflammation and insulin resistance measured by increase monocyte accumulation (cells capable of move quickly and elicit immune response in site of inflammation), accumulation of free fatty acids (inhibiting insulin-stimulated glucose uptake), proinflammatory cytokines (cytokines that promote inflammation) and reduced adiponectin (protein involved regulating glucose levels) (Uchida et al., 2012).

In addition, chronic stress-related proinflammatory cytokines has shown to cause oxidative stress in a cell (Biswas, 2016; Mittal, Siddiqui, Tran, Reddy, & Malik, 2014). Oxidative stress means the imbalance in the relative levels of oxidants and antioxidants, in which oxidants are in excess (Thomas, 2013). In oxidative stress antioxidants capacity to either remove these toxic substances or rapidly repair the damage caused by their production is exceeds (Thomas, 2013).

Reactive oxygen species (ROS) is a collective term including both oxygen radicals, that have one or more unpaired electrons in the outset orbit layer, and certain non-radicals that either are oxidizing agents or are easily converted into radicals, and thus, vary in chemical reactivity (Biswas, 2016). The most common oxidants include ROS that are natural by-products of aerobic metabolism (Thomas, 2013). In low dosage ROS also have important role in cell signalling (Martin & Barrett, 2002; Thomas, 2013). Antioxidants, in which catalase and superoxide dismutase are the most common, can be defined as molecules that delay, prevent, or remove oxidative damage in a cell (Thomas, 2013).

Oxidative stress is associated with inflammation as oxidative stress can influence intracellular signalling and accelerate the production of proinflammatory cytokines that trigger inflammation (Floyd et al., 1999; Mittal et al., 2014). As an expected consequence, chronic stress has shown to cause oxidative stress in a cell as well as cause oxidative damage to cells lipids, proteins and DNA (Aschbacher et al., 2013; Colaianna et al., 2013).

1.1.3 Stress-related factors and aging-related diseases

Stress-related factors in the present thesis are defined to include personality dimensions, mental disorders, early life stress, sleep apnea, and HPA axis reactivity. Closely related to these five main factors, we also included depressive symptoms and traumatic experiences across lifespan. All of these factors are related to stress and can cause stress, body's need for adaptation. In addition, most of these stress-related factors have been related to stress in a bidirectional manner; in addition to causing stress, stress is playing a role in the development and modifying the factor itself. Early life stress and traumatic experiences throughout lifespan can most clearly be seen as stress exposures and mental disorders, depressive symptoms and HPA axis reactivity as responses to stress.

It is well established that maladaptive stress responses as well as chronic stress poses an elevated risk for psychiatric disorders as well as aging-related disorders including chronic infections, autoimmune diseases, coronary heart diseases, hypertension and type 2 diabetes (Cohen & Williamson, 1991; Cohen et al., 2007; Pouwer et al., 2010; Rozanski et al., 1999; Spruill, 2010; Stojanovich &

Marisavljevich, 2008; Wright et al., 1998). All of the stress-related factors in the present thesis are also associated with aging-related physical diseases and to some extent with early mortality. These associations are briefly reviewed in the next section.

1.1.3.1 Personality dimensions

Personality is a combination of relatively stable traits in feeling, thinking and behaving (McCrae & Costa Jr, 1997; Roberts & DelVecchio, 2000). There are many ways to define personality, but among the most popular classifications, that have gathered increasing consensus in personality research, is the five factor model (FFM) (McCrae & Costa Jr, 1997). FFM divides personality into five continuous dimensions: neuroticism, extraversion, openness to experience, agreeableness and conscientiousness (McCrae & Costa Jr, 1997).

FFM dimensions have been associated with stress reactivity both in HPA axis and SAM axis level. Higher neuroticism, lower agreeableness and openness to experiences have been associated with smaller cortisol and cardiac reactions to psychosocial stress (Bibbey, Carroll, Roseboom, Phillips, & de Rooij, 2013). Lower extraversion and higher neuroticism are also associated with lower autonomic nervous system responses to physical stress (LeBlanc, Ducharme, & Thompson, 2004). In addition, higher conscientiousness has been associated with lower levels and higher neuroticism with higher levels of inflammation (Sutin, Terracciano et al., 2010).

Increasing evidence suggests that the FFM personality dimensions are associated with mental and physical morbidity as well as mortality. Conscientiousness is associated most widely with reduced probability and neuroticism with increased probability of variety of mental and physical diseases. Higher conscientiousness reduce the risk for major depression, substance use disorder, stroke, type 2 diabetes, and hypertension (Goodwin & Friedman, 2006; Jokela et al., 2014; Kotov, Gamez, Schmidt, & Watson, 2010). Subjects with higher neuroticism, on the other hand, have greater risk for major depression, substance use disorder, stroke, arthritis, chronic widespread pain, angina, and cardiovascular disease (Charles, Gatz, Kato, & Pedersen, 2008; Kotov et al.,

2010). In a community sample one standard deviation increase in neuroticism was associated with 15% greater chance of having metabolic syndrome (higher waist circumference, triglycerides, lipoprotein cholesterol density, blood pressure, and fasting glucose) where one standard deviation increase in agreeableness and conscientiousness were associated with 25% and 20% reduction in metabolic syndrome respectively (Sutin, Costa Jr et al., 2010).

The evidence for associations between personality traits and mortality is also strongest for conscientiousness. It has been shown that lower levels of conscientiousness measured either in childhood, across adulthood or in older age predict earlier death (Friedman et al., 1993; Hill, Turiano, Hurd, Mroczek, & Roberts, 2011; Jokela et al., 2013; Kern & Friedman, 2008; Martin, Friedman, & Schwartz, 2007; Taylor et al., 2009; Terracciano, Lockenhoff, Zonderman, Ferrucci, & Costa, 2008; Weiss & Costa Jr, 2005; Wilson, Mendes de Leon, Bienias, Evans, & Bennett, 2004). There is also evidence that lower levels of extraversion (Wilson et al., 2005) agreeableness (Weiss & Costa Jr, 2005), and openness to experience (Taylor et al., 2009) may be associated with earlier mortality. Studies on neuroticism and mortality have been more contradictory. Higher neuroticism has been shown to predict all-cause mortality in some (Terracciano et al., 2008; Wilson et al., 2005), but better survival in other (Korten et al., 1999; Weiss & Costa Jr, 2005) studies. Also sex-specific associations between neuroticism and mortality have been reported. Neuroticism has found to predict mortality in women but not in men (Friedman et al., 1993).

In sum, higher neuroticism seem to be the greatest risk factor and higher conscientiousness the greatest protective factor for physical morbidity and mortality.

1.1.3.2 Mental disorders

Mental disorders include different mental health conditions that can affect mood, thinking, behaviour and quality of life. Mental disorders are most typically classified by one of the two coding systems; International Classification of Diseases (ICD) published by the World Health Organization or Diagnostic and Statistical Manual of Mental Disorders (DSM) published by the American

Psychiatric Association (Wakefield, 1992). The current versions in use in 2016 are ICD-10 and DSM-V (American Psychiatric Association, 2013; World Health Organization, 1992).

Mental disorders and stress are tightly related. Psychosocial stress, in which life events have been the most studied, have role in onset and course of mental illnesses including depression, anxiety, schizophrenia, substance use disorders, personality disorders (Battle et al., 2004; Brady & Sinha, 2005; Herbert, 1997; Slavich & Irwin, 2014; Walker & Diforio, 1997). Several hypotheses for underlying mechanisms between stressful life events and mental disorders exist. These hypotheses are associated with stress-related activations of the HPA and SAM systems that can cause altered gene expression, receptors, neurotransmitters, volume of structures in the brain, increased production of proinflammatory cytokines, glucocorticoids and neuropeptides (Doom & Gunnar, 2013; Herbert, 1997; Lupien et al., 2009). High levels of glucocorticoids are shown to induce severe mood changes and neuropeptides, such as corticotropin releasing factor (CRF), can alter fear conditioning, impair memory consolidation, and number of executive functions including cognitive flexibility (Wolkowitz, 1994; Bangasser & Kawasumi, 2015). In addition, cytokines, (IL-1 β , IL-6, TNF- α), in which production is increased by activation of stress systems HPA and SAM, are able to activate central nervous system by passing thru blood–brain barrier and by stimulating nerve fibers in the vagus nerve (which relays information to brain systems that regulate mood, motor activity, motivation, sensitivity to social threat) causing sad mood, anhedonia, fatigue, psychomotor retardation, altered appetite and sleep, and social-behavioural withdrawal (Slavich & Irwin, 2014). In addition, prolonged activation of the HPA and SAM systems can become self-promoting over time due to neuro-inflammatory sensitization and therefore remaining active long after an actual threat has passed (Slavich & Irwin, 2014). Thus in sum, arousal of the stress systems can increase the risk for mental disorders, but the process can be bidirectional and mental disorders can also contribute to stress system activation.

Mental disorders including depression, anxiety, schizophrenia, substance use disorders and personality disorders increases the risks of typical non-communicable diseases including stroke, cardiovascular diseases and diabetes

(Frankenburg & Zanarini, 2004; Hennekens, 2005; Krishnan, 2005; Penninx, Milaneschi, Lamers, & Vogelzangs, 2013; Rugulies, 2002; Spencer & Hutchison, 1999; Vogelzangs et al., 2010).

It is well established that mental disorders increase the risks of all-cause (Harris, 1998; Miller, 2006) as well as cardio-metabolic mortality (Colton & Manderscheid, 2006; Kawachi, Sparrow, Vokonas, & Weiss, 1994; Miller, 2006; Mitchell & Lawrence, 2011). Patients with severe mental disorders including psychotic disorders and major mood disorders die on average 25 years earlier than general population (Viron & Stern, 2010). Cardiovascular diseases have shown to account for 50-60% of the deaths in the mental disorder patient group (Viron & Stern, 2010).

1.1.3.3 Early life stress

Early life stress and traumatic life events are psychosocial stressors that, at least in part, seem to have cumulative or sensitizing effects in which reoccurrence of traumatic experiences causes' progressive amplification of the response (Doom & Gunnar, 2013; Lupien et al., 2009). Depending on the traumatic event, individual and contextual factors as well as developmental stage, traumatic life event associate with altered; either hyper- or dampened- HPA axis stress reactivity (Doom & Gunnar, 2013; Heim et al., 2000; Lupien et al., 2009; Miller, Chen, & Zhou, 2007; Tyrka et al., 2008), also in the cohort used in the present thesis (Pesonen et al., 2010). The reasons for hyper- vs. hypo reactivity remain unsolved, yet the presence and chronicity of current mental disorder as well as the severity and accumulation of early and later traumatic experiences may play a role in reactivity phenotype (Shea, Walsh, MacMillan, & Steiner, 2005). Exposure to early life stress (ELS) has also been associated with higher levels of proinflammatory cytokines (Danese, Pariante, Caspi, Taylor, & Poulton, 2007; Danese et al., 2009).

Mounting empirical evidence suggests ELS and cumulative traumatic experiences increases the risks of aging-related diseases including cardiovascular diseases, type 2 diabetes, autoimmune diseases, and hypertension (Dube et al., 2009; Wegman & Stetler, 2009), also in the cohort used in the present thesis

(Alastalo et al., 2013; Alastalo et al., 2009). Severe ELS also poses a risk for psychiatric disorders as well as milder psychiatric symptoms, including depressive symptoms and externalizing behavior problems (Afifi, Brownridge, Cox, & Sareen, 2006; Danese et al., 2009; Green et al., 2010; Kendler, Karkowski, & Prescott, 1999; Lahti et al., 2012; McLaughlin, Conron, Koenen, & Gilman, 2010; Read, Os, Morrison, & Ross, 2005), also in the cohort used in the present thesis (Räikkönen et al., 2011).

There is also some evidence that accumulation of stressful life events may cause higher risk for all-cause mortality and that the death of a spouse may cause moderately higher risk for death by chronic ischemic heart disease, especially in men (Martikainen & Valkonen, 1996; Rosengren, Orth-Gomer, Wedel, & Wilhelmsen, 1993).

1.1.3.4 Sleep apnea

Sleep apnea is classified by ICD - 10 as recurrent apneas during sleep despite persistent respiratory efforts due to upper airway obstruction (American Psychiatric Association, 2013). In addition, sleep apnea often contains cardiac arrhythmias, elevation of systemic and pulmonary arterial pressures, as well as frequent partial arousals throughout sleep, resulting in sleep deprivation and daytime sleepiness (American Psychiatric Association, 2013).

Sleep apnea is associated with activations and alterations in stress response systems. Increased SAM and HPA axis activity, catecholamine CRH and cortisol release as well as elevated nocturnal cortisol levels are shown to accompany sleep apnea (Buckley & Schatzberg, 2005; Narkiewicz & Somers, 1997; Späth-Schwalbe, Gofferje, Kern, Born, & Fehm, 1991). These are believed to mainly be caused by repeated hypoxia as well as arousals during the night (Buckley & Schatzberg, 2005; Narkiewicz & Somers, 1997).

Sleep apnea has shown to also pose an elevated risk for cardiovascular disease and type 2 diabetes (Botros et al., 2009; Lam & Ip, 2007; Peker, Hedner, Norum, Kraiczi, & Carlson, 2002) as well as increased risk for all-cause and cardiac mortality (Gami et al., 2013; Marshall, Wong, Cullen, Knuiman, & Grunstein, 2014).

1.1.3.5 HPA axis reactivity

The fifth stress-related factor in the present thesis is HPA axis reactivity. As described in more detail in chapter 1.1.2., HPA axis is one of the main component in the stress system. One of the most widely used stress reactivity test is The Trier Social Stress Test (TSST) that is well-standardized psychosocial stress test shown to elicit a powerful HPA axis response (Kirschbaum, Pirke, & Hellhammer, 1993).

Altered HPA reactivity is shown to associate with mental as well as aging-related physical disorders including major depression, post traumatic disorder, anxiety, obesity, hypertension, coronary artery calcification and type 2 diabetes (Hamer & Steptoe, 2011; Hamer, Endrighi, Venuraju, Lahiri, & Steptoe, 2012; Seldenrijk, Hamer, Lahiri, Penninx, & Steptoe, 2012; Shea et al., 2005; Steptoe et al., 2014).

1.1.4 Telomeres – an underlying mechanism between stress and aging-related diseases?

It has been suggested that stress may influence physical health and mortality through different pathways including behavioural, physiological and genetic (Cohen et al., 2007; McEwen, 2008). These pathways include 1) unhealthy lifestyle such as smoking and physical inactivity, 2) altered/increased stress-related HPA and SAM activity that can change variety of physiological processes including immune, metabolism, cardiovascular and central nervous system functioning, and 3) epigenetic changes for example in glucocorticoid receptor related genes) that may cause sensitivity or prolonged effects for stressful stimulus that all can play a role in disease prognoses and early mortality (Cohen et al., 2007; McEwen, 2008). In addition genetic background, such as specific single nucleotide polymorphisms (SNPs), may modulate the effect of stress by causing insufficient cortisol recovery after psychosocial stress (Ising et al., 2008). Mechanisms between the stress-related factors and aging-related non-communicable diseases and mortality are however not fully understood.

The study by Epel et al. in 2004 was the first to show that psychological stress triggered by caregiving was associated with shorter telomere length, measured at

the end of the peripheral blood mononuclear cell chromosomes (Epel et al., 2004). Their study started a new period in telomere biology research field and encouraged other research groups to examine associations between psychological, behavioural and stress-related factors and LTL. The aim of this thesis is to expand the scant literature, and to study whether stress-related factors associate with telomere length, a novel biomarker of cellular aging.

1.2 Telomeres

1.2.1 Definition and basic functions

Telomeres are specialized repeated non-coding DNA-protein complexes (TTAGGG) that are located at the end of eukaryotic chromosomes (Figure 2) (Blackburn, 2000). Telomere length in humans vary between 500-15000 base pairs (Aubert, 2008; Samassekou, Gadji, Drouin, & Yan, 2010). Telomere complexes don't include genetic information for protein coding, yet they are key factors in several important functions related to genome stability and lifespan of a cell.

In somatic cells the ends of the chromosomes are not fully replicated during DNA synthesis, resulting in the shortening of DNA molecules with each cell division; this inability to fully replicate the 3' end of DNA is often referred as the "end replicating problem" (Aubert & Lansdorp, 2008). Without the non-coding telomere strands in the ends of chromosomes, important genetic material would be lost in replication process. Human telomeres shorten by 40-1500 base pairs by each cell division (Harley, Futcher, & Greider, 1990; Martens, Chavez, Poon, Schmoor, & Lansdorp, 2000; Notaro, Cimmino, Tabarini, Rotoli, & Luzzatto, 1997).



Figure 2. Telomeres (white) are located at the end of chromosomes. (National Institute of General Medical Science, National Institutes of Health. “How Chromosomes are Protected by Telomeres”. Licensed under: CC BY 2.0 (<https://creativecommons.org/licenses/by/2.0/>)).

Hayflick was the first one to show in human cell cultures that cells can carry out a limited number of divisions (Hayflick, 1965). He showed *in vivo* that human cells can divide 50-70 times before division limit is reached, and the cell is forced into permanent proliferative arrest called cellular senescence (Hayflick, 1965). This division limit has become known as *Hayflicks limit*. Hayflick’s findings revealed that cellular senescence depends more on the replicative history than chronological age of the cell. As telomere length shortening prevent endless cell divisions, and telomere length can be used *in vivo* to predict the cells replicative potential, telomeres have been called cellular or mitotic clocks (Allsopp et al., 1992; Aviv, 2009). More recent studies have shown that telomeres are dynamic structures that also have other important functions.

Telomere repeats form a molecular platform containing binding sites for telomeric DNA-sequence-specific binding proteins (Figure 3) (Blackburn, 2005). There are six main telomere binding proteins that are necessary for forming the sheltering telomere loop: TRF1, TRF2, Rap1, TIN2, TPP1 and POT1 (Sarek, Marzec, Margalef, & Boulton, 2015). If the telomere length sequence is long enough, 50-300 nucleotides long G rich single stranded telomere repeat at the end of the telomere tucks into the double-stranded section, and together with the binding proteins they form a protective capping, t-loop, in the end of chromosome (Blackburn, 2000; Griffith et al., 1999). In absence of telomerase enzyme minimum 12.8 telomere repeats (TTAGGG) has shown to be enough for telomeres to be able to prevent chromosome fusion (Capper et al., 2007). However, in telomerase positive cancer cells only 7 repeats of telomere sequence is needed to form protective t-loop (Xu & Blackburn, 2007). If telomere length in a chromosome becomes critically short, the protective telomere loop cannot be formed and telomere remains uncapped triggering pathways leading to cellular senescence (Blackburn, 2005).

T-loop prevents the chromosome to be detected as a DNA damage and hence the activation of genome instability-promoting processes (Blackburn, 2005; Lindqvist et al., 2015). It is enough that the shortest telomere in a cell becomes critically short to trigger the senescence responses (Hemann, Strong, Hao, & Greider, 2001). However shorter average telomere length is associated with increased probability of critically short telomeres and telomere dysfunction (Blackburn, 2000; de Lange, 2002) and thus measuring average telomere length can be useful. T-loop also prevents chromosome end-to-end fusion with another telomere sequences or broken DNA (Blackburn, 2005). The end-to-end fusion would seriously disturb the protein coding processed of the cell (Blackburn, 2005). Telomeres are also shown to detect and react to genotoxic stress, for example to cancer related oncogene activity, and by becoming dysfunctional telomeres are capable of activating cells senescence processes that in turn prohibits future cell division of possibly cancerous cells (Suram & Herbig, 2014). Telomeres also play a role in movement, and anchoring of the chromosomes to the nuclear membrane during cell division (Ozturk, 2015).

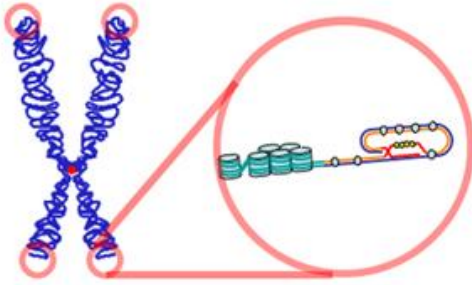


Figure 3. Telomeres are located at the end of chromosomes and together with telomere binding protein they form a protective t-loop. ("Telomere". Licensed under CC BY-SA 3.0 (<http://creativecommons.org/licenses/by-sa/3.0>))

1.2.2 Shortening and lengthening

As conserving long enough telomere length is crucial for cells, telomere length shortening is counter balanced by the telomerase enzyme that can synthesize DNA nucleotides onto telomeres, and thus lengthen the telomeric DNA (Blackburn, 2005). Telomerase is a specialized cellular ribonucleoprotein reverse transcriptase that contains protein TERT and RNA component TERC (Blackburn, 2005). TERT reverse transcribes telomere DNA using TERC as a template (Figure 4). Telomerase activity is low in human fibroblast and therefore telomerase is insufficient to prevent telomere shortening, uncapping or cell senescence in most cells (Blackburn, 2005; M. Kim, Xu, & Blackburn, 2003). However, the small amount of telomerase is not negligible, as if the telomerase activation is shut down completely cells have shown to face premature senescence (Blackburn, 2005; M. Kim, Xu, & Blackburn, 2003). Higher telomerase activity is found in malignant and tumorous cells, embryonic cells, stem cells, male germ cells and activated lymphocytes (Cong, Wright, & Shay, 2002; Counter, Gupta, Harley, Leber, & Bacchetti, 1995; N. W. Kim et al., 1994; Wright, Piatyszek, Rainey, Byrd, & Shay, 1996).

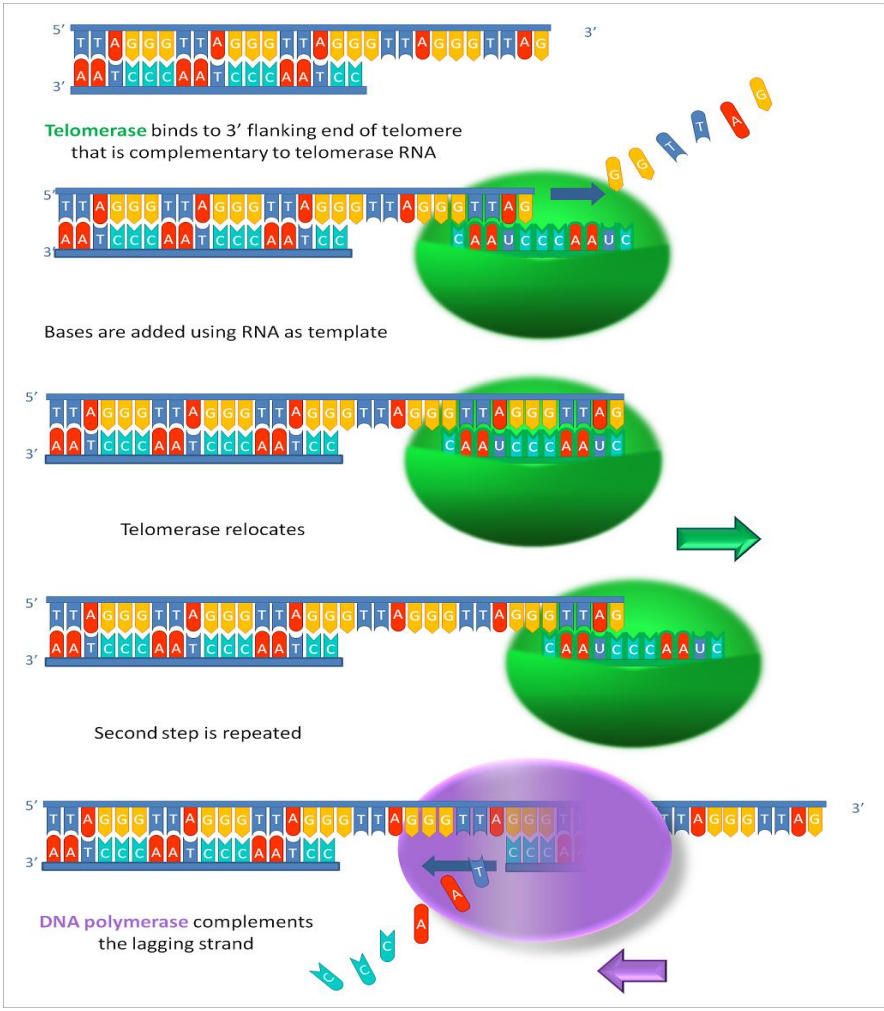


Figure 4. Telomerase synthesizes DNA nucleotides onto telomeres, and thus lengthens the telomeric DNA (Fatma Uzbas. “Working principal of telomerase” Licensed under: CC BY-SA 3.0 (<http://creativecommons.org/licenses/by-sa/3.0>)).

Telomerase activity regulation is a complex process occurring at various levels including transcription, maturation, modification, transport and localization of protein TERT and its RNA platform TERC (Cong et al., 2002). Telomerase activity is modulated in cells growth-, differentiation-, extracellular and intracellular signalling processes (Cong et al., 2002). It’s worth pointing out that

telomerase can lengthen only uncapped telomeres (Blackburn, 2001). As the telomere length shortens the probability of uncapping gives telomerase chances to act (Blackburn, 2001). Animal studies have shown that reintroducing telomerase into telomerase knock out mice with short telomeres, the mice restored their average telomere length fast (Hemann et al., 2001). This notion has raised the suggestion that telomerase may have more important role in restoring the shortest, rather than the average, telomere length (Hemann et al., 2001). Telomerase activation is not the only method for telomere elongation. Some cells that lack telomerase activity can use recombination based DNA replication method known as alternative lengthening of telomeres (ALT) (Giardini, Segatto, da Silva, Nunes, & Cano, 2014). In addition to these, there are other less studied pathways involved in telomere length maintenance; interplay among telomere binding proteins and noncoding RNA that arise from subtelomeric region (Giardini et al., 2014).

Due to these lengthening processes, LTL is dynamic structure capable of shortening and lengthening. In cross sectional level older people have shorter LTL (Frenck, Blackburn, & Shannon, 1998; Harley et al., 1990). In humans LTL is around 10 000 – 15 000 base pairs at birth and shortens at the rate of 40 – 1500 base pairs per cell division (Harley, Futcher, & Greider, 1990; Martens, Chavez, Poon, Schmoor, & Lansdorp, 2000; Notaro, Cimmino, Tabarini, Rotoli, & Luzzatto, 1997). Telomere length erosion, however, is not linear across lifespan (Aubert, Baerlocher, Vulto, Poon, & Lansdorp, 2012). Telomere shortening has shown to be the most rapid in the first year of life, become moderate between years 1-18 and to have more stable stage between years 18-50 and then shorten in moderate speed again after the age of 50 years (Aubert et al., 2012). Longitudinal studies in humans have shown that the LTL change is dependent upon baseline LTL, so that the individuals with shortest average LTL in baseline are the ones who's LTL lengthen the most often (Farzaneh-Far et al., 2010; Nordfjäll et al., 2009). In addition, LTL vary greatly between individuals, and it's not unusual that in individual level several decades older person may have longer LTL than a much younger comparison (Aubert & Lansdorp, 2008).

1.2.3 Telomere biology disorders

Maintaining sufficient telomere length have been shown to be crucial for organism. Mutations in telomere related genes have shown to cause telomere biology disorders (for example dyskeratosis congenital and Hoyeraal-Hreidasson syndromes) that are characterized by critical changes especially in highly profligate tissues and organs (Sarek et al., 2015). The incapability to maintain sufficient telomere length leads to critically shortened telomeres, accumulation of senescence cells and apoptosis as well as extensive loss of stem cells (Montanaro, Tazzari, & Derenzini, 2003), causing aging-related phenotypes including altered skin pigmentation, nail dystrophy, pulmonary fibrosis, osteoporosis, liver diseases, immunodeficiency, bone-marrow failure, growth retardation, developmental delays, cancer and early mortality (Sarek et al., 2015).

1.2.4 Telomeres, oxidative stress, inflammation and cortisol

Telomere length shortens in each cell division, however the amount of lost TTAGGG repeats varies. Higher oxidative stress levels in cell causes greater telomere loss during cell replication (Houben, Moonen, van Schooten, & Hageman, 2008). Oxidative stress have shown to shorten the telomere length dose-dependently (Kawanishi & Oikawa, 2004). As oxidative stress is so strongly associated with telomere shortening, it has even been suggested that telomere driven replicative senescence is primarily an oxidative stress response (von Zglinicki, 2002). It has been shown in vitro that treating human fibroblast with substances that reduce oxidative stress (vitamin C, free radical scavenger or tetrapeptide) the telomere erosion can be slowed down and even cells replicative potential can be prolonged (Bar-Or, Thomas, Rael, Lau, & Winkler, 2001; Furumoto, Inoue, Nagao, Hiyama, & Miwa, 1998; von Zglinicki, Pilger, & Sitte, 2000).

Even though oxidative stress and inflammation are interrelated (proinflammatory cytokines can cause oxidative stress in a cell and oxidative stress can influence intracellular signalling and accelerate the production of proinflammatory cytokines that trigger inflammation (Biswas, 2016; Mittal et al., 2014)), the associations between LTL and inflammation are somewhat

contradictory. Inflammation related C-reactive protein (CRP) and cytokine IL-6 levels have associated in shorter LTL in some studies (Deelen et al., 2014; Lu et al., 2014). However even within a single study, shorter LTL was associate with only some inflammation markers, such as CRP and Serum amyloid A proteins but not with other, such as interleukin levels or TNF- α (Wong, De Vivo, Lin, Fang, & Christiani, 2014). Study that have not found associations with CRP nor other inflammation markers also exists (Deelen et al., 2014).

Association studies between LTL and cortisol are scarce. Yet there is some preliminary evidence that cortisol may associate with shorter LTL, as shorter LTL have been associated with higher overnight urinary cortisol values (Epel et al., 2006) and T lymphocytes that have exposed to cortisol show lower telomerase activity (Choi, Fauce, & Effros, 2008). However null study also exists. Révész et al. (2014) did not find an association between LTL and salivary cortisol awakening response or LTL and evening cortisol levels. They however, reported that the highest tertile in salivary cortisol awakening response was associated with shorter LTL (Révész et al., 2014).

1.2.5 Telomere length and cellular senescence

Cells are constantly facing various intra- and extracellular stress and damaging effects, such as genotoxic stress that can either cause damage straight to cellular DNA or increase oxidative stress (Ben-Porath & Weinberg, 2004; Campisi & d'Adda di Fagagna, 2007). Cells responses to stress can vary from full recovery to tumour growth or cell death (Ben-Porath & Weinberg, 2004; Campisi & d'Adda di Fagagna, 2007). In between of these extremes is cellular senescence, in which cells can respond to damaging signals by staying alive but changing their activity so that future cell division is shut down (Childs, Durik, Baker, & van Deursen, 2015; Hayflick, 1965). It has been suggested that cellular senescence processes are beneficial for the organism in reproductive ages, due to their tumour suppressive functions that can remove potentially tumorous cells from cell cycle by preventing the cell for future divisions, but have negative impact on health in later life as the growing number of senescent cells can compromise or deface the

functioning of the related organs and even the whole organism (Campisi, 2001; Childs et al., 2015).

Cell can reach senescence phase by activation on different pathways. The two of the most important pathways are complex and interrelated tumour suppression pathways of p53 and p16INK4a (Beausejour et al., 2003; Campisi, Andersen, Kapahi, & Melov, 2011; Jacobs & de Lange, 2005). Telomeres have important, yet not exclusive, role in activating these pathways. It is well established that telomere dysfunction, that can be caused by uncapped telomeres that no longer are capable of forming protective t-loop, can trigger telomere induced senescence by both p53 and p16INK4a pathway activation (Beausejour et al., 2003; Campisi & Robert, 2014; Jacobs & de Lange, 2005; Karlseder, Broccoli, Dai, Hardy, & de Lange, 1999; Smogorzewska & de Lange, 2002; Suram & Herbig, 2014). Telomere uncapping is one of the key mechanisms in which telomeres become dysfunctional and provoke p53 activation (di Fagagna et al., 2003; Takai, Smogorzewska, & de Lange, 2003).

Briefly, p53 is any of the 15 isoforms of a protein coded by TP53 gene that is located at the chromosome 17 (Vousden & Lane, 2007). p53 is intensively studied as it has significant role in many vital processes including tumour suppression, cellular senescence, cell survival, regulation of oxidative stress, glycolytic pathways (related to glucose metabolism, endurance and overall fitness), cell differentiation, bone remodelling, repair of genotoxic damage (Vousden & Lane, 2007). The main mechanism by which p53 functions is as transcription factor, as it can activate or silence large group of genes but p53 can also interact straight with other proteins in the cell and thus more directly activate cellular senescence or apoptosis processes (Vousden & Lane, 2007). Controlling the level of p53 is vital for the organism as the overexpression leads to death already in early embryotic state (Marine et al., 2006). As earlier noted p53 production can be activated by telomere uncapping and numerous other agents, including DNA damage, chemotoxic stress, oncogene activation, hypoxia, metabolic stress, nutrient deprivation, viral infection, and psychosocial stress (Guo & Cui, 2015; Vousden & Lane, 2007). It is proposed that p53 works in two different ways to respond to stress; in case of mild stress (for example low level DNA damage), p53 activates pathways to protect the cell (activate processes that reduce ROS levels)

and in case of severe stress (for example oncogene activation or radiation), p53 activates apoptosis pathways (Vousden & Lane, 2007). p53 activated senescence/apoptosis processes have also shown to cause accelerated inflammation (IL-6 and IL-8) and oxidative stress (Coppé et al., 2008; Freund, Orjalo, Desprez, & Campisi, 2010; Johnson, Yu, Ferrans, Lowenstein, & Finkel, 1996). The association between p53 activity and mortality is not fully understood as both lower and higher levels of p53 have both positive and negative effects. Findings from animal studies that show that over-activation of TP53 genes leads to premature aging and mortality, but the activation have also shown to inhibit cancer growth and to help cell to do away with toxins (Christophorou, Ringshausen, Finch, Swigart, & Evan, 2006; Christophorou et al., 2005; Tyner et al., 2002; van Heemst et al., 2005). Also the reduction of TP53 genes have shown to cause both; increase the cancer risk as well as prompt longevity (Christophorou, Ringshausen, Finch, Swigart, & Evan, 2006; Christophorou et al., 2005; Tyner et al., 2002; van Heemst et al., 2005). It is worth pointing out that in the mice that lack telomerase activity, the loss of tissue regeneration (hair loss, hair graying, decreased wound healing) is restored if p53 is ablated (Flores & Blasco, 2009). This finding underlines the importance of p53 in aging-related phenotypes.

p16INK4a is also a telomere damage activated protein that is able to activate cells senescence and apoptosis pathways (Jacobs & de Lange, 2005). However, compared to p53, p16INK4a increases more slowly in cell culture after telomere damage and it is typically activated in cells that lack the normal p53 functioning, thus raising suggestion that p53 is the dominant and p16INK4a secondary effector pathway for telomere damage (Jacobs & de Lange, 2005; Beausejour et al., 2003).

It is also worth pointing out that non-telomeric induced senescence occurs as well. It is shown that DNA damage (double strand breaks and losing electrodes), disruptions to a chromatin organization (DNA and its packing proteins), activation of oncogenes (mutated genes that have the potential to cause cancer), strong or persistent mitogenic signals (presence of chemical substances that are able to trigger mitoses), and several types of cellular stress (for example oxidative stress) can force cell to senescence by activating p53 and/or p16 pathways

(Besson & Yong, 2001; Campisi et al., 2011; Campisi, 2000; Campisi & Robert, 2014).

Even though senescent cell has stopped dividing it remains active, yet with widespread changes in volume, shape, protein expression and secretion (Coppe et al., 2008; Coppe, Desprez, Krtolica, & Campisi, 2010). It has been shown that large number of senescent cells turn into senescence associated secretory phenotype (SASP) (Campisi & Robert, 2014). This phenotype of senescent cells can influence its surroundings significantly by increasing the level of signalling factors (including interleukins IL-6 and IL-1, chemokines (signalling proteins involved with cell movement in microenvironment) and insulin-like growth factor), extracellular proteases (enzymes that break down proteins into amino acids), and extracellular matrix (molecules in extracellular liquid that provide structural and biochemical support to the surrounding cells; for example ROS) (Coppe et al., 2010). These changes that SASP has on its surrounding microenvironment may be beneficial for organ and organism functioning in short term by activating tissue repair processes, but as a chronic condition these changes have harmful effects and it has been suggested that SASP induced changes in cellular environment is one of the key link between senescent cells and aging-related diseases (Campisi & Robert, 2014; Coppe et al., 2010).

1.2.6 Differences in telomere length between tissues

Average telomere length and the loss of telomere length across lifespan varies between individuals, tissues, cells and chromosomes (Aubert & Lansdorp, 2008). Information on telomere length in different tissues in humans is still limited. Correlative studies between blood cell telomeres and other tissues are often collected in the context of various diseases, thus the influence of the disease cannot fully be ruled out (Aubert & Lansdorp, 2008). Post mortem correlative study in humans, aged between 29 weeks to 88 years, showed that from twelve different tissues (peripheral blood leukocytes, liver, kidney, heart, spleen, brain, skin, triceps, tongue mucosa, skeletal muscle, subcutaneous fat and abdominal fat), leukocytes have the highest telomere length (TL) variability and brain tissues the lowest variability between subjects (Dlouha, Maluskova, Kralova Lesna,

Lanska, & Hubacek, 2014). Between the twelve studied tissues, leukocyte TL correlated with muscle and liver tissues but not with the other tissues (Dlouha et al., 2014). It has been shown that there is very little age dependent telomere shortening in less proliferating tissues (Gardner et al., 2007). This could partly explain the absence of correlation in TL between less and more proliferating tissues. An animal study has shown that telomere length was similar across brain, liver, testis, and spleen tissues in new-born but differed in adult mice (Prowse & Greider, 1995), suggesting that the different number of cell divisions between tissues may also cause differences in TL in adult, but not yet in new-born mice. However it has to be kept in mind that telomere length comparisons between the species is somewhat precarious as for example mice and monkeys have much longer TL and active telomerase production in many somatic cells compared to humans, whose telomerase levels in somatic cells is very limited (Kakuo, Asaoka, & Ide, 1999; Prowse & Greider, 1995).

In contrast, correlations in TL between proliferating tissues and blood vessel tissues in humans have shown to be high (De Meyer, Rietzschel, De Buyzere, Van Criekinge, & Bekaert, 2011). The correlation coefficient between TL in white blood cells, umbilical artery and skin is $r > 0.89$ in newborn (Okuda et al., 2002). Also in subjects at age of 73-95 years the associations between leukocyte TL and skin and synovial tissue TL is high (Friedrich et al., 2000). The correlation between blood cells, buccal cells and fibroblast has also shown to be high ($r > 0.65$) in patients with inherited bone marrow failure syndrome (Gadalla, Cawthon, Giri, Alter, & Savage, 2010). Also sperm TL and LTL are strongly correlated, yet sperm TL being generally longer than LTL (Aston et al., 2012; Ferlin et al., 2013).

Within the blood cells, telomere length is also very coherent. Briefly, all human blood cells originate from the hematopoietic stem cells (Wognum, Eaves, & Thomas, 2003), that are rare, one in 100 000 bone marrow cells (Hoffman, 2013). It has been suggested that hematopoietic stem cells are capable of dividing less than 100 times (Lansdorp, 1997). Telomerase enzyme is active in hematopoietic stem cells, however, the amount of enzyme is not capable of maintaining the telomere length over time (Aubert et al., 2012). White blood cells, that also are called leukocytes, are composed by five different cell types including lymphocytes (dendritic, T-, B-, and natural killer cells), monocytes, basophils,

neutrophils and eosinophils (Hoffman, 2013). The difference in telomere length between different leukocytes has shown to be small (Kimura et al., 2010). It has been shown in longitudinal analyses of human subjects aged 20-90 years that similar changes (lengthening, no change and shortening) in telomere length are present in 5 and 12 year follow up for T-cells, B-cells and monocytes (Y. Lin et al., 2015). Also the correlation between granulocytes and all leukocyte telomere length has shown to be as high as $r = 0.979$ (Kimura et al., 2010). Telomere length in lymphocytes and granulocytes are also highly ($r > 0.93$) correlated with telomere length in hematopoietic progenitor cells (Kimura et al., 2010).

In sum, it has been suggested that regulation of telomere length is partly tissue-independent and TL in proliferating tissues may serve as a proxy of telomere length in other proliferating tissues (Friedrich et al., 2000). However the generalization of TL from less proliferating tissues to more proliferating tissues, especially in later adulthood, is not supported by previous literature.

1.2.7 Differences between telomere length measurements

There are several ways to measure telomere length. Average telomere length is typically measured with telomere restriction fragment (TRF) analyses by Southern blot, quantitative polymerase chain reaction (qPCR) or fluorescence in situ hybridization (Flow-FISH) and individual telomere length with single telomere length analyses (STELA), Quantitative fluorescence in situ hybridization (Q-FISH) or 3D telomere fluorescence in situ hybridization (Samassekou et al., 2010). There are several advantages and limitations in each measurement technics. From the methods to measure average telomere length TRF method was one of the earliest technics and it has become the so called golden standard of TL measurement (Montpetit et al., 2014). It however requires large amount of DNA (500 - 5000 ng) and is labour intensive to use, making it time and money consuming (Cawthon, 2002; Montpetit et al., 2014). In addition, due to the restriction enzymes used, TRF measures also includes the subtelomeric DNA to the measurement, thus overestimating the real telomere length and making it difficult to compare to other studies that have used other methods for telomere length measurement (Montpetit et al., 2014). Advantage of qPCR

compared to TRF is that it only measured the actual telomere length, it requires much smaller amounts of DNA (< 40 ng) and labour, thus making it common and useful in large epidemiological studies (Cawthon, 2002; Montpetit et al., 2014). One of the main limitation of using qPCR is variations within and between the batches (Montpetit et al., 2014). Advantage of Flow-FISH, in turn, is that it can be used for cell type specific mean telomere length measurement, and as limitations, it is labour intensive, it requires high laboratory skills and flow sorting equipment (Aubert, Hills, & Lansdorp, 2012; Montpetit et al., 2014). In addition, unfixed cells can be challenging to process with Flow-FISH as the technique is sensitive to fixatives to reserve the cells, therefore it has been suggested that the method is more useful in samples with isolated nuclei rather than intact cells and fresh blood samples rather than stored (Aubert, Hills, & Lansdorp, 2012; Montpetit et al., 2014).

The number of comparative studies between telomere length measurement techniques are scarce. However few exists and the results have been somewhat different. A recent collaborative study compared three different telomere measurement techniques: Southern blotting, STELA and qPCR (Martin-Ruiz et al., 2015). In their study 10 human DNA samples were used for telomere length measurement with three different measurement techniques in 10 different laboratories. Relative telomere length between the laboratories measured with three techniques were highly correlated, rank correlation coefficients being between .63 - .99 (Martin-Ruiz et al., 2015). The correlations between qPCR measurements within the laboratories were $r > 0.79$ (Martin-Ruiz et al., 2015). There are also findings where reproducible result have been good for both qPCR and Southern blot; when same samples were used for telomere length measurement twice in qPCR and twice with southern blotting, the correlation between the two time points were $r > 0.9$ for both of the analyses strategies used (Aviv et al., 2011). However studies with much weaker correlations exists. Gutierrez-Rodriguez et al. compared Southern blot, Flow-FISH and qPCR in both healthy participants and participants suffering from telomere biology disorders and found only modest correlation $r = 0.3$ between telomere length measured with TRF analyses by Southern blot and qPCR in healthy participants and low correlation $r = 0.20$ in participants with telomere biology disorders (Gutierrez-

Rodrigues, Santana-Lemos, Scheucher, Alves-Paiva, & Calado, 2014). Telomere length measured with Flow-FISH and qPCR correlated also only modestly in healthy subjects whereas no correlation was found in LTL in patients within participants with telomere syndrome (Gutierrez-Rodrigues et al., 2014). Flow-FISH and TRF, however, showed correlation of $r > 0.53$ in both healthy and patient groups and as the Flow-FISH detected more sensitively and specifically, compared to qPCR, telomeres below the tenth percentile it has been suggested that flow-FISH is more appropriate measure for telomere length especially in telomere disorder diagnostic settings (Gutierrez-Rodrigues et al., 2014). Another study has also reported only weak correlation $r = 0.27$ between telomeres measured by qPCR and Southern blotting (Elbers et al., 2014).

There is also a study that compared the qPCR and southern blotting methods for telomere length measurement and found in both technics used, older age and male sex associated with shorter LTL, however, only southern blotting found a significant difference in LTL between African American and European decent individuals (Elbers et al., 2014). This finding suggests that smaller differences in LTL may be detected only by southern blotting and as the authors discuss, the higher measurement error may be causing qPCR to be less sensitive to detect small differences in TL than Southern blotting (Elbers et al., 2014). However, put in other way, it may be that some of the weak associations may not be found with qPCR but the results that are found using qPCR technic are more robust.

Different DNA extraction methods have also shown to influence the mean telomere length measured with qPCR (Cunningham et al., 2013). Mean TL was shorter if DNA was extracted by QIAmp when compared to PureGene or phenol/chloroform methods (Cunningham et al., 2013). Also, if DNA from whole blood leukocytes was extracted with Lahiri and Nurnberg method, the final TL was shorter than if extracted with QiaAmp Mini Kit (Denham, Marques, & Charchar, 2014). Telomere length was also shorter if the DNA was extracted with QIAamp DNA Blood Mini Kit from Qiagen compared to the ReliaPrep Large Volume HT gDNA Isolation Kit from Promega (Hofmann et al., 2014). However, there were no significant differences in TL between PureLink Genomic DNA Mini Kit and Lahiri and Nurnberg extracted DNA (Denham et al., 2014). In sum, it has been suggested that these discrepancies between the DNA extraction methods

may contribute to the difference in results between the studies using TL measurements (Cunningham et al., 2013).

Overall there are different advantages and limitations between the telomere length measures techniques and these differences should be considered when choosing the measurement techniques for the study. Differences in techniques as well as to some extent limited correlations between the measurement techniques should also take into account when comparing and interpreting results from different studies.

1.2.8 Factors associating with leukocyte telomere length

Studies and thus knowledge related to telomeres have increased significantly in recent decades. When there were only 439 studies found in PubMed search for “Telomere” in 1991, the number is 4 613 and 18 171 in year 2001 and 2016 respectively. To give a general understanding of the factors influencing and associating with telomere length the following chapter summarize briefly the main findings related to telomere length and its association with genetic and foetal period, aging-related diseases and mortality, socioeconomic and lifestyle factors and the stress-related factors used in the present thesis.

1.2.8.1 Genetic and foetal period

Telomere length is strongly inherited. Meta-analyses of 19 713 subjects suggest that 70% of telomere length is inherited from both mother and father (Broer et al., 2013). Genome wide association studies (GWAS) have identified several SNPs that are related to telomere length (Codd et al., 2010; Levy et al., 2010; Mangino et al., 2012; Pooley et al., 2013). Many of the LTL associated SNPs are in telomere protection or telomerase related genes (for example CTC1, TERC, TERT, OBFC1, TREL1), but associations between other genes related to synaptic transmission (PXX), immune response signalling pathway (NAF1) and gene expression related pathways (ZNF676) are reported (Codd et al., 2010; Codd et al., 2013; Mangino et al., 2012; Pooley et al., 2013). In addition same SNPs that associate with LTL have shown to be associated with higher risk physical diseases including coronary artery disease, cardiovascular disease mortality and several cancers including

lung cancer, testicular stem cell cancer, ovarian cancer and breast cancer (Bojesen et al., 2013; Burnett-Hartman et al., 2012; Codd et al., 2013).

One of the most commonly shared finding in LTL research field is that male sex is associated with shorter LTL. Large meta-analyses of 36 cohorts including 36 230 participants showed that age adjusted telomere length is shorter in men than in women regardless of age and cell type (Gardner et al., 2014). Telomeres are shorter in males than females already at birth (Aubert et al., 2012), yet not all studies have found sex differences in LTL in new-borns (Okuda et al., 2002). Benetos et al., have shown that in same-sex twins, females have longer telomere length than males, however in opposite-sex twins females do not differ from males in telomere length at birth (Benetos et al., 2014). Thus, as the authors suggest, it seems that intrauterine hormonal environment is an important factor explaining telomere length and LTL differences between the sexes in new-borns (Benetos et al., 2014). There is other evidence supporting the importance of intrauterine hormonal environment. Higher maternal estriol (E_3) estrogen levels during early pregnancy (on average 15 gestational weeks) were associated with longer buccal cell telomere length at birth (Entringer et al., 2015b).

Other prenatal factors seem to influence offspring's telomere length. Maternal folate levels in the first trimester of pregnancy associate with longer (Entringer et al., 2015a) and smoking during pregnancy with shorter cord blood telomere length of the new-born (Salihu et al., 2015). Exposure to tobacco smoke in the utero negatively associates with salivary telomere length of offspring at the age of 4-14 years (Theall, McKasson, Mabile, Dunaway, & Drury, 2013). Maternal malnutrition during pregnancy seem not to influence telomere length in a life course perspective, as the offspring whose mothers experienced malnutrition during pregnancy did not differ in LTL from the non-malnourished comparisons at the average age of 68 years (de Rooij et al., 2015).

It is well established that the offspring of an older father have longer telomere length (De Meyer et al., 2007; Kimura et al., 2008; Prescott, Du, Wong, Han, & De Vivo, 2012). This may partly be explained by the finding that telomere length in sperm elongate throughout male lifespan (De Meyer et al., 2007). The effect size of paternal age is shown to be larger than the classical gender effect (De Meyer et al., 2007). To illustrate these differences, when put into general linear

model simultaneously current age of the participant explained 5.3%, gender 1.3% and age of a father 2.6% of the variance of TL respectively (De Meyer et al., 2007). Telomere length of female oocytes, on the other hand, are shorter than in somatic cells and age of the mother is not related to offspring's telomere length (De Meyer et al., 2007; Liu et al., 2007). Telomerase activation is high in foetal period, in weeks 16-20, leading to telomere elongation in most of the tissues (Wright et al., 1996). Recent mice study has shown that paternal spermatozoa telomere length influences the offspring telomere length already in two-cell stage before the telomerase expression had started and that the TL increases more from oocyte stage to zygote stage in the offspring of longer spermatozoa telomeres (de Frutos et al., 2016). This led the authors to suggest that spermatozoa telomere length may act as a guide for telomerase-independent telomere lengthening in early development that persist after birth (de Frutos et al., 2016).

These paternal age effects suggest that in addition to genetic inheritance there may be strong epigenetic like inheritance in telomere length. Epigenetic inheritance studies however at this stage are still fully lacking.

1.2.8.2 Aging-related diseases and mortality

There is strong, yet not homogenous, evidence that shorter telomere length associate with non-communicable aging-related diseases including cardiovascular diseases, stroke and type 2 diabetes. Recent meta-analyses of 27 studies demonstrate that 1 SD decrease in LTL associate with 21% higher risk for stroke, 24% higher risk for myocardial infraction and 27% higher risk for Type 2 diabetes (D'Mello et al., 2015). However, not all studies have found associations between LTL and risk for cardiovascular disease (Ellehoj, Bendix, & Osler, 2016) or indicators of cardiovascular functioning including electrocardiograms, blood pressure, cardiovascular ultrasound and HDL and LDL levels (Zhang et al., 2014b).

Some associations between shorter LTL and risk for cancers also exist, yet findings are more conflicting than with CHD and type 2 diabetes. In meta-analyses there were associations between blood and buccal cell TL with bladder, esophageal, gastric, head and neck, ovarian, renal, and overall cancers, however,

associations between TL and lymphoma, breast, lung and colorectal cancers were inconsistent (Wentzensen, Mirabello, Pfeiffer, & Savage, 2011).

In addition longer LTL is associated with better antibody response to influenza vaccine than individuals with shorter LTL (Najarro et al., 2015) and an animal study has also shown that shorter telomere length also associate with reduced capacity of wound healing (Rudolph et al., 1999).

Longer LTL have also been associated with healthier aging (Njajou et al., 2009) and smaller age-related disease burden in elderly (Sanders et al., 2012). Physically healthy centenarians have also shown to have longer TL compared to less healthy centenarians (Terry, Nolan, Andersen, Perls, & Cawthon, 2008). Again, also studies that did not find association between LTL and “successful” or “healthy” aging, including good cognitive status as well as absence of hypertension, vascular disease, dementia, stroke, myocardial infraction, and heart failure also exists (Arai et al., 2015 Boccardi et al., 2013).

Studies in LTL and mortality are also contradictory. There are studies that have found associations between telomere length and higher all-cause mortality (Ehrlebach et al., 2009; Honig, Kang, Schupf, Lee, & Mayeux, 2012; Honig, Schupf, Lee, Tang, & Mayeux, 2006; Deelen et al., 2014), mortality from heart disease (Cawthon, Smith, O'Brien, Sivatchenko, & Kerber, 2003), cardiovascular disease in women (Epel et al., 2008), infectious disease (Cawthon et al., 2003; Fitzpatrick et al., 2011), and post stroke mortality (Martin-Ruiz et al., 2006). Also studies that have reported higher risk of mortality of twin with shorter telomere length exists (Bakaysa et al., 2007; Kimura et al., 2008). In addition, other line of research has shown that long-lived families, identified with the Family Longevity Selection Score (FLOSS), have longer LTL than individuals with lower FLOSS score (Honig et al., 2015).

However, there are many studies that have not found association between LTL and all-cause or disease specific mortality (Bischoff et al., 2006; Epel et al., 2008; Haver et al., 2015 Houben, Giltay, Rius-Ottenheim, Hageman, & Kromhout, 2011; Martin-Ruiz, Gussekloo, Heemst, Zglinicki, & Westendorp, 2005; Njajou et al., 2009 Strandberg et al., 2011). In addition, telomere attrition rate has shown to be non-related to mortality (Bendix et al., 2014).

Taking these conflicting results into account, it would be simplifying to say that telomere length is a good biomarker for healthy aging or mortality risk. Further studies are needed to disentangle in which circumstances and how big a role telomere length dynamics play in aging-related diseases and mortality. These conflicting results could indicate that there may be underlying factors related to both telomere length and mortality or some moderating effects may exist.

1.2.8.3 Socioeconomic and lifestyle factors

Also some socioeconomic and lifestyle factors have shown to be associated with LTL. Smoking, increased body weight, and unhealthy diet have been associated with shorter telomere length cross sectionally (Freitas-Simoes, Ros, & Sala-Vila, 2016; Lin, Epel, & Blackburn, 2012; Valdes et al., 2005; Weischer, Bojesen, & Nordestgaard, 2014). Associations between socioeconomic status, physical activity, alcohol consumption and LTL are more contradictory (Cherkas et al., 2006; Freitas-Simoes et al., 2016; Mundstock et al., 2015; Steptoe et al., 2011).

1.3 Hypothesized telomere pathway between stress, stress-related factors and telomere length

The mechanisms between the stress-related factors and aging-related non-communicable diseases and early mortality are not fully understood. As stress is associated with inflammation and oxidative stress (Biswas, 2016), telomeres in turn are sensitive to oxidative stress (Houben et al., 2008; Kawanishi & Oikawa, 2004) and oxidative stress together with inflammation have a role in development of many aging-related diseases (Gutierrez, Ballinger, Darley-Usmar, & Landar, 2006; Tracy, 2003; Weber, Zerneck, & Libby, 2008), it has been hypothesized that telomere length may be a novel factor partly explaining why stress is associated with aging-related diseases (Epel et al., 2004; Zhang et al., 2014a). The basis behind hypothesized telomere pathway between stress, stress-related factors and aging-related factors have been introduced in the chapter 1.2 and the summary of this hypothetical pathway is illustrated in the

figure 5. All of the stress related factors in the present thesis are related to stress and can cause or be influenced by stress system activation / alteration.

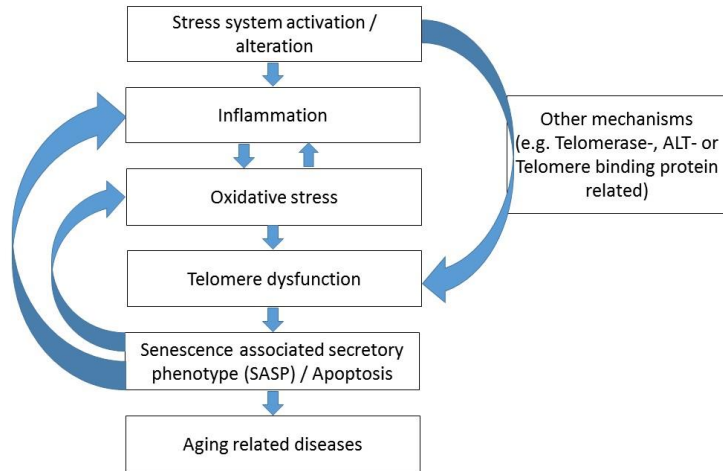


Figure 5. Hypothesized telomere pathway between stress and aging-related diseases. Chronic stress system activation and alterations in stress system are associated with interrelated chronic inflammation and oxidative stress. Other connecting mechanisms including telomerase activation, alternative lengthening of telomeres (ALT) and telomere binding protein related mechanisms may also exist. These effects can cause damage to telomeres that in turn may triggered telomere dysfunction as well as cell senescence. Accumulation of senescent cells, especially senescence associated secretory phenotype (SASP) cells, may be causing aging-related diseases.

1.3.1 Stress-related factors and telomere length

Very few studies up to date still exist that have studied associations between stress and stress-related factors with telomere length, thus, we set out to study if stress-related factors are associated with LTL. Below I give summary of the existing studies on the domains of stress and stress-related factors that are in the focus of the current PhD thesis.

1.3.1.1 Personality dimensions

Studies on FFM personality dimensions and LTL are scant. We were aware of only a few previous studies that have examined associations between personality traits and LTL. In a wide age range of adults (from 32 years to 79 years) higher neuroticism predicted shorter LTL on average 6.5 years after the measurement of neuroticism (Van Ockenburg, de Jonge, Van der Harst, Ormel, & Rosmalen, 2014). More recent study has shown that from five personality traits lower neuroticism and lower conscientiousness were associated with shorter LTL in 23-year-old ($SD = 1.7$) students (Sadahiro et al., 2015). Small prospective study including 60 women reported that teacher rated conscientiousness at average age of 10 years was associated with longer LTL 40 years later, however, the effects were not significant when adjusted for common covariates (Edmonds, Cote, & Hampson, 2015). Related to personality dimensions, other dispositional tendencies, including hostility and pessimism, were associated with shorter LTL in previous studies (Brydon et al., 2012; O'Donovan et al., 2009).

1.3.1.2 Mental disorders

The studies on mental disorders and LTL are somewhat contradictory. Shorter LTL have been associated with schizophrenia, nonaffective psychoses, mood disorders, major depressive disorder, alcohol abuse and anxiety disorders (Fernandez-Egea et al., 2009; Hartmann, Boehner, Groenen, & Kalb, 2010; Hoen et al., 2011; Kananen, 2010; Kao et al., 2008; Lung, 2007; Pavanello et al., 2011; Simon et al., 2006). However studies with null findings between schizophrenia, bipolar disorder and major depressive disorder also exists (Mansour et al., 2011; Wolkowitz, 2011) and more recent studies have shown that psychiatric patients may also have longer LTL than healthy controls (Lindqvist et al., 2015; Martinsson et al., 2013; Nieratschker et al., 2013). Recent meta-analyses, however, found LTL shortening across all psychiatric disorders (Darrow et al., 2016).

1.3.1.3 Early life stress

Previous studies testing the associations between ELS and LTL in adulthood are relatively scarce, contradictory, and they are based on retrospective self-reports of ELS. Existing studies have shown that ELS (including physical, sexual or emotional abuse, physical or emotional neglect, parental unemployment, own serious illness, losing a parent, parental mental or marital problems, lack of close relationships with adults and accumulation of adversities experienced in childhood) is associated with shorter LTL in adulthood in some (Kananen, 2010; Kiecolt-Glaser, 2011; O'Donovan et al., 2011; Surtees et al., 2011; Tyrka et al., 2010), but not in all studies (Glass, Parts, Knowles, Aviv, & Spector, 2010; Schaakxs, Verhoeven, Oude Voshaar, Comijs, & Penninx, 2015; Verhoeven, van Oppen, Puterman, Elzinga, & Penninx, 2015). In addition, there are studies on children that have shown that more objectively defined ELS was associated with shorter buccal cell telomere length (Drury et al., 2012; Shalev et al., 2013). Caregiver-reported exposure to domestic violence, frequent bullying, victimization or physical maltreatment by an adult was associated with a faster rate of buccal cell telomere shortening in children aged 5–10 years, and children who remained institutionalized longer in early childhood had shorter buccal cell telomere at age of 6 - 10 years than children whose experience of institutional care was shorter length (Drury et al., 2012; Shalev et al., 2013).

1.3.1.4 Sleep apnea

Only few studies have tested the associations between sleep apnea and LTL. Barcelo et al. have shown that individuals with severe sleep apnea have shorter LTL than individuals without a sleep apnea diagnosis (Barceló et al., 2010). More recent study has shown that also mild sleep apnea ($5 \leq$ apneas or hypopneas per hour of sleep < 15) associates with shorter LTL (Barceló et al., 2010; Shin, Yun, Yoon, & Baik, 2016). The long term impacts of sleep apnea on LTL had not been studied.

1.3.1.5 HPA axis reactivity

The studies that have tested associations between LTL and HPA axis reactivity are also scarce. The existing evidence suggested that higher stress reactivity could be associated with shorter LTL in adulthood. Only one previous study have looked at the associations between psychosocial stress reactivity and LTL in adults. Higher salivary cortisol responses to a modified Trier Social Stress Test (TSST) cells was associated with shorter telomere length of peripheral blood mononuclear cells (Tomiya et al., 2012). The study, however, included only 23 menopausal women and used modified psychosocial stress protocol (Tomiya et al., 2012) and thus more comprehensive studies were needed for generalization.

Few studies have looked at the associations between stress reactivity and telomere length in children. Higher salivary cortisol responses to a stress task including serial subtraction was associated with shorter telomere length measured in saliva in 10 - 14 year-old girls, and higher autonomic nervous system response (higher sympathetic reactivity, greater parasympathetic withdrawal) to tasks involving social, cognitive, sensory, and emotional elements was associated with shorter buccal cell telomere length in 5 - 6 year-old children (Gotlib et al., 2015; Kroenke et al., 2011).

2 Aims of the study

As there were discrepancies between the previous studies, lack of studies in elderly participants and even complete lack of well-designed studies between stress-related factors and LTL, the main aim of this thesis was to study in well characterized elderly cohort if:

1) Personality dimension, especially neuroticism and conscientiousness, are associated with LTL. We also tested if the associations varied by sex. (Study I)

2) History of hospitalization for mental disorders are associated with LTL. We also examined if antidepressant and psychotropic medication use associate with LTL and if psychotropic medication use modulated the associations of mental disorder hospitalizations and LTL. Finally, we also examined if recently reported depressive symptoms including depression, anxiety and loss of vitality associated with LTL. (Study II)

3) Objectively measured early life stress, temporary separation from both parents, associated with LTL. We also examined whether age at separation and length of separation associate with LTL. The secondary aim was to test whether self-reported emotional and physical traumatic experiences across the lifespan and age at the occurrence of the traumatic experiences were associated with LTL and whether the separation-related ELS in combination with the self-reported traumatic experiences were associated with shorter LTL. We also tested if the associations varied by sex. (Study III)

4) History of sleep apnea, severe enough to be recorded as a hospital inpatient diagnosis, was associated with LTL. We also examined if primary snoring, a classic symptom of sleep apnea most often appearing without fulfilling the diagnostic criteria for sleep apnea, associated with LTL. (Study IV)

5) Higher HPA axis reactivity, measured by salivary cortisol, plasma cortisol and plasma ACTH responses to TSST, was associated with LTL. We also explored if the associations were non-linear, or sex-specific. (Study V)

3 Methods

3.1 Participants

The participants in the present thesis came from Helsinki Birth Cohort Study (HBCS) (Eriksson, Forsen, Tuomilehto, Osmond, & Barker, 2001). The HBCS was approved by the Coordinating ethics committee of the Hospital District of Helsinki and Uusimaa. All participants gave a written informed consent. The HBCS comprises 13 345 individuals (6975 men, 6370 women) born in 1934 – 1944 in one of the two public maternity hospitals in Helsinki, Finland, who attended child welfare clinics in the city of Helsinki and who were still living in Finland in 1971 when unique personal identification numbers were assigned to all Finnish residents. Between the years 2001 - 2004 a randomly selected sample of 2003 (75.5% of 2690 invited) subjects (men $n = 928$ and women $n = 1075$) participated in a detailed clinical examination including blood sampling for LTL measurement at a mean age of 61.5 (SD = 2.9, Range = 56.7 - 69.8) years. Data available on LTL and different measures of stress varies in studies I-V. The sample size available for these studies are described in Figure 6 and in the measures-section below.

3.2 Measures

3.2.1 Leukocyte telomere length (Studies I-V)

Genomic DNA was extracted from EDTA-anti-coagulated whole peripheral blood by using a QIAamp DNA BloodMaxi Kit (Qiagen). Relative telomere length from peripheral blood DNA was determined by a quantitative real-time PCR-based method described in detail in Kananen et al. (Cawthon, 2002; Kananen, 2010). PCR reactions were performed separately for telomere and β -haemoglobin reactions in paired 384 well plates in which matched sample well positions were used. β -haemoglobin was used as a single copy reference gene.

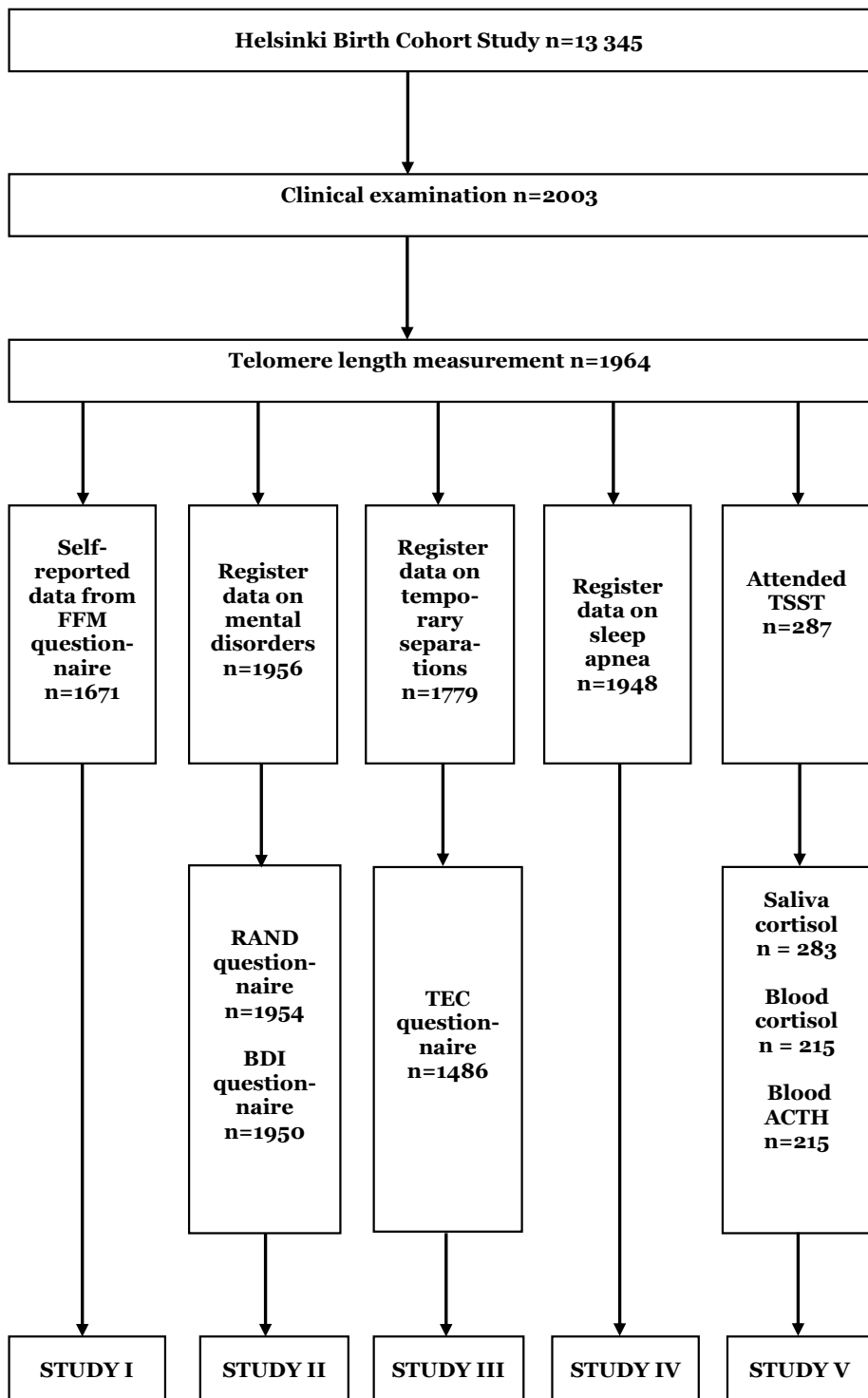


Figure 6. The selection and number of participants in each study (I-V).

Based on O'Callaghan's method, a synthetic oligomer (Sigma) dilution series, hgb-120-mer and tel14x (0.0002; 0.002; 0.02; 0.2; 1.0; 3.0 and 6.0 pg) were included on every plate to create reaction specific standard curves (O'Callaghan, Dhillon, Thomas, & Fenech, 2008). Plasmid DNA (*pcDNA3.1*) was added to each standard to maintain a constant 10 ng of total DNA concentration per reaction. Standard curves were used to perform absolute quantification of each individual sample reaction with 10 ng of template DNA. Samples and standard dilutions were transferred into 384-well plates as triplicates using DNA Hydra 96 robot and dried for 24 h at +37 °C. β -haemoglobin PCR reaction mixture consisted of 300 nM Hgb1 primer (5'-GCTTCTGACACAACGTGTGTTCACTAGC-3') and Hgb2 primer (5'-CACCAACTTCATCCACGTTCCACC-3') in a total volume of 15 μ l of iQ SyBrGreen supermix (Bio-Rad). The reaction conditions were 95 °C for 3 min followed by 35 cycles at 95 °C for 15 s, 58 °C for 20 s and 72 °C for 20 s. Telomere PCR reaction mix content was: 270 nM tel1b primer (5'-CGTTTT(GTTTGG)₅GTT-3') and 900 nM tel2b primer (5'-GGCTTG(CCTTAC)₅CCT-3'), 1% DMSO (Sigma), 0.2 mM of each dNTP (Fermentas), 1.5 mM MgCl₂ (Applied Biosystems International), 5 mM DTT (Sigma), 0.2X SYBR Green I (Invitrogen), and 1.25 U AmpliTaq Gold DNA polymerase (Applied Biosystems International) in a total volume of 15 μ l AmpliTaq Gold Buffer II. The cycling conditions were 10 min at 95°C followed by 25 cycles at 95°C for 15 s and 54°C for 2 min. Specific primer binding was controlled by Melt-curve analysis. Both reactions were performed with CFX 384 Real-Time PCR Detection System (Bio-Rad).

Quality control (QC) was carried out with the Bio-Rad CFX Manager software v.1.6. At this point triplicates with amplification curve standard deviation above 0.5 at the threshold level were omitted (n = 38). One subject had missing blood sample data and was therefore excluded. The correlation coefficient of the standard curves varied from 0.991 to 1.000. The corresponding average PCR efficiencies were 89.1 \pm 5.3 % for telomere and 95.9 \pm 1.1 % for β -haemoglobin reaction.

All plates included four genomic DNA control samples for the plate effect calibration and for monitoring repeat measures correlation coefficient of variation (CV). The quantities of the control samples were used for calculating CV values as the ratio of the standard deviation to the mean, being on average 21.0 % for the telomere reaction, 6.0 % for the β -haemoglobin reaction, and 24.8 % for their ratio (T/S). The plate effect was taken into account by normalizing the telomere signal and reference gene signal to the corresponding average of 4 control samples analysed on every qPCR plate before taking the T/S ratio. The relative telomere length for the samples was calculated in Microsoft Office Excel for Windows.

Overall, blood samples of 1964 individuals (98.1% of the 2003 participants attending clinical examination) passed the QC for LTL measurement and thus LTL data were available for 912 men and 1052 women.

3.2.2 Personality dimensions (Study I)

We used the Finnish version of the NEO-PI (Neuroticism-Extraversion-Openness Personality Inventory) (Costa & McCrae, 1985; Pulver, Allik, Pulkkinen, & Hamalainen, 1995) questionnaire to measure the FFM personality dimensions. The NEO-PI is a 181-item self-report personality inventory consisting of 48 items for neuroticism, extraversion, and openness to experience (there were two missing items on openness to experience in our questionnaire and therefore this scale comprised only 46 items in our study) and 18 items each for agreeableness and conscientiousness (Costa & McCrae, 1985; Pulver et al., 1995). Participants answered the statements using a five-point Likert scale ranging from totally disagree (0) and totally agree (4). As a result, a continuous sum score for each of the five dimensions was calculated. In brief, neuroticism measures low emotional stability and a tendency to feel anxious, sad or tense; extraversion measures energetic approach toward social and material world and positive emotion expression; openness to experience measures creativity, curiosity, and open-mindedness; agreeableness measures trust toward other people, altruism, and cooperativeness; and conscientiousness measures good impulse control that facilitates task- and goal directed behaviours, delaying

gratification and obedience of social norms (Costa & McCrae, 1985). NEO-PI also includes more detailed subcategories, facets, for neuroticism, extraversion and openness to experience; sum scores for the facets were also calculated.

Total of 1671 participants with accurate telomere length measurement filled FFM on average 1.87 (SD 0.71, Range 0.44 - 3.29) years from the clinical examination and form the analytical sample in the Study I.

3.2.3 Mental disorders (Study II)

Mental disorders severe enough to warrant hospitalization were identified from the Finnish Hospital Discharge Register (HDR). The HDR covers inpatient episodes of residents in Finland in the general and psychiatric hospitals from 1969 onwards. Mental disorders were coded by the ICD system. ICD-8 was used for the 1969 - 1986 inclusive period, ICD-9, according to the DSM Third Revision (DSM-III-R), for the years 1987 - 1995 inclusive and ICD-10 from the year 1996 to 2004. Both the primary and subsidiary hospitalization diagnoses were used, except in the case of acute substance-intoxication (ICD-9: 305 and ICD-10: F1x.0). In such cases only primary diagnoses were used because intoxication is a frequent subsidiary diagnosis in Finnish medical practice and does not automatically indicate a substance use disorder *per se*. Mental disorders were categorized according to the ICD codes into the following groups: any mental disorder, substance use disorder, (non-affective) psychotic disorders, mood disorders, anxiety disorders and personality disorders.

The register data on mental disorders were available for all the $n = 1964$ participants with telomere length measurement. We excluded 8 participants whose mental health status was ambiguous as they had been hospitalized for mental disorder between the clinical examination (carried out between years 2001 – 2004) and end of the mental disorder identification period in the end of year 2004. Accordingly the 1956 participants form the analytical sample for mental disorder analyses in the Study I. From the 1954 participants, we identified 116 mental disorder patients whose first hospitalization was before clinical examination. The 1840 participants who had not been hospitalized for mental disorders between 1969 and the clinical examination were used as the control

group. The number of specific mental disorder diagnoses does not equate to any mental disorder, because of co-morbidity.

3.2.4 Depressive symptoms (Study II)

Depressive symptoms that occurred within the two-week period prior to the clinical examination were measured by using the self-reported Beck Depression Inventory (BDI) (Beck Robert, 1988) at the clinical examination visit. The BDI consists of 21 items. Each item contains four statements that reflect varying degrees of symptom severity. Respondents were instructed to choose the number that corresponds with the statement that best describes them. The numbers ranged from zero to three, indicating the increasing severity of the symptom. Ratings were summed to calculate a total BDI score, which can range from 0 to 63.

Two subscales from the SF-36/RAND, namely the Mental Health Index (MHI) and the Vitality Scale (VS), were used to capture the following symptoms: depression, anxiety, loss of behavioural/emotional control and psychological well-being, loss of vitality and energy during the four weeks prior to the clinical examination. The five items of the MHI and the four items of the VS were measured against a 6-point scale that ranged from “all the time” (1) to “none of the time” (6). In measuring a series of depressive symptoms, the MHI has been shown to have a high sensitivity and specificity for detecting clinical depression (Arroyo et al., 2004; Berwick et al., 1991; Ware, Snow, Kosinski, & Gandek, 1993). Further, a Finnish validation study of SF-36 concluded that the VS items are also important in capturing depression in a Finnish population (Aalto, Aro, & Teperi, 1999).

Total of 1954 participant filled BDI questionnaire and 1950 MHI and VS subscales at the clinical examination visit, and they form the analytical sample for depressive symptoms in the Study II.

3.2.5 Psychotropic medication (Study II)

Information of psychotropic medication that belongs to the World Health Organizations Anatomical Therapeutic Chemical Classification System codes

N05 (psycholeptics) and N06 (psychoanaleptics), was derived from the Finnish National Social Insurance Institution register. This register covers medication reimbursement entitlements and purchases throughout the follow-up between 1995 and 2002. We identified a total of 665 individuals with psychotropic medication reimbursement entitlements and purchases. This included 391 individuals with antidepressant medication reimbursement entitlements and purchases.

3.2.6 Early life stress (Study III)

During World War II Finland fought two wars with the Soviet Union: the Winter War from November 1939 to March 1940, and the Continuation War from June 1941 to September 1944. To protect the Finnish children from the strains of these wars, approximately 70 000 children from various socio-economic backgrounds were evacuated abroad, mainly to Sweden and Denmark, to temporary foster care unaccompanied by their parents. Individuals in the present study who were separated temporarily from their parents were identified from the Finnish National Archives' register. The register, which was kept by the Ministry of Social Affairs and Health between 1939 and 1946, provides full documentation for all of the evacuated children, including the age at which the children were evacuated and the length of the evacuation (Pesonen et al., 2007; 2010). We identified 215 participants who had been exposed to temporary separation from both parents in childhood.

The register data on temporary separation from both parents was available for all the $n = 1964$ participants with telomere length measurement. In line with previous studies on early separations in HBCS (Räikkönen et al., 2011), 185 participants with ambiguous separation status (who self-reported that they had been evacuated, but whose information could not be found in the registers) were excluded. From the 1779 participants who had unambiguous data on early separation available, total of 1486 participants filled Traumatic Experiences Checklist (TEC) questionnaire and they form the analytical sample for early life stress and the cumulative trauma analyses in the Study III.

3.2.7 Traumatic experiences across lifespan (Study III)

Presence of emotional and physical traumatic experiences across the life span was measured using trauma area questions from the TEC (Elzinga et al., 2008; Nijenhuis, VanderHart, & Vanderlinden, 1999). Emotional traumas included six questions; three questions on emotional neglect and three questions on emotional abuse. Physical traumas also included six questions; three questions on physical abuse and three on threat to life/pain/bizarre punishment. Individuals answering 'yes' to the question "Did this happen to you?" on any of the six emotional or physical trauma questions were assigned to a group of having experienced any emotional or physical traumas. Participants who replied 'no' to all of the 12 trauma questions were assigned to the group of no traumatic experiences. Following the lead of Nijenhuis et al. (1998), if the participant did not recall the event, the response was coded 'no'. However, additional analyses revealed that none of the results differed significantly when participants who did not recall one or more of the events ($n = 110$) were excluded from the analyses. If a traumatic event had occurred, the participants were also requested to indicate the age(s) that it had taken place. In case the same trauma had occurred at multiple ages, the age at first occurrence was used.

From the 1779 participants with accurate telomere length measurement and unambiguous data on temporary separation available, 1486 participants filled TEC questionnaire on average 1.87 (SD = 0.71, Range = 0.44 - 3.29) years from the clinical examination.

3.2.8 Sleep apnea and snoring (Study IV)

Subjects with central or obstructive sleep apnea or primary snoring, recorded as inpatient diagnoses, were identified from the Finnish HDR between the year 1987 and the clinical examination. HDR data also was available between 1969 and 1986 based on ICD-8 criteria, but less clinical awareness existed about the importance of sleep apnea at that time; accordingly, there were no apnea diagnoses (code 783.21; no specific code for primary snoring) within our study population. Between 1987 and 1996, sleep apnea and snoring were coded by ICD-9 criteria (sleep apnea codes, 3472A and 3478X; primary snoring code, 7849C) and by ICD-

10 criteria (sleep apnea code, G47.3; primary snoring code, R06.5) from 1996 onward.

As sleep apnea often co-occurs with other sleep disorders such as insomnia, and insomnia in turn is associated with higher oxidative stress and it is reported that poor sleep quality and shorter duration are associated with shorter LTL (Gulec et al., 2012; Liang et al., 2011; Luyster, Buysse, & Strollo, 2010; Prather et al., 2011), 16 participants who were hospitalized for sleep disorders other than sleep apnea and primary snoring were excluded. From the 1948 participants, we identified 34 men (14 with snoring) and 10 women (4 with snoring) who had been hospitalized with sleep apnea (2.3% of the 1948 participants). Sleep apnea was a primary diagnosis for hospitalization for 41 (93.2%) of these subjects. All of the 20 men and 9 women hospitalized for primary snoring without sleep apnea (1.6% of the 1948 participants) had this diagnosis as the primary diagnosis of hospitalization. 1875 participants who had not been hospitalized for sleep apnea or snoring were used as the control group.

3.2.9 HPA axis reactivity (Study V)

HPA axis reactivity was measured by using The Trier Social Stress Test (TSST). TSST is a well-standardized psychosocial stress test known to elicit a powerful HPA axis response (Kirschbaum et al., 1993). After resting for 45 minutes participant were led to the laboratory where the stress protocol was performed. Participant was given 3 min to prepare a 5-min speech where they had to convince a committee of two white coated persons that his/her personal abilities make him/her the best candidate for a self-selected confidential post. After the speech, participant was asked to perform series of serial subtractions for another 5 min in front of the committee. Throughout the protocol the committee minimized all verbal and non-verbal communication with the participant. After both tasks being completed, participant was led to another room for follow up and debriefing. We obtained saliva and blood at 18 min before the TSST stressor ended and at 0, 10, 20, 30, 45, 60, and 90 min after the end of the stressor.

Salivary and plasma cortisol were measured from all samples and ACTH, which returns more rapidly to baseline level, from the first four samples. Salivary

cortisol concentrations were determined using a competitive solid-phase, time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA; Wallac, Turku, Finland). Plasma cortisol concentrations were determined by ELISA (Immuno Biological Laboratories, Hamburg, Germany) and ACTH by chemiluminescence immunofluorometric assay (Nichols Institute Diagnostics, San Clemente, CA).

Total of 287 participants (70.5% of the 407 invited) took part in the TSST on average 2.12 (SD 0.81, range 0.41 - 3.50) years from the clinical examination. Data on LTL and salivary cortisol were available on 283, and plasma cortisol and ACTH on 215 participants. The smaller sample size for plasma cortisol and ACTH resulted from adding sampling of blood later to the study protocol (Kajantie et al., 2007).

3.2.10 Covariates and confounders (Studies I-V)

Variables known to be associated with LTL and/or independent variables were treated as covariates.

Stock DNA concentration (ng / μ l) was determined from the samples of which LTL was determined. History of hospitalizations for Coronary Heart Disease (CHD) (codes: 430–434 and 436–437 from ICD-8 and 9, 438 from ICD-9, and I60–I69 from ICD-10) and stroke (codes: 410–414 from ICD-8 and ICD-9 and I21–I25 from ICD-10) were identified from the HDR between year 1969 and clinical examination in 2001-2004. Diabetes Mellitus was defined according to the World Health Organization criteria (World Health Organization, 1999) by using the standard 75-g oral glucose tolerance test at the time of clinical examination. Body mass index (BMI, kg/m²) was calculated by dividing the weight of the participant in kilograms by the square of the participant's height in meters, measured at the clinical examination. Level of education (elementary school or less/vocational school / high school diploma /university degree), current smoking status (yes vs.no), leisure time physical activity (<3 vs. \geq 3 times/week), alcohol consumption frequency (< 3 vs. \geq 3 times/week) was assessed with self-reported questionnaire in conjunction with the clinical examination. Childhood Socio Economic Status (SES) was based on father's

highest occupational status (manual worker / lower, junior clerical /middle, senior clerical /upper) extracted from birth records, child welfare clinic records and school health care records. Mother's age (years) at delivery was extracted from birth records.

3.3 Statistical analyses

In all the studies (I -V) LTL was log-transformed to attain normality and thereafter standardized to the mean of 0 and standard deviation of 1 to facilitate interpretation. The associations between FFM personality dimensions and sub-facets (if the higher order personality dimension was significantly associated with LTL) (Study I), mental disorders, psychotropic medication use and depressive symptoms (Study II), separation status and retrospectively self-reported any physical/emotional traumatic experiences (Study III), sleep apnea and snoring (Study IV) were examined using multiple linear regression analyses with LTL as the outcome variable. When using multiple linear regression to test the association between TSST summary measures of cortisol and ACTH; baseline, post-stress peak value, time-weighted area under the curve (AUCg) (calculated using the trapezoidal rule), and time-weighted AUC increment (time-weighted AUCg minus baseline), LTL was used as explanatory variable (Study V). Further analyses were run in each study. We adjusted all the analyses for age at the blood sampling for LTL, sex and stock DNA concentration (Model 1) and thereafter for CHD, stroke, diabetes mellitus, BMI, education level, smoking status and alcohol consumption (Model 2). Continuous variables of stock DNA concentration and BMI were natural log transformed to attain normality. Depending on the independent variables additional covariate adjustments were added in each study. All the regression analyses were two-tailed and carried out with PASW 18 or SPSS 21 softwares for Windows.

In the Study I, to study if sex moderated the personality dimension associations an interaction term 'sex × personality dimension / sub-facet' was placed into the regression equation. Each dimension and sub-facet were tested separately, followed by their main effects. Time difference between the blood

sample for LTL and filling in the NEO-PI were added into Model 1 and self-reported depressive symptoms into Model 2.

In the Study II, to examine whether the overall level of depressive symptoms or symptoms that exceed a cut-off limit associate with LTL, BDI, MHI and VS scores were used both as continuous and as dichotomous variables. A score of 10 was used for the BDI as the cut-off for at least mild depressive symptoms. The lowest tertile was used as the cut-off points for the MHI and the VS. By entering three out of four dummy coded interaction variables of 'mental disorder/control x medication/no-medication' into the regression equation, we examined if psychotropic medication or antidepressant medication use modulated associations between mental disorder and LTL.

In the Study III, to examine whether separation status in combination with the physical/emotional traumatic experience was associated with LTL an interaction term between 'separation status x any traumatic experiences', followed by main effects, were added in a regression equation. If the interaction term involving any traumatic experiences was significant, sub analyses with physical and emotional traumas were tested separately in the separated and non-separated groups to specify the effects. To test whether the associations varied significantly by sex interaction term 'sex x separation status x any/physical/ emotional traumatic experiences' were added in a regression equation. In the more detailed analyses of separation, the effects of age at separation and length of separation on LTL was analysed using split at the median (above median vs. not-separated; below median vs. not separated; median split was used as the distributions of age at and length of separation were significantly skewed). In the further analyses of any self-reported traumatic experiences the age at the first occurrence of the traumatic experience was analysed by split at the median (above median vs. no traumatic experiences; below median vs. no traumatic experiences; median split was used as this variable was also significantly skewed). Any severe mental disorder, self-reported depressive symptoms, father's occupational status in childhood and mother's age at delivery were added into Model 2. To account for multiple testing, the results in the Study III are reported in Bonferroni-corrected p-values ($1 - (1 - \text{observed p-value})^{y(n \text{ of tests})}$); we assumed three main effects analyses for separation (separation, age at separation, length of separation) two

main effects analyses for traumatic experiences (any experience, age at first occurrence of any trauma) resulting in six tested 'separation × trauma' interactions.

In the Study V, association between LTL and cortisol and ACTH during the TSST was tested by linear mixed model analyses with intercept defined as random factor (SPSS 21, IBM). A main effect of LTL tested if LTL associated with overall levels of cortisol and ACTH during the TSST, and an interaction 'LTL × sampling time' tested if LTL associated with cortisol and ACTH responses during the TSST. Non-linearity of the associations was tested by adding a main effect of a squared LTL variable and an interaction term 'squared LTL variable × sampling time' into the regression equation. Non-linear associations between LTL and cortisol and ACTH summary measures: baseline, post-stress peak value, time-weighted area under the curve (AUC_g), and time-weighted AUC increment were tested by adding the squared LTL variable into the linear regression equation. Time difference between the blood sample for LTL and attending TSST, and time of the day when attending TSST were added into Model 1. Self-reported current depressive symptoms, and in the sex-specific analyses in women the use of estrogen therapy, were added into Model 2. We also report Bonferroni-corrected significance levels for associations that reached significance level of $p < 0.05$ to account for multiple testing. We assumed three main effects and three interaction analyses for the three HPA axis outcomes (salivary and plasma cortisol and plasma ACTH).

4 Results

As predicted (Gardner et al., 2014), we found that within the 1964 participants with LTL measurement older age correlated with shorter LTL ($p < 0.001$), and men had shorter LTL than women ($p = 0.001$) when put into linear regression equation separately or together with all Model 1 covariates ($p \leq 0.001$). Within the 1964 participants, from the Model 2 covariates used in all of the studies (I - V) in the present thesis, smoking ($p = 0.123$), alcohol usage ($p = 0.855$), exercise habits ($p = 0.452$), education level ($p > 0.198$), CHD ($p = 0.142$), stroke ($p = 0.348$), type 2 diabetes ($p = 0.257$) or BMI ($p = 0.669$) were not associated with LTL when put into linear regression equation separately but with Model 1 covariates.

4.1 Personality dimensions (Study I)

None of the FFM personality dimensions were significantly associated with the LTL in the analyses of both sexes combined (p -values > 0.28 in Model 1, Table 1). From the interaction analyses between each personality dimension and sex, only agreeableness x sex -interaction was significant ($p = 0.001$ for Model 1). Further sex-specific analyses showed that in men higher agreeableness associated with shorter LTL ($p = 0.016$ in Model 1, Table 1), while in women lower agreeableness associated with shorter LTL ($p = 0.016$ in Model 1). Model 2 adjustments did not change the sex-specific significant associations (p -values < 0.042). There were no quadratic associations between personality dimensions and LTL within all participants (p -values > 0.051 in Model 1) or when men (p -values > 0.056 in Model 1) and women (p -values > 0.10 in Model 1) were analysed separately.

Table 1. Associations between Five factor model (FFM) personality dimensions and relative leukocyte telomere length (LTL) in Z-score units

| | All N=1664 ¹ | | Men N=739 ¹ | | Women N=925 ¹ | |
|---------------------------|----------------------------|-----|---------------------------|------|-----------------------------|------|
| | 95%CI | p | 95%CI | p | 95%CI | p |
| Neuroticism | -0.021, 0.072 | .28 | 0.003, 0.143 | .040 | -0.070, 0.051 | .76 |
| Extraversion | -0.048, 0.044 | .93 | -0.067, 0.073 | .93 | -0.072, 0.050 | .72 |
| Openness to experience | -0.048, 0.047 | .98 | -0.068, 0.071 | .97 | -0.070, 0.053 | .78 |
| Agreeableness | -0.042, 0.053 | .82 | -0.155, -0.016 | .016 | 0.014, 0.134 | .016 |
| Conscientiousness | -0.046, 0.045 | .98 | -0.118, 0.022 | .18 | -0.024, 0.096 | .24 |

¹= Agreeableness n=1665 (men 740, women 925), 95%CI =95% confidence interval, p=p-values from Model 1.

4.2 Mental disorders and depressive symptoms (Study II)

Participants hospitalized for any mental or substance use disorders had longer LTL than non-hospitalized controls (significant p-values < 0.03 in Model 2, Table 2). Moreover, taking the psychotropic medication use into account, only those any mental disorder patients who had psychotropic medication use had longer LTL than non-hospitalized controls with (p = 0.008 in Model 1, Figure 7 Panel A) and without psychotropic medication use (p = 0.02 in Model 1, Figure 7 Panel A). In contrast, those mental disorder patients who did not have psychotropic medication use did not differ from controls with or without psychotropic medication use (p-values > 0.32 in Model 1, Figure 7 Panel A). Psychotropic mediated associations were similar with antidepressant medication use (Figure 7 Panel B). When all the participants were analysed together, any psychotropic or antidepressant medication use did not associate with relative LTL (p-values > 0.79 in Model 1). Further analyses revealed that when subjects who had used antidepressants were excluded, neither the mental disorder patients with (n = 21) or without (n = 35) non-antidepressant psychotropic medication use differed from the controls (p-values > 0.47). Also, within the mental disorder patients, those with or without psychotropic medication use and also those with or without antidepressant medication use did not differ in LTL from each other (p-values >

0.16 in Model 1). Model 2 adjustments did not change any of the significant associations (p-values < 0.03).

Neither the number of psychotropic medication entitlement and purchases (p = 0.94) nor the number of antidepressant entitlement and purchases (p = 0.42) associated with LTL *per se*. The associations between psychotropic medication use and LTL were also non-significant when mental disorder patients and controls were analysed separately (p-values > 0.38).

Further analyses on time between latest any mental disorder hospitalization and LTL revealed that time from the most recent hospitalization within the mental disorder hospitalized participants group did not associate with LTL when treated as continuous variable (p = 0.72). However, when compared in tertiles, only the patients hospitalized for less than 8.3 years prior to LTL measurement had longer LTL than controls (p = 0.045 in Model 1), where in contrast, LTL did not differ from the controls for subject hospitalized 8.3 to 14 years or over 14 years (p-values > 0.10). Model 2 adjustments did not change the results.

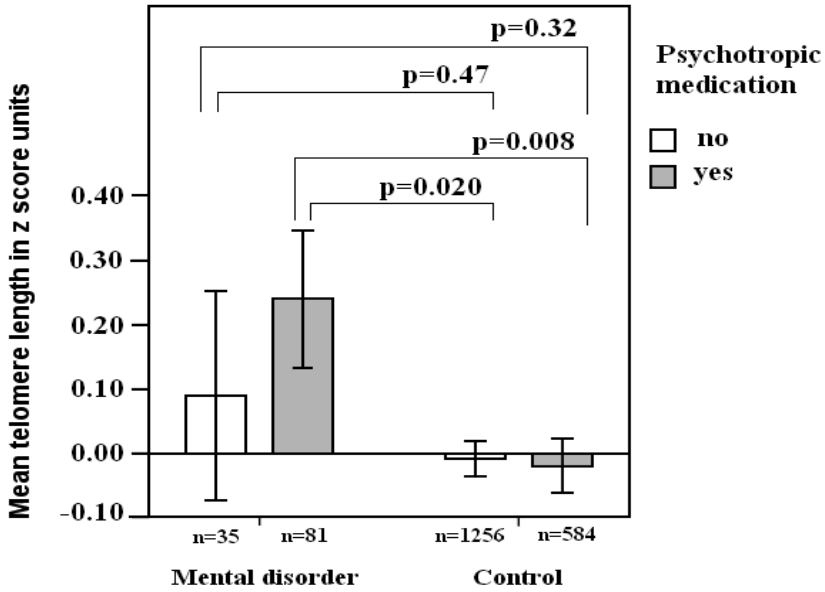
Self-reported sub-clinical symptoms of depression, anxiety and loss of vitality were not associated with relative LTL when these variables were treated either as continuous or as dichotomous variables (p > 0.30). Neither, if mental disorder patients or subjects with any psychotropic medication use were excluded (p-values > 0.13).

Table 2. Associations between mental disorders and relative leukocyte telomere length

| Mental Disorder Diagnoses | N (%) | 95%CI | p |
|---------------------------|-----------|---------------|-------|
| Any Mental Disorder | 116 (5.9) | 0.050, 0.425 | 0.013 |
| Mood Disorders | 49 (2.5) | -0.234, 0.317 | 0.77 |
| Substance Abuse Disorders | 40 (2.0) | 0.012, 0.626 | 0.042 |
| Anxiety Disorders | 30 (1.5) | -0.024, 0.678 | 0.07 |
| Psychoses | 9 (0.5) | -0.401, 0.875 | 0.47 |
| Personality Disorders | 6 (0.3) | -0.630, 0.937 | 0.70 |

N = number of individuals, 95%CI=95% confidence interval, p=p-values from Model 1.

A)



B)

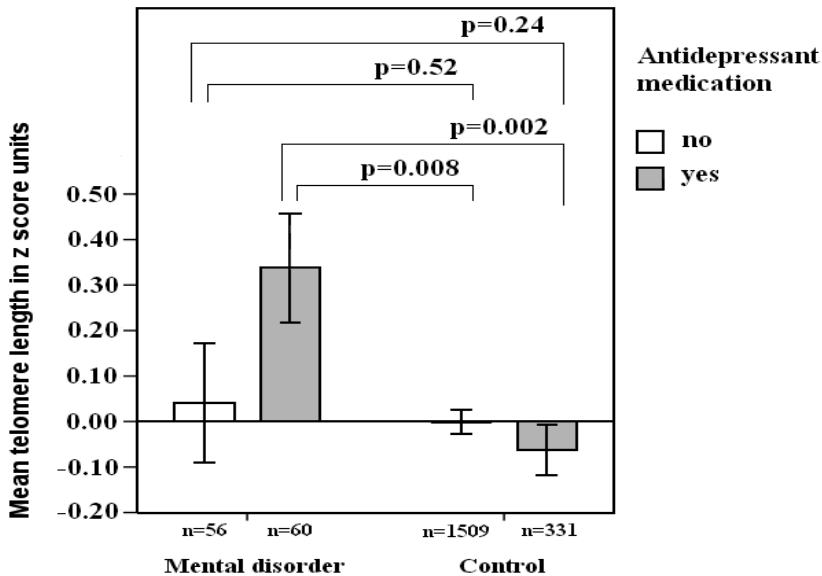


Figure 7. Patients with mental disorder severe enough to warrant hospitalization who in addition had psychotropic (Panel A) or antidepressant (Panel B) medication use had longer leukocyte telomere length compared to controls with no mental disorder hospitalizations, where in contrast mental disorder patients without psychotropic (Panel A) or antidepressant (Panel B) medication use did not differ from the controls.

4.3 Early life stress and traumatic experiences (Study III)

Separation status and traumatic experiences were related; participants who were separated had experienced any self-reported traumas more frequently than participants who were not separated ($p = 0.044$).

No significant differences in LTL were found between those who were and those who were not separated ($p = 0.39$, Table 3). Further analyses revealed that age at the separation was not associated with LTL ($p > 0.18$, Table 3). However, length of the separation associated with LTL such that participants who were separated for shorter periods than the median length of 1.4 years had shorter LTL than non-separated participants ($p = 0.027$ in Model 1, Table 3) but participants separated for longer than 1.4 years did not differ in LTL from non-separated participants ($p = 0.99$, Table 3).

Self-reported experiences of any emotional or physical traumas across the lifespan, experiences of emotional or physical traumas tested separately and age at occurrence of the first traumatic experience were not significantly associated with LTL ($p > 0.71$, Table 3).

There were significant interaction effects between childhood separations x self-reported any/any emotional/any physical traumatic experiences on LTL ($p < 0.018$ in Model 1). Further analyses revealed that when compared with the controls that were non-separated and who reported no traumatic experiences, the separated participants who reported any traumatic experiences had shorter LTL ($p = 0.037$ in Model 1, Figure 8) where in contrast participants who were only separated or who only self-reported traumatic experiences did not differ from the controls ($p > 0.10$, Figure 8). Results were similar with any emotional traumatic experiences. Model 2 adjustments did not change the results. Sex did not significantly moderate any of the associations ($p > 0.86$).

Table 3. Associations between separation status, retrospectively self-reported emotional and physical traumatic experiences and relative leukocyte telomere length.

| | N | 95% CI | p |
|--|------|----------------|-------|
| Non-separated vs. | 1271 | | |
| Separated | 215 | -0.255, 0.039 | 0.15 |
| Age at separation | | | |
| < median 4.1 years | 99 | -0.251, 0.141 | 0.58 |
| ≥ median 4.1 years | 99 | -0.424, 0.011 | 0.06 |
| Length of separation | | | |
| < median 1.4 years | 97 | -0.478, -0.067 | 0.009 |
| ≥ median 1.4 years | 97 | -0.189, 0.216 | 0.90 |
| No self-reported traumatic experiences vs. | 533 | | |
| Any emotional or physical traumatic experiences | 953 | -0.105, 0.097 | 0.94 |
| Any emotional traumatic experiences | 661 | -0.099, 0.121 | 0.85 |
| Any physical traumatic experiences | 745 | -0.107, 0.108 | 0.99 |
| Age at first occurrence of any emotional or physical traumatic experience ¹ | | | |
| < median 10.0 years | 249 | -0.179, 0.110 | 0.65 |
| ≥ median 10.0 years | 308 | -0.081, 0.184 | 0.45 |

N = number of individuals, 95% CI = 95% confidence interval, p=p-values from Model 1, ¹ = Using cut-off age before (n = 462) and after adulthood (n = 208) (< 18 years vs. > 18 years) did not change the result (p-values > 0.556); neither did dividing the age into developmentally meaningful categories of early childhood 0-5 years (n = 86), childhood 5-13 years (n = 309), adolescence 13-20 years (n = 77), adulthood over 20 years (n = 198) (p-values > 0.44).

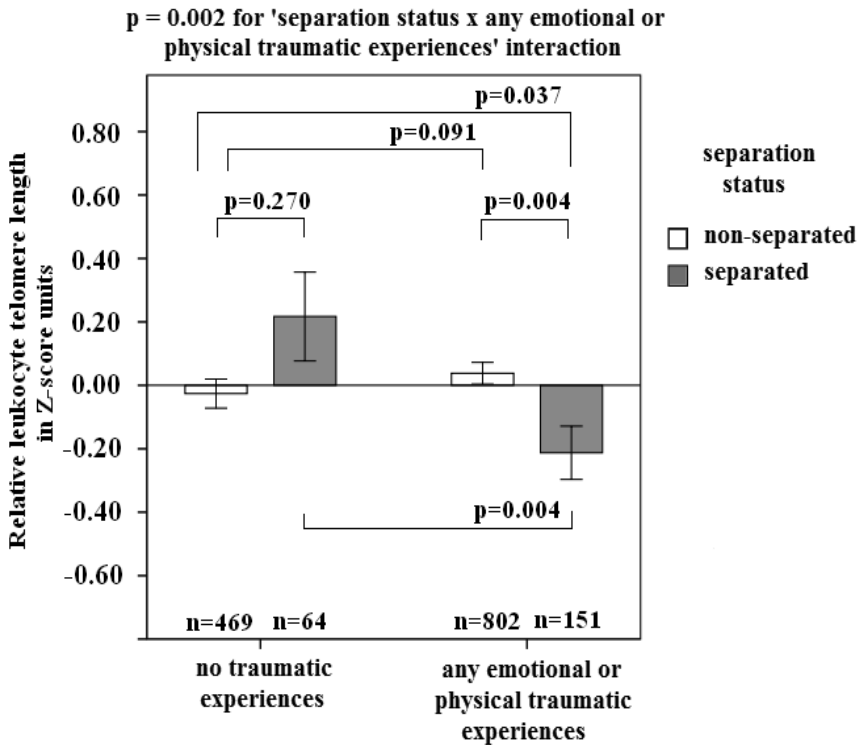


Figure 8. Associations between retrospectively self-reported any emotional or physical traumatic experiences and leukocyte telomere length (LTL) in participants who were and who were not separated from their parents in childhood. Bars represent mean LTL (\pm standard error of the mean). P-values are from Model 1.

4.4 Sleep apnea (Study IV)

As predicted, participants with sleep apnea and primary snoring were more frequently men ($p < 0.010$) and participants with apnea also had a higher BMI than controls ($p < 0.001$).

Participants with sleep apnea had shorter LTL than control participants ($p = 0.010$ for Model 1, Figure 9), whereas participants with a history of primary snoring did not differ in LTL from the controls ($p = 0.90$, Figure 9). Participants with sleep apnea with and without primary snoring did not differ in LTL either ($p = 0.56$, Figure 9). No significant interactions existed in sex x sleep apnea or sex x

primary snoring ($p > 0.13$). Age at first hospitalization for sleep apnea or primary snoring was not associated with LTL ($p > 0.40$).

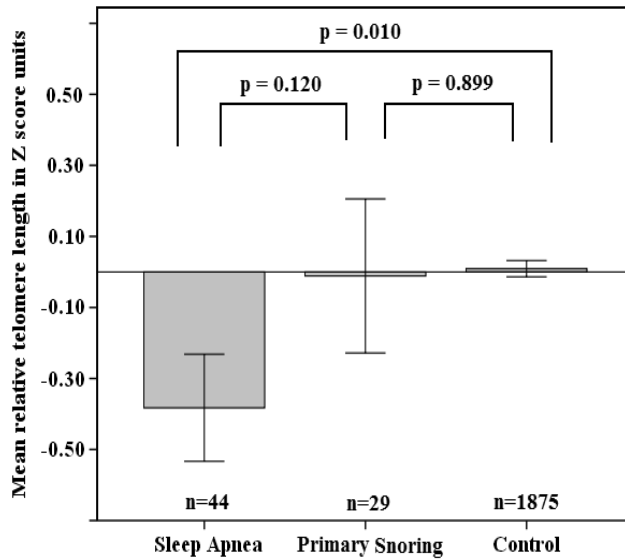


Figure 9. Associations between history of sleep apnea and primary snoring and relative leukocyte telomere length. Bars represent mean relative leukocyte telomere length in Z score units. Error bars represent standard errors of the mean. P-values are from Model 1.

4.5 HPA axis reactivity (V)

Time difference between the blood sample for LTL and attending TSST was not significantly associated with LTL ($p > 0.53$).

In the analyses of linear effects, LTL was not significantly associated with overall levels of salivary cortisol, plasma cortisol, ACTH during the TSST (p -values for LTL main effects > 0.83 in Model 1), with salivary cortisol, plasma cortisol or ACTH responses during the TSST (p -values for LTL \times sampling time-interactions > 0.70). There were no significant non-linear effects either (p -values for LTL main effects > 0.30 ; p -values for LTL \times sampling time interactions > 0.10).

There were no significant linear associations between LTL and summary measures of HPA axis activity either (p-values > 0.50, Table 5) nor were there significant interactions by sex (p-values > 0.05 for sex × LTL interactions). In the quadratic analyses two associations were significant: individuals with shorter and longer LTL had higher plasma ACTH at base-line and lower plasma ACTH AUC increment in response to TSST (p-values < 0.029 in Model 1, Table 5); however only the association with plasma ACTH AUC increment remained significant in Model 2 (p = 0.022). Neither of these non-linear associations remained significant after Bonferroni correction for multiple testing (both p-values > 0.05).

Table 5. Linear and non-linear associations between leukocyte telomere length and salivary and plasma cortisol and plasma ACTH concentrations during the Trier Social Stress Test.

| | Linear associations | | Quadratic associations | |
|-----------------------------------|---------------------|-------|------------------------|-------|
| | 95%CI | p | 95%CI | p |
| Salivary cortisol (nmol/l) | | | | |
| Baseline | -0.073, 0.076 | 0.967 | -0.368, 1.131 | 0.32 |
| Peak after stress | -0.058, 0.098 | 0.614 | -0.940, 0.624 | 0.70 |
| AUC | -0.068, 0.070 | 0.974 | -0.769, 0.611 | 0.82 |
| AUC increment | -0.060, 0.068 | 0.906 | -1.021, 0.031 | 0.07 |
| Plasma cortisol (nmol/l) | | | | |
| Baseline | -0.053, 0.059 | 0.923 | -0.175, 0.948 | 0.18 |
| Peak after stress | -0.054, 0.044 | 0.852 | -0.732, 0.264 | 0.36 |
| AUC | -0.053, 0.050 | 0.956 | -0.598, 0.450 | 0.78 |
| AUC increment | -0.057, 0.048 | 0.876 | -1.156, 0.127 | 0.12 |
| Plasma ACTH (pmol/l) | | | | |
| Baseline | -0.059, 0.083 | 0.740 | 0.088, 1.512 | 0.028 |
| Peak after stress | -0.109, 0.064 | 0.611 | -1.035, 0.718 | 0.72 |
| AUC | -0.075, 0.073 | 0.976 | -0.624, 0.886 | 0.73 |
| AUC increment | -0.069, 0.039 | 0.490 | -1.286, -0.069 | 0.029 |

AUC = time-weighted area under the curve (calculated by the trapezoidal rule), AUC increment = time-weighted area under the curve from baseline (calculated by the trapezoidal rule). P-values are from Model 1.

5 Discussion

The aim of this thesis was to study whether stress-related factors are associated with LTL, and thus, if LTL could provide insight into the mechanisms explaining why stress is associated with increased risk for aging-related diseases and early mortality. Majority of our findings did not support our hypotheses as we did not find associations between personality dimensions and LTL (Study I), mental disorders and LTL (Study II), objectively measured ELS and LTL (Study III), self-reported traumatic experiences across lifespan and LTL (Study III) or stress reactivity and LTL (Study V). In line with the study hypotheses, however, were the findings showing that the combination of objectively measured ELS and self-reported traumatic experiences (Study III) and a history of sleep apnea (Study IV) were associated with shorter LTL. Findings of this thesis also showed, unexpectedly, that patients hospitalized for mental disorders who used psychotropic medication had longer LTL than non-hospitalized controls (Study II); Also agreeable personality dimension associated with LTL in a sex-specific way such that men with more agreeable and women with less agreeable personality had shorter LTL (Study I).

5.1 Comparison to the current and previous findings

5.1.1 Personality dimensions

In the Study I, the lack of associations between FFM personality dimensions and LTL was somewhat unexpected and against our hypothesis. As previous studies had linked higher conscientiousness with longevity and better health and neuroticism with increased mortality and worse health (Charles et al., 2008; Goodwin & Friedman, 2006; Hill et al., 2011; Jokela et al., 2014; Jokela et al., 2013; Sutin et al., 2010), we expected that these personality dimensions would have been associated with LTL. Neither do these null findings in the present thesis support the previous studies where neuroticism and dispositional tendencies, including hostility and pessimism, were associated with shorter LTL (Brydon et al., 2012; O'Donovan, 2009; Van Ockenburg et al. 2014).

Our findings were also not in line with a recent study that was also testing the associations between FFM personality traits and LTL at on average 23-year-old Japanese university students and found that from the five personality traits lower neuroticism and lower conscientiousness were associated with shorter LTL (Sadahiro et al., 2015). However, our null findings of neuroticism and LTL and counterintuitive findings of lower neuroticism and shorter LTL found by Sadahiro et al. (2015) may not be that surprising when taking into account that the studies on neuroticism and mortality are also incoherent. Higher neuroticism has been shown to predict both better survival as well as all-cause mortality, in addition studies that have not found any association between neuroticism and mortality or have found neuroticism to predict survival only sex-specifically, also exists (Friedman et al., 1993; Jokela et al., 2013; Korten et al., 1999; Terracciano et al., 2008; Weiss & Costa Jr, 2005; Wilson et al., 2005). Thus it seems that neuroticism may have both protective and harmful influences on life expectancy, therefore it may not be surprising that the associations between neuroticism and LTL may vary.

Our sex-specific analyses revealed that more agreeable men and less agreeable women had shorter LTL. The recent study on FFM and LTL did not test whether sex moderated the effects (Sadahiro et al., 2015), thus our results are not yet replicated. As these sex-specific findings are still lacking replication, we cannot draw strong conclusions from our findings.

5.1.2 Mental disorders

In the Study II, it was demonstrated that a past history of any mental disorder severe enough to warrant hospitalization was associated with longer LTL and any psychotropic or antidepressant medication use modulated this association. Longer LTL was particularly characteristic of those mental disorder patients who also had any psychotropic or antidepressant medication use, as only they differed from the non-hospitalized controls. These findings are not in line with previous studies where mental disorders have either been associated with shorter LTL (Fernandez Egea, 2009; Hartmann et al., 2010a; Hoen et al., 2011; Kananen, 2010; Kao et al., 2008; Lung, Chen, & Shu, 2007; Pavanello et al., 2011; Simon,

2006) or no associations has been found (Mansour et al., 2011; Wolkowitz et al., 2011). More recent studies, however, are more in line with our findings. Recently published review article has summarized that the associations between major depressive disorder, psychotic disorders, bipolar disorders, anxiety disorders, post traumatic disorders and LTL are not concordant (Lindqvist et al., 2015). There are also studies that have replicated our counterintuitive findings that psychiatric patients may also have longer LTL than healthy controls (Martinsson et al., 2013; Nieratschker et al., 2013). The modulating effect of psychotropic medication in the present thesis, as well as results from the studies by Martinsson et al. (2013) and Nieratschker et al. (2013), suggest that the positive associations between psychiatric disorder and LTL could be mediated through the psychotropic medication use. All of the patients diagnosed with schizophrenia and longer LTL in the study of Nieratschker et al. (2013) were receiving psychotropic medication, and all of the patients with bipolar disorder and longer LTL in the study of Martinsson et al. (2013) were receiving lithium treatment. And even further, it was shown that longer lithium treatment as well as better responding to lithium therapy in bipolar disorder patient associated with longer LTL (Martinsson et al., 2013).

The mechanism behind these possibly modifying effect of psychotropic medication is however not clear. It is not known if psychotropic medication in the present thesis increased LTL, or slowed down the LTL erosion or if the mental disorder patients who also had any psychotropic or antidepressant medication use had longer telomeres at baseline. There is evidence that antidepressants may act as antioxidants in a cell (Zafir, Ara, & Banu, 2009) and thus reduce the oxidative stress in the cell. However this does not explain why LTL was longer only in the participants who had been hospitalized for severe mental disorder but not in the participants in the control group without hospitalization but who had also received psychotropic or antidepressant medication. In addition, also opposing results on psychotropic medication in mental disorder populations have been reported as individuals with past year major depression who were receiving antidepressants had shorter LTL than controls, but depressed individuals who were not taking antidepressants did not (Needham et al., 2015). Therefore it is possible that other mechanisms may explain our results.

Alternative explanations may include survival effect. Mental disorders patients in the Nordic countries have shown to live 20 and 15 years less, than their counterparts in the general population (Wahlbeck, Westman, Nordentoft, Gissler, & Laursen, 2011) and as the participants of the present study were on average 62 years at the time of the telomere measurement, it is possible that LTL is longer in those mental disorder patients who have survived to older age. There is also evidence from genome wide association studies that the same loci that associates with LTL also associates with longevity (Atzmon et al., 2010). Therefore it is possible that the same genetic basis may partly explain longer LTL and better survival also in mental disorders population.

Another explanation may be related to the fact that we did not exclude subjects with multiple mental disorder diagnoses. Because substance abuse disorders have been shown to have high comorbidity with other mental disorders (Najt, Fusar-Poli, & Brambilla, 2011), it may be that the positive associations we found between any mental disorders and LTL are partly explained by the subjects with substance abuse disorders. Recent studies on alcohol consumption and LTL are contradictory: some studies have shown that alcohol consumption is associated with shorter LTL, other have found no associations, and some of the studies have reported that higher alcohol consumption is associated with longer LTL (Latifovic, Peacock, Massey, & King, 2016; Pavanello et al., 2011; Shin & Baik, 2016; Strandberg et al., 2012; Weischer et al., 2014). The mechanisms, once more, are not well understood, even though a recent study showed that genetic risk factors may play a role in these seemingly contradictory results. It was shown that almost daily light-to-moderate alcohol consumption was associated with longer LTL only on carriers of common allele (CC) of AHDH2 gene, but not among those with the rare allele carriers when compared in LTL with participants with lower alcohol consumption level (Shin & Baik, 2016).

Our results on self-reported depression, anxiety and loss of vitality indicate that these symptoms are not associated with LTL. These results are in line with the recent studies. A recent review has shown that 12 out of 16 studies did not find association between depression (that was measured from participants that didn't have major depressive disorder diagnoses) and LTL (Lindqvist et al., 2015). This lack of association is not surprising as age-corrected LTL is thought to reflect a

cumulative measure of the history of oxidative damage that a cell has undergone (Kawanishi & Oikawa, 2004). It is possible, that measurement of symptoms of depression, anxiety and loss of vitality that past two to four weeks, may be too short a period of time for measurable LTL shortening to occur.

5.1.3 Early life stress

In the Study III, contradictory to the study hypotheses, objectively measured ELS and self-reported traumatic experiences across the lifespan were not associated with LTL. Yet, we found that participants who had experienced both, objectively measured ELS and also self-report traumatic experiences across the lifespan, had significantly shorter LTL. In the current literature, retrospectively reported ELS has been associated with shorter LTL in some (Kananen, 2010; Kiecolt-Glaser, 2011; O'Donovan et al., 2011; Surtees et al., 2011; Tyrka et al., 2010) but not all studies (Glass et al., 2010; Schaakxs et al., 2015; Verhoeven et al., 2015). Our results may shed light on these ambiguous findings. It is possible that also in the studies finding associations between ELS and shorter LTL (Kananen, 2010; Kiecolt-Glaser, 2011; O'Donovan et al., 2011; Surtees et al., 2011; Tyrka et al., 2010), the associations may be driven by a subset of individuals who also would recall traumatic experiences across the lifespan.

We cannot address the mechanism directly and therefore can only speculate that the harmful effect that cumulative traumatic experiences have on LTL may be explained through body's sensitization processes, in which the ELS sensitizes individuals such that the later stressful experiences are particularly impactful. This interpretation is supported by both animal and human studies showing that ELS can change later behavioural outcomes (for example to use less maladaptive strategies when coping with stress), higher cortisol levels and altered cortisol reactivity in response to stressful stimuli as well as increase risk for immune dysregulation across the lifespan (Fagundes, Glaser, & Kiecolt-Glaser, 2013; Heim et al., 2000; Hennessy, Paik, Caraway, Schiml, & Deak, 2011; Hyman, Paliwal, & Sinha, 2007; Yusko, Hawk, Schiml, Deak, & Hennessy, 2012) that may also be reflected in shorter LTL.

However, it is also possible that the reported cumulative effects may reflect the quality, and hence the severity, of the ELS. This interpretation would be supported by Schilling et al. (2008) who have suggested that the combination of adverse experiences may rather reflect the severity of the early experience than the actual accumulation of separate traumatic events (Schilling, Aseltine, & Gore, 2008). In this case, our results would give further support to the hypotheses that especially severe ELS associate with shorter LTL.

5.1.4 Sleep apnea

In the Study IV, it was shown that a history of sleep apnea severe enough to be recorded as an inpatient diagnosis for hospitalization was associated with shorter LTL. The results are in line with the still scant research literature which has demonstrated in a case-control setting that sleep apnea (apneas or hypopneas per hour of sleep > 10) and mild sleep apnea ($5 \leq$ apneas or hypopneas per hour of sleep < 15) were associated with shorter LTL (Barceló et al., 2010; Shin et al., 2016). Our results give further support for the hypotheses that sleep apnea is associated with shorter LTL. Sleep apnea is associated with increased ROS production in leukocytes, lower antioxidant capacity and greater oxidative stress levels (Christou, Markoulis, Moulas, Pastaka, & Gourgoulianis, 2003; Christou, Moulas, Pastaka, & Gourgoulianis, 2003; Dyugovskaya, Lavie, & Lavie, 2002). The oxidative stress may be prompted by pauses of breathing that in turn cause temporarily deprivation of oxygen supply causing a state of intermitted hypoxia, that has shown to trigger oxidative stress and mitochondrial dysfunction (Peng, Yuan, Overholt, Kumar, & Prabhakar, 2003). Thus greater oxidative stress may speed up telomere erosion in each cell division (Houben et al., 2008; Kawanishi & Oikawa, 2004).

Clinician-diagnosed snoring was not associated with LTL in our sample. There is one other study testing the associations between snoring and LTL, in which relative time spent in snoring (measured by nasal cannula which monitors the pressure fluctuations produced by snoring) was associated with shorter LTL but self-reported frequency of snoring (snoring nights / week) was not (Shin et al., 2016). Neither this study nor the study of Shin et al. (2016) found differences in

LTL between participants with sleep apnea and snoring (Shin et al., 2016). As the sample size of snorers in our cohort was relatively small ($n = 29$) and we were unable to identify the participants that snore but who were not admitted to hospitals, which may weaken our ability to find significant associations, we cannot draw strong conclusion from the lack of associations between snoring and LTL.

5.1.5 HPA axis reactivity

In the Study V, no linear nor non-linear associations between LTL and HPA axis activity during a standardized psychosocial stress test, that would have survived adjustments for covariates and correction for multiple testing, was detected. In the current literature cortisol reactivity in psychosocial stress test has been associated with shorter telomere length in two (Gotlib et al., 2015; Tomiyama et al., 2012) but not in one study (Kroenke et al., 2011). However the previous studies are not fully comparable to ours as they only include women (Tomiyama et al., 2012) or under 14 year old children (Gotlib et al., 2015; Kroenke et al., 2011). Hence, our study is still the largest study in adult men and women showing no associations between LTL and HPA axis stress reactivity and thus suggesting that differences in HPA axis reactivity does not account for large shares of variation in LTL.

These findings, however, cannot be used for concluding that physiological stress reactivity in general is not associated with LTL. HPA axis reactivity is only one part in the physiological stress cascade where complex multidimensional dynamics between HPA and SAM axis as well as immune system activities have shown to play central roles (Chrousos & Gold, 1992; Gunnar & Quevedo, 2007; Lupien et al., 2009). Accordingly, it has been demonstrated that a cumulative index of HPA axis, inflammation and autonomic nervous system stress markers may be associated with shorter LTL (Révész et al., 2014). Also the study of Kroenke et al. (2011) showed that children who had both a higher salivary cortisol and higher autonomic nervous system activity in response to psychosocial stress, had shorter telomere length than less reactive comparisons (Kroenke et al., 2011).

These findings suggest that a more broad-spectrum stress system activation may be needed to capture physiological stress and stress responses to influence LTL.

5.2 Methodological considerations

Comparing and generalizing results from scientific studies that are always methodologically slightly or substantially different, is never straightforward and critical consideration is always needed. Studies related to telomere length, however, have multiple crucial aspects that make the comparison especially challenging.

First, telomere length, by nature, is a dynamic structure, which can shorten and lengthen over time (Blackburn, 2005). In addition, the telomere length loss/gain speed is not linear across the lifespan but varies across the years (Aubert et al., 2012; Farzaneh-Far et al., 2010; Frenck et al., 1998). We also still know very little about the individual variations in telomere length across the lifespan in humans as most of the studies have been cross-sectional and the longitudinal studies following the telomere length in the same individuals are limited to a maximum of few decades and a few measurement points. Unfortunately using animal models to disentangle the open questions of telomere length dynamics may not be too informative nor generalized to humans, as the telomere length and telomerase dynamics are shown to differ greatly between the species (Kakuo et al., 1999; Prowse & Greider, 1995). In addition, related to telomere length dynamics, it is shown that the shortest telomeres are the ones to lengthen most often (Farzaneh-Far et al., 2010; Nordfjäll et al., 2009), adding another aspect to take into the consideration when drawing conclusions from studies with single or few telomere length measurement points. Consequently, these known and unknown aspects in telomere length dynamics mean that some of the association between telomere length and independent variables studied, may exist in some age groups but not necessarily in others and for these reasons generalization over the age groups is not supported. In addition, due to the dynamic structure, there may still be many unstudied factors that can impact the telomere length dynamics and thus also explain some discrepancies between the studies with telomere length measurement.

Secondly, there is a notable variability in the DNA collection and the measurement choices that also impact telomere length measurement that in turn may play a role in the result of an individual study, but more importantly, the different DNA collection and measurement methodologies may complicate generalizing the results as well as compromise comparing the results between different studies. To be more specific, studies vary in the cell types used to extract the DNA, the methods used for the DNA extraction, and the technique used for telomere length measurement. All of these factors have shown to influence the actual telomere length measurement (Aubert & Lansdorp, 2008; Cunningham et al., 2013; Denham et al., 2014; Dlouha et al., 2014; Gutierrez-Rodrigues et al., 2014; Hofmann et al., 2014; Martin-Ruiz et al., 2015). It is demonstrated that due to the choice of telomere length measurement technique, the associations between independent factor and telomere length may either be found or not (Elbers et al., 2014). The correlations between telomere length measurement between different laboratories and measurement techniques vary from low to high (Aviv et al., 2011; Gutierrez-Rodrigues et al., 2014; Martin-Ruiz et al., 2015). Put together, it needs to be kept in mind that the discrepancies between the studies using telomere length measurement can always be partly be explained by different measurement choices. Evaluating the proportion of effects in published studies is however almost impossible. Future will show whether more precisely defined, easily replicated and widely used golden standards for telomere length measurement will be developed, and if the unwanted effects caused by variation in measurement techniques will be overcome, and if the reliability of telomere length measurement across studies will thus increase. This development may help to clarify the role that telomere length shortening plays also in stress-related phenotypes. However, before that, we should keep reporting the procedures used for determining the telomere length carefully and avoid drawing too strong conclusions especially from less replicated studies. It is also difficult to know if and how much the measurement methodology related issues explained the discrepancies between our results and the related literature. The awareness of how much the measurement choices influence telomere length measurement has only started to emerge, and therefore for example reporting the DNA extraction methods are still lacking in most of the studies. Nonetheless, our findings in large

elderly birth cohort with carefully reported telomere length measurement section are important for expanding the still relatively scant literature on stress-related factors and telomere length.

Third, Telomere length biology is relatively new field of study. Yet, telomere biology is complex and LTL is dynamic making the field of study challenging. This novelty of this biomarker may easily lead to positive publication bias, in favour of those results supporting the hypothetical pathway and leading the null or unexpected associations not published. Therefore our null and unexpected findings are particularly important for widening the understanding of the multifaceted dynamics of LTL.

5.2.1 Strengths and limitation in our study

In addition to the common methodological considerations in telomere length research field, there are specific strengths and limitations in our study setting that should be taken into consideration when drawing conclusions from the results.

One of the main strengths in our studies was the use of a representative and large birth cohort of elderly adults and the ability to control for a number of covariates and confounding factors. While the list of covariates and confounders that we used was extensive, it was still not comprehensive. For instance, we cannot rule out that our existing or null associations would be explained or moderated by genetic or prenatal factors that have been linked with telomere length (Broer et al., 2013; Codd et al., 2010; Codd et al., 2013; Entringer et al., 2015b; Kimura et al., 2008; Pooley et al., 2013; Prescott et al., 2012; Salihu et al., 2015; Theall et al., 2013). As our study cohort now provides genome-wide data on DNA of the study participants, this will be a question that can be answered in future research.

We also used well validated and objective measurements. In the Study I personality traits were measured with FFM personality trait questionnaire and in the Study V psychosocial stress reactivity with TSST. In the Studies II and IV, the mental disorder, sleep apnea and snoring cases were obtained from a hospital register and thus all were clinician-diagnosed and severe enough to warrant hospitalization. In the Study III, the ELS cases, namely participants with

temporary separation from both parents, were identified from the Finnish National Archives' register.

We decided to use qPCR method for LTL measurement and one of the limitations of using it, is its relatively high inter-assay variability. However, this method is fast, cheap, and does not require much DNA and for these reasons it is commonly used in epidemiological research. There are no golden standards for acceptable cut off limits for CV in telomere assays and the number of plates and samples affect the CV values. Our 24.8% inter-assay coefficients of variation for T/S ratio (four controls on each of the twelve plates) is relatively high, yet comparable to other studies. For example Kajantie et al. (2012) have reported telomere length CV's of 28% for the HeSVA cohort (two controls on each of the four plates) and 13% for FinnTwin16 cohort (four controls on each of the four plates).

We also measured LTL only once, and without baseline LTL measurement we cannot directly address the underlying mechanisms that explain the detected significant associations between mental disorder patients who also had any psychotropic or antidepressant medication use, combination of ELS and traumatic experiences and sleep apnea. More precisely, we do not know if the participants increased LTL, or slowed down the LTL erosion or if they had longer telomeres at baseline. We also did not have telomerase enzyme measurement, so we cannot determine the effects telomerase activation plays on our significant or null results.

As we are analysing cross-sectional associations between LTL and complex stress-related variables that can activate multidimensional physiological pathways we can only speculate the possible mechanisms between the variables.

In the Study II, it should be noted that psychotropic medication, including antidepressant medication entitlements and prescription fills do not necessarily represent actual medication use as they can be prescribed for other than mental disorder treatment purposes. The hospital discharge register that was used to identify the cases have recorded diagnoses from 1969 onwards; thus the individuals in our study who were hospitalized for mental disorders only before the age of 25 - 35 remain undetected for having mental disorder. As the number of individuals with specific mental disorder diagnoses are relatively small and as

we also included subjects with multiple mental disorders diagnoses, the interpretations drawn from the associations between specific disorder diagnoses and LTL are somewhat limited. Finally, we do not have information on those mental disorders that did not warrant admission to hospital, and therefore our findings apply only to the most severe mental disorder cases.

In the Study III, we had no data available on the stressfulness of the separation period from both parents, so we do not know how much more stressful the separation was in comparison with subjects who stayed with their parent during the war. We also used retrospective self-reports of traumatic experiences. These self-reports are not free from bias related to psychopathology, mood state, personality traits or intelligence (Bradley & Mogg, 1994). However, it is suggested that recall bias produced by such factors as mood state are not very strong (Duggan, Sham, Minne, Lee, & Murray, 1998; Hardt & Rutter, 2004).

In the Study IV, we have no information on sleep apnea or primary snoring in individuals not admitted to hospitals, and therefore our study sample also may include some of these individuals. In addition, 21.4% of the cases in our cohort with sleep apnea were diagnosed with snoring before the apnea diagnosis; therefore, although snoring also occurs without sleep apnea, we cannot determine if some of these cases will fulfil the apnea diagnosis in the future. We were unable to differentiate between central and obstructive sleep apnea or test the effects of treatments (for example the use of continuous positive airway pressure) on LTL.

In the Study V, sampling of blood for LTL and attending TSST did not occur simultaneously: the time differences between the two examinations ranged between 0.4 and 3.5 years. We cannot entirely rule out that the time difference may give some explanation for the lack of associations. However, adjusting for this time difference did not alter the null associations between LTL and HPA axis stress reactivity, nor did the time difference modulate any associations between LTL and HPA axis summary measures.

5.3 Implications of the findings

5.3.1 Telomere pathway hypotheses in stress and aging-related diseases

Overall our findings do not give strong support for the hypotheses that stress and stress-related factors are associated with LTL as shorter LTL was not associated with personality dimensions, mental disorders, depressive symptoms, objectively measured ELS, self-reported traumatic experiences across the lifespan or HPA axis stress reactivity. Therefore majority of our results suggest that LTL does not provide insight into the mechanisms explaining why stress is associated with increased risk for aging-related diseases and early mortality. However, as the combination of ELS and traumatic experiences across lifespan as well as sleep apnea were associated with shorter LTL, and as current literature recognizes several methodological issues related to telomere length measurements (discussed in 5.2.) that suggests that too strong or generalized conclusions should not be drawn from single studies using LTL measurement, it is possible that LTL plays some role in stress and aging-related diseases.

It's also important to point out, that in the present thesis the effect sizes (R^2) that the stress-related factors that were associated with shorter LTL explained from the variance of LTL, were relatively small. Combination of ELS and any/emotional/physical traumatic experiences explained 0.4% to 1.2% and sleep apnea / snoring status 0.4% from the total variance of LTL respectively. These small effect sizes mean that even though some stress-related factors may associate with LTL the overall and clinical impacts remain relatively small. For this reason it can be argued that telomere length plays a relatively small role in explaining the associations between stress-related factors and aging-related diseases.

With respect to effect sizes it is however worth comparing the effect sizes between stress-related factors on LTL with some other factors that are known to be associated with LTL: In our cohort age explained 1.3% and sex 0.6% of the variance in LTL. The effect sizes are not commonly reported in LTL literature but our age and sex percentages are somewhat in line with the study of De Meyer et al. who reported that current age of the participant explained 5.3% and sex 1.3% of the variance in TL (De Meyer et al., 2007). These overall small effects,

especially in elderly adults in our cohort, are not surprising, bearing in mind the great variety of factors, including genetic, physiological and environmental, that have shown to contribute to telomere length. And also that our sample comprised of genetically homogenous individuals whose range in age at the time of LTL measurement was relatively narrow. There does not exist longitudinal data in humans following the telomere length of same individuals across lifespan but animal study on birds have shown that the birds that had relatively long telomere length at old age had long telomeres at all points they were measured, already at early ages (Heidinger et al., 2012). As telomere length varies between subjects already at birth (Okuda et al., 2002) and it is highly heritable (Broer et al., 2013) it may be that great part of telomere length is explained by genetic and prenatal factors. Recently some of the LTL researchers have come to a conclusion that “heritability and early life environment are the main determinants of LTL throughout the human life course” (Hjelmborg et al., 2015).

Accordingly, our results that at the first sight are somewhat unexpected in respect to telomere pathway hypotheses, may in the light of more recent literature not be all that surprising. There is clear evidence that in severe cases, such as in dyskeratosis congenita, which is caused by malfunctioning in telomere maintenance genes, that shortened telomeres are causing multiple somatic signs of premature senescence (Sarek et al., 2015). However within more normative population, even with the reasonably well documented association between LTL and atherosclerosis, the telomere driven mechanism behind atherosclerosis is still only hypothetical (Sanders & Newman, 2013). It is for example unclear how big a role telomere shortening plays in telomere uncapping and senescence processes. Telomere uncapping can be measured directly by p-histone γ -H2A.X (ser139) localized to telomeres (chromatin immunoprecipitation) and by telomeric repeat binding factor 2 bound to telomeres (Morgan et al., 2013). In the study of Morgan et al. telomere length measured in arterial tissue was shorter in older individuals but it did not associate either with the uncapping of the telomeres nor p53/p21 induced senescence in the same tissue (Morgan et al., 2013). Telomere uncapping, on the other hand was associated expectedly with p53/p21 induced senescence (Morgan et al., 2013). These findings suggest that *in vivo* telomere length shortening may not reflect the risk of telomere uncapping

and related p53/p21 activation. In addition, these findings question the whole role of telomere length shortening in the process of activating senescence pathways, and points out that other factors than the length of the telomeres may be more important to prompt telomere uncapping and related p53/p21 activation. The modelled lack of association between telomere length and senescence pathway activation (Morgan et al., 2013) could also partly explain the null associations between stress-related factors and LTL in the present thesis.

There is not consensus in telomere research field on either what role LTL has in health and disease, or how big is its value as a biomarker of healthy aging. Houben et al. have evaluated the use of a telomere length in biomarker of chronic oxidative stress, and even though they don't totally dismiss it, they critically warn for drawing causal relationships from association studies and suggest to take the strong genetic factor in LTL, as well as high interindividual variability and difference in LTL measurement techniques into account (Houben et al., 2008). Other researchers have ended up with similar conclusions. Sanders & Newman reason that it is still unknown whether LTL is a biomarker of aging, however, based on existing studies they suggested that even if it is, it is a weak biomarker with poor predictive accuracy compared with many traditional covariates (Sanders & Newman, 2013).

As majority of the studied stress-related factors in the present PhD thesis did not associate with shorter LTL, and the effect sizes in the few existing associations were relatively small, it seems plausible that other mechanism are more important in explaining why stress is associated with increased risk for aging-related diseases and early mortality. Some of the still unknown underlying mechanism between stress-related factors and health outcomes may be related, for example, by less studied stress induced epigenetic changes (Cattaneo et al., 2015).

5.3.2 Suggestions for further studies

This thesis has raised several suggestions for further studies. Our sex-specific associations between personality traits and LTL await replication. The potential cellular mechanisms that psychotropic medication could have within mental

disorder populations should be studied further. Prospective studies testing the impact of successful sleep apnea treatments on LTL are clearly warranted. Associations between LTL and HPA axis stress reactivity using concurrently collected samples and broad-spectrum stress system activation should be explored.

In general, telomere biology research would benefit from precisely defined, easily replicated and widely used golden standards for telomere length measurement. Longitudinal studies in humans with frequent telomere and telomerase enzyme measurement would be important for better understanding of the basic LTL dynamics. Also the still hypothesized role of telomere shortening in telomere dysfunction in normative human population is warranted. As telomere length by itself may not be that useful biomarker of cellular aging (Houben et al., 2008; Sanders & Newman, 2013), taking into account the role of telomerase enzyme or the ratio of telomere length and telomerase activity, the value of LTL measurement could increase. It has been suggested that telomerase and telomere length ratio may indicate active cell stress and/or an unsuccessful compensatory attempt of telomerase to lengthen or maintain LTL (Lindqvist et al., 2015), that may reflect overall health status more accurately than LTL. And finally, as telomere length is strongly inherited, with supplementary paternal age effects, the genetic and epigenetic inheritance studies are also well justified.

5.4 Conclusions

Results of this thesis showed that shorter LTL was not associated with personality dimensions, mental disorders, depressive symptoms, objectively measured ELS, self-reported traumatic experiences across lifespan or psychosocial stress reactivity. However, the combination of ELS and traumatic experiences across lifespan, and sleep apnea were associated with shorter LTL, with effect sizes ranging from 0.4% to 1.2%. Also two unexpected findings showed that patients hospitalized for mental disorders who had psychotropic medication use had actually longer LTL than non-hospitalized controls and that men with more agreeable and women with less agreeable personality had shorter LTL. It can be concluded that in disagreement with our study hypotheses, majority of the

studied stress-related factors did not associate with shorter LTL and the effect sizes in the few existing associations were relatively small. These results suggest that stress related factors are not strongly associated with LTL.

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