Psychobiological effects of juvenile violence exposure: effects on telomere erosion

Idan Shalev

PART 1: The evidence base

The psychobiological outcome: Telomeres

Telomeres are the repetitive TTAGGG sequence at the end of chromosomes with a main function to cap and protect the ends of chromosomes. This repetitive non-coding sequence of telomeres is conserved throughout evolution, for all vertebrates, and is thought to share a common ancestor over 400 million years ago (Meyne, Ratliff & Moyzis, 1989). Telomeres can be seen as analogous to the plastic caps at the end of shoe laces which keep the laces from unraveling. They play a major role in regulating cellular replication, and shorten with increasing age in replicating human tissues. In certain cell types, such as germ cells and stem cells, the length of telomeres is maintained by the enzyme telomerase that can add telomeric repeats to the chromosome ends. Most somatic cells, however, lack sufficient telomerase and as a consequence, telomeres shorten progressively with each cell division. Upon reaching a critically short length, cells enter a state of replicative arrest called senescence. Thus, progressive telomere shortening is thought to serve as a molecular clock for cellular replicative aging (Sahin & Depinho, 2010).

The discovery of telomeres has a long and intriguing history dating back to the early 20th century. The first hint that chromosomes ends bore special features came from analysis of fruit fly mutants. In the early 1930s Herman Muller was using high energy x-rays to produce mutants with deletions and inversions involving the ends of chromosomes. Although Muller detected many flies with a wide spectrum of genetic abnormalities, he never found mutants with deletions or inversions involving the natural ends of the chromosomes. He noted that “the terminal gene must have a special function, that of sealing the end of the chromosome, so to speak, and that for some reason a chromosome cannot persist indefinitely without having its ends thus sealed” (Muller, 1938). Muller coined the term telomere for this terminal end from the Greek, meaning simply ‘end part’. The fact that the end of chromosomes deserved a unique name was important recognition that telomeres have a specific function.

In 1910s, following experiments conducted by Alexis Carrel, it was erroneously believed that normal human cells had the capacity to divide and function indefinitely in culture (Carrel, 1912). It was thought that aging could not be the result of events that occurred within normal cells but rather the result of extracellular events. This erroneous concept was held for almost 5 decades. In 1960s, the concept of cell immortality was challenged by experiments published by Hayflick and Moorehead (Hayflick & Moorhead, 1961). It was discovered that fibroblast cells have a built-in mechanism that limit their replicative capacity, later named the 'Hayflick limit'. They theorized that the limited capacity for normal cells to divide is an expression of aging, and that it determines the longevity of the organism. Later it was found that the mechanism that limits cellular replication was located in the nucleus of the cell.

The chain of discoveries continued in the early 1970s when Olovnikov (Olovnikov, 1971) and Watson (Watson, 1972) independently recognized the 'end

replication problem', the loss of nucleotides at each cell division, suggested that telomeres are lost with each replication until a certain (Hayflick) limit, at which point the cell arrest. Subsequent research confirmed that telomeres indeed get shorter with each division and established the utility of telomeres as a molecular clock for cellular replicative aging (Blackburn & Gall, 1978; Harley, Futcher & Greider, 1990).

Telomere length is important predictor of physical health and quality of life

In the last years, telomeres are emerging as a promising new candidate in epidemiological research. Shorter telomere length (TL) and increased erosion rate are both associated with higher risk of morbidity and mortality. *In vitro* studies have established a causal link between telomere shortening and cellular senescence (Bodnar et al., 1998). Studies using animal models have provided support for the predictive utility of TL early in life on the actual lifespan of birds (Heidinger et al., 2012), and the potential to reverse tissue degeneration in aged, telomerase-deficient, mice by telomerase activation (Jaskelioff et al., 2011). Other studies have shown that shortened telomeres are associated with lower survival rate also in humans (Cawthon et al., 2003; Ehrlenbach et al., 2009b). The advent of high-throughput and cost-effective laboratory techniques that measure TL, in buccal cells or peripheral blood cells, opened the gate to new studies linking shorter TL with a broad range of risk factors that predict disease morbidity, including smoking, obesity (Buxton et al., 2011; Nordfjall et al., 2008; Valdes et al., 2005), schizophrenia (Yu et al., 2008), mood disorders (Simon et al., 2006), and psychosocial stress (Epel et al., 2004; Kiecolt-Glaser et al., 2011). It was suggested that TL is a marker for biological aging, rather than clock to chronological aging.

Literature linking juvenile violence victimization to telomere length

Childhood stress is associated with later-life physical and mental-health problems (Dong et al., 2004; Nanni, Uher & Danese, 2011; Rich-Edwards et al., 2010; Riley et al., 2010; Wegman & Stetler, 2009). Interest in the etiological pathways that mediate the effect of early-life stress on physical and mental health has focused on key biological systems, including the sympathetic nervous system, hypothalamus-pituitary-adrenal axis, immune system, and the epigenome, leading to important insights about the systemic effects of stress (2006; Danese & McEwen, 2011; Miller, Chen & Parker, 2011). But the questions of how and when childhood stress gets 'under the skin' at the cellular level, specifically in humans, remain to be answered.

In the past two years, several studies provided support for an association between TL and childhood stress. Adult participants who reported physical or emotional neglect had significantly shorter TL regardless of age, sex, smoking or body mass index (BMI) (Tyrka et al., 2010). Similarly, another study observed that shorter TL was associated with a greater number of retrospectively reported childhood adverse life-events among both anxiety disorder cases and control adults (Kananen et al., 2010). In another study of healthy older adults including dementia family caregivers, it was shown that the presence of multiple childhood adversities was related to shorter TL, regardless of age, caregiving status, sex, BMI, exercise, and sleep (Kiecolt-Glaser et al., 2011). The authors reported that the telomere difference could translate into a 7- to 15-year difference in life span

(Kiecolt-Glaser et al., 2011). In a case-control study of adults with chronic post-traumatic stress disorder (PTSD) and control subjects, the authors showed that although PTSD participants had shorter age-adjusted TL, childhood trauma seemed to account for the PTSD group difference and only participants with PTSD and exposure to multiple categories of childhood trauma had significantly shorter TL than control subjects (O'Donovan et al., 2011a). In the first study of children, greater exposure to institutional care was significantly associated with shorter TL in middle childhood (Drury et al., 2011). However, in contrast to previous findings, one study failed to replicate the association between TL and retrospective assessment of physical and sexual abuse in childhood in a large cohort of adult twins (Glass et al., 2010).

Although these studies advance understanding of the link between childhood stress and TL, they have relied on adult measures of TL and retrospective recall of stress years after the stress was experienced (Kananen et al., 2010; Kiecolt-Glaser et al., 2011; O'Donovan et al., 2011a; Tyrka et al., 2010) raising important questions about the true nature of these findings. Interpretation of findings from cross-sectional studies of TL is ambiguous in light of recent longitudinal analyses of repeated TL measurements. These studies show that TL is highly variable across different age groups, that there is a significant inter- and intra-individual variability, there is inverse correlation between baseline TL and telomere erosion, and also show that, in some individuals, telomeres can lengthen over time (Aviv et al., 2009; Chen et al., 2011b; Ehrlenbach et al., 2009a; Epel et al., 2009; Farzaneh-Far et al., 2010; Nordfjall et al., 2009; Zeichner et al., 1999). These recent findings indicate that the temporal process of telomere erosion is more complex than initially assumed, and that repeated measures (not just length at one time point) are needed to measure true telomere erosion in individuals who are experiencing stress. Moreover, given the elapsed time between the putative stress exposure and the measurement of TL, it has not been clear whether telomeres began eroding during stress exposure or whether erosion occurred years later, possibly promoted by the sequelae of childhood stress or other intervening variables. For example, given that maltreated children often grow up to be in poor physical health as adults (Danese et al., 2009; Springer et al., 2007) telomere erosion could be a consequence of later health problems, as opposed to a proximal effect of maltreatment itself.

The hypothesis that childhood violence exposure would accelerate telomere erosion in children was tested in the first prospective-longitudinal study in children, while they experienced stress (Shalev, 2012). Violence was assessed as exposure to maternal domestic violence, frequent bullying victimization and physical maltreatment by an adult. Participants were 236 children (42% with one or more violence exposures) recruited from the Environmental Risk (E-Risk) Longitudinal Twin Study, a nationally-representative UK 1994-1995 birth cohort. Each child’s mean relative TL was measured simultaneously in baseline and follow-up DNA samples. Based on evidence that the effects of stress are cumulative (Felitti et al., 1998) the hypothesis was that cumulative exposure to violence will be associated with accelerated TL erosion. Indeed, compared to their counterparts, children who experienced 2 or more kinds of violence exposure showed significantly more telomere erosion between age-5 baseline and age-10 follow-up measurements, even after adjusting for sex, socio-economic status and BMI. This finding provided the first evidence that stress-related accelerated telomere erosion can be observed already at young age while children are experiencing stress (Shalev, 2012).

Caveats and open questions in the study of juvenile violence victimization and telomere length

Although these recent findings support the hypothesis of stress-related telomere erosion, even at young age, there are caveats and open questions that require further research. First, although studies have reported on the association between different kinds of violence exposure and telomere erosion, the question of what type of violence matter the most is not entirely clear (e.g., physical abuse, domestic violence, bullying victimization, sexual abuse, emotional abuse, physical neglect or emotional neglect). As some studies suggest, the effect of stress might be seen most clearly when stress is measured in a cumulative way (Danese & McEwen, 2011; Felitti et al., 1998; Shalev, 2012). This raises an important caution in stress research, more generally, that all exposures are not necessarily alike and that considerable care needs to be take when attempting to synthesize and interpret research about the psychobiological sequelae of stress exposures. As more studies accumulate, it will also be possible to interrogate the specific type and features of a stressor that matter most in relation to telomere erosion (e.g., duration, severity, physical harm, perceived threat).

Second, longitudinal reports using repeated measurements of TL have revealed that in some individuals telomeres can lengthen (Aviv et al., 2009; Chen et al., 2011b; Ehrlenbach et al., 2009a; Epel et al., 2009; Farzaneh-Far et al., 2010; Nordfjall et al., 2009; Shalev, 2012). Interestingly, most 'lengtheners' in those studies have shorter TL at baseline raising important questions about the dynamics of telomere length in living cells (Aviv et al., 2009; Ehrlenbach et al., 2009a; Nordfjall et al., 2009). Observed lengthening could result from (a) measurement error, (b) regression to the mean, (c) differences in cells sampled at different ages, or (d) meaningful telomere dynamics involving; telomerase activity, alternative lengthening of telomeres or telomere trimming (the rapid shortening of long telomeres) (Cesare & Reddel, 2010; Pickett & Reddel, 2012; Slatter et al., 2012; Svenson et al., 2011). Several studies have suggested a theoretical explanation for telomere dynamics. TL appears to oscillate over short periods of time which levels out at longer follow-up measures (Chen et al., 2011a; Svenson et al., 2011). Others theorized that there is an upper limit on TL and that TL is maintain in an equilibrium in cells that have a telomere elongation mechanism. Thus, since TL can predict health outcomes, the mechanisms that regulate TL are of great interest and demand more investigation.

Third, there are tradeoffs regarding the best ways to measure TL (Nakagawa, Gemmell & Burke, 2004). The three main methods to assess telomere length are (a) Southern blot analysis of the terminal restriction fragments (TRF) (Allshire, Dempster & Hastie, 1989), (b) Quantitative RT-PCR to measure the ratio between a single-copy gene and telomeric repeat region (T/S ratio) (Cawthon, 2002), and (c) Fluorescence *in situ* hybridization (FISH) methods; Quantitative-FISH (Q-FISH) and flow-FISH (Poon & Lansdorp, 2001; Rufer et al., 1998). All three methods measure the average length of telomeres in a specific cell population. The southern blot method measure telomere length in kilobases of not only the telomeric region (TTAGGG sequence) but also the sub-telomeric region. In contrast, both the T/S ratio and FISH methods measure the average telomere length of the telomeric region. The T/S ratio is compared to a standard

reference sample and thus the ratio of one individual to another will correspond to the relative telomere lengths of their DNA. FISH methods can also determine the specific telomere length in distinct cell populations or measure the specific length in individual cell or chromosome. However, FISH methods, especially the Q-FISH, are highly technical which limits their use. The principal advantage of the T/S ratio over the other methods is that it can be performed at high throughput and low-cost. However, this method can suffer from higher rate of measurement error compare with the other methods. Taken together, although all methods are highly correlated (compared to TRF method; R^2=0.67-0.85 for T/S ratio, R^2=0.87 for flow-FISH, R^2=0.90 for Q-FISH) (Aubert, Hills & Lansdorp, 2012; Aviv et al., 2011), each method has advantages and disadvantages and there is still a debate about the optimal way to measure TL for a given study. In addition, the use of different methods makes it difficult to compare mean values between studies or to establish normative reference values for a particular age group.

Lastly, another question concerns the measurement of TL in different tissues (Hewakapuge et al., 2008). Because of ethical difficulties obtaining blood from children in the community, most studies in children have used buccal cells (Drury et al., 2011; Kroenke et al., 2011; Shalev, 2012), instead of the peripheral blood cells more commonly used in studies of adults. However, buccal swabs may not only yield buccal cells, and it is possible that infiltration of immune cells due to poor oral hygiene or infection can alter the oral cell composition which has different telomere dynamics than buccal cells. Moreover, even when estimating TL from blood cells, different population of cells (e.g., granulocytes, T cells, B cells, natural killer cells) may give rise to false estimates of TL (Ouyang et al., 2007; Rufer et al., 1998). For example, telomerase is active to a different degree in different cells and tissues which may affect the length of telomeres (Autexier & Greider, 1996). TL might also vary among different cells and tissues due to factors such as cell turnover rates, stem cell capacity, exposure to oxidative damage, or dynamic regulation of telomeres (Monteiro et al., 1996; Rufer et al., 1999b; Weng et al., 1995). There are limited studies on the comparability between different tissues (Butler et al., 1998; Friedrich et al., 2000; Thomas, NJ & Fenech, 2008). In the case of buccal cells and blood cells, positive correlations have been reported between TL from both type of cells (Gadalla et al., 2010). In addition, buccal cell TL has been associated with age-related disease (Broberg et al., 2005), although one report showed no significant correlation between TL from buccal cells and white blood cells (Thomas et al., 2008). Recent reports suggest that estimating TL from buccal cells can detect effects of stress, although validation in larger studies that measure TL from multiple tissues is needed.

What are the mechanisms linking juvenile violence victimization to accelerated telomere erosion?

Previous studies have raised the question of what biological processes explain the association between stress and telomere erosion. Currently there are no studies that address the question of causality. Most of the insights about mechanisms associated with telomere erosion originate from research on oxidative stress and inflammation, indicating both as important influences on TL (Ilmonen, Kotrschal & Penn, 2008; O'Donovan et al., 2011b; Shiels et al., 2011; von Zglinicki, 2002). Telomeres are sensitive to damage by oxidative stress, as demonstrated by experiments showing increased erosion under

conditions of high reactive oxygen species *in vitro* (von Zglinicki, 2002). Intriguingly, a recent study showed that up to half of the DNA damage foci in stress-induced senescence, whether it is caused endogenously by oxidative stress or exogenously by DNA-damaging agents, are located at telomeres irrespective of telomerase activity (Hewitt et al., 2012). The results of this study imply that telomeres are important targets for stress both *in vitro* and *in vivo*. Inflammation is associated with increased proliferation of immune cells and, as a consequence, with more telomere erosion (Goronzy, Fujii & Weyand, 2006). Childhood stress predicts elevated inflammation (Danese et al., 2007), suggesting a possible cause for the increased telomere erosion observed in victims of violence. Research is needed to test whether effects of stress on telomere erosion are mediated by oxidative stress, immune-system changes or other factors in children.

PART 2: Implications for prevention and intervention

By now, studies have implicated age-related TL as important determinant of morbidity and mortality, with most of the information arising from studies of adult or old populations. However, although the word 'aging' is visualized with old age and older people, 'aging' in the sense of TL is a life-course phenomenon that begins at birth. There is some evidence to suggest rapid TL erosion of infants very soon after birth, and for the first years of life, corresponding with the rapid growth rates and high production and turnover of cells (Rufer et al., 1999a; Zeichner et al., 1999). This erosion then continues in a steadier and moderate rate into childhood and adulthood and into old age (Eisenberg, 2011). The manifestation of age-related diseases is seen mostly at old age but the aging process can be view as a life-time course. Given that children who are victims of violence show faster erosion rate of TL (Shalev, 2012), early intervention and prevention strategies can ameliorate the acceleration of aging processes early in life.

The fostering efforts to reduce the burden of child maltreatment and later-life physical and mental-health problems include multilevel strategies from the molecular, psychological, behavioral and policy perspectives. From the cellular perspective, the growing field of telomeres, and the better understanding of the processes that underlie stress-related accelerated aging, is a potentially promising new avenue for prevention and intervention. Studies suggest that not all individuals age at the same speed and there are several factors that contribute to the acceleration of the aging processes with a strong indication of stress as a prominent factor.

There are several indications that healthy lifestyle factors and stress-coping interventions can alter the rate of telomere erosion and improve our wellbeing. Evidence from genetically modified mouse model demonstrates that activation of telomerase can reverse many of the hallmarks of aging (Jaskelioff et al., 2011). Studies of humans have provided further support. Longer telomeres have been associated with; years of healthy life (Njajou et al., 2009), physical activity during leisure time (Bendix et al., 2011; Cherkas et al., 2008) and the buffering effect of exercise on stress (Puterman et al., 2010), multivitamin use (Xu et al., 2009), higher folate levels (Paul, 2011) and higher vitamin D levels (Richards et al., 2007). Moreover, in one study, healthy diet and having high social support attenuated the relationship between short TL and presence of heart disease (Diaz & Samani, 2010). In another study, reduction of psychological distress was associated

with increase telomerase activity (Daubenmier et al., 2011). Finally, 3-month meditation intervention was associated with higher telomerase activity and improved health profiles (Jacobs et al., 2011). Taken together, healthy lifestyle factors, psychological support and stress-reduction interventions can improve the rate of cellular aging and ameliorate the deleterious effects that stress and violence have on our telomeres.

Research recommendation

In conclusion, the literature provides evidence that stress-related accelerated telomere erosion can be observed already in childhood. This suggests the importance of integrating telomeres as stress markers in research to evaluate the effects of maltreatment on young victims. TL measurement is now offered to adults as a diagnostic tool to monitor health and predict disease risk (Wolinsky, 2011). It is conceivable that research may eventually implicate TL measurement in clinical pediatrics. However, extreme caution should be taken as more research is needed to uncover mechanisms that govern TL dynamics. Notwithstanding, therapeutic strategies aims at the deceleration of telomeres are potentially important target for treatment of young victims of violence.

References:

(2006). Resilience in children. Proceedings of a conference sponsored by the New York Academy of Sciences and Brown Medical School. February 26-28, 2006. Arlington, Virginia, USA. *Ann N Y Acad Sci 1094*, 1-368.

Allshire, R. C., Dempster, M. & Hastie, N. D. (1989). Human telomeres contain at least three types of G-rich repeat distributed non-randomly. *Nucleic Acids Res 17*(12), 4611-27.

Aubert, G., Hills, M. & Lansdorp, P. M. (2012). Telomere length measurement-Caveats and a critical assessment of the available technologies and tools. *Mutat Res 730*(1-2), 59-67.

Autexier, C. & Greider, C. W. (1996). Telomerase and cancer: Revisiting the telomere hypothesis. *Trends in Biochemical Sciences 21*(10), 387-91.

Aviv, A., Chen, W., Gardner, J. P., Kimura, M., Brimacombe, M., Cao, X., Srinivasan, S. R. & Berenson, G. S. (2009). Leukocyte telomere dynamics: longitudinal findings among young adults in the Bogalusa Heart Study. *Am J Epidemiol 169*(3), 323-9.

Aviv, A., Hunt, S. C., Lin, J., Cao, X., Kimura, M. & Blackburn, E. (2011). Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. *Nucleic Acids Res 39*(20), e134.

Bendix, L., Gade, M. M., Staun, P. W., Kimura, M., Jeune, B., Hjelmborg, J. V. B., Aviv, A. & Christensen, K. (2011). Leukocyte telomere length and physical ability among Danish Twins age 70+. *Mech Ageing Dev 132*(11-12), 568-72.

Blackburn, E. H. & Gall, J. G. (1978). A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. *J Mol Biol 120*(1), 33-53.

Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., Harley, C. B., Shay, J. W., Lichtsteiner, S. & Wright, W. E. (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science 279*(5349), 349-52.

Broberg, K., Bjork, J., Paulsson, K., Hoglund, M. & Albin, M. (2005). Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. *Carcinogenesis 26*(7), 1263-71.

Butler, M. G., Tilburt, J., DeVries, A., Muralidhar, B., Aue, G., Hedges, L., Atkinson, T. & Schwartz, H. (1998). Comparison of chromosome telomere integrity in multiple tissues from subjects at different ages. *Cancer Genetics and Cytogenetics 105*(2), 138-44.

Buxton, J. L., Walters, R. G., Visvikis-Siest, S., Meyre, D., Froguel, P. & Blakemore, A. I. (2011). Childhood obesity is associated with shorter leukocyte telomere length. *J Clin Endocrinol Metab 96*(5), 1500-5.

Carrel, A. (1912). On the permanent life of tissues outside of the organism. *The Journal of Experimental Medicine 15*(5), 516.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Res 30*(10), e47.

Cawthon, R. M., Smith, K. R., O'Brien, E., Sivatchenko, A. & Kerber, R. A. (2003). Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet 361*(9355), 393-5.

Cesare, A. J. & Reddel, R. R. (2010). Alternative lengthening of telomeres: models, mechanisms and implications. *Nat Rev Genet 11*(5), 319-30.

Chen, W., Kimura, M., Kim, S., Cao, X., Srinivasan, S. R., Berenson, G. S., Kark, J. D. & Aviv, A. (2011a). Longitudinal versus Cross-sectional Evaluations of Leukocyte Telomere Length Dynamics: Age-Dependent Telomere Shortening is the Rule. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences 66*(3), 312-9.

Chen, W., Kimura, M., Kim, S., Cao, X., Srinivasan, S. R., Berenson, G. S., Kark, J. D. & Aviv, A. (2011b). Longitudinal versus cross-sectional evaluations of leukocyte telomere length dynamics: age-dependent telomere shortening is the rule. *J Gerontol A Biol Sci Med Sci 66*(3), 312-9.

Cherkas, L. F., Hunkin, J. L., Kato, B. S., Richards, J. B., Gardner, J. P., Surdulescu, G. L., Kimura, M., Lu, X., Spector, T. D. & Aviv, A. (2008). The association between physical activity in leisure time and leukocyte telomere length. *Archives of Internal Medicine 168*(2), 154-8.

Danese, A. & McEwen, B. S. (2011). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiol Behav*.

Danese, A., Moffitt, T. E., Harrington, H., Milne, B. J., Polanczyk, G., Pariante, C. M., Poulton, R. & Caspi, A. (2009). Adverse childhood experiences and adult risk factors for age-related disease: depression, inflammation, and clustering of metabolic risk markers. *Arch Pediatr Adolesc Med 163*(12), 1135-43.

Danese, A., Pariante, C. M., Caspi, A., Taylor, A. & Poulton, R. (2007). Childhood maltreatment predicts adult inflammation in a life-course study. *Proc Natl Acad Sci U S A 104*(4), 1319-24.

Daubenmier, J., Lin, J., Blackburn, E., Hecht, F. M., Kristeller, J., Maninger, N., Kuwata, M., Bacchetti, P., Havel, P. J. & Epel, E. (2011). Changes in stress, eating, and metabolic factors are related to changes in telomerase activity in a randomized mindfulness intervention pilot study. *Psychoneuroendocrinology*.

Diaz, V. A. & Samani, N. J. (2010). Effect of healthy lifestyle behaviors on the association between leukocyte telomere length and coronary artery calcium (vol 106, pg 659, 2010). *American Journal of Cardiology 106*(11), 1674-.

Dong, M., Giles, W. H., Felitti, V. J., Dube, S. R., Williams, J. E., Chapman, D. P. & Anda, R. F. (2004). Insights into causal pathways for ischemic heart disease: adverse childhood experiences study. *Circulation 110*(13), 1761-6.

Drury, S. S., Theall, K., Gleason, M. M., Smyke, A. T., De Vivo, I., Wong, J. Y., Fox, N. A., Zeanah, C. H. & Nelson, C. A. (2011). Telomere length and early severe social deprivation: linking early adversity and cellular aging. *Mol Psychiatry*.

Ehrlenbach, S., Willeit, P., Kiechl, S., Willeit, J., Reindl, M., Schanda, K., Kronenberg, F. & Brandstatter, A. (2009a). Influences on the reduction of relative telomere length over 10 years in the population-based Bruneck Study: introduction of a well-controlled high-throughput assay. *International Journal of Epidemiology 38*(6), 1725-34.

Ehrlenbach, S., Willeit, P., Kiechl, S., Willeit, J., Reindl, M., Schanda, K., Kronenberg, F. & Brandstatter, A. (2009b). Influences on the reduction of relative telomere length over 10 years in the population-based Bruneck Study: introduction of a well-controlled high-throughput assay. *International Journal of Epidemiology 38*(6), 1725-34.

Eisenberg, D. T. (2011). An evolutionary review of human telomere biology: the thrifty telomere hypothesis and notes on potential adaptive paternal effects. *Am J Hum Biol 23*(2), 149-67.

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D. & Cawthon, R. M. (2004). Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A 101*(49), 17312-5.

Epel, E. S., Merkin, S. S., Cawthon, R., Blackburn, E. H., Adler, N. E., Pletcher, M. J. & Seeman, T. E. (2009). The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging (Albany NY) 1*(1), 81-8.

Farzaneh-Far, R., Lin, J., Epel, E., Lapham, K., Blackburn, E. & Whooley, M. A. (2010). Telomere length trajectory and its determinants in persons with coronary artery disease: longitudinal findings from the heart and soul study. *Plos One 5*(1), e8612.

Felitti, V. J., Anda, R. F., Nordenberg, D., Williamson, D. F., Spitz, A. M., Edwards, V., Koss, M. P. & Marks, J. S. (1998). Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med 14*(4), 245-58.

Friedrich, U., Griese, E., Schwab, M., Fritz, P., Thon, K. & Klotz, U. (2000). Telomere length in different tissues of elderly patients. *Mech Ageing Dev 119*(3), 89-99.

Gadalla, S. M., Cawthon, R., Giri, N., Alter, B. P. & Savage, S. A. (2010). Telomere length in blood, buccal cells, and fibroblasts from patients with inherited bone marrow failure syndromes. *Aging (Albany NY) 2*(11), 867-74.

Glass, D., Parts, L., Knowles, D., Aviv, A. & Spector, T. D. (2010). No correlation between childhood maltreatment and telomere length. *Biol Psychiatry 68*(6), e21-2; author reply e3-4.

Goronzy, J. J., Fujii, H. & Weyand, C. M. (2006). Telomeres, immune aging and autoimmunity. *Exp Gerontol 41*(3), 246-51.

Harley, C. B., Futcher, A. B. & Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature 345*(6274), 458-60.

Hayflick, L. & Moorhead, P. S. (1961). The serial cultivation of human diploid cell strains. *Exp Cell Res 25*, 585-621.

Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B. & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proc Natl Acad Sci U S A 109*(5), 1743-8.

Hewakapuge, S., van Oorschot, R. A., Lewandowski, P. & Baindur-Hudson, S. (2008). Investigation of telomere lengths measurement by quantitative real-time PCR to predict age. *Leg Med (Tokyo) 10*(5), 236-42.

Hewitt, G., Jurk, D., Marques, F. D. M., Correia-Melo, C., Hardy, T., Gackowska, A., Anderson, R., Taschuk, M., Mann, J. & Passos, J. F. (2012). Telomeres are favoured targets of a

persistent DNA damage response in ageing and stress-induced senescence. *Nature Communications 3*, 708.

Ilmonen, P., Kotrschal, A. & Penn, D. J. (2008). Telomere attrition due to infection. *Plos One 3*(5), e2143.

Jacobs, T. L., Epel, E. S., Lin, J., Blackburn, E. H., Wolkowitz, O. M., Bridwell, D. A., Zanesco, A. P., Aichele, S. R., Sahdra, B. K., MacLean, K. A., King, B. G., Shaver, P. R., Rosenberg, E. L., Ferrer, E., Wallace, B. A. & Saron, C. D. (2011). Intensive meditation training, immune cell telomerase activity, and psychological mediators. *Psychoneuroendocrinology 36*(5), 664-81.

Jaskelioff, M., Muller, F. L., Paik, J. H., Thomas, E., Jiang, S., Adams, A. C., Sahin, E., Kost-Alimova, M., Protopopov, A., Cadinanos, J., Horner, J. W., Maratos-Flier, E. & Depinho, R. A. (2011). Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature 469*(7328), 102-6.

Kananen, L., Surakka, I., Pirkola, S., Suvisaari, J., Lonnqvist, J., Peltonen, L., Ripatti, S. & Hovatta, I. (2010). Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. *Plos One 5*(5), e10826.

Kiecolt-Glaser, J. K., Gouin, J. P., Weng, N. P., Malarkey, W. B., Beversdorf, D. Q. & Glaser, R. (2011). Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. *Psychosom Med 73*(1), 16-22.

Kroenke, C. H., Epel, E., Adler, N., Bush, N. R., Obradovic, J., Lin, J., Blackburn, E., Stamperdahl, J. L. & Boyce, W. T. (2011). Autonomic and adrenocortical reactivity and buccal cell telomere length in kindergarten children. *Psychosom Med 73*(7), 533-40.

Meyne, J., Ratliff, R. L. & Moyzis, R. K. (1989). Conservation of the Human Telomere Sequence (Ttaggg)N among Vertebrates. *Proceedings of the National Academy of Sciences of the United States of America 86*(18), 7049-53.

Miller, G. E., Chen, E. & Parker, K. J. (2011). Psychological stress in childhood and susceptibility to the chronic diseases of aging: Moving toward a model of behavioral and biological mechanisms. *Psychol Bull*.

Monteiro, J., Batliwalla, F., Ostrer, H. & Gregersen, P. K. (1996). Shortened telomeres in clonally expanded CD28(-)CD8(+) T cells imply a replicative history that is distinct from their CD28(+)CD8(+) counterparts. *Journal of Immunology 156*(10), 3587-90.

Muller, H. (1938). The remaking of chromosomes. *Collecting Net 13*(1), 181-98.

Nakagawa, S., Gemmell, N. J. & Burke, T. (2004). Measuring vertebrate telomeres: applications and limitations. *Mol Ecol 13*(9), 2523-33.

Nanni, V., Uher, R. & Danese, A. (2011). Childhood Maltreatment Predicts Unfavorable Course of Illness and Treatment Outcome in Depression: A Meta-Analysis. *Am J Psychiatry*.

Njajou, O. T., Hsueh, W. C., Blackburn, E. H., Newman, A. B., Wu, S. H., Li, R. L., Simonsick, E. M., Harris, T. M., Cummings, S. R., Cawthon, R. M. & Study, H. A. (2009). Association Between Telomere Length, Specific Causes of Death, and Years of Healthy Life in Health, Aging, and Body Composition, a Population-Based Cohort Study. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences 64*(8), 860-4.

Nordfjall, K., Eliasson, M., Stegmayr, B., Melander, O., Nilsson, P. & Roos, G. (2008). Telomere length is associated with obesity parameters but with a gender difference. *Obesity (Silver Spring) 16*(12), 2682-9.

Nordfjall, K., Svenson, U., Norrback, K. F., Adolfsson, R., Lenner, P. & Roos, G. (2009). The individual blood cell telomere attrition rate is telomere length dependent. *PLoS Genet 5*(2), e1000375.

O'Donovan, A., Epel, E., Lin, J., Wolkowitz, O., Cohen, B., Maguen, S., Metzler, T., Lenoci, M., Blackburn, E. & Neylan, T. C. (2011a). Childhood trauma associated with short leukocyte telomere length in posttraumatic stress disorder. *Biol Psychiatry 70*(5), 465-71.

O'Donovan, A., Pantell, M. S., Puterman, E., Dhabhar, F. S., Blackburn, E. H., Yaffe, K., Cawthon, R. M., Opresko, P. L., Hsueh, W. C., Satterfield, S., Newman, A. B., Ayonayon, H. N., Rubin, S. M., Harris, T. B. & Epel, E. S. (2011b). Cumulative inflammatory load is associated with short leukocyte telomere length in the Health, Aging and Body Composition Study. *Plos One 6*(5), e19687.

Olovnikov, A. (1971). Principle of marginotomy in template synthesis of polynucleotides]. *Doklady Akademii Nauk SSSR 201*(6), 1496.

Ouyang, Q., Baerlocher, G., Vulto, I. & Lansdorp, P. M. (2007). Telomere length in human natural killer cell subsets. *Ann N Y Acad Sci 1106*, 240-52.

Paul, L. (2011). Diet, nutrition and telomere length. *Journal of Nutritional Biochemistry 22*(10), 895-901.

Pickett, H. A. & Reddel, R. R. (2012). The role of telomere trimming in normal telomere length dynamics. *Cell Cycle 11*(7).

Poon, S. S. & Lansdorp, P. M. (2001). Quantitative fluorescence in situ hybridization (Q-FISH). *Curr Protoc Cell Biol Chapter 18*, Unit 18 4.

Puterman, E., Lin, J., Blackburn, E., O'Donovan, A., Adler, N. & Epel, E. (2010). The Power of Exercise: Buffering the Effect of Chronic Stress on Telomere Length. *Plos One 5*(5).

Rich-Edwards, J. W., Spiegelman, D., Lividoti Hibert, E. N., Jun, H. J., Todd, T. J., Kawachi, I. & Wright, R. J. (2010). Abuse in childhood and adolescence as a predictor of type 2 diabetes in adult women. *Am J Prev Med 39*(6), 529-36.

Richards, J. B., Valdes, A. M., Gardner, J. P., Paximadas, D., Kimura, M., Nessa, A., Lu, X. B., Surdulescu, G. L., Sivaminathan, R., Spector, T. D. & Aviv, A. (2007). Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. *American Journal of Clinical Nutrition 86*(5), 1420-5.

Riley, E. H., Wright, R. J., Jun, H. J., Hibert, E. N. & Rich-Edwards, J. W. (2010). Hypertension in adult survivors of child abuse: observations from the Nurses' Health Study II. *J Epidemiol Community Health 64*(5), 413-8.

Rufer, N., Brummendorf, T. H., Kolvraa, S., Bischoff, C., Christensen, K., Wadsworth, L., Schulzer, M. & Lansdorp, P. M. (1999a). Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *Journal of Experimental Medicine 190*(2), 157-67.

Rufer, N., Brummendorf, T. H., Kolvraa, S., Bischoff, C., Christensen, K., Wadsworth, L., Schulzer, M. & Lansdorp, P. M. (1999b). Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *Journal of Experimental Medicine 190*(2), 157-67.

Rufer, N., Dragowska, W., Thornbury, G., Roosnek, E. & Lansdorp, P. M. (1998). Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. *Nat Biotechnol 16*(8), 743-7.

Sahin, E. & Depinho, R. A. (2010). Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature 464*(7288), 520-8.

Shalev, I., Moffitt, T.E., Sugden, K., Williams, B., Houts, R.M., Danese, A., Mill, J., Arseneault, L., Caspi, A. (2012). Exposure to Violence During Childhood is Associated with Telomere Erosion from 5 to 10 Years of Age: A Longitudinal Study. *Molecular Psychiatry* (Accepted).

Shiels, P. G., McGlynn, L. M., MacIntyre, A., Johnson, P. C., Batty, G. D., Burns, H., Cavanagh, J., Deans, K. A., Ford, I., McConnachie, A., McGinty, A., McLean, J. S., Millar, K., Sattar, N., Tannahill, C., Velupillai, Y. N. & Packard, C. J. (2011). Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid cohort. *Plos One 6*(7), e22521.

Simon, N. M., Smoller, J. W., McNamara, K. L., Maser, R. S., Zalta, A. K., Pollack, M. H., Nierenberg, A. A., Fava, M. & Wong, K. K. (2006). Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biol Psychiatry 60*(5), 432-5.

Slatter, T. L., Tan, X., Yuen, Y. C., Gunningham, S., Ma, S. S., Daly, E., Packer, S., Devenish, C., Royds, J. A. & Hung, N. A. (2012). The alternative lengthening of telomeres pathway may operate in non-neoplastic human cells. *J Pathol 226*(3), 509-18.

Springer, K. W., Sheridan, J., Kuo, D. & Carnes, M. (2007). Long-term physical and mental health consequences of childhood physical abuse: results from a large population-based sample of men and women. *Child Abuse Negl 31*(5), 517-30.

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

Thomas, P., NJ, O. C. & Fenech, M. (2008). Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease. *Mech Ageing Dev 129*(4), 183-90.

Tyrka, A. R., Price, L. H., Kao, H. T., Porton, B., Marsella, S. A. & Carpenter, L. L. (2010). Childhood maltreatment and telomere shortening: preliminary support for an effect of early stress on cellular aging. *Biol Psychiatry 67*(6), 531-4.

Valdes, A. M., Andrew, T., Gardner, J. P., Kimura, M., Oelsner, E., Cherkas, L. F., Aviv, A. & Spector, T. D. (2005). Obesity, cigarette smoking, and telomere length in women. *Lancet 366*(9486), 662-4.

von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends Biochem Sci 27*(7), 339-44.

Watson, J. D. (1972). Origin of concatemeric T7 DNA. *Nat New Biol 239*(94), 197-201.

Wegman, H. L. & Stetler, C. (2009). A meta-analytic review of the effects of childhood abuse on medical outcomes in adulthood. *Psychosom Med 71*(8), 805-12.

Weng, N. P., Levine, B. L., June, C. H. & Hodes, R. J. (1995). Human naive and memory T lymphocytes differ in telomeric length and replicative potential. *Proc Natl Acad Sci U S A 92*(24), 11091-4.

Wolinsky, H. (2011). Testing time for telomeres. Telomere length can tell us something about disease susceptibility and ageing, but are commercial tests ready for prime time? *EMBO Rep 12*(9), 897-900.

Xu, Q., Parks, C. G., Deroo, L. A., Cawthon, R. M., Sandler, D. P. & Chen, H. L. (2009). Multivitamin use and telomere length in women. *American Journal of Clinical Nutrition 89*(6), 1857-63.

Yu, W. Y., Chang, H. W., Lin, C. H. & Cho, C. L. (2008). Short telomeres in patients with chronic schizophrenia who show a poor response to treatment. *J Psychiatry Neurosci 33*(3), 244-7.

Zeichner, S. L., Palumbo, P., Feng, Y., Xiao, X., Gee, D., Sleasman, J., Goodenow, M., Biggar, R. & Dimitrov, D. (1999). Rapid telomere shortening in children. *Blood 93*(9), 2824-30.