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Cold parenting is associated with cellular aging in offspring: A retrospective study

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Abstract

Background: Early life stress is a known risk factor for diseases and premature death. We tested whether parenting style impacts telomere length (TL), a cellular aging biomarker.

Methods: Information on parents' style of parenting was obtained from 199 participants in the Adventist Health Study-1 (AHS-1) who 27+ years later also enrolled in the AHS-2 where blood was collected for relative TL (rTL) assessment.

Results: Subjects describing their mothers' parenting style as cold had on average 25% smaller rTL compared to subjects not reporting a cold mother (1.89 vs 2.53). This association was greatest among those with less education, and those who stayed overweight/obese or put on weight during follow-up.

Conclusions: These results support previous findings that early life stress may have health implications by promoting cellular aging, and expands these stressors to include cold parenting during an individuals' formative years. Higher education and normal weight seem to provide some resilience.

Keywords

Telomeres; Cold Parenting; Epigenetics; Early Life Stress; Adventist Health Study; Cohort Study

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Conflicts of interest

Declarations of interest: none.

Introduction

Poor mental and physical health over the life-course have been linked to psychosocial stressors, such as family violence and other signs of unstable relations [1–4]. Early life stresses from risky family processes, characterized by marital conflict and aggression, and cold, unsupportive and neglectful family relationships, threatens the emotional security of children, weakening their coping and behavioral mechanisms in response to daily challenges, and reducing the emotional availability or sensitivity of parents, causing increased restrictiveness and compromising parental supervision, affection and communication with the child [5–7]. Findings indicate that such parenting may initiate affective, behavioral and cognitive deficits in children, compromising their “ability to modulate and express emotion, social competency, aggression, coping, and information-processing skills”, and rendering them prone to risk-taking behaviors (e.g. smoking, drug and alcohol abuse, sexual promiscuity) and poor, chronic health outcomes resulting from injuries, ischemic heart disease, cancer, chronic lung disease, skeletal fractures and liver disease [6, 7]. Several studies have found that early life stress increases, in a dose-dependent manner, the risk of many somatic and mental diseases as well as of premature death [8–15]. In recent years, telomere length (TL) has increasingly been used as a biomarker for accelerated aging and disease risk associated with such stressors [16]. In their review of the association between early life stress and telomeres, Price et al concluded that “early findings raise hopes that telomere length might serve as a deep biomarker of early-life stress in terms of damage done, future vulnerability and efficacy of therapeutic interventions” [8]. Telomeres are specialized nucleoprotein complexes that cap chromosomal ends, promoting chromosomal stability by protecting these ends from being recognized as double-stranded DNA breaks [17, 18]. However, linear chromosomes are incompletely replicated by DNA polymerases, causing telomere shortening with every cell division, and finally resulting in cell senescence [19]. A causal link between telomere biology and aging is provided by the “telomere syndromes”, a group of rare diseases resembling premature aging, caused by mutations in telomere maintenance genes [20]. Significant TL shortening has been found to be associated with inter-partner violence and childhood trauma, as well as with major depressive disorders, pessimism and hostility [10, 21–32]. In a study of mothers who were caregivers of chronically sick children, mothers with perceived high stress levels displayed shorter TL in their peripheral blood mononuclear cells than mothers without such stress [33]. Maternal psychosocial stress during pregnancy has also been associated with shorter TL in young adulthood of her offspring, and in one study, was reported to have a significant, independent, linear effect on newborn leucocyte TL (LTL) accounting for 25% of the variance in adjusted LTL [34–36]. An unpredictable home environment characterized by lack of warmth and emotional support has been found to produce “a cascade of psychological vulnerabilities” and to be associated with shorter TL both in children and later in adulthood, and with increased risk of cardiovascular disease, diabetes, cancer, stroke and autoimmune disease [37, 38]. In a cross-sectional study from Saudi-Arabia a threatening parenting style of beating and insults was associated with increased risk of adult cancer, asthma and cardiac disease [39]. In African American adolescents, a high parent-child conflict level and low parent-child warmth were associated with shorter TL at 5 year follow-up, but participation in a family-based parenting skills intervention program resulted in

longer TLs, indicating a protective effect of less conflict and more warmth on cellular aging [40, 41]. However, other studies have not found a relationship between early life stressors and TL [42, 43].

The present study examines the association between subjects' perception of the parenting style (cold/warm/neither) of their parents and the subjects' measured telomere length approximately 25–30 years later.

Materials and Methods

Cohort and sample collection

This is a retrospective cohort study consisting of 199 subjects who enrolled in both the Adventist Health Study-1 (AHS-1) (enrollment 1976–1977) and the AHS-2 about 25 years later (enrollment 2002–7) and, in addition, attended a AHS-2 follow-up clinic where blood was collected for later analysis. The AHS-1 was designed to examine the association between lifestyle and other factors on the risk of cancer, coronary heart disease and all-cause mortality in a prospective cohort study of 34,198 non-Hispanic white California Adventists who completed a comprehensive diet, lifestyle, psycho-social and medical history questionnaire in 1976. Further details of the AHS-1 are published elsewhere [44, 45].

The AHS-2 was designed to investigate the effects of lifestyle choices on cancer and other health outcomes in black and white Adventists throughout all 50 US states as well as Canada. About 96,000 subjects, aged 30 years and older, were recruited from 2002–2007 and as part of enrollment they completed a large baseline diet, lifestyle and medical history questionnaire. The AHS-2 has been described in detail elsewhere [45, 46].

A total of 5,700 subjects, the “overlap” population, participated in both the AHS-1 and AHS-2 [47]. As part of the AHS-2, a Church Clinic Pilot study [48] and a large Calibration Study [49] were conducted where blood was collected and buffy coat frozen in liquid nitrogen for later analyses. Among this “overlap” population, 234 subjects participated in at least one of these two clinics. Frozen buffy coat available from 205 of these subjects was used to assess telomere length. However, we failed to obtain DNA suitable for PCR analysis from 6 subjects, leaving only 199 subjects with sufficient buffy coat to enable TL assessment, and these constitute our study population. Figure 1 shows the timeline for the collection of the various data used in this study.

Exposure variables

At enrollment into the AHS-1 in 1976, subjects completed a baseline lifestyle and medical history questionnaire, which also included some psycho-social items, including questions on their perception of their mothers'/fathers' parenting style as well as their parents' presence or absence during their first 15 years of life. Parenting style thus included perceiving a cold mother/ father, a warm mother/father and a permanently absent mother/father.

The following question was used as the exposure variable for this study:

Please think back about your home during the first 15 years of your life. Mark all of the items which describe your parents or guardians during the MAJORITY of your first 15 years of life.

MOTHER or FEMALE GUARDIAN:

Warm and understanding toward me

Somewhat cold and detached

PERMANENTLY absent from our home

The same question was asked about the father or male guardian.

Outcome variable

The outcome variable for this study is the mean relative telomere length (rTL) assessed across subjects. The rTL was determined using the relative T/S ratio, assessed as telomere to single-copy gene Ct values according to Cawthon [50]. Leukocytes from the buffy coat collected at one of the two AHS-2 clinics from 2003–2006 was used for the rTL assessment (40,41).

Relative Telomere length (rTL) assessment—DNA was extracted from buffy coat samples using Quick-gDNA Blood MiniPrep Kit (Zymo Research). qPCR was performed as described [50] using BioRad CFX96 Touch Real-Time Detection System. For the quantification of telomere (T), primers tel 1, GGTTTTGTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT and tel 2, TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA were used at 270 nM and 900 nM concentrations, respectively. To measure the single copy number human gene 36B4 (S), we used primers 36B4u, CAGCAAGTGGGAAGGTGTAATCC; and 36B4d, CCCATTCTATCATCAACGGGTACAA at concentration of 300 nM and 500 nM, respectively. For each DNA, a sample master mix was prepared for eight separate 25 µl reactions without adding primers using SYBR Green 2xm (Bio-Rad). 20 µl of Master mix aliquots were added to wells of a 96-well plate and mixed with 5 µl mix of either T or S specific primers. After 10 min incubation at 95°C to activate Taq DNA polymerase, PCR was run for 40 cycles of 95°C for 15 s, then 58°C for 1 min. Raw data was analyzed by CFX Manager software (Bio-Rad). All samples for both amplicons were done in triplicate. In addition to the samples, each 96 well plate included negative controls and three samples of genomic DNA derived from the CaSki cell line with concentrations of 12.5, 25, and 50 ng to evaluate the PCR efficiency (E=103%, slope = -3.2435 for the telomere and E = 96.8%, slope = -3.401 for the single gene amplicon). The average inter- and intra-assays CVs for telomere amplicon were 1.01% and 4.7%, correspondingly, when we used reference CaSki DNA. The average inter- and intra-assays CVs for the 36B4 gene were 0.45% and 0.95% in the same experiments. The T/S ratio for the sample DNA and DNA from CaSki cell line, which was used as a reference DNA, was calculated by subtracting the average of the 36B4 gene C_t value from the average of the telomere C_t value (C_t of sample DNA). We determined rTL by subtracting the average of T/S value for the reference DNA (C_t) from the T/S ratio of the sample from each study subject, according to the method described by

Cawthon using the 2^{-Ct} formula [50]. Supporting qPCR data is available upon reasonable requests.

Co-variables

All co-variables were obtained from the AHS-2 baseline questionnaire during enrollment from 2002–2007, 25–30 years after subjects had enrolled in the AHS-1. Covariates included age (continuous), gender, years of education (Bachelor or higher vs. less than Bachelor), BMI (kg/m^2) (<25 vs. ≥ 25), physical activity (min/wk), smoking history (Never/Ever), dietary pattern (vegetarian vs. non-vegetarian) and number of prevalent comorbidities (0–1; 2–3; 4–9). Co-morbidities included various cardiovascular diseases, cancer, various respiratory diseases, various metabolic diseases (e.g. diabetes mellitus and hypo-/hyperthyroidism), chronic gastrointestinal disease, various neurologic diseases (e.g. Parkinson's disease, Multiple sclerosis), rheumatic/immunologic diseases, chronic degenerative arthritis, kidney disease and HIV.

In addition to testing interaction by educational level, BMI and diet using data from the AHS-2, we also performed two *a priori* sensitivity analyses. Because it is well known that the Adventists tend to become more vegetarian as they age [51], we used dietary information from the AHS-1 to assess its interaction with cold mother. Finally, for BMI, we created a long-term BMI variable to assess interaction with change in BMI using data from AHS-1 and AHS-2 to define 3 levels: consistent normal BMI, consistent overweight/obese and change from normal to obese.

Statistical Methods—Guided multiple imputations using the Hmisc package [52] provided data for a small number of missing dietary values and of co-variables used in the multivariable linear regression analysis [53]. Beta coefficients and their variances were calculated from twenty imputed datasets, and combined to form composite beta coefficients and variances [54]. Least squares means of rTL were later derived from these.

Descriptive statistics were assessed using means and frequencies from the imputed datasets. We also compared the AHS-1 descriptive factors of study subjects with those of the AHS-1 subjects who did not participate in the AHS-2 (Appendix, Tables 1a–b), as well as with the rest of the “overlap” population who enrolled in both AHS-1 and AHS-2 (Appendix, Tables 2a–c).

The rTLs were not normally distributed, so were log transformed prior to analyses. Minutes/week of exercise was also log transformed. Multivariable linear regression was used to estimate mean log rTL for each exposure using Least Squares Means, adjusting for age, gender, years of education, BMI, physical activity, smoking history, diet and number of comorbidities. To obtain rTL, the log rTL was anti-logged at the end of the analyses.

Possible interaction with cold mother by other variables thought to be related to rTL was tested by adding a multiplicative term to the multivariable analyses. These variables included dichotomous levels of education, BMI and diet pattern. If the interaction was statistically significant, the effect of cold mother was assessed separately in dichotomous strata of these variables.

Lastly, the sensitivity analyses for these interactions used the same multivariable model, but the potentially interacting term was AHS-1 data for diet and change from normal to overweight/obese for the body mass variable.

Results

AHS-2 baseline characteristics of the study subjects are given in Table 1. This is an older and well-educated study population where 55% were 70+ years of age and 65% were college graduates or higher and 60% were females. They also seemed to be more health conscious than the full AHS-2 cohort [46] with about 73% being vegetarians and 72% regarding themselves as physically active. In spite of this, 56% were overweight or obese.

When comparing our study subjects with the rest of the AHS-1 population (Appendix, Tables 1a–b), we find that, at enrollment in 1976–77, there was no difference except that our study subjects were younger, 45 vs. 55 years, respectively, a higher proportion were vegetarian (73.2 vs. 50.8%), a higher proportion had a college degree or higher (60.1 vs. 26.5%) and their BMI was lower (23 vs. 25). There was no statistically significant difference in the proportion reporting cold or warm mother/father or a permanently absent mother/father. However, probably partly because of younger age, the study subjects reported fewer prevalent diseases than the rest of the AHS-1 population (Appendix, Table 1b).

On the other hand, when comparing study subjects with the rest of the “overlap” population, e.g. those who participated in both AHS-1 and AHS-2 (Appendix, Tables 2a–c), there was no differences in age or in BMI. The study subjects were, however, more likely to be vegetarian (73.2 vs. 65.7%) and to have a Bachelor’s degree (64.6 vs. 40.9%). Except for Irritable Bowel Syndrome (4.0 vs. 1.8%) we found no difference between prevalence of co-morbidities reported on the baseline AHS-2 questionnaire nor on the mean number of co-morbidities, 2.37 vs. 2.55, respectively, among the study population and the rest of the “overlap” population.

The multivariable-adjusted least squares mean rTL was 25% smaller ($r=-0.54$) ($p=0.002$) among the subjects who described their mother as being cold compared to those who did not have a cold mother (Table 2, Fig. 2). Those describing their mother as warm and/or not cold had a similar 28% higher rTL ($r=0.24$) as compared to those describing their mother as cold and/or not warm. For the father we found the same trends, but the estimates were much smaller and statistically non-significant. If the mother or father had been permanently absent, the rTL was also smaller, 30% ($r=-0.35$) and 12% ($r=-0.13$), respectively. However, very few of our study subjects had experienced having one absent parent. Thus the 95% CI’s were very wide and these estimates did not reach statistical significance. Age was, as expected, associated with a monotonic reduction in rTL with 19% smaller rTL ($r=-0.21$) among those 80+ year olds compared to the reference group (50–59 year olds) ($p_{trend}=0.007$). No significant association was found between education or BMI and rTL.

When testing for interaction with co-variables known to influence health, there was a significant interaction for both educational level ($p=0.034$) and BMI ($p=0.038$). In subjects with less than a Bachelor degree, there was a 42% smaller rTL among those who reported

having a cold mother compared to those who did not characterize their mother as cold (Table 3). However, among those with a Bachelor degree or higher, there was only a non-significant 10% smaller rTL associated with having a cold mother. Among those who had low or normal weight (BMI<25) in AHS-2, there was no difference in rTL of having a cold vs. not cold mother. However, among those who were overweight/obese, those having a cold mother had 37% smaller rTL compared to those not having a cold mother ($p=0.001$). There was a smaller, and non-significant, interaction effect of diet pattern (vegetarian/non-vegetarian) associated with cold mother. However, when using the diet information from earlier in life, at the AHS-1 baseline questionnaire, there was a 40% smaller rTL among non-vegetarians who had a cold mother ($p=0.009$), whereas the effect of a cold mother was only 17% ($p=0.132$) smaller rTL among the vegetarians. For BMI change from AHS-1 to AHS-2, those who had put on significant weight and reported a cold mother had 41% smaller rTL compared to those not reporting a cold mother ($p=0.008$). Among those who had been overweight/obese at both studies and reported having a cold mother, the rTL was 32% smaller as compared to those not having a cold mother ($p=0.022$) whereas no effect of cold mother was found among those who had maintained normal weight throughout both studies.

Discussion

Our findings of smaller rTL among subjects who indicate that they were raised by a cold mother is in line with other cohort [11] and cross-sectional [22, 23, 25, 43, 55, 56] studies that have looked at the effect of early-life stress (ELS) on TL. Savolainen et al. studied about 1500 subjects who had been separated from their parents during childhood. At an average age of about 60 years, these subjects were asked about traumatic experiences across the lifespan. Although the authors were not able to detect a detrimental effect on TL of early life parental separation or of self-reported traumatic life experiences, they did find that early parental separation modified the effect of traumatic life experiences. Those who had experienced both early parental separation and traumatic life experiences had significantly shorter TL [11]. They speculate that their findings could possibly reflect a sensitization process mediated by pro-inflammatory activity and hypothalamic-pituitary-adrenal (HPA) hormones, similar to what has been observed in animal studies where early maternal separation changed later behavioral outcomes and cortisol levels in response to stressful stimuli [57]. Verhoeven et al. studied 42-year old subjects and found that only recent life stress events that occurred less than 6 years ago were associated with shorter TL, whereas childhood life events and childhood trauma by themselves were not related to shorter TL [43]. However, other cross-sectional studies have found a clear relationship between adverse childhood experiences and TL. Among Finnish 50-year olds, Kananen et al. found a dose-response association between a number of childhood adversities and TL, with parental unemployment and own serious illness having the greatest impact [22]. Similarly, Tyrka et al. found that adults reporting a history of childhood maltreatment had significantly shorter TLs than those without such experiences [55]. Kiecolt-Glaser et al. reported that multiple childhood adversities shortened the TL of the study subjects, equivalent to 7–15 years [56]. Surtees et al. in their EPIC-Norfolk cohort study of 4,400 females aged 41–80 years, found an inverse dose-response association between mean rTL and reported adverse circumstances in childhood where separation from mother for more than one year was one of the important

factors [23]. Also, Osler et al. in a cross-sectional study of 324 men in the Danish Metropolit cohort found that the number of stressful events in childhood was associated with shorter TL, and the relation was especially strong for being placed away from home [25]. The authors found that part of the association was mediated by depressive mood and low grade inflammation as measured by C-reactive protein (CRP).

We are not aware of other studies that have specifically evaluated the association between upbringing by cold parents and rTL. However, findings indicate that growing up in “risky families” characterized by marital conflict and aggression, and cold, unsupportive and neglectful family relationships are associated with psychological vulnerabilities and with shorter TL both in children and later in adulthood, and with increased risk of cardiovascular disease, diabetes, cancer, stroke, hypertension, autoimmune disease and all-cause mortality [7, 37–41]. We did not find any statistically significant relationship with rTL in subjects characterizing their father as cold or absent even though there was a similar, although much weaker, pattern as that found for the cold and absent mother. This may be due to the family structure and function at the time when these study subjects were growing up in the 1920s to 1960s. They most likely had a father as the bread-winner of the family, with the mother staying at home [58]. In such a home, we would expect the mother’s influence on her small children to be of a larger magnitude as compared to that of the father, who had to earn a family wage and rarely had time to engage meaningfully with the children [58].

Interestingly, the association between being a cold mother and rTL in offspring was not evident among those with a Bachelor degree or higher. This could be due to higher resilience among subjects with higher education or that the relation between cold motherhood and rTL in the offspring is modified by obtaining a higher education. We also found that this association was modified by BMI and was only present among overweight and obese subjects, and among those who had gained significant weight from AHS-1 to AHS-2. Others have reported an inverse association between adiposity and TL [59–62], but to our knowledge, no other study has reported effect modification by BMI on the association between adverse childhood events and TL. However, accumulating evidence indicates that oxidative stress and chronic inflammation, both typical features of obesity, accelerate telomere shortening [63, 64]. No statistically significant effect modification was found by dietary pattern as reported in AHS-2. However, when instead using diet pattern as reported 25–30 years earlier, in the AHS-1, the association between cold mother and rTL was only evident among those who were non-vegetarian. This could, in part, be due to the fact that Adventists tend to adopt a vegetarian life-style as they age, and vegetarian status in older subjects in AHS-2 may therefore not reflect their lifestyle at younger age [51]. We may therefore conclude that the negative influence of cold motherhood on rTL was primarily seen in the presence of one of the other adverse factors such as poor education, high BMI and a less healthy diet. Thus, it is possible that factors such as higher education, normal BMI and a healthy diet may have a buffering effect on early life experiences.

Compared to the general population a disproportionate part of our analytic subjects were vegetarians, largely non-smokers and consumed very little alcohol. Findings indicate that consumption of antioxidant-rich foods derived from plants is associated with maintained TL, while meat and meat products, refined flour cereals and sugar-sweetened beverages are

associated with telomere attrition, even though data on alcohol consumption still are controversial [65–71]. Our analytic subjects are also highly educated and leaner than average, both of which have been associated with maintained TL [72, 73]. Even though we have controlled for these differences, the possibility still exists of residual confounding.

Our findings of about 25% smaller rTL among subjects who perceived their mother as cold underscores the possible importance of the home environment for the long-term health of the children. On the other hand, our findings also suggest the possibility that a warm maternal influence may provide resiliency to factors that would otherwise be detrimental. Consistent with this idea, a buffering effect of maternal warmth on the effects of early-life low socioeconomic status on pro-inflammatory signaling in adulthood, was reported by Chen et al., indicating a possible biological contributor to resilience [74, 75]. Brody et al. found that high parent-child conflict and low parent-child warmth were associated with shorter TL, and Robles et al. in the same population showed that children whose parents participated in a family-based parenting skills intervention showed longer TL as compared to a control group [37, 38]. Supportive data is also provided by a cross-sectional study (Asok et al.) which reported that parental responsiveness moderated the association between early-life stress and TL, and a study by Bellis et al. also supported the view that the effects of adverse childhood experiences may be attenuated by a supportive care-giver [76, 77].

While an adverse caregiving environment in early childhood is known to increase the risk for many psychiatric as well as somatic diseases, the mechanisms underlying this development are poorly understood [8]. However, new findings linking TL to many of these conditions indicate that telomere biology may be an important factor [43]. ELS may cause glucocorticoid dysregulation by modulating the expression of calcium channels mRNA in subjects depending on their genotypes [78]. Studies have reported an association between TL and glucocorticoid dysregulation, with stress exposure resulting in HPA hyperactivity, reduced telomerase activity, increased inflammation, oxidative stress damage and TL shortening [79–83]. *In vitro* studies have found that oxidative stress results in accelerated TL shortening [84, 85], and that antioxidants can reverse this [86, 87]. Verhoeven et al. [43] report findings from the Netherlands Study of Depression and Anxiety (NESDA) indicating that childhood trauma had an impact on psychiatric status [88], brain structure [89] and metabolic health in their sample population [90]. Thus, the impact of childhood trauma during a developmental and vulnerable period has potentially lifelong ramifications.

Strengths

This study has several strengths. First, the exposure variable, although self-reported, was assessed 25–30 years earlier than the collection of blood used to assess the outcome variable, rTL. Also, because the study subjects were part of two large prospective cohort studies, there were a number of potential co-variables available for adjustment, including a large number of co-morbidities that were adjusted for. Since Adventists use very little alcohol and are non-smokers, with 80% having never smoked, this population has little or no confounding by these two major risk factors which are known to affect TL [71, 91–93].

Limitations

One limitation of the current study is that both the exposure variable and the co-variables are self-reported. However, the exposure variable of parental style was obtained 25–30 years prior to the collection of the biological sample (blood) as well as the demographic and medical history information. Unfortunately, we do not have prior or follow-up data on TL measurements, which would have made it possible to estimate the rate of change over time. Although the AHS-2 study included Blacks, the AHS-1 only included non-Hispanic whites. Thus, we do not know whether the association between being a cold mother and rTL in the offspring would be different among Blacks or other racial groups. Another limitation is that we do not have information on other critical life events among these subjects.

This study consists of a survival population in that only those AHS-1 participants who were still alive and who chose to participate in the AHS-2 were eligible for this study. Further, only those among this overlap population who agreed to attend a follow-up clinic, where blood was drawn, were actually available to test the association between upbringing and telomere length later in life. We do not think this has made a difference, since the subjects who participated in the AHS-1 in the mid-70s were not aware that their responses would be used for later rTL assessment. Despite using a survival cohort for our study, we found that there was a significant reduction in rTL among participants who were raised by a cold mother. If anything, our estimates may be underestimates of the true association, given that this survival cohort may also have greater resilience. On the other hand, given that this is a highly educated population with a healthy lifestyle that is leaner than average, generalizability may be compromised. It is, however, difficult to predict whether the less health conscious general population would be more or less affected by having been raised by a cold mother.

Conclusion

In conclusion, we found that subjects who report having had a mother, during their formative years, who they perceived as cold, have about 25% smaller rTL than those not describing their mother as cold. A somewhat smaller rTL was also observed for those reporting a cold father. The association between a cold mother parenting style and rTL was modified by educational level and BMI and to a lesser degree by dietary pattern. Further studies are needed to determine whether our findings are causal and to identify which factors are important for attenuating or buffering these possible effects of unfavorable childhood environments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Afifi TO, et al., Population attributable fractions of psychiatric disorders and suicide ideation and attempts associated with adverse childhood experiences. *Am J Public Health*, 2008 98(5): p. 946–52. [PubMed: 18381992]
2. Kessler RC, et al., Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br J Psychiatry*, 2010 197(5): p. 378–85. [PubMed: 21037215]
3. Afifi TO, et al., Child abuse and physical health in adulthood. *Health Rep*, 2016 27(3): p. 10–8.
4. Amemiya A, et al., Adverse childhood experiences and higher-level functional limitations among older Japanese people: results from the JAGES study. *J Gerontol A Biol Sci Med Sci*, 2017.
5. Davies PT and Cummings EM, Marital conflict and child adjustment: an emotional security hypothesis. *Psychol Bull*, 1994 116(3): p. 387–411. [PubMed: 7809306]
6. Troxel WM and Matthews KA, What are the costs of marital conflict and dissolution to children's physical health? *Clin Child Fam Psychol Rev*, 2004 7(1): p. 29–57. [PubMed: 15119687]
7. Repetti RL, Taylor SE, and Seeman TE, Risky families: family social environments and the mental and physical health of offspring. *Psychol Bull*, 2002 128(2): p. 330–66. [PubMed: 11931522]
8. Price LH, et al., Telomeres and early-life stress: an overview. *Biol Psychiatry*, 2013 73(1): p. 15–23. [PubMed: 22831981]
9. Shalev I, et al., Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: a longitudinal study. *Mol Psychiatry*, 2013 18(5): p. 576–81. [PubMed: 22525489]
10. Drury SS, et al., The association of telomere length with family violence and disruption. *Pediatrics*, 2014 134(1): p. e128–37. [PubMed: 24936002]
11. Savolainen K, et al., Associations between early life stress, self-reported traumatic experiences across the lifespan and leukocyte telomere length in elderly adults. *Biological Psychology*, 2014 97: p. 35–42. [PubMed: 24530884]
12. Wentzensen IM, et al., The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*, 2011 20(6): p. 1238–50. [PubMed: 21467229]
13. Zhao J, et al., Association between telomere length and type 2 diabetes mellitus: a meta-analysis. *PLoS One*, 2013 8(11): p. e79993. [PubMed: 24278229]
14. D'Mello MJ, et al., Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis. *Circ Cardiovasc Genet*, 2015 8(1): p. 82–90. [PubMed: 25406241]
15. Brown DW, et al., Adverse childhood experiences and the risk of premature mortality. *Am J Prev Med*, 2009 37(5): p. 389–96. [PubMed: 19840693]
16. Starkweather AR, et al., An integrative review of factors associated with telomere length and implications for biobehavioral research. *Nurs Res*, 2014 63(1): p. 36–50. [PubMed: 24335912]
17. Blackburn EH, Switching and signaling at the telomere. *Cell*, 2001 106(6): p. 661–73. [PubMed: 11572773]
18. Smogorzewska A and de Lange T, Regulation of telomerase by telomeric proteins. *Annu Rev Biochem*, 2004 73: p. 177–208. [PubMed: 15189140]
19. Sapolsky RM, Organismal stress and telomeric aging: an unexpected connection. *Proc Natl Acad Sci U S A*, 2004 101(50): p. 17323–4. [PubMed: 15579535]
20. Armanios M and Blackburn EH, The telomere syndromes. *Nat Rev Genet*, 2012 13(10): p. 693–704. [PubMed: 22965356]
21. Humphreys J, et al., Telomere Shortening in Formerly Abused and Never Abused Women. *Biol Res Nurs*, 2011.
22. Kananen L, et al., Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. *PLoS One*, 2010 5(5): p. e10826. [PubMed: 20520834]
23. Surtees PG, et al., Life Stress, Emotional Health, and Mean Telomere Length in the European Prospective Investigation into Cancer (EPIC)-Norfolk Population Study. *J Gerontol A Biol Sci Med Sci*, 2011.

24. Puterman E, et al., Lifespan adversity and later adulthood telomere length in the nationally representative US Health and Retirement Study. *Proc Natl Acad Sci U S A*, 2016 113(42): p. E6335–E6342. [PubMed: 27698131]
25. Osler M, et al., Stressful life events and leucocyte telomere length: Do lifestyle factors, somatic and mental health, or low grade inflammation mediate this relationship? Results from a cohort of Danish men born in 1953. *Brain Behav Immun*, 2016 58: p. 248–253. [PubMed: 27470228]
26. Hartmann N, et al., Telomere length of patients with major depression is shortened but independent from therapy and severity of the disease. *Depress Anxiety*, 2010 27(12): p. 1111–6. [PubMed: 21053332]
27. Verhoeven JE, et al., Depressive and Anxiety Disorders Showing Robust, but Non-Dynamic, 6-Year Longitudinal Association With Short Leukocyte Telomere Length. *Am J Psychiatry*, 2016 173(6): p. 617–24. [PubMed: 26940806]
28. Ridout KK, et al., Depression and telomere length: A meta-analysis. *J Affect Disord*, 2016 191: p. 237–47. [PubMed: 26688493]
29. O'Donovan A, et al., Pessimism correlates with leukocyte telomere shortness and elevated interleukin-6 in post-menopausal women. *Brain Behav Immun*, 2009 23(4): p. 446–9. [PubMed: 19111922]
30. Ikeda A, et al., Pessimistic orientation in relation to telomere length in older men: the VA normative aging study. *Psychoneuroendocrinology*, 2014 42: p. 68–76. [PubMed: 24636503]
31. Brydon L, et al., Hostility and Cellular Aging in Men from the Whitehall II Cohort. *Biol Psychiatry*, 2011.
32. Watkins LE, et al., Hostility and telomere shortening among U.S. military veterans: Results from the National Health and Resilience in Veterans Study. *Psychoneuroendocrinology*, 2016 74: p. 251–257. [PubMed: 27689898]
33. Epel ES, et al., Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A*, 2004 101(49): p. 17312–5. [PubMed: 15574496]
34. Entringer S, et al., Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proc Natl Acad Sci U S A*, 2011 108(33): p. E513–8. [PubMed: 21813766]
35. Entringer S, et al., Maternal psychosocial stress during pregnancy is associated with newborn leukocyte telomere length. *Am J Obstet Gynecol*, 2013 208(2): p. 134 e1–7. [PubMed: 23200710]
36. Marchetto NM, et al., Prenatal stress and newborn telomere length. *Am J Obstet Gynecol*, 2016 215(1): p. 94 e1–8. [PubMed: 26829506]
37. Brody GH, Yu T, and Shalev I, Risky family processes prospectively forecast shorter telomere length mediated through negative emotions. *Health Psychol*, 2017 36(5): p. 438–444. [PubMed: 27831704]
38. Robles TF, et al., Emotions and family interactions in childhood: Associations with leukocyte telomere length emotions, family interactions, and telomere length. *Psychoneuroendocrinology*, 2016 63: p. 343–50. [PubMed: 26551267]
39. Hyland ME, Alkhalaf AM, and Whalley B, Beating and insulting children as a risk for adult cancer, cardiac disease and asthma. *J Behav Med*, 2013 36(6): p. 632–40. [PubMed: 23054177]
40. Beach SR, et al., Nonsupportive parenting affects telomere length in young adulthood among African Americans: mediation through substance use. *J Fam Psychol*, 2014 28(6): p. 967–72. [PubMed: 25485673]
41. Brody GH, et al., Prevention effects ameliorate the prospective association between nonsupportive parenting and diminished telomere length. *Prev Sci*, 2015 16(2): p. 171–80. [PubMed: 24599483]
42. Jodczyk S, et al., No Association between Mean Telomere Length and Life Stress Observed in a 30 Year Birth Cohort. *Plos One*, 2014 9(5).
43. Verhoeven JE, et al., The Association of Early and Recent Psychosocial Life Stress With Leukocyte Telomere Length. *Psychosomatic Medicine*, 2015 77(8): p. 882–891. [PubMed: 26374947]
44. Mills PK, et al., Prospective study of exogenous hormone use and breast cancer in Seventh-day Adventists. *Cancer*, 1989 64(3): p. 591–7. [PubMed: 2743253]

45. Thorpe DL, et al., Effects of meat consumption and vegetarian diet on risk of wrist fracture over 25 years in a cohort of peri- and postmenopausal women. *Public Health Nutr*, 2008 11(6): p. 564–72. [PubMed: 17686206]
46. Butler TL, et al., Cohort profile: The Adventist Health Study-2 (AHS-2). *Int J Epidemiol*, 2008 37(2): p. 260–5. [PubMed: 17726038]
47. Thorpe DL, et al., The effect of vigorous physical activity and risk of wrist fracture over 25 years in a low-risk survivor cohort. *J Bone Miner Metab*, 2006 24(6): p. 476–83. [PubMed: 17072740]
48. Chan J, et al., Feasibility of running clinics to collect biological specimens in a nationwide cohort study--Adventist Health Study-2. *Ann Epidemiol*, 2007 17(6): p. 454–7. [PubMed: 17395486]
49. Fraser GE, et al., Biomarkers of Dietary Intake Are Correlated with Corresponding Measures from Repeated Dietary Recalls and Food-Frequency Questionnaires in the Adventist Health Study-2. *J Nutr*, 2016 146(3): p. 586–94. [PubMed: 26843587]
50. Cawthon RM, Telomere measurement by quantitative PCR. *Nucleic Acids Res*, 2002 30(10): p. e47. [PubMed: 12000852]
51. Martins MCT, et al., A New Approach to Assess Lifetime Dietary Patterns Finds Lower Consumption of Animal Foods with Aging in a Longitudinal Analysis of a Health-Oriented Adventist Population. *Nutrients*, 2017 9(10).
52. Harrell FE, Dupont C, et al., Hmisc: Harrell Miscellaneous. R package version 3.17–4 2016; Available from: <https://CRAN.R-project.org/package=Hmisc>.
53. Fraser GE and Yan R, A multivariate method for measurement error correction using pairs of concentration biomarkers. *Ann Epidemiol*, 2007 17(1): p. 64–73. [PubMed: 17140813]
54. Schafer JL, Multiple imputation: a primer. *Stat Methods Med Res*, 1999 8(1): p. 3–15. [PubMed: 10347857]
55. Tyrka AR, et al., Childhood maltreatment and telomere shortening: preliminary support for an effect of early stress on cellular aging. *Biol Psychiatry*, 2010 67(6): p. 531–4. [PubMed: 19828140]
56. Kiecolt-Glaser JK, et al., Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. *Psychosom Med*, 2011 73(1): p. 16–22. [PubMed: 21148804]
57. Hennessy MB, et al., Proinflammatory activity and the sensitization of depressive-like behavior during maternal separation. *Behav Neurosci*, 2011 125(3): p. 426–33. [PubMed: 21500883]
58. Barnett RC and Hyde JS, Women, men, work, and family. An expansionist theory. *Am Psychol*, 2001 56(10): p. 781–96. [PubMed: 11675985]
59. Lee M, et al., Inverse association between adiposity and telomere length: The Fels Longitudinal Study. *Am J Hum Biol*, 2011 23(1): p. 100–6. [PubMed: 21080476]
60. Nordfjall K, et al., Increased abdominal obesity, adverse psychosocial factors and shorter telomere length in subjects reporting early ageing; the MONICA Northern Sweden Study. *Scand J Public Health*, 2008 36(7): p. 744–52. [PubMed: 18647789]
61. Chen S, et al., Short leukocyte telomere length is associated with obesity in American Indians: the Strong Heart Family study. *Aging (Albany NY)*, 2014 6(5): p. 380–9. [PubMed: 24861044]
62. Wulaningsih W, et al., Investigating the associations between adiposity, life course overweight trajectories, and telomere length. *Aging (Albany NY)*, 2016 8(11): p. 2689–2701. [PubMed: 27650676]
63. Suzuki K, et al., Relationship between obesity and serum markers of oxidative stress and inflammation in Japanese. *Asian Pac J Cancer Prev*, 2003 4(3): p. 259–66. [PubMed: 14507248]
64. Carulli L, et al., Telomere length elongation after weight loss intervention in obese adults. *Mol Genet Metab*, 2016 118(2): p. 138–42. [PubMed: 27157420]
65. Gong Y, et al., Higher adherence to the 'vegetable-rich' dietary pattern is related to longer telomere length in women. *Clin Nutr*, 2018 37(4): p. 1232–1237. [PubMed: 28673689]
66. Rafie N, et al., Dietary patterns, food groups and telomere length: a systematic review of current studies. *Eur J Clin Nutr*, 2017 71(2): p. 151–158. [PubMed: 27530475]
67. Tucker LA, Consumption of Nuts and Seeds and Telomere Length in 5,582 Men and Women of the National Health and Nutrition Examination Survey (NHANES). *J Nutr Health Aging*, 2017 21(3): p. 233–240. [PubMed: 28244560]

68. Freitas-Simoes TM, Ros E, and Sala-Vila A, Nutrients, foods, dietary patterns and telomere length: Update of epidemiological studies and randomized trials. *Metabolism*, 2016 65(4): p. 406–15. [PubMed: 26975532]
69. Tucker LA, Dietary Fiber and Telomere Length in 5674 U.S. Adults: An NHANES Study of Biological Aging. *Nutrients*, 2018 10(4).
70. Latifovic L, et al., The Influence of Alcohol Consumption, Cigarette Smoking, and Physical Activity on Leukocyte Telomere Length. *Cancer Epidemiol Biomarkers Prev*, 2016 25(2): p. 374–80. [PubMed: 26656293]
71. Wulaningsih W, et al., Smoking, second-hand smoke exposure and smoking cessation in relation to leukocyte telomere length and mortality. *Oncotarget*, 2016 7(37): p. 60419–60431. [PubMed: 27509177]
72. Adler N, et al., Educational attainment and late life telomere length in the Health, Aging and Body Composition Study. *Brain Behav Immun*, 2013 27(1): p. 15–21. [PubMed: 22981835]
73. Flegal KM, et al., Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. *JAMA*, 2012 307(5): p. 491–7. [PubMed: 22253363]
74. Chen E, et al., Maternal warmth buffers the effects of low early-life socioeconomic status on pro-inflammatory signaling in adulthood. *Mol Psychiatry*, 2011 16(7): p. 729–37. [PubMed: 20479762]
75. Feder A, Nestler EJ, and Charney DS, Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci*, 2009 10(6): p. 446–57. [PubMed: 19455174]
76. Asok A, et al., Parental responsiveness moderates the association between early-life stress and reduced telomere length. *Dev Psychopathol*, 2013 25(3): p. 577–585. [PubMed: 23527512]
77. Bellis MA, et al., Does continuous trusted adult support in childhood impart life-course resilience against adverse childhood experiences - a retrospective study on adult health-harming behaviours and mental well-being. *BMC Psychiatry*, 2017 17(1): p. 110. [PubMed: 28335746]
78. Klaus K, et al., Interactive effects of early life stress and CACNA1C genotype on cortisol awakening response. *Biol Psychol* 10.1016/j.biopsycho.2018.05.002
79. Tomiyama AJ, et al., Does cellular aging relate to patterns of allostasis? An examination of basal and stress reactive HPA axis activity and telomere length. *Physiol Behav*, 2012 106(1): p. 40–5. [PubMed: 22138440]
80. Wikgren M, et al., Short telomeres in depression and the general population are associated with a hypocortisolemic state. *Biol Psychiatry*, 2012 71(4): p. 294–300. [PubMed: 22055018]
81. Choi J, Fauci SR, and Effros RB, Reduced telomerase activity in human T lymphocytes exposed to cortisol. *Brain Behav Immun*, 2008 22(4): p. 600–5. [PubMed: 18222063]
82. O'Donovan A, et al., Cumulative inflammatory load is associated with short leukocyte telomere length in the Health, Aging and Body Composition Study. *PLoS One*, 2011 6(5): p. e19687. [PubMed: 21602933]
83. Cafueri G, et al., Endothelial and smooth muscle cells from abdominal aortic aneurysm have increased oxidative stress and telomere attrition. *PLoS One*, 2012 7(4): p. e35312. [PubMed: 22514726]
84. von Zglinicki T, Oxidative stress shortens telomeres. *Trends Biochem Sci*, 2002 27(7): p. 339–44. [PubMed: 12114022]
85. Kurz DJ, et al., Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci*, 2004 117(Pt 11): p. 2417–26. [PubMed: 15126641]
86. Makpol S, et al., Tocotrienol-rich fraction prevents cell cycle arrest and elongates telomere length in senescent human diploid fibroblasts. *J Biomed Biotechnol*, 2011 2011: p. 506171. [PubMed: 21541185]
87. Makino N, et al., Antioxidant therapy attenuates myocardial telomerase activity reduction in superoxide dismutase-deficient mice. *J Mol Cell Cardiol*, 2011 50(4): p. 670–7. [PubMed: 21195081]
88. Hovens JG, et al., Childhood life events and childhood trauma in adult patients with depressive, anxiety and comorbid disorders vs. controls. *Acta Psychiatr Scand*, 2010 122(1): p. 66–74. [PubMed: 19878136]

89. van Harmelen AL, et al., Reduced medial prefrontal cortex volume in adults reporting childhood emotional maltreatment. *Biol Psychiatry*, 2010 68(9): p. 832–8. [PubMed: 20692648]
90. van Reedt Dortland AK, et al., Personality traits and childhood trauma as correlates of metabolic risk factors: the Netherlands Study of Depression and Anxiety (NESDA). *Prog Neuropsychopharmacol Biol Psychiatry*, 2012 36(1): p. 85–91. [PubMed: 22001949]
91. Huzen J, et al., Telomere length loss due to smoking and metabolic traits. *J Intern Med*, 2014 275(2): p. 155–63. [PubMed: 24118582]
92. Strandberg TE, et al., Association between alcohol consumption in healthy midlife and telomere length in older men. The Helsinki Businessmen Study. *Eur J Epidemiol*, 2012 27(10): p. 815–22. [PubMed: 22875407]
93. Pavanello S, et al., Shortened telomeres in individuals with abuse in alcohol consumption. *Int J Cancer*, 2011 129(4): p. 983–92. [PubMed: 21351086]

Highlights

- Parenting style is associated with shorter telomere length in offspring
- Perceived cold mother was associated with 25% shorter telomere length
- The effect of cold mother was attenuated among higher educated subjects
- The effect of cold mother on telomere length was stronger in obese subjects
- Father's parenting style had a similar direction but smaller and non-significant

Figure 1.
Timeline of study.

Figure 2.
Telomere length (rTL*) in offspring by parental style.

Table 1.

Demographic characteristics of subjects at enrollment into AHS-2.

*Characteristic	N (%)
Age, Mean (SD)	70.6 (9.7)
Age-groups	
50–59	34 (17.1)
60–69	55 (27.6)
70–79	78 (39.2)
80+	32 (16.1)
Gender	
Female	120 (60.3)
Male	79 (39.7)
Education	
HS or less	17 (8.5)
Some college	54 (27.1)
College graduate	47 (23.6)
Graduate degree	81 (40.7)
Number of comorbidity histories	
1	73 (36.7)
2–3	74 (37.2)
4	52 (26.1)
Diet pattern	
Vegetarian	124 (62.3)
Non-vegetarian	75 (37.2)
Diet switching (between AHS-1 and AHS-2)	
Consistently being vegetarian	111 (55.8)
Consistently being non-vegetarian	43 (21.6)
Switched diet pattern between studies	45 (22.6)
BMI (kg/m ²)	
Underweight (<18.5)	3 (1.5)
Normal (18.5 – <25)	85 (42.7)
Overweight (25 – <30)	73 (36.7)
Obese (≥ 30)	38 (19.1)
BMI change between AHS-1 and AHS-2	
Consistent (or decreased to) normal BMI	86 (43.2)
Consistent overweight/obese BMI	66 (33.2)
Became obese from a normal BMI	47 (23.6)
Physical activity (min/wk)	
Low (0)	55 (27.6)
Med (0 – <60)	52 (26.1)
High (≥ 60)	92 (46.2)
Smoking	

*Characteristic	N (%)
Ever	11 (5.5)
Never	188 (94.5)

* Characteristics were based on AHS-2, except where specified.

Table 2.

Multivariable adjusted* mean rTL by characteristic.

Characteristic	N	Mean rTL (95% CI)	p-value
Mother cold			
Yes	23	1.89 (1.54, 2.31)	0.002
No	176	2.53 (2.36, 2.72)	
Father cold			
Yes	51	2.29 (2.03, 2.59)	0.170
No	148	2.53 (2.34, 2.74)	
Mother absent**			
Yes	3	1.74 (1.05, 2.90)	0.183
No	193	2.47 (2.30, 2.65)	
Father absent			
Yes	13	2.18 (1.67, 2.84)	0.343
No	186	2.48 (2.32, 2.67)	
Mother warm and/or non-cold			
Yes	171	2.54 (2.37, 2.73)	0.010
No	28	1.99 (1.67, 2.37)	
Father warm and/or non-cold			
Yes	135	2.59 (2.29, 2.70)	0.660
No	64	2.42 (2.16, 2.70)	
Age Groups***			
50–59	34	2.82 (2.40, 3.31)	Ref.
60–69	55	2.69 (2.38, 3.04)	0.721
70–79	78	2.20 (1.97, 2.46)	0.015
80+	32	2.27 (1.94, 2.66)	0.071
*** <i>p(trend): 0.007</i>			

* Adjusted (except for Age Groups) for AHS-2 information on: age + diet pattern (vegetarian/non-vegetarian) + gender + education + exercise (min/wk) + BMI + number of comorbidities + smoking.

** Full model except number of comorbidities (due to small numbers).

*** Adjusted for all factors except age.

Table 3.

Multivariable adjusted mean rTL for cold mother by strata of effect modifiers, AHS-2.

	Cold Mother	Mean rTL (95% CI)	p-value
Education ^a			0.034 [*]
BA or higher	Yes	2.27 (1.64, 3.13)	0.439
	No	2.52 (2.27, 2.80)	
Less than BA	Yes	1.51 (1.01, 2.25)	0.002
	No	2.60 (2.25, 3.00)	
Diet pattern ^b			0.392 [*]
Vegetarian	Yes	1.99 (1.45, 2.71)	0.084
	No	2.50 (2.23, 2.80)	
Non-vegetarian	Yes	1.71 (1.13, 2.60)	0.019
	No	2.59 (2.25, 2.96)	
BMI ^c			0.038 [*]
BMI<25	Yes	2.45 (1.59, 3.77)	0.964
	No	2.47 (2.17, 2.82)	
BMI>25	Yes	1.66 (1.22, 2.24)	0.001
	No	2.62 (2.34, 2.93)	

^a Adjusted for the following AHS-2 variables: age + diet pattern + gender + exercise + BMI + number of comorbidities + smoking.^b Model ^a plus education, minus diet pattern^c Model ^a plus education, minus BMI^{*} Interaction p-value