Polyunsaturated fatty acid status and methylmercury exposure are not associated with leukocyte telomere length in mothers or their children in the Seychelles Child Development Study[1-3]

Alison J Yeates[4*], Sally W Thurston[5], Huiqi Li[6], Maria S Mulhern[4], Emeir M McSorley[4], Gene E Watson[5], Conrad F Shamlaye[7], JJ Strain[4], Gary J Myers[5], Philip W Davidson[5], Edwin van Wijngaarden[5] and Karin Broberg[8]

[4]Nutrition Innovation Centre for food and HEalth (NICHE), School of Biomedical Sciences, Ulster University, Coleraine, Cromore Road, Co. Londonderry, BT52 1SA, UK

[5] University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave, Box 671, Rochester, NY 14642, USA

[6] Division of Occupational and Environmental Medicine, Lund University, 22185 Lund, Sweden

[7]The Child Development Centre, Ministry of Health, Mahé, Republic of Seychelles, Seychelles

[8]Institute of Environmental Medicine (IMM), Karolinska Institute, SE-171 77 Stockholm, Sweden

Running Title: Fatty acids, methylmercury and telomere length
Word Count: 6424; **Number of Figures:** 1; **Number of Tables:** 3
Supplementary material: No online supporting materials have been submitted
Author list for indexing: Yeates, Thurston, Li, Mulhern, McSorley, Watson, Shamlaye, Strain, Myers, Davidson, van Wijngaarden and Broberg

*To whom correspondence should be addressed: Dr Alison J Yeates, Ulster University, Cromore Road, Coleraine, BT52 1SA, UK, Tel: + 44 (0) 28 701 23147, Fax: +44 (0) 701 , Email: a.yeates@ulster.ac.uk

[1] This research was supported by grants from the US National Institute of Environmental Health Sciences, National Institutes of Health (R01-ES010219, R01-ES015578, P30 ES001247); the European Union (Sixth Framework Programme; PHIME; FOOD-CT-2006-016253); the Swedish Research Council FORMAS; and the Government of Seychelles.

[2]Author disclosure: A.J.Yeates, S.W.Thurston, H.Li, M.S.Mulhern, E.M.McSorley, G.E.Watson, C.F.Shamlaye, J.J.Strain, G.J.Myers, P.W.Davidson, E.van Wijngaarden and K.Broberg have no conflicts of interest.

[3]Abbreviations used: AA (Arachidonic acid), ALA (alpha- linolenic acid), LA (linoleic acid), MeHg (methylmercury), PROCESS (Pediatric Review of Children's Environmental Support and Stimulation), Seychelles Child Development Study (SCDS), Socioeconimic status (SES), TL (telomere length)

ABSTRACT

Background Leukocyte telomere length (TL) is associated with age-related diseases and early mortality, but there is a lack of data on determinants of TL in early life. Evidence suggests that dietary intake of marine n-3 polyunsaturated fatty acids (PUFA) is protective of telomere attrition. Yet the effect of methylmercury (MeHg) exposure, also found in fish, on TL is unknown.

Objective The aim of this study was to investigate associations between prenatal PUFA status, MeHg exposure and TL in mothers and children in the Seychelles, where fish consumption is high.

Methods Blood samples collected from 229 mothers (at 28wk gestation and delivery) and children (at 5y of age) in the Seychelles Child Development Study Nutrition Cohort 1 were analyzed for PUFA concentrations. Prenatal Hg was measured in maternal hair collected at delivery. Postnatal Hg was also measured in children's hair samples, using a cumulative metric derived from values obtained at 3-5y of age. Relative TL was measured in blood obtained from mothers at delivery, in cord blood, and in children at 5y of age by quantitative PCR. Linear regression models were used to investigate associations between PUFA status, MeHg exposure and TL.

Results Neither prenatal PUFA status or MeHg exposure were associated with TL of the mother or child, nor with TL attrition rate. However a higher prenatal n-6/n-3 PUFA ratio was significantly associated with longer TL in the mothers (β= 0.001, *P*= 0.048). Child PUFA status and MeHg exposure were not associated with child TL. However greater values of family Hollingshead socioeconomic status (SES) at 9mo of age were significantly associated with longer TL in cord blood (β=0.005, *P*= 0.03).

Conclusions We found no evidence that PUFA status or MeHg exposure are determinants of TL, in either the mother or child. However, our results support the hypothesis that family SES may be associated with child TL.

KEYWORDS: Polyunsaturated fatty acid status, methylmercury exposure, telomere length, pregnancy, maternal infant nutrition, fish consumption, Seychelles Child Development Study

INTRODUCTION

Telomeres, composed of TTAGGG repeats of DNA, act as a protective cap at the end of chromosomes and are essential for chromosome stability and replication [1]. Telomeres shorten with each cell division cycle [2] and as such, shortened telomere length (TL) has been used as an indicator of cell senescence and biological aging [3]. Damage to, or excessive shortening of telomeres in peripheral blood has been associated with accelerated aging and diseases featuring inflammation and oxidative stress, such as cardiovascular disease [4, 5] and cancer [6, 7]. Although TL is largely genetically determined, several environmental influences, such as physical and psychological stress, smoking, body composition and socioeconomic status (SES), are reported to influence TL [8-10]. Furthermore, several recent studies have reported associations between various dietary components and TL, suggesting that modifying the diet may promote longevity [11-13]. There are consistent reports that a Mediterranean dietary pattern, characterized by high fruit and vegetable intake, is associated with greater TL in various populations [14, 15]. Specific nutrients have also been studied in relation to TL. Higher dietary intakes of long chain n-3 PUFA, which have anti-inflammatory properties, have been associated with longer TL in adults [16-18]. The balance between the n-3 PUFA and n-6 PUFA families may also be important in relation to effects on inflammation and TL. A randomized controlled trial with n-3 PUFA supplementation reported that TL increased with decreasing n-6/n-3 PUFA ratios and concluded that further study of this relationship was important in order to better understand disease prevention through dietary modification [18].

Childhood is the time period of greatest telomere loss in leucocytes, with studies of humans from birth to 90 years of age indicating the greatest attrition in the first years of life [19-21]. Little information exists regarding the natural history of telomere processes in children and it

remains relatively unknown at what lifestage dietary or environmental exposures may affect TL [22]. However, given the wide interindividual variation in TL at birth and the fact that attrition of TL begins with the first cycle of cell division, it is likely that early life exposures may have an important effect on TL and susceptibility to age-related diseases throughout life; similar to the concept of epigenetics [23, 24].

To our knowledge, no study has yet investigated the effects of exposure to methylmercury (MeHg) from fish consumption on TL. It is understood that MeHg is a toxin which can induce systemic oxidative stress and inflammation, both of which are associated with an accelerated rate of TL shortening. However fish is also a rich source of n-3 PUFA which may counteract MeHg-induced inflammation and oxidative stress [25, 26]. We have previously reported on the importance of considering the prenatal PUFA status when examining associations between MeHg exposure and neurodevelopment [27, 28]. In order to clarify the effects of prenatal PUFA status and MeHg exposure, through fish consumption, on TL and to increase understanding on determinants of TL at birth and attrition during early life, we set out to investigate associations between PUFA status, MeHg exposure and TL in mothers and their children in the Seychelles Child Development Study (SCDS) first Nutrition Cohort (NC1). Our primary aim was to investigate the effect of prenatal PUFA status and MeHg exposure on TL of the mother and child, with our secondary aim to examine postnatal PUFA and MeHg as potential determinants of child TL at birth and early life.

METHODS

Study population

The SCDS is an observational study conducted in the Republic of Seychelles. It was established to investigate the effects of prenatal exposure to MeHg, through maternal fish consumption during pregnancy, on child neurodevelopment. The NC1 cohort recruited a total of 300 mothers at their first antenatal appointment on the island of Mahé during 2001, with full details of recruitment and the study setting described previously [27]. Maternal height and weight were measured when mothers were enrolled to the study, and in children at 5y of age, from which BMI was calculated as weight (kg)/ height $(m)^2$. Smoking and alcohol use during pregnancy were each measured as a dichotomous variable (some/none). Birth weight (g) and gestational age (weeks) were determined at the child's birth. Family SES was estimated using the Hollingshead Four-Factor Social Status Index, measured when the child was 9mo of age and again when the child was 5y of age. The Hollingshead Index was modified to assess data on the primary caregiver's education and occupation (mother, father, both, or other) [29], where higher codes indicated higher educational attainment or occupational status [30]. We combined occupational and educational codes through a weighted formula into a continuous score [30]. Home environment was assessed using the Pediatric Review of Children's Environmental Support and Stimulation (PROCESS). The study was reviewed and approved by the Seychelles Ethics Board and by the Research Subjects' Review Boards at the University of Rochester.

Blood collection

Blood samples were collected from mothers at 28 weeks gestation and at delivery. Children's cord blood samples were collected at birth. Blood samples were also collected from the

children from the forearm when they were aged approximately 5y. All blood samples were venous, non-fasting and collected in EDTA-containing tubes. Whole blood, serum and plasma aliquots were obtained and stored at -80ºC until analysis.

PUFA measurement

Maternal and child blood samples were maintained and shipped at -80ºC to Ulster University, Coleraine for analysis of PUFA status. The description of this protocol has been described in full elsewhere [31]. In brief, total lipids were extracted from maternal serum samples using a modified method of Folch *et al*. [32]. Fatty acid methyl esters were prepared by addition of boron trifluoride in methanol (Sigma-Aldrich Co, Ltd) and analyzed using a Thermo-Finnegan TRACE MS with Xcaliber software (ThermoFinnegan, UK). Precision was ensured by running a reference sample in each batch analysis for which the coefficient of variance (CV) was $\leq$10%. The limit of detection was 0.01mg/ml. Fatty acids were detected and quantified with reference to an external linear calibration curve which included two standards, C17:0 and C21:0, which were also added to unknown samples as internal standards prior to extraction as recommended by Schreiner (2005) [33]. The correlation coefficient of the calibration curve was r^2 =0.99. Total serum fatty acids were analyzed in maternal blood to account for the majority of fatty acids being transported to the fetus as triglycerides during pregnancy. The geometric mean of the maternal PUFA values measured at 28 weeks and delivery was used in these analyzes [27]. As previously described, serum concentrations of long chain n-3 PUFA measured in NC1 mothers were low, which may be the result of potential oxidation of samples during blood processing [34].

Similarly, blood samples collected from the children at 5y of age were subject to PUFA analysis by the same method, but we characterized plasma phospholipid PUFA status in this

age group and quantified concentrations with an Agilent GC-MS with Chemstation software (Agilent, UK). In both methods, heptadecaenoic acid (C17:0) and heneicosaenoic acid (C21:0) were used as internal standards, added prior to lipid extraction. We quantified in absolute amounts (mg/mL) concentrations of alpha-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), docosahexaenoic acid (DHA, C22:6 n-3), linoleic acid (LA, C18:2 n-6) and arachidonic acid (AA, C20:4 n-6). For models using prenatal PUFA status we summed total n-3 PUFA (ALA+EPA+DHA) and total n-6 PUFA (LA + AA). However, for models using postnatal PUFA status, owing to low levels of ALA being detected in children's 5y blood samples, we replaced the sums of n-3 PUFA and n-6 PUFA with EPA+DHA and AA respectively and used the AA/DHA ratio in place of the n-6/n-3 PUFA ratios.

MeHg measurement

Prenatal MeHg exposure was estimated by measuring total mercury (Hg) in maternal hair samples collected at delivery using atomic absorption spectroscopy at the University of Rochester, as previously described [28]. The limit of detection was 0.5ng Hg per sample aliquot and CV was 2.1%. Method accuracy was assessed throughout the analyses by inclusion of standard reference material for hair (IAEA-085 and IAEA-086, International Atomic Energy Agency). The University of Rochester Mercury Analytical Laboratory participated in the recent quality assessment of mercury laboratories with the COPHES/DEMOCOPHES project and served as a reference laboratory for analysis of hair mercury[35]. Hair was not cleaned prior to analysis, as our previous studies have not shown external contamination to be prevalent and cleaning hair has been associated inimitable results[36]. Because Hg was measured in the longest hair segment available from maternal hair grown during pregnancy (assuming growth of 1.1 cm/month), this measure represents

exposure during the entire pregnancy. Children's hair samples were obtained at evaluations before age 3 and at approximately 5y of age. Postnatal Hg exposure was estimated by measuring total Hg in the one cm closest to the scalp. For this analysis we estimated the cumulative (area under the curve) postnatal Hg exposure between the 3 and 5y time points, which is reported as ppm-years.

TL measurement

Whole blood samples were shipped at -80ºC from Ulster University, Coleraine to Lund University, Sweden for leukocyte TL measurement. We measured TL in blood samples from the mothers at delivery, and from their children in cord blood and at 5y of age. TL was measured in the 229 mothers (and their children) who had both measures of maternal hair Hg and maternal PUFA. DNA was extracted with Qiagen mini kit (Qiagen, Hilden, Germany) at the DNA/RNA genotyping Lab, SWEGEN Resource Center for Profiling Polygenic Disease, Lund University, Malmö, Sweden. TL quantification was determined by quantitative polymerase chain reaction (qPCR) as described in detail [37]. In short, an aliquot of 5µl sample DNA (3ng/µl) was added to each reaction (end volume 20µl). A standard curve, a reference DNA and a negative control were included in each run. For each standard curve, one calibrator DNA sample was diluted serially by 2-fold per dilution to produce 7 concentrations of 0.25-16 ng/µl. Each sample, standard curve, reference and negative control were run in duplicates. Master mixes were prepared, containing 0.5U *Taq* Platina (Invitrogen, Carlsbad, CA), 1×PCR Buffer, 0.8mM dNTPs, 1.75mM $MgCl_2$, 0.3mM SybrGreen I (Invitrogen), 1×Rox (Invitrogen), and either telomere primers (0.45 µM of each primer), or hemoglobin beta chain (*HBB*) primers (0.45 µM for each primer). The PCR was performed on a real-time PCR machine (7900HT, Applied Biosystems, Foster City, CA, USA). R^2 for each standard curve was >0.99. Standard deviations (for Ct values) were accepted at <0.2.

The TL is an arbitrary value that was obtained through calculating the ratio of telomere repeat copy number to single-copy gene numbers (T/S) for each individual using the formula T/S = $2^{-\Delta Ct}$, where $\Delta Ct = Ct_{telomere} - Ct_{HBB}$. This ratio was then divided by the ratio of the reference DNA. Reference samples were included in each run and demonstrated a CV of 8.0%, based on 11 runs. The TL attrition rate was calculated as the ratio of the scaled 5y child TL to the scaled cord blood TL, where scaling divided the TL at that age by the maximum TL at the same age. Since TL shortens with age, this ratio estimates the relative attrition rate, but only when cord TL and child 5y TL are measured on the same scale. Scaling each measure was necessary to preserve this interpretation.

Statistical analysis

Complete data were available for a total of 229 mothers and their children for which at least one TL was measured. Linear regression models were fit to investigate pre-specified associations between TL and covariates as shown in **Table 1**. Three models investigated prenatal PUFA and Hg as potential determinants of TL in both the mother and child, whilst two models considered the child's postnatal PUFA and Hg exposure. We adjusted for PUFA status in two ways: in primary models as prenatal n-3 PUFA and n-6 PUFA, or postnatal (DHA+EPA) and AA, and in secondary models, as ratios of prenatal n-6/n-3 PUFA or postnatal AA/DHA.

All models adjusted for possible confounders chosen *a priori* based on the literature. As shown in Table 1, models that used prenatal PUFA status adjusted for maternal age, maternal BMI, smoking during pregnancy (yes/no) and alcohol during pregnancy (yes/no), while models that used postnatal PUFA status adjusted for child 5y BMI and home environment. Models investigating child TL, cord TL or their ratio adjusted for child's sex, and the model for cord TL also adjusted for birth weight and gestational age. Finally, all models adjusted for

193 SES either as measured at 9 months (maternal TL or cord TL) or at 5y (models that use 5y
194 child TL).

195 Model assumptions were checked using standard methods, and included checking whether the
196 residuals had constant variance, were normally distributed, and had an approximate linear
197 relationship with each continuous covariate. We also checked for outliers, and for influential
198 observations as defined by Cook's distance. If model assumptions were violated we refit the
199 model using a transformation of the outcome that better satisfied assumptions. All tests were
200 two-sided and a *P* value <0.05 considered as significant.

201 TL in cord blood and the TL attrition rate required a logarithmic transformation to better meet
202 model assumptions. There were no unduly influential or unduly outlying observations in any
203 models. Due primarily to missing data on one or more TL measure and missing data on child
204 PUFA status, models for maternal TL, cord TL, child TL at 5y, and TL attrition rate were fit
205 on data from *n*=216, *n*=183, *n*=202 (adjusted for maternal markers; *n*=178 when adjusted for
206 child markers) and *n*=141 respectively.

207

208 **Results**

209 Maternal and child characteristics are presented in **Table 2**. The average TL decreased from
210 1.18 ± 0.5 in cord blood to 0.71 ± 0.1 at 5y of age and was lowest in mothers at an average of
211 0.64 ± 0.11. The mean TL attrition rate was 0.47 (SD= 0.14), with a range of -0.16 to 0.73.
212 TL across the three time-points were only weakly correlated (r= -0.02 for maternal and cord
213 TL, r= 0.06 for maternal and child's TL at 5y, and r= 0.14 for cord and child's 5y TL, *P*>0.05
214 for all correlations).

No significant associations were found between prenatal and postnatal PUFA status, hair Hg and any of the TL measures with the exception of the n-6/n-3 PUFA ratio in the mothers, where greater n-6/n-3 PUFA status was significantly associated with longer TL (β= 0.001, *P*=0.048, **Table 3**).

Family SES at 9 months was significantly positively associated with TL in cord blood (β=0.005, *P*=0.03, **Figure 1**). A positive trend was noted between family SES at 5y and TL at 5y of age, however this relationship was non-significant (β=0.001, *P*=0.08). At age 5y, TL was almost significantly longer among girls than boys (β=0.026, *P*=0.08), and in models adjusting for maternal factors, a positive trend was noted between maternal age and TL of the children at 5y of age (β=0.002, *P*=0.07); however both associations were found to be non-significant. These associations are from models that adjusted for maternal n-3 and n-6 PUFA, but similar associations were also found when adjusting for the n-6/n-3 PUFA ratio. No other covariates significantly predicted TL

Discussion

This study focused on TL in early life which, as an indicator of cellular ageing, may be related to a range of health outcomes including risk of developmental disorder in adolescence [38] and age-associated diseases, such as cardiovascular disease, in later life [2, 39]. Many populations depend on fish as their primary source of nutrition, and are therefore exposed to MeHg whilst also consuming n-3 PUFA. To our knowledge there are no longitudinal studies confirming a beneficial effect of fish consumption to TL, either in adults or children. However several studies of dietary data have indicated a protective effect of a Mediterranean diet, which is expected to feature high fish intakes, on TL in adults [14, 15]. We hypothesized

238 that prenatal PUFA status and MeHg exposure would have conflicting associations with TL,
239 both of the mother and child, through their opposing roles in inflammation and oxidative
240 stress. We found no clear evidence for associations between either prenatal or postnatal PUFA
241 status, MeHg exposure and TL in Seychellois mothers and their children, despite a uniquely
242 high fish intake in this cohort.

243 However we did observe that a higher prenatal n-6/n-3 PUFA ratio was associated with longer
244 TL in mothers. This finding was unexpected given that a higher n-6/n-3 PUFA ratio is
245 generally, but not always, indicative of greater inflammatory insult in the body. Previous
246 studies have reported a protective effect of supplementation with long chain n-3 PUFA on
247 telomere shortening in adults [16]. However the relationships between PUFA and TL remain
248 controversial and not fully understood, particularly in pregnancy [12, 40]. One intervention
249 study with long chain n-3 PUFA supplementation found that every one unit decrease of n-6/n-
250 3 PUFA ratio was associated with a 20 base pair increase of TL [18]. Yet, there was no
251 significant difference in the change in TL between placebo and treatment groups in their
252 study. A further intervention study for 6 months with a relatively small sample size found a
253 positive trend for longer TL with greater n-3 PUFA status, but no significant differences in
254 TL between groups of elderly adults taking either EPA+DHA, DHA or LA supplements [17].

255 The mechanism for a relationship between PUFA and TL is proposed to be via action of the
256 lipid metabolites derived from PUFA (e.g. eicosanoids, resolvins and protectins) which differ
257 in inflammatory properties according to whether their precursor is of the n-3 or n-6 PUFA
258 family. It is possible that our finding of a longer TL with greater maternal n-6/n-3 PUFA is
259 population-specific, given that the Seychelles cohort may have a unique genetic background
260 for PUFA metabolism (FADS genotype) as we have previously reported[41]. It is evident that
261 the relationship between PUFA and TL is more complex than previously understood and this

relationship may be further complicated by altered lipid metabolism during pregnancy. Therefore it would be of interest for future studies to consider the influence of various genotypes regulating PUFA metabolism when investigating associations between PUFA and TL.

This is the first time to our knowledge that the relationship between MeHg exposure and TL has been investigated. A major mechanism of MeHg toxicity in the body is exerted through promotion of inflammation and oxidative stress [42]. Therefore our finding of a lack of association with TL in either mothers or children is encouraging in that it suggests MeHg exposure from fish consumption in the Seychelles is not having a detrimental effect on cell aging.

We observed that a higher family SES, as measured at 9mo of age, was associated with longer TL of infants at birth. The association between child TL and SES at 5y of age was somewhat less strong and was not statistically significant. Other studies have shown that lower SES and social disadvantage during childhood are associated with shorter TL, both in childhood and in adulthood [43-45]. Our results confirm the importance of the early home environment for TL in children; a relationship that may have lifelong health effects for children in the Seychelles. It is possible that a higher family SES score is an indicator of other environmental factors which could influence the TL, such as a higher quality diet. A focused examination of the postnatal diet of children may eludicate dietary determinants of TL, and potentially explain why we did not find an association between SES at 5y with TL at the same age. Therefore the clinical implications of a longer TL in early life may relate to lower risk of developmental disorder in adolescence [38, 46] and a variety of conditions in later life [2]. To date, the majority of research conducted in this area ascribe these relationships to the balance between

oxidative stress and antioxidant defenses known to regulate DNA replication and senescence [47, 48].

In all samples TL was measured and calculated based on the same reference DNA, therefore the values were comparable between different groups. We observed that TL in cord blood was the longest, and in mothers the shortest. This pattern supports the general idea that TL could be a biomarker for biological age [39]. However, in mothers, there was no evidence of an association between TL and maternal age. The telomere attrition rate between newborn and 5 year-old children was surprisingly large, most likely reflecting the rapid growth, which requires prolific cell division. Robertson *et al* found the largest telomere attrition in the first year of life with a more constant rate of loss thereafter[21]. This high attrition rate could also explain the surprisingly low correlations between TL among mothers and children. We found one child with TL lengthening between birth and 5y, a phenomenon which has been observed by others [49, 50]. It is therefore possible that telomere lengthening processes may be part of overall oscillations in TL and we speculate that this phenomenon may represent fluctuations in cell types, which it was not possible to account for in our analysis. This represents one of few studies reporting TL in children and as such further investigation is warranted to determine the effect of early life exposures including diet to TL and telomere attrition.

This study has several strengths. The mother-child cohort allows investigation of various influential factors on TL, both in the mothers and the offspring up to 5y of age. The study population had high fish consumption [51], resulting in a concurrent high intake of n-3 PUFA and high exposure to MeHg. Therefore, any possible effects of these factors should have been detected in this study. This study also has limitations. Despite best efforts to prevent, it is possible that delayed blood processing of maternal samples in this cohort may have resulted in selective oxidation of the more susceptible long chain PUFA among a random subset of

serum samples. This may account for the relatively low n-3 PUFA concentrations and the higher n-6/n-3 PUFA ratio observed in mothers. As we have previously commented, this may induce non-differential measurement error with the result that observed associations in models examining prenatal PUFA status within the current study are likely to be closer to the null hypothesis than the true associations [34].

In conclusion, we found no clear evidence that prenatal or postnatal PUFA status or MeHg exposure are determinants of TL in our high fish-eating mother-child cohort. However, our results support the hypothesis that early life family SES may influence TL in the child.

Acknowledgments

All authors have read and approved the final version of the manuscript. A.J.Y had full access to all data in the study, with the exception of Hg data, assisted with data interpretation and prepared the manuscript. K.B. conceived the overall research concept and designed the analysis plan with S.W.T. and A.J.Y. C.F.S., G.J.M., J.J.S and P.W.D. were responsible for overall SCDS NC1 study design and involved in fieldwork and data collection. S.W.T. designed and conducted the statistical analysis and assisted with data interpretation. H.L. conducted the telomere length analysis and assisted with data interpretation. G.E.W. takes responsibility for the integrity of the Hg data. A.J.Y., M.S.M., E.M.M. and J.J.S. conducted PUFA analysis and assisted with data interpretation. J.J.S, P.W.D, E.vW, C.F.S and G.J.M provided overall *s*tudy supervision. A.J.Y. had final responsibility for final content of the manuscript.

References

1. Blackburn EH: **Structure and function of telomeres.** *Nature* 1991, **350:**569-573.
2. Blasco MA: **Telomeres and human disease: ageing, cancer and beyond.** *Nat Rev Genet* 2005, **6:**611-622.
3. Raynaud CM, Sabatier L, Philipot O, Olaussen KA, Soria JC: **Telomere length, telomeric proteins and genomic instability during the multistep carcinogenic process.** *Crit Rev Oncol Hematol* 2008, **66:**99-117.
4. Balasubramanyam M, Adaikalakoteswari A, Monickaraj SF, Mohan V: **Telomere shortening & metabolic/vascular diseases.** *Indian J Med Res* 2007, **125:**441-450.
5. Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ: **Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study.** *Lancet* 2007, **369:**107-114.
6. Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, Shay JW, Luo S, Hong WK, Spitz MR: **Telomere dysfunction: a potential cancer predisposition factor.** *J Natl Cancer Inst* 2003, **95:**1211-1218.
7. Ma H, Zhou Z, Wei S, Liu Z, Pooley KA, Dunning AM, Svenson U, Roos G, Hosgood HD, 3rd, Shen M, Wei Q: **Shortened telomere length is associated with increased risk of cancer: a meta-analysis.** *PLoS One* 2011, **6:**e20466.
8. Shalev I, Entringer S, Wadhwa PD, Wolkowitz OM, Puterman E, Lin J, Epel ES: **Stress and telomere biology: a lifespan perspective.** *Psychoneuroendocrinology* 2013, **38:**1835-1842.
9. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, Aviv A, Spector TD: **Obesity, cigarette smoking, and telomere length in women.** *Lancet* 2005, **366:**662-664.
10. Needham BL, Adler N, Gregorich S, Rehkopf D, Lin J, Blackburn EH, Epel ES: **Socioeconomic status, health behavior, and leukocyte telomere length in the National Health and Nutrition Examination Survey, 1999-2002.** *Soc Sci Med* 2013, **85:**1-8.
11. Paul L: **Diet, nutrition and telomere length.** *J Nutr Biochem* 2011, **22:**895-901.
12. Cassidy A, De Vivo I, Liu Y, Han J, Prescott J, Hunter DJ, Rimm EB: **Associations between diet, lifestyle factors, and telomere length in women.** *Am J Clin Nutr* 2010, **91:**1273-1280.
13. Rafie N, Golpour Hamedani S, Barak F, Safavi SM, Miraghajani M: **Dietary patterns, food groups and telomere length: a systematic review of current studies.** *Eur J Clin Nutr* 2017, **71:**151-158.
14. Crous-Bou M, Fung TT, Prescott J, Julin B, Du M, Sun Q, Rexrode KM, Hu FB, De Vivo I: **Mediterranean diet and telomere length in Nurses' Health Study: population based cohort study.** *BMJ* 2014, **349:**g6674.
15. Garcia-Calzon S, Martinez-Gonzalez MA, Razquin C, Aros F, Lapetra J, Martinez JA, Zalba G, Marti A: **Mediterranean diet and telomere length in high cardiovascular risk subjects from the PREDIMED-NAVARRA study.** *Clin Nutr* 2016, **35:**1399-1405.
16. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, Whooley MA: **Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease.** *JAMA* 2010, **303:**250-257.

17. O'Callaghan N, Parletta N, Milte CM, Benassi-Evans B, Fenech M, Howe PR: **Telomere shortening in elderly individuals with mild cognitive impairment may be attenuated with omega-3 fatty acid supplementation: a randomized controlled pilot study.** *Nutrition* 2014, **30:**489-491.
18. Kiecolt-Glaser JK, Epel ES, Belury MA, Andridge R, Lin J, Glaser R, Malarkey WB, Hwang BS, Blackburn E: **Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: A randomized controlled trial.** *Brain Behav Immun* 2013, **28:**16-24.
19. Frenck RW, Jr., Blackburn EH, Shannon KM: **The rate of telomere sequence loss in human leukocytes varies with age.** *Proc Natl Acad Sci U S A* 1998, **95:**5607-5610.
20. Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M, Lansdorp PM: **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.** *J Exp Med* 1999, **190:**157-167.
21. Robertson JD, Gale RE, Wynn RF, Dougal M, Linch DC, Testa NG, Chopra R: **Dynamics of telomere shortening in neutrophils and T lymphocytes during ageing and the relationship to skewed X chromosome inactivation patterns.** *Br J Haematol* 2000, **109:**272-279.
22. Kark JD, Goldberger N, Kimura M, Sinnreich R, Aviv A: **Energy intake and leukocyte telomere length in young adults.** *Am J Clin Nutr* 2012, **95:**479-487.
23. Barnes SK, Ozanne SE: **Pathways linking the early environment to long-term health and lifespan.** *Prog Biophys Mol Biol* 2011, **106:**323-336.
24. Shalev I: **Early life stress and telomere length: investigating the connection and possible mechanisms: a critical survey of the evidence base, research methodology and basic biology.** *Bioessays* 2012, **34:**943-952.
25. Choi AL, Mogensen UB, Bjerve KS, Debes F, Weihe P, Grandjean P, Budtz-Jorgensen E: **Negative confounding by essential fatty acids in methylmercury neurotoxicity associations.** *Neurotoxicol Teratol* 2014, **42:**85-92.
26. Mahaffey KR, Sunderland EM, Chan HM, Choi AL, Grandjean P, Marien K, Oken E, Sakamoto M, Schoeny R, Weihe P, et al: **Balancing the benefits of n-3 polyunsaturated fatty acids and the risks of methylmercury exposure from fish consumption.** *Nutr Rev* 2011, **69:**493-508.
27. Davidson PW, Strain JJ, Myers GJ, Thurston SW, Bonham MP, Shamlaye CF, Stokes-Riner A, Wallace JM, Robson PJ, Duffy EM, et al: **Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy.** *Neurotoxicology* 2008, **29:**767-775.
28. Strain JJ, Yeates AJ, van Wijngaarden E, Thurston SW, Mulhern MS, McSorley EM, Watson GE, Love TM, Smith TH, Yost K, et al: **Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: associations with child development at 20 mo of age in an observational study in the Republic of Seychelles.** *Am J Clin Nutr* 2015, **101:**530-537.
29. Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, Cernichiari E, Needham L, Choi A, Wang Y, et al: **Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study.** *JAMA* 1998, **280:**701-707.
30. Hollingshead AB: **Four Factor Index of Social Status.** In *Unpublished working paper*: Yale University; 1975.

31. Strain JJ, Davidson PW, Bonham MP, Duffy EM, Stokes-Riner A, Thurston SW, Wallace JM, Robson PJ, Shamlaye CF, Georger LA, et al: **Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study.** *Neurotoxicology* 2008, **29:**776-782.
32. Folch J, Lees M, Sloane Stanley GH: **A simple method for the isolation and purification of total lipides from animal tissues.** *J Biol Chem* 1957, **226:**497-509.
33. Schreiner M: **Quantification of long chain polyunsaturated fatty acids by gas chromatography. Evaluation of factors affecting accuracy.** *J Chromatogr A* 2005, **1095:**126-130.
34. Strain JJ, Davidson PW, Thurston SW, Harrington D, Mulhern MS, McAfee AJ, van Wijngaarden E, Shamlaye CF, Henderson J, Watson GE, et al: **Maternal PUFA status but not prenatal methylmercury exposure is associated with children's language functions at age five years in the Seychelles.** *J Nutr* 2012, **142:**1943-1949.
35. Esteban M, Schindler BK, Jimenez JA, Koch HM, Angerer J, Rosado M, Gomez S, Casteleyn L, Kolossa-Gehring M, Becker K, et al: **Mercury analysis in hair: Comparability and quality assessment within the transnational COPHES/DEMOCOPHES project.** *Environ Res* 2015, **141:**24-30.
36. Nuttall KL: **Interpreting hair mercury levels in individual patients.** *Ann Clin Lab Sci* 2006, **36:**248-261.
37. Li H, Jonsson BA, Lindh CH, Albin M, Broberg K: **N-nitrosamines are associated with shorter telomere length.** *Scand J Work Environ Health* 2011, **37:**316-324.
38. Costa Dde S, Rosa DV, Barros AG, Romano-Silva MA, Malloy-Diniz LF, Mattos P, de Miranda DM: **Telomere length is highly inherited and associated with hyperactivity-impulsivity in children with attention deficit/hyperactivity disorder.** *Front Mol Neurosci* 2015, **8:**28.
39. Sanders JL, Newman AB: **Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither?** *Epidemiol Rev* 2013, **35:**112-131.
40. Das UN: **Telomere length and polyunsaturated fatty acids.** *Nutrition* 2014, **30:**1218-1221.
41. Yeates AJ, Love TM, Engstrom K, Mulhern MS, McSorley EM, Grzesik K, Alhamdow A, Wahlberg K, Thurston SW, Davidson PW, et al: **Genetic variation in FADS genes is associated with maternal long-chain PUFA status but not with cognitive development of infants in a high fish-eating observational study.** *Prostaglandins Leukot Essent Fatty Acids* 2015, **102-103:**13-20.
42. Farina M, Aschner M, Rocha JB: **Oxidative stress in MeHg-induced neurotoxicity.** *Toxicol Appl Pharmacol* 2011, **256:**405-417.
43. Cohen S, Janicki-Deverts D, Turner RB, Marsland AL, Casselbrant ML, Li-Korotky HS, Epel ES, Doyle WJ: **Childhood socioeconomic status, telomere length, and susceptibility to upper respiratory infection.** *Brain Behav Immun* 2013, **34:**31-38.
44. Mitchell C, Hobcraft J, McLanahan SS, Siegel SR, Berg A, Brooks-Gunn J, Garfinkel I, Notterman D: **Social disadvantage, genetic sensitivity, and children's telomere length.** *Proc Natl Acad Sci U S A* 2014, **111:**5944-5949.
45. Needham BL, Fernandez JR, Lin J, Epel ES, Blackburn EH: **Socioeconomic status and cell aging in children.** *Soc Sci Med* 2012, **74:**1948-1951.
46. Henje Blom E, Han LK, Connolly CG, Ho TC, Lin J, LeWinn KZ, Simmons AN, Sacchet MD, Mobayed N, Luna ME, et al: **Peripheral telomere length and hippocampal volume in adolescents with major depressive disorder.** *Transl Psychiatry* 2015, **5:**e676.

47. Glade MJ, Meguid MM: **A glance at ... telomeres, oxidative stress, antioxidants, and biological aging.** *Nutrition* 2015, **31:**1447-1451.
48. von Zglinicki T: **Oxidative stress shortens telomeres.** *Trends Biochem Sci* 2002, **27:**339-344.
49. Wojcicki JM, Shiboski S, Heyman MB, Elwan D, Lin J, Blackburn E, Epel E: **Telomere length change plateaus at 4 years of age in Latino children: associations with baseline length and maternal change.** *Mol Genet Genomics* 2016, **291:**1379-1389.
50. Shalev I, Moffitt TE, Sugden K, Williams B, Houts RM, Danese A, Mill J, Arseneault L, Caspi A: **Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: a longitudinal study.** *Mol Psychiatry* 2013, **18:**576-581.
51. Bonham MP, Duffy EM, Wallace JM, Robson PJ, Myers GJ, Davidson PW, Clarkson TW, Shamlaye CF, Strain JJ: **Habitual fish consumption does not prevent a decrease in LCPUFA status in pregnant women (the Seychelles Child Development Nutrition Study).** *Prostaglandins Leukot Essent Fatty Acids* 2008, **78:**343-350.

Table 1. Description of linear regression models, their outcomes and covariates

Outcome	Exposure	Covariates
TL in mothers	Prenatal n-3 PUFA Prenatal n-6 PUFA Prenatal n-6/n-3 PUFA[1] Prenatal Hg	Maternal age; Maternal BMI; Smoking; Alcohol; 9mo family SES
Log(TL in cord blood)	Prenatal n-3 PUFA Prenatal n-6 PUFA Prenatal n-6/n-3 PUFA[1] Prenatal Hg	Maternal age; Maternal BMI; Smoking; Alcohol; 9mo family SES; Child sex; Birth weight; Gestational age
TL at 5y of age	Prenatal n-3 PUFA Prenatal n-6 PUFA Prenatal n-6/n-3 PUFA[1] Prenatal Hg	Maternal age; Maternal BMI; Smoking; Alcohol; 5y family SES
TL at 5y of age	Postnatal EPA+DHA Postnatal AA Postnatal AA/DHA[2] Postnatal Hg	Child sex; child BMI; Home environment; 5y family SES
Log(TL attrition rate)	Postnatal EPA+DHA Postnatal AA Postnatal AA/DHA[2] Postnatal Hg	Child sex; child BMI; Home environment; 5y family SES

[1] Ratio replaced n-3 PUFA and n-6 PUFA in secondary prenatal model; [2] Ratio replaced EPA+DHA and AA in secondary postnatal model

Table 2. Characteristics of 229 mother-child pairs with at least one TL measurement.

	N	Mean	SD	Range
Mothers				
Age (years)	229	27.2	5.93	15.0 – 42.0
BMI (kg/m^2) at enrollment	228	25.77	6.38	15.52 – 50.03
Gestational age (weeks)	229	38.75	1.34	34.0 – 41.0
Family SES at 9mo	229	33.93	11.01	13.0 – 63.0
Family SES at 5y	225	31.48	11.06	8.0 – 63.0
Hair Hg (ppm)	229	5.70	3.69	0.19 – 18.49
Serum n-3 PUFA (mg/mL)	229	0.03	0.01	0.01 – 0.06
Serum n-6 PUFA (mg/mL)	229	1.22	0.20	0.66 – 1.72
Serum n-6/n-3 PUFA ratio	229	40.2	11.7	13.2 – 90.4
TL (T/S)[1]	218	0.64	0.11	0.39 – 0.98
Children				
Sex (male/female)	229	113/116		
Birth weight (kg)	229	3.24	0.47	1.87 – 4.45

BMI (kg/m^2) at 5y	220	14.96	1.98	11.61 – 27.16
Home environment (PROCESS score)	229	152.14	14.63	113.0 – 190.0
Postnatal Hg (ppm-years)	220	12.83	7.32	2.52 – 68.58
Cord TL (T/S)[1]	184	1.18	0.5	0.47 – 4.66
Plasma AA (mg/mL) at 5y	201	0.05	0.01	0.02 – 0.07
Plasma EPA + DHA (mg/mL) at 5y	201	0.04	0.01	0.01 – 0.07
Plasma AA/DHA ratio at 5y	201	1.51	0.34	0.82 – 2.8
TL at 5y (T/S)[1]	209	0.71	0.1	0.45 – 0.99
Telomere attrition rate (T/S)[1]	141	0.47	0.14	-0.16 – 0.73

Data presented are mean, SD and range. SES: socioeconomic status; PROCESS: Pediatric Review of Children's Environmental Support and Stimulation.
[1] Ratio of telomere repeat copy number to single-copy gene numbers (T/S)

Table 3. Associations between TL in different life stages, PUFA status and Hg exposure from covariate-adjusted linear regression models.

Outcome	Exposure covariate	Beta	SE	*P*-value[1]
TL in mothers (n=216)	Prenatal n-3 PUFA	-1.70	0.93	0.07
	Prenatal n-6 PUFA	-0.011	0.039	0.78
	Prenatal Hg	0.001	0.002	0.58
	Prenatal n-6/n-3 PUFA	0.001	0.001	**0.048**
	Prenatal Hg	0.001	0.002	0.71
Log(TL in cord blood) (n=183)	Prenatal n-3 PUFA	4.38	3.20	0.17
	Prenatal n-6 PUFA	-0.031	0.14	0.82
	Prenatal Hg	-0.001	0.007	0.88
	Prenatal n-6/n-3 PUFA	-0.002	0.002	0.39
	Prenatal Hg	0.001	0.007	0.93
TL at 5y (n=202)	Prenatal n-3 PUFA	0.081	0.92	0.93
	Prenatal n-6 PUFA	-0.020	0.040	0.62
	Prenatal Hg	-0.002	0.002	0.23
	Prenatal n-6/n-3 PUFA	0.000	0.001	0.69
	Prenatal Hg	-0.003	0.002	0.17
TL at 5y (n=178)	Postnatal EPA+DHA	-1.19	1.06	0.26
	Postnatal AA	0.82	0.87	0.35
	Postnatal Hg	0.001	0.001	0.26
	Postnatal AA/DHA	0.026	0.022	0.25
	Postnatal Hg	0.001	0.001	0.27
Log(TL attrition rate) (n=141)	Postnatal EPA+DHA	-2.49	4.11	0.55
	Postnatal AA	-1.33	3.63	0.72
	Postnatal Hg	0.006	0.004	0.16
	Postnatal AA/DHA	-0.002	0.088	0.98
	Postnatal Hg	0.005	0.004	0.20

[1]Significant *P* values are bolded

Figure legends:

Figure 1: Association between the logarithm of the cord telomere length and family Hollingshead SES index measured when the child was 9mo of age. The superimposed lines show the slopes and 95% confidence intervals from the covariate-adjusted regression.