**Periconceptional environment predicts leukocyte telomere length in a cross-sectional study of 7-9 year old rural Gambian children**

*Kim Maasen1,2,3, Philip T James4, Andrew M Prentice2, Sophie E Moore 2,5, Caroline H Fall6, Giriraj R Chandak7, Modupeh Betts2,8, Matt J Silver2 #\* and Jessica L Buxton 9,10 #\**

*#Joint senior authors*

*\*Corresponding authors:*

Matt SilverMatt.Silver@lshtm.ac.uk

Jessica Buxton J.Buxton@kingston.ac.uk

**Author Affiliations**

1. Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands. 2. ﻿Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine, Atlantic Boulevard, Fajara, Banjul, The Gambia.

3. Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands.

4. Department of Population Health, London School of Hygiene and Tropical Medicine, UK.

5.﻿ Department of Women and Children’s Health, King’s College London, London, UK.

6. ﻿MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton, UK.

7. ﻿**G**enomic **R**esearch on **C**omplex diseases (GRC Group), CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India.

8. Division of Infection and Immunity, University College London, London, England, UK.

9. School of Life Sciences, Pharmacy and Chemistry, Kingston University, UK.

10. Genetics and Genomic Medicine Programme, UCL Great Ormond Street Institute of Child Health, UK.

**Abstract**

Early life exposures are important predictors of adult disease risk. Although the underlying mechanisms are largely unknown, telomere maintenance may be involved. This study investigated the relationship between seasonal differences in parental exposures at time of conception and leukocyte telomere length (LTL) in their offspring. LTL was measured in two cohorts of children aged 2 yrs (N=487) and 7–9 yrs (N=218). The association between date of conception and LTL was examined using Fourier regression models, adjusted for age, sex, leukocyte cell composition, and other potential confounders. We observed an effect of season in the older children in all models [likelihood ratio test (LRT) ²2=7.1, p=0.03; fully adjusted model]. LTL was greatest in children conceived in September (in the rainy season), and smallest in those conceived in March (in the dry season), with an effect size (LTL peak–nadir) of 0.60 z-scores. No effect of season was evident in the younger children (LRT ²2=0.87, p=0.65). The different results obtained for the two cohorts may reflect a delayed effect of season of conception on postnatal telomere maintenance. Alternatively, they may be explained by unmeasured differences in early life exposures, or the increased telomere attrition rate during infancy.

**Keywords**

Telomeres, early life programming, DOHaD, The Gambia, Sub-Saharan Africa, ageing, conception

**Introduction**

The developmental origins of health and disease (DOHaD) paradigm holds that early life factors are important determinants of disease risk in adult life 1,2.The processes involved in gamete maturation, conception and early embryogenesis appear particularly vulnerable to environmental exposures (reviewed in 3). The biological mechanisms underlying associations between the periconceptional environment and future health remain poorly understood, although persistent epigenetic changes may play a role 4-7. Another molecular process through which the effects of early life factors may exert enduring changes to cellular and organismal phenotypes is the maintenance of telomeres: nucleoprotein structures that protect the ends of linear chromosomes. A potential role for telomere biology in the developmental programming of adult disease is supported by preliminary evidence from both animal and human studies 8-11.

Vertebrate telomeres consist of variable numbers of a tandem repeat sequence, (TTAGGG)n, bound to the shelterin protein complex 12. In germ cells and embryonic tissues, telomere length is maintained through the addition of *de novo* telomeric DNA repeats to the chromosome ends by telomerase. However, this enzyme is generally not active in differentiated cells postnatally. Therefore, in proliferative tissues, telomeres shorten with each cell division due to the ‘end replication problem’, a process accelerated by oxidative stress 13,14 and inflammation 15, and potentially ameliorated by antioxidants in the diet and vitamin intake 16-18.

Shorter age-adjusted leukocyte telomere length (LTL) in humans is associated with increased risk of several non-communicable diseases, notably cardiometabolic conditions 19,20. LTL is also associated with several known risk factors for ill health, including obesity and smoking 21 and with genetic factors 22,23. However, the proportion of variation in adult LTL explained by these factors is relatively small compared to the wide variation in telomere length that exists in newborns 24,25. Research into the early life determinants of telomere length implicates the prenatal environment 26-32.

In the present study, we sought to investigate the relationship between the periconceptional environment and telomere length in children born in the rural Kiang West region of The Gambia. This region is marked by a distinct tropical seasonality, with an intense rainy season from July to September, coinciding with a period of dietary restriction (the ‘hungry’ season) but a greater availability and consumption of green and leafy vegetables lasting for several months. The dry season lasts for the remainder of the year, with February to April being peak dry season. As a result, conceptions and births in this setting occur against very different exposures to parental energy balance, diet composition, maternal nutritional status and infections 33-35. Seasonal changes in early life in this population have been linked to differences in mortality, and to epigenetic changes in offspring 36-41. We therefore tested the hypothesis that LTL is associated with timing of conception in children from two established Gambian population cohorts.

**Results**

Details of the two cohorts studied (ENID and EMPHASIS) are given in the Methods and characteristics of the participants are summarised in Table 1. We obtained relative LTL measurements (as ‘T/S’ ratios, using monochrome multiplex quantitative PCR, see Methods for details) for 487 children in the ENID cohort (median T/S ratio 1.8, IQR 1.5-2.1) and 218 children in the EMPHASIS cohort (median T/S ratio 1.1, IQR 0.9-1.3). The decrease in median T/S ratio between the cohorts (95% CI [0.63-0.74]) is consistent with the fact that telomere length declines with age.

**Association between date of conception and leukocyte telomere length**

ENID cohort (2 year old children)

In our primary analysis, we found no evidence for a seasonal effect on LTL when comparing a crude model with seasonality modelled as a single pair of Fourier terms with a baseline (intercept-only) model (LRT ²2=4.24, p=0.12; Table 2, Model 1; Fig. 1). In subsequent secondary analyses adjusted for additional covariates (Fig. 1 and Supplementary Fig. S3), there was weak evidence for a seasonal effect in models adjusted for maternal BMI (LRT ²2=5.48, p=0.06; Table 2, Model 4; Fig. 1) and birthweight (LRT ²2=5.86, p=0.05; Table 2, Model 5; Fig. 1). Adjustment for estimated white cell composition had the largest effect on Fourier coefficient estimates, as indicated by a large difference in the timing of seasonal phase (Table 2, Model 3; Fig. 1 and Supplementary Table S1). Note that sample size was much reduced for this model since DNA methylation data for estimation of cellular fractions was only available for a limited number of samples (n=204). Note also that use of more than 1 pair of Fourier terms to model seasonality did not improve model fits (data not shown).

Effect of white blood cell composition on LTL – ENID cohort

To further explore the individual effect of white blood cell composition on LTL, we compared a full model with cell composition estimates as predictors to a baseline (intercept-only) model. This indicated no cell composition effect (LRT ²2=2.19, p=0.82). Coefficients for individual cell proportions also indicated no independent cell type effects (p-values from 0.21-0.87).

EMPHASIS cohort (7-9 year old children)

For the EMPHASIS cohort, a primary crude analysis with seasonality once again modelled as a single pair of Fourier terms found evidence for a seasonal effect on LTL (LRT ²2=8.27, p=0.016; Table 3, Model 1; Fig. 2). The modelled T/S ratio mean reached a maximum for individuals conceived in September (rainy season), and a minimum in March (dry season), with an effect size (amplitude = T/S mean z-score maximum – minimum) of 0.60 SD. (Fig. 2). Evidence for a seasonality effect was also evident in models adjusted for child age and sex (²2=7.89, p=0.019, amplitude=0.56; Table 3, Model 2; Fig. 2) and in further models additionally adjusted for cell composition, maternal BMI, birthweight and maternal supplementation (Table 3, Models 3-5; Fig. 2; Supplementary Table S2, Model 6; and Supplementary Fig. S4). In every case the temporal nature (phase) of the seasonal association did not change, with no evidence for a change in estimated Fourier regression coefficients, and modelled T/S mean maxima in September, and minima in March (Fig. 2 and Supplementary Table S2). Use of more than 1 pair of Fourier terms to model seasonality did not improve model fits (data not shown).

Other early-life predictors of LTL in ENID and EMPHASIS cohorts

For both cohorts, we additionally explored the individual effect of maternal BMI, birthweight, maternal folate (ENID cohort only), maternal supplementation and infant supplementation (ENID only) on LTL. After accounting for potential seasonality effects, we found no evidence of associations with LTL in either of the cohorts (Supplementary Table S3 and Supplementary Figures S2 and S3).

**Discussion**

This study explored whether the periconceptional environment is associated with blood telomere length in children living in a rural area of The Gambia. We show that timing of conception in this highly seasonal environment predicts LTL in children aged 7-9 years. Children in this cohort (EMPHASIS) who were conceived mid-dry season (March) have the shortest mean LTL, while those conceived towards the end of the rainy season (September) have the longest mean LTL. This effect is not explained by differences in white blood cell composition at the time of blood collection, maternal periconceptional BMI, child birthweight, or supplementation of mothers or infants. Interestingly, we found no evidence for an association between timing of conception and LTL in children aged 2 years from the ENID cohort despite a greater sample size.

 Our main finding, that children conceived mid-dry season have shorter telomeres at age 7-9 than their peers conceived mid-rainy season, is the first reported evidence for potential early life influences on telomere length in Gambian children. Our previous preliminary study of young men (18-23y) in this population found that individuals born in the rainy season tended to have shorter CD8+ lymphocyte telomeres than those born in the dry season 11. Telomere length (TL) at any given age is determined both by initial TL at birth and TL attrition over time. Taken together, our studies indicate that season of conception/birth is associated with TL in young adults and older children. This suggests that the effect of periconceptional environment on telomere maintenance processes, if it exists, may be delayed until mid-childhood onwards.

Accelerated telomere shortening in leukocytes during childhood may be due to a greater burden of infectious disease, as demonstrated by studies reporting substantial telomere shortening after malaria infection 42-44. Furthermore, a study conducted in the Philippines found an association between higher rates of early-life infections and shorter adult blood telomere length 45. It is possible that our findings may also reflect a long-term effect of differences in infectious disease burden during infancy, although this would not explain the lack of an association between LTL and season of conception in the younger cohort. In addition, such an effect would be at least partly mitigated by exclusive breastfeeding, rates of which are high in this population 46.

Our finding that timing of conception predicts telomere length in mid-childhood, but not infancy, in this population could also potentially be due to elements of our study design. The two cohorts differed in timing of sample collection (Fig. 1). In the EMPHASIS cohort all blood samples were collected in a 4-month window spanning the middle of the dry season, whereas in the ENID cohort samples were collected throughout the year when the child reached the age of 2 years. Therefore, in the ENID cohort date of conception is very strongly correlated with date of sample collection (see Fig. 31), so any effect of conception timing maybe confounded by an effect related to timing of sample collection. Previous research carried out in Costa Rica, which also has distinct rainy and dry seasons, found that season of blood collection is associated with adult LTL and that this may mask the effects of other factors 47,48. These findings could reflect seasonal differences in leukocyte cell composition, although we found no evidence for this in our sample. Alternatively, the lack of association between LTL and season of conception in the younger cohort may be partly or wholly explained by the faster telomere attrition rate known to exist in infancy, compared to that in older children and adults. This could have affected our ability to resolve differences in telomere length between the individuals in our younger cohort.

Previous studies report that higher maternal pre-pregnancy BMI and lower maternal folate concentrations during pregnancy are associated with shorter newborn telomere length 49,50. Low birthweight is also associated with shorter telomere length in 5-year-old children 51. We found no evidence of confounding of our main observations by these or other *in utero* exposures. We also found no evidence of independent associations of these early-life predictors with LTL, although there was weak evidence of an association between increased periconceptional maternal BMI prior and shorter LTL prior to adjustment for date of conception.

Our study has several strengths. First, the seasonal ‘experiment of nature’ setting of this well-documented population allowed us to examine the effect of distinct differences in periconceptional environment. Second, the availability of relatively large numbers of samples from two established cohorts enabled us to explore determinants of telomere length at two stages in early life. Third, the availability of anthropometric and biochemical measurements facilitated adjustment for multiple potential confounders and the exploration of potential mediators. A limitation of our study is that we measured relative, rather than absolute telomere length, so were not able to estimate the effect of timing of conception in base-pairs.

 In summary, we show that timing of conception predicts leukocyte telomere length in 7-9 year old children, with the shortest LTL for children conceived mid-dry season and the longest LTL for those conceived towards the end of the rainy season. This association was not explained by other (pre)pregnancy or early-life factors, such as maternal periconceptional BMI, child birthweight, maternal and infant supplementation, or maternal folate concentration. To the best of our knowledge, this is the first study to report an association between date of conception and telomere length in children in a West-African setting. Most studies into the determinants and consequences of short telomeres have so far been conducted in high income countries, so their relevance to healthy ageing in nutritionally deprived individuals who face a high burden of infectious disease is unknown. The identification of novel factors associated with telomere length in Gambian children may help elucidate potential mechanisms through which prenatal and early-life exposures affect adult health in this and other populations. Future longitudinal studies would provide greater insight into telomere attrition rate throughout life, and help identify determinants of healthy development and ageing.

**Methods**

**Study population**

DNA for LTL measurements was prepared using stored blood samples from two established population cohorts from the Kiang West region of The Gambia. This population, consisting of approximately 15,000 individuals, depends predominantly on subsistence farming, and has been described in detail previously 52.

ENID cohort

The ENID (Early Nutrition and Immune Development, ISRCTN49285450) trial was a 2x2x2 factorial randomised, partially blinded trial in which pregnant women (< 20 weeks gestation) and their infants (from 6 to 18 months of age) received nutritional supplementation 53 (See Supplementary Methods for details). Venous blood samples were collected when the child turned 2 years old, between 2012-2016 (see Fig. 31). In total, blood samples were available from 581 children.

EMPHASIS cohort
The Gambian arm of the EMPHASIS (Epigenetic Mechanisms linking Pre-conceptional nutrition and Health Assessed in India and Sub-Saharan Africa; ISRCTN14266771) study followed up children of mothers who were enrolled in the Peri-conceptional Multiple Micronutrient Supplementation Trial (PMMST) 54,55 (See Supplementary Methods for details). The present study utilised DNA collected from venous blood samples from the EMPHASIS children at age 7-9 yrs. All blood samples were collected mid-dry season between February and May 2016 (see Fig. 31). In total, blood samples were available from 287 children.

Further details on study participants from both cohorts are given in Table 1. Both trials were approved by the joint Gambia Government / MRC Unit The Gambia Ethics Committee and informed consent was obtained from all participants (ENID: SCC1126v2; EMPHASIS: SCC1441v2; LTL study: L2016.03).

**Assessment of date of conception**

Estimated date of conception was obtained using gestational age data, estimated by ultrasound at the first (‘booking’) clinic visit.

**Blood collection and DNA extraction**

Venous blood was collected into EDTA tubes and stored at -80 °C. For the ENID study, genomic DNA was extracted from peripheral blood using the salting-out method 56 for the majority of samples (n=450 - of total analysed samples after exclusions) and the Nucleon kit (Nucleon Bacc2 DNA extraction kits, Scientific Laboratory Supplies Ltd, Nottingham, UK) for the remainder of the samples (n=37). Mean LTL did not differ between the two extraction methods (p=0.75). For the EMPHASIS samples, genomic DNA was extracted using the Qiagen DNA Blood Midi Kit (Qiagen, Crawley, UK). All DNA samples were resuspended or eluted in RT-PCR water, and dilutions of 10ng/l were prepared using RT-PCR Grade Water (Thermo Fisher Scientific) approximately 2-6 weeks before carrying out telomere measurements. Additionally, in the ENID study whole venous blood from mothers was collected after an overnight fast for maternal folate measurements. Blood was collected into EDTA tubes and stored at -80 °C.

**Telomere length measurement**

Mean relative LTL was measured using monochrome multiplex quantitative PCR (MMqPCR) in genomic DNA samples with minor modifications 57 (see Supplementary Methods).

After amplification and data collection, the CFX manager software (Bio-Rad Laboratories) was used to generate standard curves from the reference DNA dilutions, one for the telomere amplicon (T) and one for the single-copy gene amplicon (S). A “T/S ratio” was then calculated for each DNA sample - a relative measure of the amplification of the telomeric DNA sequence compared to that of the single copy gene (albumin gene – see supplementary methods for primer sequences).

After excluding samples that failed to amplify and those with a coefficient of variation of >15% between duplicates, the mean coefficient of variation for the T/S measurements was 5.5% for the ENID samples and 4.7% for the EMPHASIS samples. Inter-assay coefficients of variation, calculated using the reference sample and control samples common to each plate, were 8.6% and 16.9% for ENID and EMPHASIS samples respectively. For the three control samples, aged 2, 26, and 72 years, mean T/S ratios were 1.90, 1.54 and 1.09 respectively, showing an expected decrease in T/S ratio over age.

Sufficient DNA and reagents were available for telomere measurements in a total of 581 children from the ENID cohort and 287 children from the EMPHASIS cohort. After exclusions, T/S measurements were available for statistical analyses for 487 children (84%) from the ENID cohort and 218 children (76%) from the EMPHASIS cohort (see Supplementary Fig.s S1 and S2 for details).

**Statistical methods**

All LTL measurements (T/S ratio) were converted to a per plate z-score, in order to account for a batch effect observed in measurements obtained for both cohorts (ANOVA, p<0.001). The main outcome variable (LTL z-score) was normally distributed (by visual inspection of histogram) in the EMPHASIS cohort, and in the ENID cohort after Boxcox transformation of LTL z-scores. Skewed predictor variables (maternal BMI and maternal folate status) were log-transformed, and fitted models had normally distributed residuals, with no evidence of homoscedasticity or multicollinearity.

The association between date of conception and LTL was examined in multiple linear regression models using Fourier terms to capture seasonal variation 58,59. Fourier regression-based approaches allow for decomposition of any periodic function into a linear combination of simple oscillating functions (sines and cosines) parametrized by coefficients (the Fourier coefficients). This approach allows the modelling of cyclical variation with no prior assumptions about the frequency, phase or amplitude of any seasonal effects.

In our analysis, seasonality was modelled using pairs of Fourier terms (predictors), with the number of pairs determined using a likelihood ratio test (LRT). We began with a crude primary model that included seasonality modelled as Fourier terms only. We subsequently performed sensitivity analyses by separately adding potential confounders of the relationship between seasonality and LTL. The confounders that we considered were the child’s age, sex, white blood cell composition (estimated from DNA methylation data using the Houseman method 60), maternal periconceptional BMI, child birthweight, maternal and infant supplementation, and maternal folate concentration (in ENID cohort only) (see Supplementary Methods). Missing data for some of these variables meant that sample numbers varied between the models considered.

Lastly, to explore the individual effect of other early-life exposures on LTL, we performed multiple linear regression analyses with maternal BMI, birthweight, supplementation status, and maternal folate as predictors. All models were adjusted for age and sex of children. Predictors with a known seasonal variation (maternal BMI, birthweight, and maternal folate) were additionally adjusted for seasonality using one pair of Fourier terms. All analyses were performed using R version 3.3.0 (www.cran.r-project.org).

**Tables**

Table 1 – General characteristics of the ENID and EMPHASIS study participants

|  |  |  |
| --- | --- | --- |
|  | ENID (N=487) | EMPHASIS (N=218) |
| Age (years)1 | 2.0 (2.0-2.0) | 9.0 (8.6-9.2) |
| Sex (% male) | 50 | 55 |
| Maternal folate concentration (nmol/L)1 |  |  |
|  Method A (Architect system) | 12.8 (9.8-16.7) | - |
|  Method B (EDTA plasma folate) | 13.2 (9.3-18.9) | - |
| Maternal BMI1 | 20.4 (19.0-22.3) | 20.8 (19.3-22.9) |
| Child birthweight (grams) 2 | 3023 ± 402 | 3069 ± 417 |
| Maternal intervention arm3 |  |  |
|  FeFol | 120 (25) | - |
|  MMN | 130 (27) | - |
|  PE | 118 (24) | - |
|  PE + MMN | 119 (24) | - |
|  UNIMMAP | - | 102 (47) |
|  Placebo | - | 116 (53) |
| Infant intervention arm, n (%) |  |  |
|  LNS 3 | 247 (51) | - |
|  + MMN | 240 (49) | - |

1 median (IQR)

2 mean ± SD

3 n (%)

BMI, Body Mass Index, FeFol, iron and folic acid; MMN, multiple micronutrient; PE, protein energy; LNS, lipid-based nutritional supplement; UNIMMAP, ﻿UNICEF/WHO/United Nations University Multiple Micronutrient Preparation

Table 2 – ENID cohort. Association between LTL (T/S mean z-score) and seasonality using Fourier regression models.

|  |  |  |  |
| --- | --- | --- | --- |
|  | N | LRT 2 (2 df) | LRT p-value |
| Model 1 - crudeseasonality modelled with one pair of Fourier terms1Baseline: intercept only | 487 | 4.24 | 0.12 |
| Model 2 – adjusted for age and sex1 pair Fourier terms + age + sexBaseline: age and sex only | 487 | 4.24 | 0.11 |
| Model 3 – adjusted for age, sex and estimated cell composition1 pair Fourier terms + age + sex + cell compositionBaseline: age + sex + cell composition only | 204 | 0.87 | 0.65 |
| Model 4 – adjusted for age, sex and maternal BMI1 pair Fourier terms + age + sex + maternal BMIBaseline: age + sex + maternal BMI only | 345 | 5.48 | 0.06 |
| Model 5 – adjusted for age + sex + birthweight1 pair Fourier terms + age + sex + birthweightBaseline: age + sex + birthweight | 395 | 5.86 | 0.05 |

1 In each case, model fit is assessed by comparing the full and baseline models using a likelihood ratio test (LRT).LRT confirmed that two/three pairs of Fourier terms did not improve model fit. BMI, Body Mass Index

|  |  |  |  |
| --- | --- | --- | --- |
|  | N | LRT 2(2 df) | LRT p-value |
| Model 1 - crudeseasonality modelled with one pair of Fourier terms1Baseline: intercept only | 218 | 8.27 | **0.016** |
| Model 2 – adjusted for age and sex1 pair Fourier terms + age + sexBaseline: age and sex only | 218 | 7.89 | **0.019** |
| Model 3 – adjusted for age, sex and estimated cell composition1 pair Fourier terms + age + sex + cell compositionBaseline: age + sex + cell composition only | 214 | 7.10 | **0.029** |
| Model 4 – adjusted for age, sex and maternal BMI1 pair Fourier terms + age + sex + maternal BMIBaseline: age + sex + maternal BMI only | 218 | 7.96 | **0.019** |
| Model 5 – adjusted for age + sex + birthweight1 pair Fourier terms + age + sex + birthweightBaseline: age + sex + birthweight | 200 | 6.51 | **0.039** |

Table 3 - EMPHASIS cohort. Association between LTL (T/S mean z-score) and seasonality using Fourier regression models.

1 In each case, model fit is assessed by comparing the full and baseline models using a likelihood ratio test (LRT).LRT confirmed that two/three pairs of Fourier terms did not improve model fit. BMI, Body Mass Index

**Data availability**

The datasets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

**References**

1 Hales, C. N. & Barker, D. J. The thrifty phenotype hypothesis. *Br. Med. Bull.* **60,** 5-20 (2001).

2 Heindel, J. J. *et al.* Developmental origins of health and disease: Integrating environmental influences. *Endocrinology.* **156,** 3416-3421 (2015).

3 Fleming, T. P. *et al.* Origins of lifetime health around the time of conception: Causes and consequences. *Lancet.* **391,** 1842-1852 (2018).

4 Heijmans, B. T. *et al.* Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 17046-17049 (2008).

5 Lillycrop, K. A. & Burdge, G. C. Epigenetic mechanisms linking early nutrition to long term health. *Best Pract. Res. Clin. Endocrinol. Metab.* **26,** 667-676 (2012).

6 Vaiserman, A. M. Epigenetic programming by early-life stress: Evidence from human populations. *Dev. Dyn.* **244,** 254-265 (2015).

7 James, P. *et al.* Candidate genes linking maternal nutrient exposure to offspring health via DNA methylation: A review of existing evidence in humans with specific focus on one-carbon metabolism. *Int. J. Epidemiol.* **47,** 1910-1937 (2018).

8 Tarry-Adkins, J. L., Martin-Gronert, M. S., Chen, J. H., Cripps, R. L. & Ozanne, S. E. Maternal diet influences DNA damage, aortic telomere length, oxidative stress, and antioxidant defense capacity in rats. *FASEB J.* **22,** 2037-2044 (2008).

9 Wang, R. *et al.* Earthquake experience at different trimesters during pregnancy is associated with leukocyte telomere length and long-term health in adulthood. *Front Psychiatry.* **8,** 208 (2017).

10 Entringer, S., de Punder, K., Buss, C. & Wadhwa, P. D. The fetal programming of telomere biology hypothesis: An update. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **373,** (2018).

11 Ngom, P. T. *et al.* Thymic function and t cell parameters in a natural human experimental model of seasonal infectious diseases and nutritional burden. *J. Biomed. Sci.* **18,** 41 (2011).

12 de Lange, T. Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes Dev.* **19,** 2100-2110 (2005).

13 von Zglinicki, T. Oxidative stress shortens telomeres. *Trends Biochem. Sci.* **27,** 339-344 (2002).

14 Kurz, D. J. *et al.* Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J. Cell Sci.* **117,** 2417-2426 (2004).

15 Zhang, J. *et al.* Ageing and the telomere connection: An intimate relationship with inflammation. *Ageing Res Rev.* **25,** 55-69 (2016).

16 Pusceddu, I. *et al.* Prospective study of telomere length and line-1 methylation in peripheral blood cells: The role of b vitamins supplementation. *Eur. J. Nutr.* **55,** 1863-1873 (2016).

17 Richards, J. B. *et al.* Higher serum vitamin d concentrations are associated with longer leukocyte telomere length in women. *The American journal of clinical nutrition.* **86,** 1420-1425 (2007).

18 Pusceddu, I. *et al.* One-carbon metabolites and telomere length in a prospective and randomized study of b- and/or d-vitamin supplementation. *Eur. J. Nutr.* **56,** 1887-1898 (2017).

19 D'Mello, M. J. *et al.* Association between shortened leukocyte telomere length and cardiometabolic outcomes: Systematic review and meta-analysis. *Circ. Cardiovasc. Genet.* **8,** 82-90 (2015).

20 Zhao, J. *et al.* Short leukocyte telomere length predicts risk of diabetes in american indians: The strong heart family study. *Diabetes.* **63,** 354-362 (2014).

21 Valdes, A. M. *et al.* Obesity, cigarette smoking, and telomere length in women. *Lancet.* **366,** 662-664 (2005).

22 Codd, V. *et al.* Identification of seven loci affecting mean telomere length and their association with disease. *Nat. Genet.* **45,** 422-427, 427e421-422 (2013).

23 Dorajoo, R. *et al.* Loci for human leukocyte telomere length in the singaporean chinese population and trans-ethnic genetic studies. *Nature Communications.* **10,** 2491 (2019).

24 Factor-Litvak, P. *et al.* Leukocyte telomere length in newborns: Implications for the role of telomeres in human disease. *Pediatrics.* **137,** (2016).

25 Aviv, A. & Shay, J. W. Reflections on telomere dynamics and ageing-related diseases in humans. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **373,** (2018).

26 Entringer, S. *et al.* Maternal folate concentration in early pregnancy and newborn telomere length. *Ann. Nutr. Metab.* **66,** 202-208 (2015).

27 Louis-Jacques, A. F. *et al.* A positive association between umbilical cord rbc folate and fetal tl at birth supports a potential for fetal reprogramming. *Nutr. Res.* **36,** 703-709 (2016).

28 Hjort, L. *et al.* Telomere length is reduced in 9- to 16-year-old girls exposed to gestational diabetes in utero. *Diabetologia.* **61,** 870-880 (2018).

29 Entringer, S. *et al.* Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proceedings of the National Academy of Sciences.* **108,** E513-E518 (2011).

30 Kim, J.-H. *et al.* Higher maternal vitamin d concentrations are associated with longer leukocyte telomeres in newborns. *Matern. Child Nutr.* **14,** e12475 (2018).

31 Zhang, L. *et al.* Prenatal cadmium exposure is associated with shorter leukocyte telomere length in chinese newborns. *BMC Med.* **17,** 27 (2019).

32 Mannan, T. *et al.* Associations of arsenic exposure with telomere length and naïve t cells in childhood—a birth cohort study. *Toxicol. Sci.* **164,** 539-549 (2018).

33 Rayco-Solon, P., Moore, S. E., Fulford, A. J. & Prentice, A. M. Fifty-year mortality trends in three rural african villages. *Trop. Med. Int. Health.* **9,** 1151-1160 (2004).

34 Rayco-Solon, P., Fulford, A. J. & Prentice, A. M. Differential effects of seasonality on preterm birth and intrauterine growth restriction in rural africans. *Am. J. Clin. Nutr.* **81,** 134-139 (2005).

35 Bates, C. J., Prentice, A. M. & Paul, A. A. Seasonal variations in vitamins a, c, riboflavin and folate intakes and status of pregnant and lactating women in a rural gambian community: Some possible implications. *Eur. J. Clin. Nutr.* **48,** 660-668 (1994).

36 Moore, S. E. *et al.* Season of birth predicts mortality in rural gambia. *Nature.* **388,** 434 (1997).

37 Moore, S. E. *et al.* Prenatal or early postnatal events predict infectious deaths in young adulthood in rural africa. *Int. J. Epidemiol.* **28,** 1088-1095 (1999).

38 Dominguez-Salas, P. *et al.* DNA methylation potential: Dietary intake and blood concentrations of one-carbon metabolites and cofactors in rural african women. *Am. J. Clin. Nutr.* **97,** 1217-1227 (2013).

39 Dominguez-Salas, P. *et al.* Maternal nutrition at conception modulates DNA methylation of human metastable epialleles. *Nature Communications.* **5,** 3746 (2014).

40 Silver, M. J. *et al.* Independent genomewide screens identify the tumor suppressor vtrna2-1 as a human epiallele responsive to periconceptional environment. *Genome Biol.* **16,** 118 (2015).

41 Kühnen, P. *et al.* Interindividual variation in DNA methylation at a putative pomc metastable epiallele is associated with obesity. *Cell Metab.* **24,** 502-509 (2016).

42 Asghar, M. *et al.* Cellular aging dynamics after acute malaria infection: A 12-month longitudinal study. *Aging cell.* **17,** e12702 (2018).

43 Asghar, M. *et al.* Chronic infection. Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science.* **347,** 436-438 (2015).

44 Asghar, M. *et al.* Parallel telomere shortening in multiple body tissues owing to malaria infection. *Proc. Biol. Sci.* **283,** (2016).

45 Eisenberg, D. T. A., Borja, J. B., Hayes, M. G. & Kuzawa, C. W. Early life infection, but not breastfeeding, predicts adult blood telomere lengths in the philippines. *Am. J. Hum. Biol.* **29,** (2017).

46 Eriksen, K. G. *et al.* Following the world health organization's recommendation of exclusive breastfeeding to 6 months of age does not impact the growth of rural gambian infants. *The Journal of Nutrition.* **147,** 248-255 (2016).

47 Rehkopf, D. H. *et al.* Seasonal variation of peripheral blood leukocyte telomere length in costa rica: A population-based observational study. *Am. J. Hum. Biol.* **26,** 367-375 (2014).

48 Rosero-Bixby, L. *et al.* Correlates of longitudinal leukocyte telomere length in the costa rican longevity study of healthy aging (creles): On the importance of DNA collection and storage procedures. *PLoS One.* **14,** e0223766 (2019).

49 Martens, D. S., Plusquin, M., Gyselaers, W., De Vivo, I. & Nawrot, T. S. Maternal pre-pregnancy body mass index and newborn telomere length. *BMC Med.* **14,** 148 (2016).

50 Entringer, S. *et al.* Maternal folate concentration in early pregnancy and newborn telomere length. *Ann. Nutr. Metab.* **66,** 202-208 (2015).

51 Raqib, R. *et al.* Low birth weight is associated with altered immune function in rural bangladeshi children: A birth cohort study. *Am. J. Clin. Nutr.* **85,** 845-852 (2007).

52 Hennig, B. J. *et al.* Cohort profile: The kiang west longitudinal population study (kwlps)—a platform for integrated research and health care provision in rural gambia. *Int. J. Epidemiol.* **46,** e13-e13 (2015).

53 Moore, S. E. *et al.* A randomized trial to investigate the effects of pre-natal and infant nutritional supplementation on infant immune development in rural gambia: The enid trial: Early nutrition and immune development. *BMC Pregnancy Childbirth.* **12,** 107 (2012).

54 Owens, S. *et al.* Periconceptional multiple-micronutrient supplementation and placental function in rural gambian women: A double-blind, randomized, placebo-controlled trial. *Am. J. Clin. Nutr.* **102,** 1450-1459 (2015).

55 Chandak, G. R. *et al.* Protocol for the emphasis study; epigenetic mechanisms linking maternal pre-conceptional nutrition and children’s health in india and sub-saharan africa. *BMC Nutrition.* **3,** 81 (2017).

56 Miller, S. A., Dykes, D. D. & Polesky, H. F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16,** 1215-1215 (1988).

57 Cawthon, R. M. Telomere length measurement by a novel monochrome multiplex quantitative pcr method. *Nucleic Acids Res.* **37,** e21-e21 (2009).

58 Fulford, A. J., Rayco-Solon, P. & Prentice, A. M. Statistical modelling of the seasonality of preterm delivery and intrauterine growth restriction in rural gambia. *Paediatr. Perinat. Epidemiol.* **20,** 251-259 (2006).

59 Nabwera, H. M., Fulford, A. J., Moore, S. E. & Prentice, A. M. Growth faltering in rural gambian children after four decades of interventions: A retrospective cohort study. *Lancet Glob Health.* **5,** e208-e216 (2017).

60 Houseman, E. A. *et al.* DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics.* **13,** 86 (2012).

**Main table and figure legends**

**Table 1 – General characteristics of the ENID and EMPHASIS study participants.
1 median (IQR)**

2 mean ± SD

3 n (%)

BMI, Body Mass Index, FeFol, iron and folic acid; MMN, multiple micronutrient; PE, protein energy; LNS, lipid-based nutritional supplement; UNIMMAP, ﻿UNICEF/WHO/United Nations University Multiple Micronutrient Preparation

**Table 2 – ENID cohort. Association between LTL (T/S mean z-score) and seasonality using Fourier regression models.**
1 In each case, model fit is assessed by comparing the full and baseline models using a likelihood ratio test (LRT).LRT confirmed that two/three pairs of Fourier terms did not improve model fit. BMI, Body Mass Index

**Table 3 - EMPHASIS cohort. Association between LTL (T/S mean z-score) and seasonality using Fourier regression models.**
1 In each case, model fit is assessed by comparing the full and baseline models using a likelihood ratio test (LRT). LRT confirmed that two/three pairs of Fourier terms did not improve model fit. BMI, Body Mass Index

**Figure 1 - ENID cohort.**
Modelled associations between LTL (Boxcox transformed T/S mean z-score) and seasonality using Fourier regression. Grey shaded areas are 95% confidence intervals.

**Figure 2 - EMPHASIS cohort.**
Modelled associations between LTL (T/S mean z-score) and seasonality using Fourier regression. Grey shaded areas are 95% confidence intervals.

**Figure 3 – Date of conception versus date of collection ENID and EMPHASIS cohort.**
These are correlated for the ENID cohort since the majority of samples are collected when the children are 2 years old. (J-D, Jan-Dec)

**List of abbreviations**

BMI: Body mass index

DOHaD: Developmental origins of health and disease

EMPHASIS: Epigenetic Mechanisms linking Pre-conceptional nutrition and Health Assessed in India and Sub-Saharan Africa

ENID: Early Nutrition and Immune Development

FeFol: iron and folic acid

LNS: lipid-based nutritional supplement

LRT: Likelihood ratio test

LTL: Leukocyte telomere length

MMN: multiple micronutrtient

MMqPCR: Monochrome multiplex quantitative polymerase chain reaction

PE: protein energy

PMMST: Peri-conceptional Multiple Micronutrient Supplementation Trial

S: single-copy gene amplicon

T: telomere amplicon

TL: telomere length

UNIMMAP: UNICEF/WHO/United Nations University Multiple Micronutrient Preparation

**Declarations**

Ethics approval and consent to participate

Both trials were approved by the joint Gambia Government / MRC Unit The Gambia Ethics Committee and informed consent was obtained from all participants (ENID: SCC1126v2; EMPHASIS: SCC1441v2; LTL study: L2016.03). All methods were performed in accordance with the relevant guidelines and regulations.

Competing interests

The authors declare that they have no competing interests

Funding

This study was funded by the Bill and Melinda Gates Foundation grant 0PP1135346. The EMPHASIS study was jointly funded by MRC, DFID and the Department of Biotechnology (DBT), Ministry of Science and Technology, India under the Newton Fund initiative (MRC grant no.: MR/N006208/1 and DBT grant no.: BT/IN/DBT-MRC/ DFID/24/GRC/2015–16). ﻿The ENID Trial was jointly funded by the UK Medical Research Council (MRC) and the Department for International Development (DFID) under the MRC/DFID Concordat agreement (MRC Programme MC-A760- 5QX00). The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

MJS, JLB and AMP conceived and designed the study. KM performed the laboratory work and the statistical analysis, with additional sample processing by MB and additional data analysis by PTJ. JLB guided the laboratory work. MJS and JLB guided the statistical analysis and writing. SEM, CHF, GRC and MJS provided samples and data. All authors critically revised the paper, contributed to the interpretation of the results and approved the manuscript.

Acknowledgements

We would like to thank the children and their parents in The Gambia who participated in the ENID and EMPHASIS cohorts, and the staff of Medical Research Council Unit The Gambia for their work on collecting these data.

**Supplementary files**

Supplementary file - Online supplementary material. **Supplementary tables:** Supplementary table S1 – Seasonality Fourier term regression coefficients for ENID cohort regression models described in Table 2; Supplementary table S2 – Seasonality Fourier term regression coefficients for EMPHASIS cohort regression models described in Table 3; Supplementary table S3 – Multiple linear regression of early life predictors on LTL for samples from the ENID and EMPHASIS cohorts. **Supplementary figures:** Supplementary figure S1 – Flowchart ENID samples; Supplementary figure S2 – Flowchart EMPHASIS samples. Supplementary figure S3 - Modelled associations between LTL and supplementation, maternal folate in ENID cohort; Supplementary figure S4 – Modelled association between LTL and supplementation in EMPHASIS in cohort. **Supplementary methods:** Supplementation details ENID cohort; Supplementation details EMPHASIS cohort; Leukocyte telomere length measurements; Covariates used for adjustment; Primer sequences albumin gene primers.