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Prenatal undernutrition and leukocyte telomere length in late adulthood: the Dutch famine birth cohort study[1]

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ABSTRACT

Background: Energy restriction in prenatal life has detrimental effects on later life health and longevity. Studies in rats have shown that the shortening of telomeres in key tissues plays an important role in this association.

Objective: The aim of the current study was to investigate leukocyte telomere length in relation to prenatal famine exposure.

Design: The Dutch famine birth cohort consists of 2414 term singleton men and women who were born between 1943 and 1947 in Amsterdam around the time of the famine. At a mean age of 68 y, telomere length and the percentage of short telomeres was assessed in a subsample of 131 cohort members, of whom 45 were born before the famine (control), 41 were exposed to famine during early gestation, and 45 were conceived after the famine (control). Median telomere length was determined in peripheral blood leukocytes by a high-throughput quantitative fluorescent in situ hybridization–based technology.

Results: Leukocyte telomere length and the percentage of short telomeres did not differ between those exposed to famine during early gestation and those unexposed during gestation. A lower socioeconomic status at birth, frequent consumption of alcohol (specifically consumption of spirits), a history of cancer, and a lower self-reported health status were significantly associated with shorter leukocyte telomere length (all $P \leq 0.03$). Currently having a job was significantly associated with a smaller percentage of short telomeres ($P = 0.04$).

Conclusion: The results of the current study suggest that prenatal exposure to famine is not associated with the shortening of telomeres in peripheral blood leukocytes at age 68 y. *Am J Clin Nutr* doi: 10.3945/ajcn.115.112326.

Keywords: developmental programming, prenatal famine, telomere, late life; aging

INTRODUCTION

Energy restriction during postnatal life has been shown to be an effective way to slow age-related disease processes and enhance longevity across species (1). Studies in which mice were subjected to mild dietary restriction suggested that the lower intake of calories increased life span by improving the maintenance of telomeres (2, 3). Telomeres are hexameric DNA repeat sequences that cap the end of chromosomes. In telomerase-negative cells, telomeres decrease in length by a predictable amount with each cell division. Telomeres also shorten in length as a consequence of oxidative damage. When telomere length becomes critically short, the cell is signaled to stop replicating and enter cellular senescence. Reduced telomere integrity has been associated with the incidence of age-related diseases and longevity (4–6).

When energy or protein restriction in animal experiments occurs prenatally (during gestation), its effect on age-related diseases and longevity is completely reversed and results in a phenotype with a high risk of diabetes and cardiovascular disease and a decreased life span (7). Again, these effects are associated with changes in telomere length. Studies in rats have shown that protein restriction during gestation diminishes telomere length in tissues such as pancreatic islets, kidney, and aorta and that these rats ultimately have a reduced life span (8–10).

The Dutch famine birth cohort study can be seen as a natural quasi-experiment resembling the animal studies on prenatal energy restriction. The Dutch famine was a 5-mo period that started at the end of 1944 during which the Western part of the Netherlands was struck by a severe famine. At the peak of the famine, rations were reduced to ~400 kcal/d. Still, some women became pregnant and infants who were born in the Wilhelmina Gasthuis in Amsterdam were followed up to study their health in later life (11). Results from the Dutch famine birth cohort study are strikingly similar to those from experimental studies on the long-term effects of poor maternal nutrition. It was shown that those who were conceived during the famine—and had thus been undernourished during the earliest stages of their development—have an increased risk of a range of age-associated diseases, including impaired glucose tolerance, cardiovascular

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disease, and breast cancer (11–15). Moreover, women who were exposed to famine during early gestation also died younger, as recently shown by increased mortality up to the age of 63 y (16).

Early gestation also seems to be a critical period for the establishment of telomeres. A study in human fetuses has shown that there is a rapid reduction in telomere length in fetal tissue, followed by a slow erosion from gestational age 6 to 11 wk, after which a relatively stable or slightly shortened telomere length was maintained until birth, indicating early gestation to be a critical period for the setting of telomere length (17).

In the current study, we hypothesized that—in concurrence with the evidence from animal experiments—exposure to undernutrition during early gestation is associated with shortened telomeres in all human tissues. We therefore measured leukocyte telomere length and the percentage of short telomeres in a subsample of the Dutch famine birth cohort at age 68 y comparing those exposed to famine in early gestation with those prenatally unexposed to famine.

METHODS

Participants

Participants were selected from the Dutch famine birth cohort. This cohort comprises 2414 men and women who were born as term singletons between 1 November 1943 and 28 February 1947 in the Wilhelmina Gasthuis in Amsterdam, Netherlands. Infants whose medical record was missing (n = 27) or those with a gestational age shorter than 259 d (n = 239) were excluded. The selection procedure of the cohort was further described in detail elsewhere (11). At the start of the current study in 2012, 1307 (54%) cohort members were alive, were still living in the Netherlands, and their current address was known. Birth weight did not differ between eligible and noneligible cohort members (3357 vs. 3333 g; P = 0.22). The study was approved by the local medical ethics committee and carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Exposure to famine

The official daily food rations for the general population aged ≥21 y were used to define exposure to famine (18). A person was considered to be prenatally exposed to famine if the average daily food ration of the mother during any 13-wk period of gestation contained <1000 kcal. On the basis of this definition, infants born between 7 January 1945 and 8 December 1945 had been exposed in utero. In keeping with previous publications on this cohort, we delineated periods of 16 wk each to differentiate between those exposed in late gestation (born between 7 January and 28 April 1945), in midgestation (born between 29 April and 18 August 1945), and in early gestation (born between 19 August and 8 December 1945) (19). People born before 7 January 1945 and people conceived after 8 December 1945 were considered to be unexposed to famine in utero and acted as control groups. Because most effects of prenatal famine exposure on later life health, which we previously showed occurred in those who were exposed to the famine in early gestation and early gestation seems to be a critical period for the establishment of telomere length, we focused the current study on this group and did not include those exposed to famine in late or midgestation (17, 19).

Sample selection

We aimed to include a total of 150 people in the study: 50 people from the groups of those born before the famine, those exposed in early gestation, and those conceived after the famine. We randomly drew equal samples from each of the 3 groups until the number of 50 people agreeing to participate was reached. We arrived at a final number of 151 participants of an eligible group of 268 cohort members (56%). Participation rates were similar in those born before the famine and those exposed in early gestation (54% vs. 51%, respectively) and somewhat higher in those conceived after the famine (66%). A total of 132 individuals agreed to have their blood drawn. Blood preparation before telomere length analysis failed in one participant, which left 131 subjects for analysis. Recruitment started in October 2012. Data collection started on 2 November 2012 and finished on 18 September 2013.

Study variables

Socioeconomic status (SES) at birth and birth outcomes were extracted from medical birth records. SES at birth was based on the head of the household having a manual or a nonmanual occupation (dichotomous variable). In 2012–2013, participants were visited at home, where a trained research assistant conducted a standardized interview and took measurements. The interview yielded information about employment, SES, lifestyle, medical history, and self-reported health. SES was defined according to the International Socio-Economic Index for occupational status–92, which is based on the participant's or their partner's occupation, whichever status was highest (20). We asked each participant about current smoking and whether he or she consumed alcohol. We considered drinking at least one glass of alcohol per week a positive answer. Alcohol consumption was further divided into consumption of beer, wine, or spirits. We computed pack-years of smoking as the number of cigarette packs (20 cigarettes) smoked per day × number of years smoking. Self-reported health was based on the answer of the participant to the following question: "How do you rate your health in general?" (1 = excellent, 2 = very good, 3 = good, 4 = fair, 5 = poor). Furthermore, height was measured with a portable stadiometer and weight with a portable Tefal scale. BMI was computed as weight (kg)/height $(m)^2$.

Telomeres

Peripheral blood mononuclear cells were isolated from fresh anticoagulated whole blood by using Histopaque (Sigma) gradient centrifugation for 30 min at 400 × g without brake. The peripheral blood mononuclear cell layer was washed twice with phosphate-buffered saline. Cells were cryopreserved in 90% fetal calf serum/10% DMSO and stored in liquid nitrogen for later telomere analysis. To measure telomere length and the percentage of short telomeres in peripheral blood leukocytes, a high-throughput quantitative fluorescent in situ hybridization (Q-FISH) technique was used (Life Length Inc.). This method is based on a quantitative fluorescence in situ hybridization method modified for cells in interphase (21). Q-FISH allows

the quantification of individual telomeres and measurement of the whole telomere length distribution. In brief, telomeres are hybridized with a fluorescent peptide nucleic acid probe that recognizes 3 telomere repeats (sequence: Alexa488-OO-CCCTAACCCTAACCCTAA; Panagene). Images of nuclei and telomeres are captured by a high-content screen system. The intensity of the fluorescent signal from the telomeric peptide nucleic acid probe that hybridizes to a given telomere is linearly proportional to the length of the telomere. Intensities of fluorescence are translated to telomere lengths by comparing the obtained intensities of fluorescence with a standard regression curve built with control cell lines of known telomere length. Every sample was analyzed in 5 replicates. If one of these replicates deviated significantly from the rest, it was discarded for further analysis by using a generalized extreme Studentized deviate algorithm. We accepted a maximum CV between these 4 or 5 replicates of 10%, but the CV mostly was <5%. As a measure of telomere length, the median telomere length of the entire (nonsymmetrical) telomere length distribution in each individual was used. The percentage of cells with critically short telomeres was assessed by measuring the percentage of nuclei with telomeres shorter than 4.5 kb corresponding to the 10–20% percentile of the telomere length distribution.

Statistical analyses

In line with previous publications on this cohort, we compared those exposed to the famine during gestation with those unexposed to famine during gestation. In the current study, we compared those exposed to famine in early gestation with those born before the famine and those conceived after the famine. We applied linear and logistic regression analyses to test differences between exposed and unexposed groups in general, birth, adult, and telomere characteristics. We applied linear regression analyses to test associations between general, birth and adult characteristics (predictors) and telomere characteristics (outcomes). We adjusted our analyses for potential confounders by adding these variables to the regression models. As potential confounding variables, we included sex, age, and variables that showed an association with telomere length and/or percentage of short telomeres with $P < 0.15$ based on linear regression analysis. Because we previously reported a sex-specific effect of prenatal famine exposure on mortality, we also tested a famine × sex interaction for telomere length and percentage of short telomeres by adding a sex × famine interaction term to our regression models (16). We considered differences between groups to be statistically significant if P values were <0.05. All data were analyzed with IBM SPSS Statistics version 21 software.

RESULTS

Study group characteristics

Data were available for 131 participants in the study, of whom 60 were men (46%) and 71 were women (54%). Of these 131 subjects, 45 were born before the famine (34%), 41 were exposed to famine in early gestation (31%), and 45 were conceived after the famine (34%) (**Table 1**). The mean ± SD age was 67.6 ± 0.9 y, and, at the time of participation in the study, >75% of the study population had retired. No significant differences in any of the maternal, birth, and adult characteristics were found between the exposed and unexposed groups (Table 1).

Telomeres and general characteristics

The mean ± SD leukocyte telomere length was 8.53 ± 1.07 kb for the whole study group, and the mean percentage of short telomeres was 21.7% ± 4.7% (Table 1). **Table 2** shows that, in general, sex, age, birth weight, BMI, smoking, and pack-years were not significantly associated with telomere length, although there was a trend toward shorter telomere length with increasing age: −0.17 (95% CI: −0.38 to 0.03) kb/y. SES at birth was significantly associated with shorter telomere length (for effect sizes, see Table 2). If the head of the household at birth had a manual occupation, indicating a lower SES, telomeres in adult life were shorter. Leukocyte telomeres were also significantly shorter in those with regular consumption of alcohol, those with a history of cancer, and those who reported to feel less healthy. Further analysis showed that regular consumption of beer or wine was not associated with telomere length, but consumption of spirits was strongly associated with shorter telomere length: −0.7 (95% CI: −1.2 to −0.2) kb. Current employment was the only variable significantly associated with the percentage of short telomeres. Participants who were still at work at the time of the study had a smaller percentage of short telomeres. A trend toward a significant association between a history of cancer and percentage of short telomeres was found. Participants who had ever been diagnosed with cancer seemed to have a larger percentage of short telomeres.

Telomeres and prenatal famine exposure

Mean leukocyte telomere length did not differ between those exposed to famine during early gestation (8.39 kb) and those unexposed to famine during gestation (8.59 kb); the mean difference was −0.19 kb (95% CI: −0.59, 0.21) (Table 1). The percentage of short telomeres also did not differ between the exposed (22.3%) and unexposed (21.4%) groups; the mean difference was 0.9% (95% CI: −0.9%, 2.7%). We found no evidence for an interaction between sex and famine exposure on telomere length ($P = 0.82$) or percentage of short telomeres ($P = 0.58$). Adjustment for the analyses on famine exposure and telomere length/percentage of short telomeres for age, sex, SES at birth, current employment, alcohol consumption, history of cancer, or self-reported health status did not change our findings; the mean differences were −0.10 (95% CI: −0.49, 0.23) kb and 0.3% (95% CI: −1.6%, 2.1%), respectively.

DISCUSSION

The results of the current study do not support our study hypothesis that prenatal exposure to the Dutch famine affects telomere length in all tissues, including leukocytes. The findings showed that leukocyte telomere length and the percentage of short leukocyte telomeres did not differ between those exposed to famine during early gestation and those unexposed to the famine during gestation at age 68 y.

We previously reported an increased risk of age-associated diseases and higher female mortality among those exposed to famine in early gestation (11–16). Animal experimental evidence

TABLE 1
Maternal, birth, and adult characteristics and telomere characteristics according to famine exposure status

		Exposure to famine				
General characteristics	*n*	Born before	In early gestation	Conceived after	P^1	Total
n		45	41	45		131
Age, y	131	68.7 ± 0.4[2]	67.4 ± 0.2	66.7 ± 0.4	0.12	67.6 ± 0.9
Female, %	131	60	49	53	0.40	54
Birth characteristics						
SES[3] at birth, manual, %	109	79	58	68	0.11	68
Gestational age, d	113	286 ± 11	288 ± 10	287 ± 14	0.56	287 ± 12
Birth weight, g	131	3413 ± 463	3494 ± 510	3396 ± 504	0.37	3433 ± 490
Adult characteristics						
Current employment, %	131	22	27	18	0.38	22
SES	131	52 ± 14	47 ± 13	48 ± 15	0.26	49 ± 14
Smoking, %	131	2	15	11	0.15	9
Pack-years of smoking[4]	130	2.7	9.0	3.0	0.15	4.5
Alcohol consumption >1 glass/wk, %	131	64	73	71	0.54	70
BMI, kg/m^2	131	28.1 ± 3.5	28.4 ± 5.2	29.7 ± 5.1	0.60	28.8 ± 4.7
Self-reported health status[5]	131	2.8 ± 0.8	3.0 ± 0.9	2.9 ± 0.8	0.29	2.9 ± 0.8
Ever had cancer, %	131	20	17	13	0.95	17
Telomere characteristics						
Telomere length, kb	131	8.44 ± 1.00	8.39 ± 1.11	8.73 ± 1.09	0.34	8.53 ± 1.07
Short telomeres, %	130	21.6 ± 4.9	22.3 ± 4.6	21.2 ± 4.7	0.31	21.7 ± 4.7

[1]*P* for difference exposed compared with combined unexposed groups based on regression analysis (born before and conceived after famine).
[2]Mean ± SD (all such values).
[3]SES, socioeconomic status.
[4]Values are medians.
[5]Based on self-reported health in 5 categories: 1 = excellent, 2 = very good, 3 = good, 4 = fair, 5 = bad.

suggests that compromised telomere integrity may have mechanistically contributed to this association, although another possibility is that the increased negative health consequences after prenatal famine exposure would have resulted in compromised telomere length, because it remains an open debate whether telomere length is a contributor to or merely a marker of disease (8–10). However, in the current study we were unable to show any effects of undernutrition during gestation on leukocyte telomere length in humans later in life, as opposed to the evidence of studies in rats in which mothers were protein restricted during pregnancy and that showed decreased telomere length in kidney, pancreatic islet, and aortic cells in the offspring (8–10). A number of reasons to explain this divergence are possible. First, we measured telomere length in leukocytes as opposed to more metabolically important tissues. Telomere length and rate of shortening differ greatly between different

TABLE 2
Regression coefficients for mean telomere length and percentage of short telomeres in association with general, lifestyle, and health characteristics

		Mean telomere length, kb		Short telomeres, %	
	n	B (95% CI)	P^1	B (95% CI)	P^1
Age, y	131	−0.17 (−0.38, 0.03)	0.09	−0.27 (−1.16, 0.62)	0.54
Sex (M/F)	131	0.12 (−0.25, 0.49)	0.53	−0.33 (−1.96, 1.30)	0.69
SES[2] at birth, manual (no/yes)	109	−0.53 (−0.95 to −0.10)	0.02	1.44 (−0.39, 3.27)	0.12
Birth weight, kg	131	0.01 (−0.37, 0.39)	0.96	0.27 (−1.39, 1.93)	0.75
SES current	131	0.01 (−0.01, 0.02)	0.16	−0.03 (−0.09, 0.03)	0.35
Current employment (no/yes)	131	0.30 (−0.15, 0.74)	0.19	−2.08 (−4.02 to −0.14)	0.04
Smoking (no/yes)	131	0.16 (−0.49, 0.80)	0.62	−0.18 (−2.99, 2.63)	0.90
Pack-years of smoking	130	0.00 (−0.01, 0.01)	0.72	0.03 (−0.01, 0.06)	0.15
Alcohol (no/yes)	131	−0.47 (−0.86 to −0.74)	0.02	1.43 (−0.32, 3.17)	0.11
BMI, kg/m^2	131	0.01 (−0.03, 0.05)	0.77	0.04 (−0.14, 0.21)	0.69
Ever cancer (no/yes)	131	−0.55 (−1.04 to −0.06)	0.03	2.09 (−0.05, 4.22)	0.06
Self-reported health status[3]	131	−0.33 (−0.55 to −0.12)	0.002	0.77 (−0.19, 1.72)	0.11

[1]Based on linear regression analysis.
[2]SES, socioeconomic status.
[3]Based on self-reported health in 5 categories: 1 = excellent, 2 = very good, 3 = good, 4 = fair, 5 = bad.

tissues. This could be due to differences in numbers of rounds of replication of cells in a particular tissue or could be due to differences in vulnerability to oxidative stress. Consistent with this suggestion, a recent study showed that telomere length in peripheral leukocytes is not strongly correlated with telomere length in other organs, such as the brain, heart, and kidney (22). Unfortunately, we could not compare our study results with those of an equivalent study in animals because, to the best of our knowledge, there is no published study that investigated leukocyte telomere length after prenatal undernutrition in animals. So, although we did not find differences in the integrity of telomeres in leukocytes between famine-exposed and -unexposed groups, telomeres within specific organs may have been different (8–10).

Second, the absence of an effect of prenatal famine exposure on telomere length may have been due to selective participation of cohort members in the current study. The previously shown excess mortality in women exposed to famine in early gestation and the disability associated with the increased prevalence of disease in men and women exposed in early gestation may have resulted in selective participation of people who were alive and fit enough to participate in the current study at age 68 y (16, 19). Table 1 also shows that, although not significantly different from the control group, exposed individuals seem to be still at work more often, which suggests that they may be a fitter, healthier group. Unfortunately, we do not have detailed data on glucose metabolism and coronary heart disease at this age; thus, we could not make a comparison between the exposure groups.

Another possible explanation for why we did not find differences is because of the relatively small size of our study sample. However, a post hoc power analysis showed that we had 80% power to detect differences of 0.5 kb in telomere length, which corresponds to the 540-bp difference that was reported between groups prenatally exposed and unexposed to severe maternal stress (23). Also, the Q-FISH–based method we applied has a high sensitivity, and other findings in the current study do largely seem to coincide with the existing literature. There was a trend toward a significant negative association between telomere length and age. The fact that the association was not strong was probably due to the very small variation in age in our sample, i.e., 66–69 y. In line with the work by Kajantie et al. (24), we also could not show an association between birth weight—an important marker of the prenatal environment—and leukocyte telomere length. Kajantie et al. showed in 3 large birth cohorts that low birth weight did not predict shorter leukocyte telomere length, providing a strong case for the absence of such an association. They did find an association between childhood economic status and telomere length. Subjects whose fathers were manual workers had shorter telomeres than did those whose fathers had lower or upper middle class occupations. Our data showed exactly the same association. Participants who were born in a family in which the head of the household had a manual occupation had shorter telomeres than did those whose father or mother had a nonmanual occupation. Other studies have shown associations between low childhood SES and short telomere length, although it remains unclear which factors within a low-SES child contribute to the telomere erosion (25, 26). Needham et al. (25) investigated health behaviors as a mediator, but could not show that vegetable consumption, levels of activity, or BMI contributed to the association. Long-term exposure to chronic stress may be an alternative explanation (27), although this seems a difficult measure to capture, as shown by a recent study by Jodczyk et al. (28). In this large longitudinal study, the authors could not show associations between 29 different measures of life course adversity or stress, which occurred before the age of 25 y, and leukocyte telomere length (28).

Also in congruence with previous publications, the current study results showed an association between shorter telomeres and the frequent consumption of alcohol, specifically spirits, and an association with a history of cancer diagnosis (5, 29). Interestingly, we also found an association between self-reported health and telomere length that, to our knowledge, has not been reported before. Participants who perceived their health as being most poor had shorter leukocyte telomeres. This finding seems to correspond with the fact that self-reported health is a strong predictor of future mortality (30).

A final explanation for our null finding could be that prenatal undernutrition does not affect telomere integrity in humans. Given the evidence from animal experiments, we consider this an unlikely possibility, especially because other adverse prenatal insults have clearly been shown to affect telomere length. For example, a study by Entringer et al. (23) showed that offspring of mothers who experienced a severe stressor during pregnancy had shorter leukocyte telomere length in adult life compared with offspring of control mothers. It remains a possibility however, that the effects of prenatal energy restriction on telomeres differ from those of other poor prenatal circumstances and also do not parallel the effects observed in rats.

In summary, the current study results suggest that prenatal exposure to famine is not associated with the shortening of telomeres in peripheral blood leukocytes at age 68 y. Many different methodologic shortcomings potentially explain our findings. Alternatively, prenatal undernutrition may not affect leukocyte telomere length in later life. On the other hand, we showed for the first time that a general association exists between leukocyte telomere length and self-reported health.

The authors' responsibilities were as follows—SRdR, TJR, SEO, and MS: designed the research; SRdR, AMMvP, CMK, SKMvD, and MHV: conducted the research; SRdR: analyzed the data, wrote the manuscript, and had primary responsibility for the final content; and TJR, SEO, AMMvP, RCP, and MS: critically reviewed the manuscript and provided essential input. The funder had no role in the current study, other than as a financial contributor. The authors declared no conflicts of interest.

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