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High plasma folate is negatively associated with leukocyte telomere length in Framingham offspring cohort

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Abstract

Purpose—Shortening of telomeres, the protective structures at the ends of eukaryotic chromosomes, is associated with age-related pathologies. Telomere length is influenced by DNA integrity and DNA and histone methylation. Folate plays a role in providing precursors for nucleotides and methyl groups for methylation reactions and has the potential to influence telomere length.

Method—We determined the association between leukocyte telomere length and long-term plasma folate status (mean of 4 years) in Framingham Offspring Study (n=1044, female =52.1%, mean age 59 y) using data from samples collected before and after folic acid fortification. Leukocyte telomere length was determined by Southern analysis and fasting plasma folate concentration using microbiological assay.

Results—There was no significant positive association between long-term plasma folate and leukocyte telomere length among the Framingham Offspring Study participants perhaps due to their adequate folate status. While the leukocyte telomere length in the second quintile of plasma folate was longer than that of the first quintile, the difference was not statistically significant. The leukocyte telomere length of the individuals in the fifth quintile of plasma folate was shorter than

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Conflict of interest

The authors declare that they have no conflict of interest

that of those in the second quintile by 180 bp ($P < 0.01$). There was a linear decrease in leukocyte telomere length with higher plasma folate concentrations in the upper 4 quintiles of plasma folate (P for trend = 0.001). Multivitamin use was associated with shorter telomeres in this cohort ($P = 0.015$).

Conclusions—High plasma folate status possibly resulting from high folic acid intake may interfere with the role of folate in maintaining telomere integrity.

Keywords

Telomere length; folate; multivitamins; folic acid fortification

Introduction

Shortening of the length of telomeres, the protective structures at the ends of eukaryotic chromosomes, has been associated with aging and various age-related diseases including cardiovascular disease, cancer, Alzheimer disease and Parkinson disease [1–5]. Telomere length is influenced by DNA integrity [6] and DNA and histone methylation [7–9]. Damage in telomeric DNA is not repaired efficiently and this can lead to shortening of telomeres [6, 10]. Short telomeres lose their protective function, leading to end to end fusion of chromosomes, and chromosome rearrangements [6, 11]. Folate plays a major role in purine and pyrimidine synthesis [12] and deficiency of folate results in DNA damage [13]. Folate also plays a role in providing methyl groups for the synthesis of methionine, the precursor of S-adenosyl methionine, which is the methyl donor for DNA and histone methylation. Loss of DNA and histone methylation results in loss of telomere length regulation and results in unusually long telomeres without the normal heterochromatin structure which are prone to recombinations [7–9]. Thus folate nutritional status has the potential to influence telomere length. We had previously shown a U-shaped relationship between telomere length of peripheral mononuclear cells and plasma folate status in men [14]. Here longer telomeres were observed both at very low folate status and when folate status was adequate. Since low folate status is associated with DNA hypomethylation in peripheral mononuclear cells [15], the longer telomeres observed at under low folate status is possibly due to loss of telomere length regulation due to DNA hypomethylation and hence not an advantageous feature. A positive relationship between plasma folate and telomere length was observed in a small group of older men [16] and in a cohort that was predominantly women [17]. Folic acid fortification was adopted in the US in 1998 leading to a significant increase in the folate status of the US population [18]. Here we determined the association between leukocyte telomere length and plasma folate status using data from Framingham Offspring Study that included both men and women. A significant proportion of the individuals in our study cohort had been exposed to folic acid fortification, which allowed us to determine if the higher folate status of those exposed to fortification is reflected in their telomere length.

Materials and methods

Subjects

The Framingham Offspring Study was initiated in 1971 and included 5124 men and women, who were the offspring or the spouses of the original Framingham Heart Study cohort [19]. Examinations are conducted and data collected approximately every four years in this cohort. Leukocyte telomere length data were available for 1244 participants of Framingham Offspring Study at the sixth examination (1995–1998). Of these, 200 subjects were excluded in this study due to missing values for folate, BMI, serum creatinine or alcohol intake. This study was approved by the Institutional Review Board of Tufts Medical Center and Boston University Medical Center and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all the subjects. The characteristics of the participants in this study are given in Table 1.

Determination of leukocyte telomere length

Leukocyte telomere length [20] was determined from the mean terminal restriction fragment length of chromosomes using a Southern blot procedure for biologically unrelated participants of Framingham Offspring Study at the sixth examination [20].

Plasma analyses

All plasma analyses were conducted on fasting samples. Plasma folate concentration was determined by microbiological assay [21]. When folic acid fortification of cereal grains was adopted in the US, fortification was phased-in during the period between September 1996 and July 1997. Thus exposure to folic acid fortification of those who were examined between October 1996 and August 1997 was not certain [22]. The participants who were examined after September 1997 were exposed to folic acid fortification and thus had an increase in plasma folate status over varying durations prior to sample collection [22]. For these reasons, we used the mean of plasma folate concentration from the fifth (1991–1995) and sixth (1995–1998) examinations which better reflects their long-term plasma folate status for the analyses. Serum concentration of creatinine was determined by the Jaffe reaction [23], vitamin B12 using a radioimmunoassay (Quantaphase II; Bio-Rad, Hercules, CA) and pyridoxal 5'-phosphate (PLP) the active form of vitamin B6 using the tyrosine decarboxylase method [24].

Statistical analyses

We used general linear modeling (analysis of covariance) to determine the association between leukocyte telomere length and long-term plasma folate (in quintile categories), multivitamin use and folic acid fortification status (pre vs post). Adjustments (Tukey) were made for multiple comparisons between long-term plasma folate categories. Linear trend across categories of long-term plasma folate was determined by assigning each participant the median value for the category and modeling this value as a continuous variable. The following covariates that have been shown to influence telomere length were included in the analysis; age, gender, smoking, alcohol use, menopause, serum creatinine, BMI. We also

considered plasma concentrations of vitamin B12 and PLP as covariates in the analyses since they are involved in the one carbon metabolism in which folate plays a central role. But, plasma concentrations of vitamin B12 and PLP were not associated with leukocyte telomere length in this model and hence were not used in further analyses. There was no interaction between gender and long-term folate and hence data analyses were not stratified by gender. When the association of leukocyte telomere length with folic acid fortification was determined, only the individuals who were examined before fortification began and after fortification was complete were included in the analyses, those who were examined during the period when fortification was phased-in were excluded from the analysis. 4 participants with missing multivitamin use data were excluded when the relationship between multivitamin use and telomere length was examined. The analyses were conducted using SAS (version 9.2). Associations with a *P* value of ≤ 0.05 were considered significant.

Results

The age-related difference in mean leukocyte telomere length in the study population was a decrease of 22 bp per year of age. There was no significant positive association between plasma folate and leukocyte telomere length. While the leukocyte telomere length in the second quintile of plasma folate was longer than that of the first quintile, the difference was not statistically significant. The mean leukocyte telomere length of the individuals in the fifth quintile of plasma folate (6.80 ± 0.04 kb $\pm$ SE) was significantly shorter than that of those in the second quintile (6.98 ± 0.04 kb $\pm$ SE) ($P < 0.01$) (Fig. 1). Since the overall relationship between plasma folate and leukocyte telomere length was not linear, we performed additional secondary analyses to determine the association between plasma folate and telomere length in the upper 4 quintiles. There was a linear decrease in telomere length across the second through fifth quintiles of plasma folate concentrations (Fig. 1, *P* for trend = 0.001). Concentration of plasma folate was negatively associated with its biochemical marker total homocysteine in plasma ($r = -0.2426$, $P < 0.0001$).

We considered 2 variables that could influence plasma folate concentration among the study participants; exposure to folic acid fortification and multivitamin use (Online resources 1 and 2). The mean leukocyte telomere length of individuals examined before fortification was 6.95 ± 0.04 , kb $\pm$ SE and those examined after folic acid fortification was 6.88 ± 0.04 , kb $\pm$ SE, but this difference was not statistically significant ($P = 0.14$), (Online resource 2). Multivitamin users had a shorter leukocyte telomere length when compared to non-users in this cohort (6.84 ± 0.04 vs 6.93 ± 0.03 kb $\pm$ SE; $P = 0.015$), (Online resource 2). Among the individuals in the fifth quintile of long-term plasma folate where leukocyte telomere length was shortest, 83% were multivitamin users (Online resource 1). Since multivitamins contain other components like iron that can negatively influence telomere length [25] we conducted the analysis of leukocyte telomere length vs folate quintiles after excluding multivitamin users. The decrease in telomere length across second through fifth quintile of plasma folate persisted even when multivitamin users were omitted from the analysis (*P* for trend < 0.01).

Discussion

Folate is essential for providing nucleotide precursors necessary for maintaining DNA integrity which in turn influences telomere length [6]. Prior studies have shown an association between telomere length and plasma folate status [14, 16, 17], though one recent study did not find any association between plasma folate or folate intake and telomere length [26]. In the current study of Framingham Offspring cohort, samples had been collected before and after the mandatory folic acid fortification of flour and cereal grains in the US in 1998. Folic acid fortification significantly improved the plasma folate status of this cohort [18] and based on the previous studies we expected to see a positive association between plasma folate concentration and telomere length in this cohort. However there was no significant positive association between plasma folate and telomere length in this cohort. The leukocyte telomere length in the second quintile of long-term plasma folate was longer than that of the first quintile but the difference was not statistically significant (Fig. 1). Contrary to our hypothesis, at higher concentrations, plasma folate appeared to have a negative influence on telomere length (Fig 1). The mean leukocyte telomere length of individuals in the fifth quintile of long-term plasma folate was significantly shorter than those in the second quintile by 180 bp (Fig 1). As a reference for this difference in telomere length due to nutritional status, the difference in leukocyte telomere length due to age, which is a major determinant of telomere length, was a decrease of 22 bp per year of age. The age related difference in telomere length in this study is similar to what has been previously reported for other cohorts [27].

Current literature shows that there is a bell shaped relationship between plasma folate/folate intake and optimal health where both low and high plasma folate/folate intake are associated with negative health outcomes. A similar effect has been reported for selenium intake and health outcomes [28]. Under low folate status there is an increase in risk for neural tube defects and many chronic diseases including many types of cancer, cardiovascular disease and cognitive dysfunction [29–33]. Supplementary folate nutrition has been shown to prevent occurrence of neural tube defects [33] and many countries adopted folic acid fortification for this reason. Folic acid is a synthetic form of the vitamin used in fortified foods and supplements due to its stability. Among men and women over the age of 50 in the 2003–2006 National Health and Nutritional Examination Survey, the mean folic acid intake from supplements alone was 436 ± 21.4 $\mu\text{g/d}$ and 5% had a folic acid intake above the tolerable upper level of 1000 $\mu\text{g/d}$ [34]. In recent years, several studies have shown that high plasma folate and high folic acid intake, presumably in excess of what is required by the body, can also be associated with negative health outcomes as noted below. High plasma folate and multivitamin use are associated with increased risk for breast cancer in women [35–37]. Among female breast cancer survivors, those who took folic acid supplements or multivitamins but had otherwise poor quality diet was at higher risk for mortality [38]. While adequate folate nutrition is critical for embryonic development, maternal intake of excess folic acid is associated with embryonic developmental disorders in wild type mice and increased incidence of neural tube defects in some mouse models of neural tube defects [39–41]. In a lesion model of central nervous system injury, administration of folic acid resulted in neuronal regeneration, but use of higher concentrations folic acid had a negative

influence on neuronal regeneration [42]. Deficiency of folate impairs DNA synthesis and results in anemia [43, 44]. At the other end of the spectrum, presence of unmetabolized folic acid in plasma that is indicative of high folic acid intake is associated with higher incidence of anemia in humans [45], which suggests impairment of DNA synthesis. Telomere length is determined by DNA integrity [6] and if high folic acid intake impairs DNA synthesis it can adversely influence telomere length.

The high plasma folate concentration in Framingham Offspring cohort is the result of high folic acid intake from fortified foods and supplements (Choumenkovich and Selhub, unpublished data). Folic acid fortification appears to have a negative impact on leukocyte telomere length in this cohort, but the effect was not statistically significant. After folic acid fortification, prevalence of individuals who take more than the tolerable upper level for folic acid increased among supplement users in the Framingham Offspring cohort [22]. At the sixth examination of this cohort, 40.7 % of the participants were multivitamin users (Table 1). Multivitamin use was associated with shorter leukocyte telomere length in this study ($P=0.015$). This result is different from that of Xu et al [25] who showed that multivitamin use is associated with longer telomeres in women. The cohort of Xu et al was comprised of healthy sisters of breast cancer cases [25]. The results of Xu et al might be partially explained by the possibility that the sisters of breast cancer patients who themselves are at a higher risk for cancer development, might adopt a healthier life style (eg: less inclination to smoke) and diet which may also include multivitamin use [25, 46]. A healthy life style and diet are positively associated with telomere length [47, 48]. In Framingham Offspring cohort, multivitamin use was probably not associated with a healthier lifestyle since smoking and alcohol use was not significantly different between multivitamin users and non-users (Online resource 2).

Though the mechanism behind the deleterious effect of excess folic acid intake in humans and animals is not known at present, it has been shown that the enzymes of folate pathway can be inhibited by various folate derivatives [49, 50]. The direction of the metabolic flux in folate pathway is to some extent regulated by concentration of different forms of folate [49, 50]. Folic acid intake in excess of the metabolic capacity of the cell may lead to accumulation of folate derivatives. The enzyme activities thus regulated or inhibited by folate derivatives include, thymidylate synthase, methylene tetrahydrofolate dehydrogenase (MTHFD), 5,10- methenyl tetrahydrofolate cyclohydrolase (also carried out by MTHFD) and dihydrofolate reductase [49, 50]. Inhibition of MTHFD, dihydrofolate reductase and thymidylate synthase all contribute to reduced availability of nucleotide precursors and the nucleotide thymidine and thus causing DNA damage, which can potentially result in shorter telomeres. Thus the shorter telomeres in the individuals with high plasma folate concentration could be the result of DNA damage resulting from folic acid intake beyond what is optimal for cellular functions.

The strength of our study includes a community-based cohort that is composed of both men and women, with a wide range of plasma folate concentrations. This cohort also had individuals who were exposed to folic acid fortification and those who were not, allowing us to determine the influence of fortification on leukocyte telomere length. The Framingham Offspring cohort is predominantly Caucasian, and the results of this study may not be

applicable to other populations. The median age of this cohort was 54.4 years. We have previously shown that the negative association between the presence of unmetabolized folic acid in plasma and immune function is seen only in older individuals [51]. It is possible effect of excess folic acid intake on telomere length might be different in a younger population.

In conclusion, high plasma folate was associated with shorter leukocyte telomere length. Shortening of telomeres is associated with age-related dysfunctions. While adequate folate nutrition is necessary to maintain DNA integrity and hence that of telomeres, the results of this study suggest that high folate status possibly resulting from high folic acid intake from multivitamins and fortified foods may interfere with the role of folate in maintaining telomere integrity. Additional studies have to be conducted to determine the mechanism behind shortening of telomeres under high folate status and if it is associated with any health outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Leukocyte telomere length in participants of Framingham Heart Study Offspring cohort according to quintiles of long-term plasma folate concentration. General linear modeling was used to determine the association between leukocyte telomere length and long-term plasma folate. Values are mean and SE. Labeled mean values with unlike letters differ, P value <0.01 . * P for trend = 0.001 across second to fifth quintiles. The data were adjusted for age, gender, menopause, smoking, BMI, smoking, alcohol use and serum creatinine

Table 1Characteristics of the study population ¹

Age, y	59.0 (45, 75)
Female	544 (52.1)
Post-menopausal female	446 (82.0)
Multivitamin users	423 (40.7)
Current smokers	134 (12.8)
BMI, kg/m ²	27.2 (21.3, 38.5)
Alcohol use, g/d	4.7 (0, 43.0)
Folic acid fortification status	
No	319 (30.6)
Yes	431 (41.3)
Not known	294 (28.1)
<i>Plasma /serum concentrations of metabolites</i>	
Plasma folate nmol/L (n=1044)	
Exam 5 (1991–1995)	13.1 (4.2, 46.0)
Exam 6 (1995–1998)	22.6 (7.0, 61.4)
Mean of exams 5&6 (long-term)	18.8 (6.8, 49.9)
Vitamin B-12 pmol/L	293 (158.3, 544.7)
PLP (nmol/L)	63.3 (24.9, 275.6)
Hcy µmol/L	9.0 (5.7, 14.7)
Creatinine (µmol/L)	102.5 (78.7, 133.5)

¹ n=1044, except for 4 missing values for multivitamin, values are median (5th, 95th percentiles) or n (%)