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Plasma micronutrient levels and telomere length in children

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ABSTRACT

Objective: Telomeres are long hexamer (TTAGGG) repeats at the ends of chromosomes, and contribute to maintenance of chromosomal stability. Telomere shortening has been linked to cancers and other chronic diseases in adults, although evidence for causal associations is limited. The aim of this study was to determine whether nutritional factors are associated with telomere length (TL) in children.

Methods: We conducted a cross-sectional study of nutritional factors and TL in 437 children between 2009 and 2011. Healthy children ages 3, 6, and 9 y provided blood samples, and their parents completed a food frequency questionnaire and a telephone interview about relevant environmental exposures. TL and blood micronutrient levels were measured, and genotyping at 10 loci was undertaken. Associations between the micronutrients and other variables were assessed using linear regression.

Results: No significant main or interactive effects of age or sex were seen. After adjustment for age, sex, parental education, and month of blood collection, TL was inversely associated with plasma zinc, and shorter in children with the homozygous mutant genotype of the *RFC* G80A (rs1051266) polymorphism.

Conclusions: To the best of our knowledge, this is the first investigation of the association between telomere length and micronutrients in healthy children. The reason for the inverse relationship of TL with zinc is unknown but could be the result of an increase in telomere sequence deletions caused by labile zinc induction of oxidative stress. These findings should be corroborated in other studies before nutritional recommendations might be considered.

Introduction

Telomeres are long hexamer (TTAGGG) repeats located at the ends of mammalian chromosomes that, together with the associated protein telosome structure, act to maintain chromosomal integrity and stability. However, telomeres shorten with age due to the incomplete replication of DNA at chromosome ends with each cell division and DNA strand breaks incurred by oxidative stress. Eventually, telomeres shorten to the extent that they are unable to protect the ends of chromosomes from degradation, leading to accelerated cell senescence and death. Telomere degradation and/or dysfunction promote chromosomal instability via telomere end fusion and the generation of breakage–fusion–bridge cycles within chromosomes [1,2]. These threats to genomic stability may result in abnormal karyotypes, altered gene dosage, loss of heterozygosity, or gene amplification, which

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in turn cause abnormal gene expression including activation of oncogenes—or inactivation of tumor suppressor genes—important initiating events in carcinogenesis. Telomere shortening has been linked to several types of cancer (including lung, breast, colon, prostate, and certain leukemias); these associations have been the subject of two recent meta-analyses [3,4]. Although most studies have been cross-sectional or retrospective, some prospective studies have found telomere shortening to be predictive of the risk for cancer [5–7], adverse cardio-vascular events [8,9], and mortality [5,6,10,11].

There is increasing evidence that nutritional factors are associated with telomere length (TL) in adults. A recent longitudinal study reported an inverse relationship between marine ω-3 fatty acid levels in the blood and the rate of telomere attrition [12]. Other cross-sectional studies have reported positive associations between TL and multivitamin use and dietary intake of vitamins C and E [13], folate [14,15], vitamin D [16], and fiber [17]. In vitro studies have shown that age-related telomere shortening can be slowed by enrichment of intracellular vitamins C [18] and E [19]. Shorter telomeres have been associated with higher intake of processed meat [20] and alcohol [21], and higher body mass index (BMI) in adults [22,23]. There also is evidence that minerals involved in antioxidant response, such as zinc and selenium, may influence TL and telomere base damage, but the shape of the dose–response relationship within the physiological range has yet to be determined [24–26].

Virtually all previous studies of factors associated with TL and other forms of DNA damage have been conducted in adults, and the focus of the few studies conducted in children to date has been on the effects of environmental pollutants and socioeconomic status [27,28] rather than nutritional factors. Given that diseases of adulthood may originate early in life [29] and that lifelong dietary habits are established in childhood [30], it is important to examine whether nutrition during childhood is associated with telomere shortening. Here, we present the results of the first comprehensive study of blood micronutrient levels and TL in healthy children.

Materials and methods

A cross-sectional study of nutritional factors and DNA damage in children was conducted in Western Australia between 2009 and 2011. Parents of healthy children ages 3, 6, and 9 y were invited to participate via mail to childcare centers and schools, and through community-based advertisements. Almost all volunteers were accepted into the study; only children with asthma, diabetes, cancer, arthritis, or epilepsy were not eligible. Parents of 464 children provided informed consent: 155 were age 3 y, 155 were 6, and 154 were 9 y. The 9-y-olds also were required to provide consent. The study was approved by the Department of Education and Training, and the University of Western Australia Human Research Ethics committee.

In a telephone interview, parents provided information about the family's demographic characteristics, the child's health, and relevant exposures. Parents were mailed instructions about giving the child a simple breakfast on the day of the scheduled blood collection, and recording exactly what was eaten; toast, milk, butter, margarine and/or jam, and water were allowed. Parents were also mailed a tube of anaesthetic cream to apply to the child's arm 1 h before the appointment. Height was measured in cm to 1 decimal point using a portable stadiometer (Seca, Medical Scales and Measuring Systems, United Kingdom) and weight was measured in g (to 100 g) using a digital bathroom scale (HD-327, Tanita Corporation, Japan).

The phlebotomist collected 18 mL of blood on either 1 or 2 d. Blood samples were maintained at steady temperature (5°C–10°C) in Labtop coolers and couriered to the laboratory in insulated boxes (DGP BioTherm 25, Singapore); temperature during transportation was tracked with a digital thermometer placed with the samples. Approximately 1 mL of whole blood was used to measure vitamin B_3 (niacin number) and red cell folate. The remaining blood was spun at 3000 *g* for 20 min at 4°C and 3.8 mL of the plasma collected were used to measure folate, vitamins B_{12}, and D, α-tocopherol and carotenoids (lutein, retinol, lycopene, α-carotene, and β-carotene), and minerals (selenium, calcium, magnesium, and zinc) levels. Red cell folate, plasma folate, and vitamin B_{12} were measured with a chemiluminescent microparticle folate or vitamin B_{12} binding protein assay, ARCHITECT (Abbott Laboratories, Abbott Park, IL, USA). Vitamin D was measured by an enzyme immunoassay before 2010 and an automated chemiluminescent assay after 2010 (both from Immuno Diagnostic Systems Ltd, Boldon, UK). Vitamin B_3 (niacin number), was measured with a validated colorimetric enzymatic assay [31,32]. α-Tocopherol and carotenoids (lutein, retinol, lycopene, α-carotene, and β-carotene) were measured by high-performance liquid chromatography, and minerals were assessed by inductively coupled plasma mass spectrometry (calcium, magnesium, and zinc) and inductively coupled plasma optical emission spectrometry (selenium). Peripheral blood lymphocytes were isolated using Ficoll-paque Plus separation medium (Pharmacia Biotech, Uppsala, Sweden), prepared for cryopreservation in fetal bovine serum plus 10% dimethyl sulfoxide, frozen and stored at to 80°C until required. DNA was isolated from lymphocytes using a QIAGEN DNeasy Kit with minor modifications to prevent DNA oxidation, as previously described [33]. Absolute TL was measured in duplicate samples by determining the number of TTAGGG hexamer repeats using quantitative real-time polymerase chain reaction as previously described [34]. TL values in kb/diploid genome were calculated using a synthesised TTAGGG 84 mer oligonucleotide to generate a standard curve; the number of diploid genome copies per reaction was determined using the single copy gene *36B4*. If the results for duplicate samples differed by more than one quantification cycle value, the results were discarded and the assay repeated. Using our internal control (1301 cell line DNA), we estimated that the interexperimental variability was ≤7% (n = 55) and the intraexperimental variability 1.1% (n = 34).

Genotyping was performed at the following loci according to standard protocols: methylenetetrahydrofolate reducatase (*MTHFR*) C677T (rs1801133), *MTHFR* A1298C (rs1801131), 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) A2756G (rs1805087), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) A66G (rs1801394), X-ray repair cross-complementing protein (*XRCC1*) Arg399 Gln (rs25487), reduced folate carrier (*RFC*) G80A (rs1051266), glutathione-*S*-transferase (*GST*) mu, *GST* theta, and *XRCC3* Thr241Met (rs861539). These loci were chosen because they had previously been shown to be associated with chromosome instability [35, 6], but only *MTHFR* C677T had been previously investigated in relation to TL (in adults) [14]. MTHFR, MTR, MTRR, and RFC enzymes are required for bioavailability and metabolism of folate and vitamin B_{12}, and the chosen polymorphisms are known to reduce the activity of these enzymes [37,38]. Thus, genotype may affect the availability of folate for DNA synthesis/repair and *S*-adenyl methionine (SAM) for maintenance of DNA methylation, which are essential for genome and telomere integrity [35,39]. The MTHFR enzyme is required for the conversion of the 5,10 methylenetetrahydrofolate (MTHF), the folate form required for thymidine synthesis, to 5-MTHF, the folate form required for conversion of homocysteine to methionine from which the common methyl donor SAM is synthesized [40]. Synthesis of methionine is catalyzed by MTR, which requires vitamin B_{12} as cofactor in its reduced form, which is maintained in this state by the activity of the MTRR enzyme [41]. Additionally entry of 5-MTHF from plasma and interstitial fluids into cells occurs through the activity of RFC [42]. XRCC1 and XRCC3 are required for the repair of DNA base lesions and DNA single and double-strand breaks caused by oxidative stress, which lead to telomere dysfunction and attrition [36,43]. GST is an enzyme required for detoxification of genotoxic chemicals by catalyzing conjugation with reduced glutathione [39,44]. The polymorphisms we studied for the *XRCC* and *GST* genes involve mutations that reduce the activity of their protein products [39,43,44].

Statistical analysis

The following variables were considered possible predictors of TL: blood micronutrient levels, supplement use, child's BMI, *Z*-score, parents' ages at the child's birth, socioeconomic status (parental education, household income), ethnicity, blood cortisol and cotinine levels, environmental exposures (X-rays, immunizations, pesticide exposure, history of fevers, medication use, sunburn, parental smoking), and genotype. We sought to identify the smallest subset of these variables that still had good predictive ability for TL using multivariate linear regression. Analyses were conducted in SPSS 22 (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp, Armonk, NY, USA).

As TL is potentially related to age, sex, and month of blood collection, these variables were entered into all models. All nutrient, demographic, environmental, and genotype variables were then added one at a time to assess the relationship with TL. Nutrient variables with a univariate $P \leq 0.10$ were assessed for inclusion in a combined model, and for interactions with genotypes. Genotype main effects or interactions that indicated larger or smaller effect sizes for heterozygotes than both wildtype and variant homozygotes were excluded. All variables or interactions of interest were then added to a combined model and retained if the variable block caused a significant change in R^2, or (for ordinal variables and bi-allelic genotypes), had a significant trend *P*-value of the coefficient.