Clinical Nutrition 34 (2015) 694–699

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Dietary total antioxidant capacity is associated with leukocyte telomere length in a children and adolescent population

Sonia García-Calzón [a], Adriana Moleres [a], Miguel A. Martínez-González [b, c], J. Alfredo Martínez [a, c], Guillermo Zalba [d], Amelia Marti [a, c, *], GENOI members[1]

[a] Department of Nutrition, Food Science and Physiology, University of Navarra, Pamplona, Spain
[b] Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain
[c] CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Spain
[d] Department of Biochemistry and Genetics, University of Navarra, Pamplona, Spain

ARTICLE INFO

Article history:
Received 8 January 2014
Accepted 29 July 2014

Keywords:
Antioxidants
Diet
Oxidative stress
Telomeres
Young population

SUMMARY

Background & aims: Oxidative stress and inflammation seem to be potential underlying mechanisms for telomere attrition. A lack of specific antioxidants is believed to increase free radical damage and a greater risk for telomere shortening. Our aim was to evaluate the relationship between diet and leukocyte telomere length in a cross-sectional study of children and adolescents. We hypothesized that dietary total antioxidant capacity would be positively associated with telomere length.
Methods: Telomere length was measured by quantitative real-time polymerase chain reaction in 287 participants (55% males, 6–18 years), who were randomly selected from the GENOI study.
Results: A positive correlation between dietary total antioxidant capacity and telomere length ($r = 0.157$, $p = 0.007$) was found after adjustment for age and energy intake. However, higher white bread consumption was associated with shorter telomeres ($\beta = -0.204$, $p = 0.002$) in fully-adjusted models. Interestingly, those individuals who had simultaneously higher dietary total antioxidant capacity and lower white bread consumption significantly presented the longest telomeres. Moreover, the multivariable-adjusted odds ratio for very short telomeres was 0.30 for dietary total antioxidant capacity ($p = 0.023$) and 1.37 for white bread ($p = 0.025$).
Conclusion: It was concluded that longer telomeres were associated with higher dietary total antioxidant capacity and lower white bread consumption in Spanish children and adolescents. These findings might open a new line of investigation about the potential role of an antioxidant diet in maintaining telomere length.

1. Introduction

Telomeres are tandem TTAGGG repeats of DNA that, together with associated protein factors, protect the ends of chromosomes and become shorter during repeated DNA replication [1]. Thus leukocyte telomere length (LTL) has been proposed as a biomarker of biological age [1]. Moreover, TL has been linked to the risk for several diseases, such as cancer and cardiovascular diseases [2]. Specifically, inflammation, oxidative stress and ageing are endogenous factors causing telomere shortening [1,3]. Notably, telomeres are highly sensitive to the hydroxyl radical, which causes DNA breakage, leading to the clipping of greater stretches of telomeres with each replication of haematopoietic stem cells, which is ultimately expressed in shortened LTL [4].

Interestingly, several studies have suggested that LTL is a dynamic factor being modifiable by lifestyle practises [5]. Among the determinants accompanying accelerated telomere attrition, smoking and unhealthy dietary habits are commonly reported [1,6]. Recently, Sun et al. [7] showed that a healthy lifestyle is associated with longer LTL in US women, whereas other factors including smoking, low physical activity, unhealthy dietary patterns and alcohol consumption may be associated with shortening of the telomeres.

Non-standard abbreviations: LTL, leukocyte telomere length; TAC, total antioxidant capacity; BMI-SDS, Standard deviation Score for body mass index; FFQ, food frequency questionnaire.

* Corresponding author. Department of Nutrition, Food Science and Physiology, University of Navarra, C/Irunlarrea s/n CP, 31008 Pamplona, Navarra, Spain. Tel.: +34 948 425600; fax: +34 948 425619.
E-mail address: amarti@unav.es (A. Marti).

[1] Other members of GENOI are: MC Ochoa, M. Chueca, M. Oyarzabal, A. Patiño, MC Azcona-Sanjulián, R. Pelach and M. J. Moreno-Aliaga.

http://dx.doi.org/10.1016/j.clnu.2014.07.015

There is scarce information about the effect of dietary components on LTL. Several investigations tried to explain the underlying mechanisms by which nutrients and bioactive dietary components may influence telomere length [8]. The Mediterranean dietary pattern has been widely considered as a model of healthy eating and some studies have shown its protective role on LTL [9,10]. Notably, a high consumption of vegetables and fruits [6,11], and a higher intake of omega-3 fatty acids [3] or fibre [12] were associated with longer telomeres, meanwhile a higher intake of saturated fatty acids [6] or a higher consumption of processed meats [13,14] were associated with shortening of the telomeres. So, it has been suggested that a diet rich in antioxidants may maintain the LTL and therefore decrease biological ageing [2]. However, so far no studies have analysed the association between diet and dietary total antioxidant capacity (TAC) with LTL in a youth population. Hence, the aim of the present study was to assess the effect of nutrients, food groups and dietary TAC on LTL in a subsample of Spanish children and adolescents from the GENOI study. We hypothesized that dietary TAC would be positively associated with LTL.

2. Materials and methods

2.1. Participants

The GENOI study (Grupo Navarro de Estudio de la Obesidad Infantil; GENOI) was composed of 451 Spanish children and adolescents (49% boys), aged 5–18 years. The study group included 160 obese, 132 overweight and 159 normal-weight individuals. Obesity was defined according to Cole et al. criteria [15]. Non-obese participants were healthy subjects coming to the community health centres for routine medical examination or to be vaccinated. From the GENOI total sample, participants with missing data or DNA missing samples were excluded from the telomere length analysis. Thus a subsample of 287 Spanish children and adolescents (55% males, aged 6–18 yr), were analysed in the study. This population included 51% obese (BMI-SDS>2.2), 12% overweight (BMI-SDS between 1.1 and 2.2) and 37% normal-weight participants (BMI-SDS<1.1). Moreover, 32% (25% of the boys and 40% of the girls) of the children and adolescents presented insulin resistance (Table 1). The subjects were recruited from the Paediatrics Departments of Complejo Hospitalario de Navarra, Clínica Universidad de Navarra and other Primary Care Centres in Navarra (Spain). Exclusion criteria were exposure to hormonal treatment or development of secondary obesity due to endocrinopathy or serious intercurrent illness. Further aspects of the methods and design of the GENOI study have previously been detailed elsewhere [16].

All parents and subjects who were 12 years of age and older provided written informed consent, whereas children younger than 12 years gave verbal consent to participate in the study. The study protocol was performed in accordance with the ethical standards of the Declaration of Helsinki (as revised in Hong Kong in 1989, in Edinburgh in 2000 and in South Korea in 2008), and was approved by the ethics committee of the University of Navarra.

Weight and height were measured with an electronic scale (type SECA 861; SECA, Birmingham, UK) and a telescopic height measuring instrument (type SECA 225; SECA), respectively, to establish Standard deviation Score for body mass index (BMI-SDS) according to the criteria of Cole et al. [15] Venous blood samples were collected after an overnight fast to obtain DNA samples.

2.2. Leukocyte telomere length assessment

Genomic DNA was extracted from human peripheral blood samples using the MasterPure DNA purification kit for Blood Version II (Epicenter Biotechnologies, Madison, WI, USA) and was stored at −80 °C until processing. LTL was measured in genomic DNA, using RT-PCR, as described by Cawthon [17]. This method measures concentrations of telomere repeat copy number (*T*) and single-copy gene (Ribosomal Protein Large PO) copy number (*S*) as a reference for each sample.

PCRs were performed separately for *T* and *S* reactions in paired 384-well plates on an ABI-Applied Biosystems 7900 HT thermal cycler (Applied Biosystems, CA, USA). QuantiTect Sybr Green PCR kit (Qiagen, Valencia, CA, USA) was used as master mix and the total reaction volume was 10 μL containing 10 ng of genomic DNA. The final telomere primer concentrations were as follows: for telomere amplification tel1, 675 nmol/L and for tel2, 1350 nmol/L; and for the amplification of the single copy gene RPLPO: hRPLPO1, 800 nmol/L; hRPLPO2, 800 nmol/L. The primer sequences (Sigma–Aldrich, St.Louis, MO, USA) were tel1 (5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′), tel2 (5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′), hRPLPO1 (5′-CCCATTCTATCATCAACGGGTACAA -3′) and hRPLPO2 (5′-CAGCAAGTGGGAAGGTGTAATCC -3′). The T/S ratio for each sample was obtained as a measure of relative LTL and it was calculated as follows $2^{CT(\text{telomeres})}/2^{CT(\text{single copy gene})} = 2^{-\Delta CT}$ [17]. For quality control, all samples were run in triplicate and checked for concordance between triplicate values. In order to achieve a robust consistence, samples showing a high variation (more than 10%) were rerun and reanalysed. The intra-assay coefficient of variation between triplicates was 1.5% and the inter-assay coefficient of variation between plates was 2.8%. The variation coefficient was calculated as the ratio between the average standard deviations and the average means of the triplicates. A calibration curve of the same DNA sample of reference (64–0.25 ng in 2 fold dilutions) was included for each measurement as a standard, to control the day to day variations. Standard curve with linearity $R^2 > 0.98$ was accepted.

Table 1
Characteristics of the 287 children included in the study.

Participant characteristics	
Age, years	11.5 (2.5)
Sex, %males	55.5
BMI-SDS[a)]	2.3 (2.2)
BMI-SDS, % normalweight/moderately obese/obese	37/12/51
HOMA-IR	3.2 (2.4)
IR, %[a)]	32.1
Telomere length	2.6 (0.5)
Total energy intake, kcal/d	3019.2 (757.2)
Carbohydrate, %E	46.4 (5.5)
Protein, % E	15.9 (2.4)
Fat, % E	37.7 (4.8)
MUFA	15.5 (2.9)
PUFA	5.7 (1.5)
SFA[a)]	13.0 (2.5)
Dietary TAC, mmol[a)]	8.4 (4.1)
Glycaemic load, units	264.1 (92.8)
Foods, g/d	
Fruits	206.1 (197.7)
Vegetables	325.3 (205.4)
Fish	54.2 (33.1)
Meat	202.9 (61.0)
Dairy products	371.5 (268.2)
Legumes	36.8 (53.8)
SSSB[a)]	233.1 (348.9)
Cereals	174.7 (118.3)
White bread	131.6 (118.4)

Values are means (standard deviations) unless otherwise stated.
[a)] BMI-SDS: Standard Deviation Score for body mass index, TAC: total antioxidant capacity, SFA: saturated fatty acids, SSSB: sweets and sugar sweetened beverages, IR: insulin resistant.