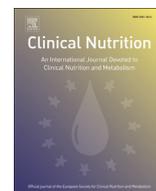




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Original article

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

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### 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.

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### 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.

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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

**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 <sup>a)</sup>	2.3 (2.2)
BMI-SDS, % normalweight/moderately obese/obese	37/12/51
HOMA-IR	3.2 (2.4)
IR, % <sup>a)</sup>	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 <sup>a)</sup>	13.0 (2.5)
Dietary TAC, mmol <sup>a)</sup>	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 <sup>a)</sup>	233.1 (348.9)
Cereals	174.7 (118.3)
White bread	131.6 (118.4)

Values are means (standard deviations) unless otherwise stated.

<sup>a)</sup> 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.

Version II (Epicenter Biotechnologies, Madison, WI, USA) and was stored at  $-80^{\circ}\text{C}$  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  $\mu\text{L}$  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'-TCCCAGCTATCCCTATCCCTATCCCTATCCCTATCCCTA-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  $2^{\text{CT}(\text{telomeres})}/2^{\text{CT}(\text{single copy gene})} = 2^{-\Delta\text{CT}}$  [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.

### 2.3. Dietary assessment

Trained researchers conducted face to face interviews with participants and their parents based on standardized procedures. A semi-quantitative food frequency questionnaire (FFQ), previously validated in Spain [18], containing 132 food items was filled in, in order to evaluate dietary patterns. The questionnaire was divided into the following categories: dairy products, meat and eggs, fish, fruits and vegetables, legumes, potatoes and cereals, nuts, oils and fat, sweets and sugar sweetened beverages. For each food item, an average portion size was specified, and participants and their parents were asked how often they had consumed that unit throughout the previous year. The glycaemic load for each item was calculated as the total carbohydrate content of each item weighted by its glycaemic index, using published tables for glycaemic index [19].

The dietary TAC value was calculated by adding the TAC values from the ferric reducing antioxidant power assay of each food as previously reported [20] and was expressed as TAC in mmol/100 g food. To assign a value to TAC providing foods not available in previous reports, the data for a similar food item (e.g. same botanical group) were used as a proxy. When TAC values of cooked food were not available, TAC values of fresh food were used to calculate the TAC value [21].

### 2.4. Statistical analysis

LTL was ln-transformed to achieve a better normal distribution conformation. We calculated the Pearson correlation coefficient ( $r$ ) between LTL and dietary TAC after adjustment for age and total energy intake using the residual method.

We fitted a crude model (without any adjustment) and a multivariable adjustment model to assess the effect of macronutrients and food groups on LTL after controlling for the following potential confounders: age, sex, BMI-SDS and total energy intake (Kcal/d). Moreover, dietary TAC and white bread intakes were separately stratified into quintiles, and means and 95% confidence intervals (CI) of LTL were compared in fully adjustment models. Tests for ANOVA and for linear trend across the quintiles were conducted and the lowest consumption of dietary TAC and white bread was taken as the reference group. In addition, to assess the joined effect of dietary TAC and white bread intake, we have cross-classified both exposures, taking as reference category having a low dietary TAC and a high consumption of white bread.

Finally, logistic regression models were performed to calculate the association between dietary TAC or white bread intake and the odds to have the LTL lower than the 10th percentile. Odds ratios (OR) and their 95% CI were determined in a crude and multiple adjusted models according to the increase of 6 mmol (approximately 1 bar of dark chocolate) of dietary TAC [20] and the increase of 1 serving per day (1 serving = 60 g) of white bread consumption.

## 3. Results

The main characteristics of the 287 participants (55% males) are shown in Table 1. The mean age of the children was 11.5 y (SD: 2.5) and the mean BMI-SDS was 2.3 (SD: 2.2). The subjects presented an appropriate macronutrient distribution intake.

A positive correlation was found between dietary TAC and LTL ( $r = 0.157$ ,  $p = 0.007$ ) after adjustment for age and energy intake (Fig. 1). This relationship remained statistically significant in multivariable models ( $\beta = 0.173$ ,  $p = 0.007$ ). The consumption of legumes ( $\beta = 0.132$ ,  $p = 0.032$ ) and the intake of PUFA ( $\beta = 0.136$ ,  $p = 0.019$ ) were also associated with LTL, meanwhile a higher cereal consumption was associated with shorter telomeres ( $\beta = -0.201$ ,

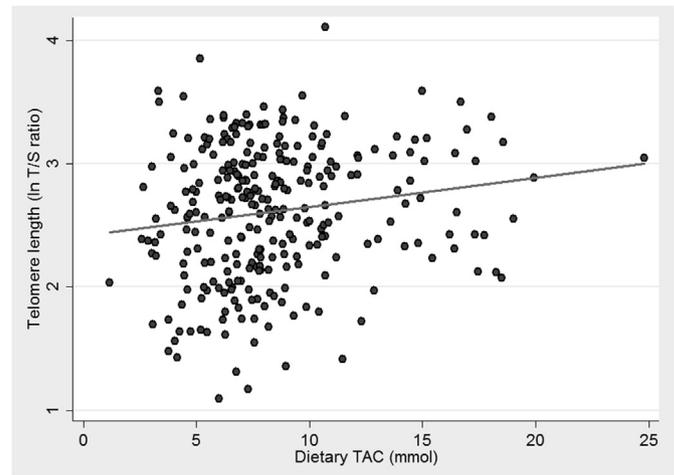


Fig. 1. Correlation between leukocyte telomere length and dietary TAC. There is a highly significant and positive association between LTL and dietary TAC ( $r = 0.157$ ,  $p = 0.007$ ) after adjusting for age and total energy intake using the residual method.

$p = 0.002$ ). Interestingly, glycaemic load was inversely associated with LTL ( $\beta = -0.395$ ,  $p = 0.003$ ). In addition, when analysing the cereals group, white bread appeared to have the major adverse effect on LTL ( $\beta = -0.204$ ,  $p = 0.002$ ) in fully adjusted models (Table 2).

Specifically, we tested for the differences in LTL between quintiles groups of dietary TAC and white bread intake (Fig. 2). Notably, the higher the dietary TAC intake, the longer the telomere ( $p_{ANOVA} = 0.031$ ;  $p_{trend} = 0.005$ ) whilst the higher the white bread consumption, the shorter the telomere ( $p_{ANOVA} = 0.036$ ;  $p_{trend} = 0.002$ ) after adjusting for potential confounders. Therefore, in those subjects with more than 15 mmol of dietary TAC, the LTL was 0.29 higher (95% CI, 0.08–0.50) compared to those who were in the first quintile (Fig. 2a). On the other hand, among children with almost no white bread intake, the LTL was 0.35

Table 2

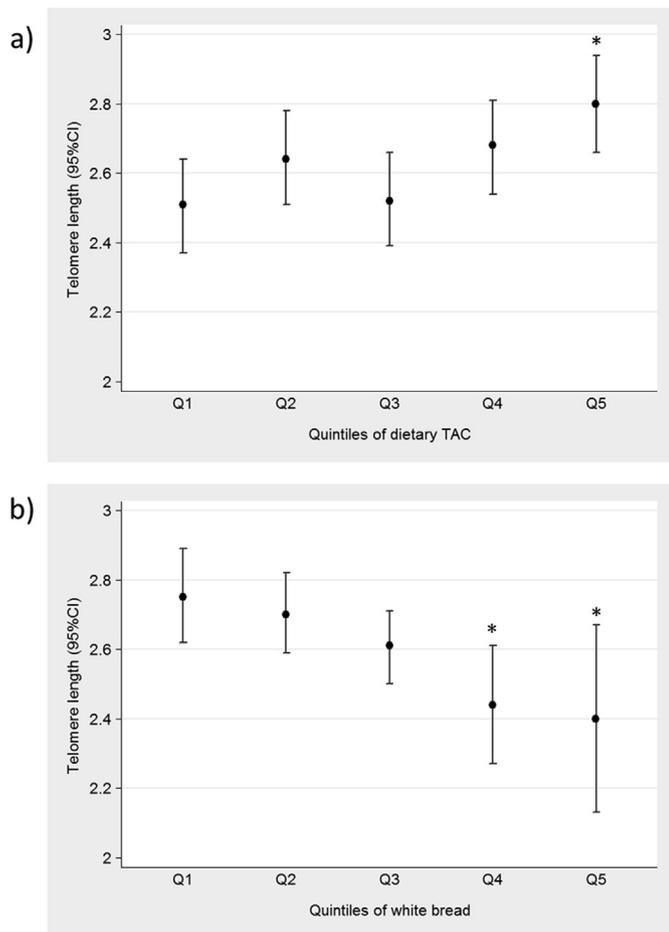
Linear regression between telomere length and total energy intake (kcal/d), dietary TAC (mmol), macronutrients (%E) and foods (g/d).

	Telomere length			
	Unadjusted model		Adjusted for covariates <sup>a)</sup>	
	$\beta$ (SE)	$p$ -value	$\beta$ (SE)	$p$ -value
Energy, kcal/g	-0.057 (0.001)	0.339	0.020 (0.001)	0.735
Dietary TAC, mmol <sup>b)</sup>	0.099 (0.008)	0.092	0.173 (0.008)	0.007
Glycaemic load, units	-0.131 (0.001)	0.021	-0.395 (0.001)	0.003
Carbohydrates, % E	-0.093 (0.006)	0.112	-0.055 (0.005)	0.346
Protein, % E	0.022 (0.013)	0.714	-0.026 (0.013)	0.656
Fat, % E	0.096 (0.007)	0.104	0.075 (0.006)	0.197
MUFA	0.080 (0.011)	0.183	0.034 (0.011)	0.565
PUFA	0.106 (0.021)	0.074	0.136 (0.020)	0.019
SFA <sup>b)</sup>	0.082 (0.013)	0.165	0.099 (0.013)	0.090
Foods (g/d)				
Legumes	0.117 (0.001)	0.047	0.132 (0.001)	0.032
Vegetables	0.015 (0.001)	0.805	0.040 (0.001)	0.499
Fruits	0.063 (0.001)	0.284	0.041 (0.001)	0.489
Fish	-0.030 (0.001)	0.611	-0.015 (0.001)	0.794
Meat	0.043 (0.001)	0.469	0.050 (0.001)	0.421
Dairy products	-0.088 (0.001)	0.136	0.015 (0.001)	0.830
SSSB <sup>b)</sup>	0.006 (0.001)	0.929	0.022 (0.001)	0.741
Cereals	-0.209 (0.001)	<0.001	-0.201 (0.001)	0.002
White bread	-0.209 (0.001)	<0.001	-0.204 (0.001)	0.002

Values are expressed as  $\beta$  coefficient regression (standard error).

<sup>a)</sup> Adjusted for age, sex, BMI-SDS and total energy intake (kcal/d).

<sup>b)</sup> TAC: total antioxidant capacity, SFA: saturated fatty acids, SSSB: sweets and sugar sweetened beverages.



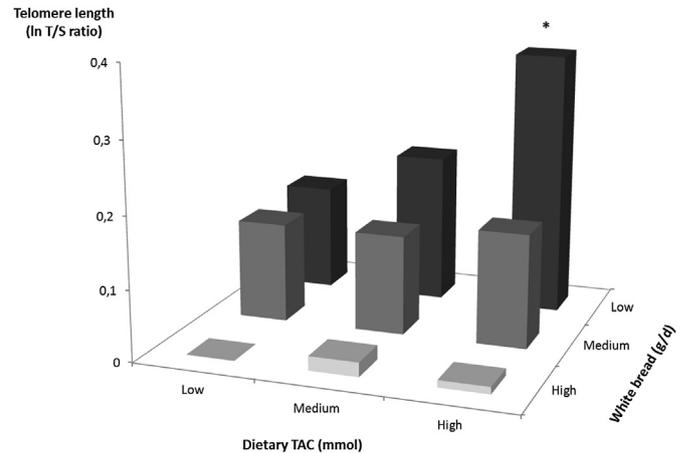
**Fig. 2.** Telomere length by quintiles of dietary TAC (a) and white bread (b) after adjusting for age, sex, BMI-SDS and total energy intake. Quintiles cut-offs were as follows 4.5, 6.1, 7.4, 9.0, 15.0 mmol for dietary TAC and 0.08, 4.8, 6.0, 7.0, 8.0 g/d for white bread consumption. Regarding dietary TAC  $p_{ANOVA} = 0.031$ ,  $p_{trend} = 0.005$  and  $*p_{Q5 \text{ vs. } Q1} = 0.006$ , and in relation to white bread consumption  $p_{ANOVA} = 0.036$ ,  $p_{trend} = 0.002$  and  $*p_{Q5 \text{ vs. } Q1} = 0.025$ ,  $*p_{Q4 \text{ vs. } Q1} = 0.004$ .

lower (95% CI,  $-0.65$  to  $-0.04$ ) compared to those in the highest quintile (Fig. 2b). Moreover, when combining dietary TAC and white bread intakes into three categories, those who simultaneously showed a higher dietary TAC and a lower consumption of white bread significantly presented longer telomeres ( $B = 0.37$ , 95% CI:  $0.09$ – $0.64$ ) than subjects in the group with the lowest dietary TAC and the highest intake of white bread, after multi-variable adjustments (Fig. 3).

The OR of having very low LTL (<10th percentile) was 0.30 according to the increase of 6 mmol (equivalent to 1 bar of dark chocolate) of dietary TAC ( $p = 0.023$ ) and 1.37 for the increase of 1 serving/d of white bread consumption ( $p = 0.025$ ) in fully adjusted models (Table 3).

#### 4. Discussion

In this cross-sectional study of 287 Spanish children and adolescents, we observed that a higher dietary TAC and a lower consumption of cereals, specifically white bread consumption, were associated with longer telomeres. Moreover, there was a 70% lower risk of having very short telomeres according to the increase of 6 mmol (1 bar of dark chocolate) of dietary TAC, meanwhile there was a 37% higher risk of presenting lower LTL for an increase of 1 serving per day of white bread consumption. Interestingly, this is



**Fig. 3.** Mean relative telomere length according to categories of dietary TAC and white bread intake. Adjusted for age, sex, BMI-SDS and total energy intake. Values are expressed as B coefficient regression taking the lowest dietary TAC and the highest white bread consumption as the reference group. Categories for dietary TAC were  $<6.3$  (low), 6.3 to 8.6 (medium) and  $>8.6$  (high) mmol, meanwhile cut offs for white bread intake were as following:  $>150$  (high), 60 to 150 (medium) and  $<60$  (low) g/d.  $*P = 0.010$ .

the first study analysing the effect of diet on LTL in a children and adolescent population.

Dietary TAC is an integrated parameter that considers the overall capacity of all the antioxidants present in food and it has been shown to be inversely associated with risks of chronic diseases such as cardiovascular disease [22]. Previous works by Puchau and other authors [21,23] have calculated the dietary TAC in a reliable manner from FFQ data in adult and children populations. In our study, we proceeded similarly using the FFQ from the SUN study which has been previously validated for estimating nutrient intake [18] and the dietary TAC value [21]. In addition, dietary TAC has been described as a good predictor of dietary and plasma antioxidant status [24], what has been also reported in young adults [25]. In accordance with these findings, we considered dietary TAC as a useful tool for investigating the association between antioxidant status of the diet and LTL.

Telomere attrition expressed in blood cells can serve as a biomarker of the cumulative oxidative stress and inflammation [1]. In fact, telomeres were demonstrated highly sensitive to damage by oxidative stress due to their high content of guanines [26]. It has been suggested that the mechanism involved could be attributable to the detrimental action of hydroxyl radicals which might cause DNA damage in telomere length with each replication of haematopoietic stem cells that is finally expressed in shortened LTL [4]. Nevertheless we cannot discard also a relevant role of peripheral T and B lymphocytes on telomere shortening. In fact the latter pool

**Table 3**

Risk of very low telomere length (<10th percentile) according to the increase of 6 mmol (1 bar of dark chocolate) of dietary TAC and to the increase of 1 serving/day of white bread consumption.

	OR (95% CI)	P-value
Dietary TAC (mmol) <sup>b)</sup>		
Unadjusted	0.42 (0.12–1.04)	0.059
Multiple-adjusted <sup>a)</sup>	0.30 (0.06–0.81)	0.023
White bread (servings/d)		
Unadjusted	1.27 (1.06–1.53)	0.009
Multiple-adjusted <sup>a)</sup>	1.37 (1.04–1.81)	0.025

<sup>a)</sup> Adjusted for age, sex, BMI-SDS, energy intake (kcal/d).

<sup>b)</sup> TAC: total antioxidant capacity.

might contribute more to leukocyte telomere attrition than those derived from haematopoietic stem cells [27]. Since telomere shortening is induced by a chronic increase in the systemic burden of oxidative stress, several studies have reported that antioxidants may prevent telomere attrition [28].

In the present study we found that dietary TAC is associated with longer telomeres. In a report of the GENOI study, dietary TAC was strongly positively correlated with vegetables, fruit, legumes and vitamins ( $p < 0.001$ ) [21]. While some studies found no association between LTL and vegetable and fruit intake [12,14], others have shown longer telomeres in individuals with higher vegetable or fruit intake [11].

Despite we did not detect any association between LTL and vegetables or fruits, which are rich antioxidant foods, a strongly relationship was found with dietary TAC. Due to these findings, we hypothesized that the effect on LTL of the overall antioxidants together, expressed as dietary TAC, was greater than the sum of its individual parts because of their synergistic interactions. Similarly, it is thought that the Mediterranean dietary pattern may have a stronger influence on LTL than considering food groups separately [9]. Indeed, in a previous investigation from our group we found an increase in LTL after 5 years of an intervention study with a Mediterranean diet, in the context of the PREDIMED trial [10].

Another novel feature of our study is that a high intake of white bread was strongly associated with shorter telomeres. There are no previous reports regarding this association, but in disagreement with our study Nettleton et al. [14] found no correlation between refined grains (includes white bread) and LTL in 840 Hispanic adults. White bread has a very high glycaemic load since it is commonly used as the reference food in determining the glycaemic index for the foods [19]. In fact, in this study we found a very strong association between glycaemic load and white bread intake ( $r = 0.634$ ,  $p < 0.001$ ; data not shown), and an inverse association between LTL and glycaemic load has also been observed. Observational and interventional studies suggested that the intake of high glycaemic load foods contribute to the increase of oxidative stress indicators [29] and inflammatory markers [30]. Hence, we speculated that a high intake of white bread could be associated with a higher oxidative stress and inflammation leading to shorter telomeres.

This study has several strengths. We fitted multiple-adjusted models to minimise small differences among individuals and potential confounders. This is the first study showing that a higher intake of dietary TAC is associated with a greater LTL in a children and adolescent population, suggesting that antioxidants from the diet could potentially decrease telomere attrition. On the other hand, the present study has some limitations. First, the cross-sectional design limits the potential to discern causative relationships. Second, there is a possibility of a measurement error as the participants and/or their parents self-reported their dietary intake. However, a registered dietician carefully collected the dietary information through individual sessions and the referred FFQ was previously validated and used in many studies. Third, the study sample consisted of Spanish children and adolescents, of whom 50% were obese; our results could not be applied to the general population.

In conclusion, our cross-sectional study shows that dietary TAC, as a novel measurement of antioxidant intake, is associated with longer telomeres, meanwhile a higher white bread consumption leads to telomere shortening in a population of Spanish children and adolescents. These findings open a new line of investigation about the potential role of an antioxidant diet in maintaining the telomere. Nevertheless, further research is needed to confirm these findings.

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#### Statement of authorship

The authors' contributions were as follows: SG contributed to the data collection, performed the experiments, analysis and writing of the manuscript; AdM performed the experiments and analysed the data. GZ was responsible for the design, the experiments and analyse the data. AmM, M.A.M and J.A.M were responsible of the follow-up, design, financial management and editing of the manuscript. All the authors actively participated in the manuscript preparation, as well as revise and approved the final manuscript.

#### Conflict of interest

None declared.

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