



## Trabajo Original

Obesidad y síndrome metabólico

### Green tea supplementation promotes leukocyte telomere length elongation in obese women

*La suplementación con té verde promueve la elongación de los telómeros de leucocitos en mujeres obesas*

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#### Abstract

**Introduction:** inflammation and oxidative stress are factors that may play a substantial role in telomere attrition. In line of this, obesity is associated with telomere shortening. Green tea had anti-inflammatory and antioxidant effects and may alter telomere length (TL).

**Objectives:** we evaluated the effect of decaffeinated green tea supplementation in obese women on TL.

**Methods:** we conducted a cross-sectional interventional study with ten obese (body mass index [BMI] > 40 kg/m<sup>2</sup>) and eight normal weight (BMI > 18.5 and < 24.9 kg/m<sup>2</sup>) women (age between 27 and 48 years). The supplementation was carried out with capsules (each contained 450.7 mg of epigallocatechin-3-gallate) during eight weeks. Anthropometric and dietary intake assessment, and blood collection (for biochemical and TL analysis by quantitative PCR) were performed before and after supplementation. Normal weight patients were evaluated at a single moment.

**Results:** we observed a significant increase on TL after supplementation ( $1.57 \pm 1.1$  to  $3.2 \pm 2.1$  T/Sratio;  $p < 0.05$ ). Moreover, we found shorter TL in obese patients (day 0) when compared to normal weight individuals ( $3.2 \pm 1.9$  T/Sratio;  $p < 0.05$ ) and an inverse association between TL and BMI, even after age adjustment ( $\beta = -0.527$ ;  $r^2 = 0.286$ ;  $IC = -0.129, -0.009$ ).

**Conclusion:** obesity is related to shorter telomeres. Green tea supplementation during eight weeks promotes telomere elongation in obese women.

#### Key words:

Obesity.  
Epigallocatechin-3-gallate. Green tea.  
Telomere length.  
Supplementation.

#### Resumen

**Introducción:** la inflamación y el estrés oxidativo son factores que pueden jugar un papel importante en el desgaste de los telómeros. En línea con esto, la obesidad está asociada con el acortamiento de los telómeros. El té verde tiene efectos antiinflamatorios y antioxidantes y puede alterar la longitud de los telómeros (LT).

**Objetivos:** evaluamos el efecto de la suplementación de té verde descafeinado en la LT en mujeres obesas.

**Métodos:** realizamos un estudio intervencionista de corte transversal con 10 mujeres obesas (IMC > 40 kg/m<sup>2</sup>) y 8 con peso normal (IMC > 18,5 y < 24,9 kg/m<sup>2</sup>) (edad entre 27 y 48 años). La suplementación se llevó a cabo con cápsulas (cada una contenía 450,7 mg de epigallocatequina-3-galato) durante 8 semanas. La evaluación de la ingesta antropométrica y dietética y la recolección de sangre (para análisis bioquímicos y LT por PCR cuantitativa) se realizaron antes y después de la administración de suplementos. Los pacientes de peso normal fueron evaluados en un solo momento.

**Resultados:** observamos un aumento significativo en LT después de la suplementación ( $1,57 \pm 1,1$  a  $3,2 \pm 2,1$  T/S ratio;  $p < 0,05$ ). Además, encontramos LT más corta en pacientes obesos (día 0) en comparación con individuos de peso normal ( $3,2 \pm 1,9$  T/S ratio;  $p < 0,05$ ) y una asociación inversa entre LT e IMC, incluso después del ajuste de edad ( $\beta = -0,527$ ;  $r^2 = 0,286$ ;  $IC = -0,129, -0,009$ ).

**Conclusión:** la obesidad está relacionada con los telómeros más cortos. La administración de suplementos de té verde durante 8 semanas promueve la elongación de los telómeros en mujeres obesas.

#### Palabras clave:

Obesidad.  
Epigallocatequina-3-galato. Té verde. Telómero.  
Suplementación.

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## INTRODUCTION

Telomeres consist of long stretches of 5'-TTAGGG-3' repeats associated with specific proteins and are located at the end of chromosomes in eukaryotic cells (1,2), promoting chromosomal stability (2) by preventing attrition, end-to-end fusions and chromosomal rearrangements (3). In this context, it is well established that telomere length (TL) of leukocytes is a reliable marker of biological aging (4).

Inflammation and oxidative stress are factors that may play a substantial role in telomere attrition (5). Given that lifestyle factors affect oxidative stress and inflammation pathways, recent studies have showed that they might relate to telomere biology (6). On the other hand, telomere shortening, which is sensitive to the level of oxidative stress, has been associated with chronic diseases such as hypertension, insulin resistance and, mainly, obesity (7). However, the relationship between TL and obesity is still controversial in literature (8,9). Shorter TL was associated with obesity by some authors (10), but other studies showed no association (11,12). Moreover, recently, weight loss has been associated with telomere lengthening (13).

In line with this, recent studies showed a correlation between TL and energy intake (inverse correlation) (14) and dietary antioxidant intake, particularly  $\beta$ -carotene (direct correlation) (15). Supporting the evidence that antioxidant nutrients are related to longer TL, several studies have reported that the intake of antioxidant-rich foods, such as nuts and coffee, is positively related to TL (16).

Emerging evidences have demonstrated that green tea and its most abundant catechin (polyphenol epigallocatechin-3-gallate, EGCG) have anti-inflammatory (17) and antioxidant (18) effects. Thus, considering the association between telomere and oxidative status, green tea components may alter TL. The aim of this study was to evaluate the effect of decaffeinated green tea supplementation in obese women on TL and aid the knowledge of molecular mechanisms of EGCG to improve the treatment of obesity.

## MATERIAL AND METHODS

### PATIENTS

We conducted a cross-sectional interventional study in which ten obese (body mass index [BMI] > 40 kg/m<sup>2</sup>) and eight normal weight (BMI > 18.5 and < 24.9 kg/m<sup>2</sup>) women aged between 27 and 48 years were recruited. Patients were recruited from a previous study of our research group (data not published) that occurred between February 2015 and November 2015. Patients with alterations in liver biomarkers (glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, gamma-glutamyl transpeptidase and alkaline phosphatase) were not included. Patients with a history of metabolic diseases such as diabetes, Cushing syndrome, hypo or hyperthyroidism and dyslipidemias, smokers and patients in use of medications such as antidepressants, antiobesity or hormone therapy, were excluded.

The present study was approved by the Research Ethics Committee of the Clinical Hospital of Ribeirão Preto Medical School, University of São Paulo, SP, Brazil (CAAE: 30247414.6.0000.5440) and conducted according to the Declaration of Helsinki. All participants provided a written informed consent.

### GREEN TEA SUPPLEMENTATION PROTOCOL

The decaffeinated green tea supplementation was carried out with capsules. Each capsule contained 1,009.6 mg of green tea extract and 450.7 mg of EGCG. Other adjuvants included in the capsule were vegetable celluloses, magnesium stearate and silica. All capsules were purchased from the company Solaray<sup>®</sup>, USA (single lot). Patients were instructed to use two capsules per day in the morning for eight weeks.

The patients were attended at the Metabolic Unit of the University Hospital at Ribeirão Preto Medical School, São Paulo University, in three different moments: baseline (day 0), after four weeks (day 28) and after eight weeks (day 56). At day 0, the patients received 56 green tea capsules and the first assessment was performed. At day 28, they returned to the hospital to attend a conference on the capsules and received 56 capsules more. At day 56, they came to the final assessment and another capsules conference was performed. Indeed, patients were contacted weekly by telephone as a form of control of the capsules ingestion.

To ensure the green tea effect and control possible bias, patients were instructed not to change their dietary pattern and physical activity habits.

### DATA COLLECTION

For obese patients supplemented with green tea, data collection occurred on day 0 and day 56. Normal weight patients were evaluated at a single moment. In all the stages, anthropometric and dietary intake assessment and blood collection (for biochemical analysis and the TL) were performed.

### ANTHROPOMETRIC EVALUATION

After emptying the bladder, weight was measured with a Filizola<sup>®</sup> digital scale type platform with 300 kg capacity and 0.2 kg precision, with the participant barefoot and wearing light clothing. Height was measured with a vertical rod with 0.5 cm graduations.

### DIETARY INTAKE ASSESSMENT

Dietary intake was evaluated by a 24-h recall (two were applied on weekdays and one on the weekend). A specific nutrition (Nut-Win-CIS Program of Nutrition Support) was used to calculate the amount of dietary calories, carbohydrate, protein and lipid intake.

## BIOCHEMICAL ASSESSMENT

After 12 hours of fasting, a trained professional collected patients' peripheral blood. Total cholesterol (TC), low-density lipoprotein (LDL cholesterol), high-density lipoprotein (HDL cholesterol), and triglycerides (TG) levels were determined by automated colorimetric, and plasma level of glucose was determined by an enzymatic method.

## QUANTIFICATION OF TELOMERE LENGTH BY QUANTITATIVE REAL-TIME PCR

The DNA was automatically extracted using the Maxwell<sup>®</sup> MDx (Promega Corporation, Madison, WI) instrument and the Maxwell<sup>®</sup> 16 Blood DNA Purification kit, which uses paramagnetic bead separation method. The methodology was modified from original protocol. Blood samples were collected in EDTA tubes, centrifuged at 2,000 x g for ten minutes and 300 ul of buffy coat was used for the DNA purification.

TL was measured according to the protocol described by Cawthon in 2002 (19) by quantifying the relative average of the telomeres by qPCR. The thermal cycler used was the 7500 Fast Real Time PCR System (Applied Biosystems<sup>®</sup>). For the reaction, the SYBR<sup>®</sup> Green PCR Mastermix kit (Qiagen) was used in a final volume of 20 ul. The set of primers used is described in table I. The concentration of primers and sample were modified from the original protocol: 700 nM of each primer and 20 nM DNA were used (these values were selected from the stander curve). The specificity was confirmed through the melting curve in all reactions. The assays were conducted in triplicate. The relative quantification of TL was determined using the telomere to single copy gene ratio (T/S) by calculating the  $\Delta C_t (C_t^{(telomeres)}/C_t^{(single\ gene)})$ . The ratio of T/S for each sample was calculated from the following formula:  $2^{-\Delta C_t(telomere) - \Delta C_t(single\ gene)} = 2^{-\Delta \Delta C_t}$ , following the parameters of Phillip Scheinberg et al. (2010). In this assay, the 36B4 (ribosomal protein large PO) gene was used as a reference for the single copy gene. For the calculation of  $2^{-\Delta \Delta C_t}$  in this assay, each sample was normalized to the average T/S ratio of a reference sample, using the stander curve and validation sample as reference.

## STATISTICAL ANALYSIS

As a preliminary analysis, the Shapiro-Wilk test was used to assess the normality of the data. Paired t test was used to com-

pare variables at day 0 and day 56. The independent t test was used to compare variables between groups. The Pearson correlation between all the variables and TL, and multiple regression analysis with the TL as dependent variable and the other continuous variables (weight, BMI, age, biochemical variables) as the independent ones were performed. The significance level used for the tests was set at  $p < 0.05$ . All analyses were performed by using SPSS Statistics 21.0 (SPSS Inc.).

## RESULTS

Table II summarizes the general features of the patients at baseline and after eight weeks of supplementation. There are no significant weight losses or BMI changes with the intervention. However, significant reductions in cholesterol total and LDL cholesterol levels were observed. All biochemical variables were within the normal range already at baseline.

As expected, there was no change in energy and macronutrients intake during supplementation.

As shown in figure 1, TL was markedly increased after green tea supplementation. Indeed, we found shorter TL in obese patients (day 0) when compared to normal weight individual. There was an inverse correlation between TL and BMI (Fig. 2); however, TL was not correlated with biochemical variables (data not shown). The linear regression analysis confirms the influence of BMI on TL, even after age adjustment ( $\beta = -0.527$ ;  $r^2 = 0.286$ ; IC = -0.129, -0.009).

## DISCUSSION

In our study, a significant increase in TL in obese patients after eight weeks of green tea supplementation was observed. Furthermore, our study is the first to evaluate the TL after this type of intervention. Interestingly, our results showed that BMI influences TL and obese patients have shorter telomeres.

It is still unclear whether telomere shortening is a cause or a consequence of obesity. The oxidative stress and chronic inflammation associated to the obesity condition play an important role on telomere loss (20) and may accelerate telomere attrition (5,21). Elevated reactive oxygen species (ROS) levels may attack G triplets in telomeres, leading to DNA cleavage and, consequently, to telomere shortening and cellular dysfunction (20,22). However, while most of the studies reported a significant association with obesity and shorter TL (9,23,24), a few did not observe any correlation (25-27). In addition, Muezzinler et al. (2014) (24) suggest that there is a biologically plausible inverse association between BMI and leukocyte TL.

Our data are consistent with previously published studies that show telomere lengthening after different strategies to control obesity (13,28,29). In this context, it has been shown that the Mediterranean diet promotes weight loss and longevity, changing TL (28,29). In addition, Carulli et al. (2016) (13) evidenced telomere elongation after six months of bioenteric intragastric balloon.

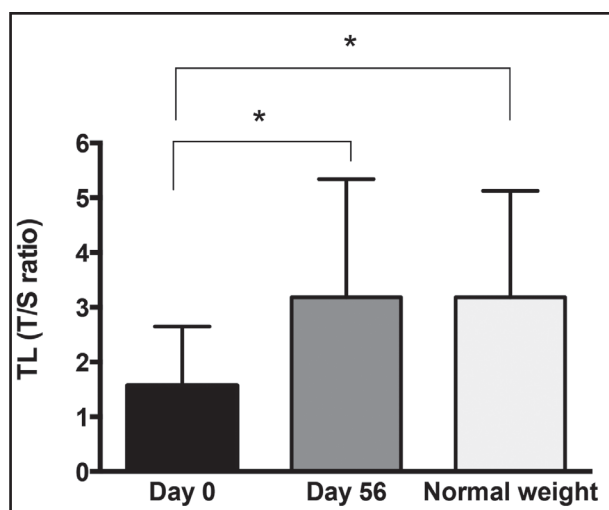
**Table I. Primers designed for telomere quantification**

Primer	Sequence
Tel 1	5'-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3'
Tel 2	5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3'
36B4u	5'-CCCATTCTATCATCAACGGGTACAA-3'
36B4d	5'-CAGCAAGTGGGAAGGTGTAATCC-3'

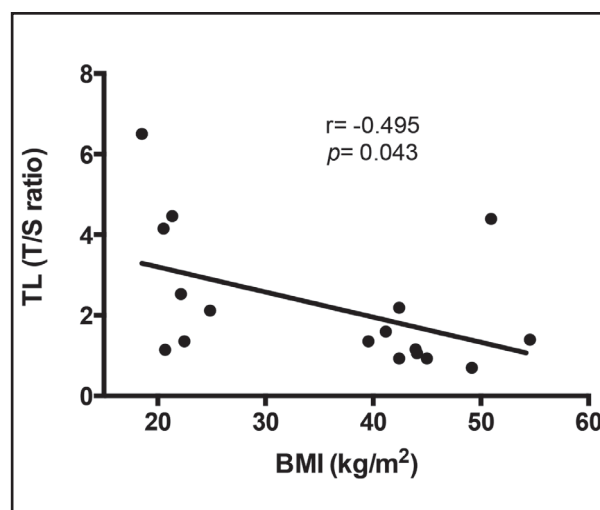
**Table II.** General characteristics of obese patients at baseline and after green tea supplementation and normal weight individuals

	Obese patients (n = 10)			Normal weight patients (n = 8)	p value <sup>†</sup>
	Day 0	Day 56	p value <sup>*</sup>		
Weight (kg)	119.7 ± 17.8 <sup>‡</sup>	120 ± 18	0.772	55.2 ± 5.6	0.000
BMI (kg/m <sup>2</sup> )	45.3 ± 4.7	45.4 ± 5	0.695	21.5 ± 2	0.000
Glycemia (g/dl)	92.2 ± 6.9	92.4 ± 8.5	0.932	84.6 ± 10.6	0.116
Total cholesterol (g/dl)	192.8 ± 33.2	182.4 ± 31.2	0.017 <sup>§</sup>	154.6 ± 10.6	0.065
HDL cholesterol (g/dl)	45.2 ± 7.7	42.7 ± 4.2	0.184	47 ± 9.7	0.711
LDL cholesterol (g/dl)	128.2 ± 20.8	120.2 ± 20.1	0.002	94.4 ± 31.8	0.024
Triglycerides (g/dl)	114.4 ± 46.2	116.3 ± 60.3	0.837	65.6 ± 25.5	0.049
AST (U/l)	17.1 ± 2.7	16.1 ± 2.3	0.162	24.4 ± 7.4	0.013
ALT (U/l)	19.7 ± 6	17.6 ± 4.8	0.060	26.4 ± 19.9	0.335
GGT (U/l)	31.1 ± 10.3	26.7 ± 8.2	0.009	33.8 ± 18.1	0.715
Alkaline phosphatase (U/l)	167.4 ± 33.7	174.3 ± 29	0.410	140.2 ± 39.6	0.187
Energy intake (kcal)	2,438 ± 344	2,309.4 ± 155.7	0.302	1,701.7 ± 628.6	0.008
Protein intake (g)	96.9 ± 19.9	102.2 ± 27.3	0.640	60.7 ± 17.4	0.002
Carbohydrate intake (g)	320 ± 68.6	260 ± 60.6	0.060	221.7 ± 85.8	0.036
Lipid intake (g)	87.3 ± 25.4	93.4 ± 1.4	0.391	71.5 ± 39.7	0.254

BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyl transferase. <sup>\*</sup>Paired t test for comparison between day 0 and day 56 (before and after eight weeks of green tea supplementation). <sup>†</sup>Independent t test for comparison between groups (obese at day 0 and normal weight patients). <sup>‡</sup>Mean ± standard deviation (SD). <sup>§</sup>Bold values indicate  $p < 0.05$ .

**Figure 1.**

TL in obese patients before (day 0) and after (day 56) green tea supplementation and in normal weight patients.  $p < 0.05$ . TL: telomere length.

**Figure 2.**

Inverse correlation between TL and BMI. TL: telomere length; BMI: body mass index.

In spite of the association between weight loss and TL changes in the literature, it is noteworthy that green tea supplementation for eight weeks was not enough to modify weight; however, it modified TL, showing that this changes may be due to tea's compounds.

Green tea flavonoids are an important source of catechins, which are strong antioxidants (30), and epigallocatechin-3-gallate (EGCG) has been attributed to decrease oxidative stress development (31). The antioxidant properties of green tea, especially of its major compound, EGCG, are directly connected to the number and

the position of the hydroxyl (-OH) groups distribute on aromatic ring in the molecule (32). It has been shown that the hydroxyl group contributed to antioxidant activity. This effects was attribute by the electron-donating hydroxyl groups location, the presence of -OH in the position 5- and 7- in the A ring, and the presence of the catechol group (3,4-dihydroxyl) in B ring was directly associated with the antioxidant activity. Another effect that can strongly modulate the potential as free radical scavengers of the catechins is the presence of the gallate group linked in the ring C (33,34).

The relationship between TL and nutrients, foods, and dietary patterns is well documented in the literature. Thus, we know that dietary intake during the period of supplementation may be a factor that interferes with TL. However, there were no changes in energy and macronutrients intake during the intervention period. Moreover, no correlation was found between food intake and TL. Therefore, we assume that the changes found in TL were due to green tea supplementation.

A potential limitation of the present study needs to be considered and is represented by the relatively limited sample size due to the peculiar intervention design of the study.

In summary, the results from this study show that obesity is related to shorter telomeres and, in obese women, green tea supplementation during eight weeks promotes telomere elongation. This indicates that obesity may accelerate the aging process and supports the evidence that the intake of antioxidant-rich foods and beverages affect longevity and health.

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## ETHICAL APPROVAL

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the Declaration of Helsinki of 1964 and its later amendments or comparable ethical standards.

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