



Telomere length elongation after weight loss intervention in obese adults



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ARTICLE INFO

Article history:

Received 12 February 2016

Received in revised form 10 April 2016

Accepted 11 April 2016

Available online 22 April 2016

Keywords:

Obesity

Weight loss

Bioenteric intragastric balloon

Telomere

Telomere length

ABSTRACT

Introduction: Telomeres may be considered markers of biological aging, shorter telomere length is associated with some age-related diseases; in several studies short telomere length has also been associated to obesity in adults and adolescents. However the relationship between telomere complex functions and obesity is still not clear. Aim of the study was to assess telomere length (TL) in adults' obese subjects before and after weight loss obtained by placement of bioenteric intragastric balloon (BIB) for 6 months.

Methods: We enrolled 42 obese subjects before and after BIB placement as weight loss intervention. Blood samples were collected in order to obtain DNA from leukocyte to measure TL by quantitative PCR.

Results: Data were analyzed only in 37 subjects with complete data; all presented important body weight loss (124.06 ± 26.7 vs 105.40 ± 23.14 , $p < 0.001$) and more interesting they presented a significant increase in TL (3.58 ± 0.83 vs 5.61 ± 3.29 , $p < 0.001$). Moreover we observed a significant positive correlation between TL elongation and weight loss ($r = 0.44$, $p = 0.007$) as well as an inverse correlation between TL at baseline and TL elongation ($r = -0.35$, $p = 0.03$). The predictors of TL elongation were once again weight loss and short TL at baseline (respectively $p = 0.007$ and $p = 0.003$).

Conclusions: Our study shows that weight loss is associated to telomere lengthening in a positive correlation: the greater weight loss the greater telomere lengthening; moreover telomere lengthening is more significant in those subjects with shortest telomeres at baseline.

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1. Introduction

Telomeres consist of repetitive DNA sequences (TTAGGG) and are located at the ends of linear chromosomes. They stabilize and protect chromosomes from erosion and from being mistaken for double-strand DNA breaks [1]. During each cell division, telomeres shorten due to the “end-replication problem” that is the DNA polymerase's inability to fully replicate the 3' end of chromosomes. In order to limit telomere attrition, germline and some somatic cells express telomerase, a reverse transcriptase that maintains telomere length by synthesizing new DNA sequences and adding them to the end of the chromosome [2]. Telomerase is an enzymatic protein complex including the telomerase reverse transcriptase (TERT) and the telomerase RNA component (TERC) used as a template to synthesize telomere DNA. When telomeres are too short, they signal the arrest of cell proliferation.

Telomere length (TL) varies between individuals and depends on TL at birth, reflecting a genetic inheritance and on the magnitude of telomere erosion from birth onwards [3] which depends on cell replication rate as well as on aging related exposure to agents that produce DNA damage. Oxidative stress and inflammation are major contributors to

aging and aging related diseases and play an important role in telomere attrition [4–6]. It is well known that obesity is a state of chronic inflammation and great oxidative stress [7].

Available data on the relationship between obesity and TL in adults have shown controversial results; some studies reported an inverse association of TL with obesity [8,9,11], but others did not [12,13] as also recently reported in a review and meta-analysis [14]. Very recently two studies showed a relationship between TL elongation with weight loss as well as with a great adherence to Mediterranean diet [15,16]. To the best of our knowledge there are no data on the relation between weight loss obtained with BIB intervention and telomere length. The Bioenteric Intragastric Balloon is an alternative gastric restrictive procedure. It is a smooth, spherical, saline-filled, silicone elastomer with a radiopaque filling valve. It is inserted endoscopically and left inflated within the stomach. Insertion can be performed under conscious sedation. It is intended to reduce weight by limiting food consumption. The BIB is not permanent and should be removed endoscopically after 6 months to reduce the risk of long-term complications, such as balloon perforation or migration and peptic ulceration [17]. The aim of this study was to assess the relationship between TL and weight loss by means of placement of Bioenteric Intragastric Balloon (BIB) for 6 months in severe obese adults. We hypothesized that a greater weight loss would lead to a greater elongation of TL as has been previously observed in adult and adolescent studies [10,15].

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2. Material and methods

2.1. Study population

The study population included 42 severe obese adults undergoing BIB placement for six months as weight loss intervention together with a lifestyle education program supported by a multidisciplinary team of nutritionists and psychologists. In the present study, we present data before BIB placement and after removal at 6 months. Anthropometric measurements and blood samples were collected for each patient before BIB placement and after its removal, they also underwent an abdominal US in order to evaluate the presence of fatty liver and to calculate the fatty liver indicator as previously described [18]. General characteristics and anthropometric measures of the study population at baseline and after 6 months, are shown in Table 1. Patients were not diabetics. Written consent to participate to the study was obtained from each participant. The study protocol was performed in accordance with the ethical standards of the Declaration of Helsinki and the study protocol was approved by the Institutional Ethics Committee.

2.2. Telomere length assay

Telomere length assay were performed according to Cawthon method as previously described [19,20]. Briefly Telomere length was evaluated with a quantitative polymerase chain reaction (q-PCR)-based assay using SYBR Green PCR mastermix (Applied Biosystems, Carlsbad, CA, USA) in a real-time iCycler IQ system (Bio-Rad Laboratories, Hercules, CA, US). Telomere DNA was amplified using 900 nM Telg primer (5'-ACACTAAGGTTTGGGTTTGGGTTTGGGTTAGTGT-3') and 900 nM Telc primer (5'-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAA-CA-3'); genomic DNA was challenged with 900 nM albu primer (5'-CGCGCGCGCGCGCGCGGCTGGGCGGAAA GCTGCACAGAATCCCTTG-3') and 900 nM albd primer (5'-GCCCGGCCCGCGCGCCGTCC CGCCGGAA AAGCATGGTCGCTGTT-3') to amplify the single copy albumin gene. Telomere length and albumin copy number were evaluated in a single qPCR reaction and samples (20 ng DNA) were measured in five replicates. The thermal cycling profile was: Stage 1: 15 min at 95 °C; Stage 2: 2 cycles of 15 s at 94 °C, 15 s at 49 °C; and Stage 3: 32 cycles of 15 s at 94 °C, 10 s at 62 °C, 15 s at 74 °C with signal acquisition, 10 s at 84 °C, 15 s at 88 °C with signal acquisition. Following amplification, dissociation curves were analyzed to confirm the specificity of the reaction.

Table 1

General characteristics of study population at baseline and after weight loss with BIB treatment.

Data are expressed as mean $\pm$ SD. Abbreviations. SBP: Systolic blood pressure, DBP: diastolic blood pressure, CRP: C reactive protein, TL: telomere length, FLI: fatty liver index, AST: aspartate transaminase, ALT: alanine transaminase ,GGT: gamma glutamyl transferase, ALP: alkaline phosphatase, BIL: bilirubin.

<i>n</i> = 37	PRE BIB	POST BIB	P
Age	40.61 ± 10.12	–	
Sex (M/F)	14/23	–	
Weight (kg)	124.06 ± 26.7	105.40 ± 23.14	0.000
BMI (m ² /cm)	43.9 ± 8.01	39.97 ± 7.72	0.000
Glycaemia	96.92 ± 20	91.00 ± 10.48	0.032
TOT-cholesterol (mg/dl)	190.63 ± 31.07	180.82 ± 32.98	0.086
HDL-cholesterol (mg/dl)	42.39 ± 8.09	43.23 ± 11.62	0.013
LDL-cholesterol (mg/dl)	132.62 ± 28.9	123.59 ± 26.13	0.07
Triglycerides (mg/dl)	138.54 ± 47.42	107.97 ± 39.53	0.000
SBP (mm Hg)	142.32 ± 17.68	127.00 ± 15.65	0.002
DBP (mm Hg)	82.85 ± 11.41	78.00 ± 11.91	0.153
CRP (mg/l)	2.87 ± 12.8	1.05 ± 1.17	0.036
TL	3.58 ± 0.83	5.61 ± 3.29	0.000
FLI	4.53 ± 2.37	2.65 ± 2.25	0.000
AST (U/l)	26.37 ± 12.04	20.80 ± 5.47	0.002
ALT(U/l)	34.50 ± 21.16	23.64 ± 13.78	0.000
GGT(U/l)	28.80 ± 16.66	20.00 ± 13.32	0.000
ALP (mU/ml)	68.11 ± 15.5	64.11 ± 22.43	0.208
BIL (mg/dl)	0.98 ± 0.53	0.94 ± 0.50	0.608

Dissociation curves and Ct values were generated by the iQTM5 Software (Bio-Rad Laboratories). The relative telomere/single copy gene ratio (T/S-ratio value) was calculated as described by as an expression of the relative average LTL in genomic DNA samples.

3. Statistical analysis

As a preliminary analysis, one-sample Kolmogorov-Smirnov test was performed, in order to detect deviations from the normal distribution in the studied variables. The test did not allow in rejecting the null hypothesis for any of the variables studied, and therefore a normal distribution was assumed for the subsequent analyses.

We used the paired *t*-test for comparing the parameters at baseline and after 6 months, expressed as mean $\pm$ SD. With the present patient population, power analysis showed that a predicted observation of a 50% variation in TL from an initial length of 3.5 and a SD of 2 [15,19] could be associated with a type-II error (risk of a false negative result) of 0.01, when keeping a significance level of 0.05.

We also calculated the Pearson correlation coefficient (r) between all the variables and performed a multiple stepwise regression analysis with the variation in TL as dependent variable and the other continuous variables (weight change, pretreatment TL, weight, BMI, age, systolic blood pressure, cholesterol, triglyceride, fatty liver index) as the independent ones.

In order to define gender-related differences in the presentation of the data, correlation analysis was repeated separately for males and females. Furthermore, analysis of covariance was performed, keeping TL variation as the dependent variable, with sex as the fixed factor and the others (age, baseline TL, baseline weight, weight variation) as covariates.

Statistical analyses were performed using SPSS 17 software on a PC workstation. The significance level for all the analyses was set at $P \leq 0.05$. Power analysis was performed with G*Power 3.1 software.

4. Results

The results were analyzed only in a series of 37 subjects having complete data available. Table 1 summarizes the general features of the 37 study subjects at baseline and after 6 months at BIB removal.

Baseline TL was not correlated with age nor with most of the metabolic variables investigated (BMI, total cholesterol, HDL-Cholesterol) ($p > 0.1$, data not shown). A significant correlation was detected between TL and serum triglycerides ($r = -0.36$, $P = 0.028$).

As shown in Table 1, all subjects did significantly lose body weight (124.06 ± 26.7 vs 105.40 ± 23.14 , $P < 0.001$) after BIB placement, which was accompanied by a significant improvement in BMI (43.9 ± 8.01 vs 39.97 ± 7.72 , $P < 0.001$), glycaemia (96.92 ± 20 vs 91.00 ± 10.48 , $p = 0.03$), CRP (2.87 ± 12.8 vs 1.05 ± 1.17 , $p = 0.036$).

We observed significant reductions in HDL-cholesterol (42.39 ± 8.09 vs 43.23 ± 11.62 , $P = 0.013$) and triglycerides (138.54 ± 47.42 vs 107.97 ± 39.53 , $P < 0.001$), but not in total-cholesterol (190.63 ± 31.07 vs 180.82 ± 32.98 , $P = 0.086$), that was within the normal range already at baseline, and in LDL-cholesterol (132.62 ± 28.9 vs 123.59 ± 26.13 , $P = 0.07$). Systolic blood pressure was also reduced after weight loss (142.32 ± 17.68 vs 127.00 ± 15.65 , $P = 0.002$) whereas diastolic blood pressure did not change (82.85 ± 11.41 vs 78.00 ± 11.91 , $P = 0.153$).

TL was markedly increased after weight loss (3.58 ± 0.83 vs 5.61 ± 3.29 , $P < 0.001$).

Fatty Liver Index (FLI), that reflects severity of fatty liver, was significantly reduced after weight loss (4.53 ± 2.37 vs 2.65 ± 2.25 , $P < 0.001$) as well as AST and ALT (respectively, 26.37 ± 12.04 vs 20.80 ± 5.47 , $P = 0.002$ and 34.50 ± 21.16 vs 23.64 ± 13.78 , $P < 0.001$) and ALP, GGT and bilirubin.

As shown in Fig. 1 we observed a positive significant correlation between TL elongation and weight loss suggesting that a greater weight

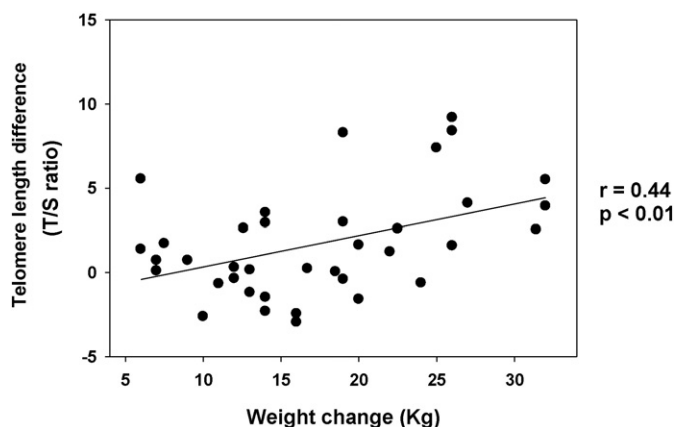


Fig. 1. Telomere length change after weight loss as a function of weight loss change. The scatter plot shows the entire sample ($n = 37$) with each dot representing one individual.

loss was paralleled by a greater telomere elongation ($r = 0.44$, $P = 0.007$).

Moreover as shown in Fig. 2, the telomere elongation was inversely correlated with TL at baseline suggesting that those subjects with longer TL at baseline had a lower elongation despite the weight loss and that the elongating rate was more important in those subjects with short telomere at baseline ($r = -0.35$, $P = 0.03$).

When analyzing the data for men and women separately we observed the same significant results for women in the correlation between TL elongation and weight loss ($r = 0.44$, $p = 0.003$) and in the inverse correlation between telomere elongation and TL at baseline ($r = -0.536$, $p = 0.008$). No significant correlation for men was observed in the correlation between TL elongation and weight loss ($r = 0.429$, $P = 0.126$) nor in the inverse correlation between telomere elongation and TL at baseline ($r = -0.010$, $p = 0.974$). Furthermore, analysis of covariance with sex as the fixed factor and the other variables as covariates showed a significant difference between genders ($F = 3.29$, $P = 0.017$).

At a stepwise regression analysis with TL elongation as dependent variable and all the others features as independent variables we observed that weight loss change and short TL at baseline were the only predictors of TL elongation (model 1, with baseline TL as a predictor: $F = 6.84$, $P = 0.014$; model 2, with baseline TL and weight variation as predictors: $F = 6.85$, $P = 0.004$).

5. Discussion

In this study conducted on 37 obese subjects, a significant increase in TL after 6 months of weight loss intervention was observed. Our results

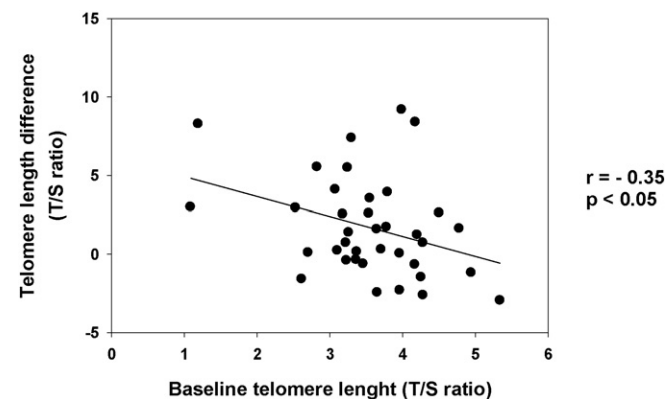


Fig. 2. Telomere length change after weight loss as a function of telomere length at baseline. The scatter plot shows the entire sample ($n = 37$) with each dot representing one individual.

are consistent with previously published studies on the benefits of a healthy lifestyle such as Mediterranean diet and weight loss on telomere length [15,16], therefore in promoting health and longevity.

Interestingly, we observed that TL elongation correlated positively with the body weight loss indicating that the lengthening rate was most pronounced in individuals with a greater weight loss. We showed that weight loss interventions contribute not only on prevention of telomere shortening but also and more important on telomere elongation. The same significant results were observed also when adjusting for age, sex and BMI, indicating these important modulating factors play no role in telomere lengthening in the presence of important weight loss.

When analyzing the data separately for men and women, results were confirmed significant for women but not for men. This could be partly due to the smaller number of men analyzed, compared with women (14 vs 23). At any rate, a gender-specific effect of the changes observed cannot be ruled out, as suggested by analysis of variance; this is also consistent with evidence in the literature showing longer telomeres in females, possibly due to hormonal influences [21,22].

These data are also in concordance with other studies on adults and adolescents on TL and weight loss obtained with life style modifications [15,16].

Garcia-Calzon et al. [15] in the “EVASYON STUDY” showed that weight loss intervention was accompanied by a significant increase of TL in overweight/obese adolescents and that TL at baseline was a better predictor of weight loss.

Moreover in a very recent study Crous-Bou et al. [16] in a population based cohort study showed that a greater adherence to Mediterranean diet was associated with longer telomeres. The mechanism responsible for telomere lengthening may be the reduction in oxidative stress and inflammation which associates to weight loss; it is well established that oxidative stress and chronic inflammation are typical features of obesity and have been reported to accelerate telomere attrition [5–7]. Telomere DNA is very sensitive to damage by oxidation suggesting that oxidative stress plays an important role on telomere loss and that replicative senescence may be considered a stress response blocking the growth of cells that might be at high risk of mutation [23]. Surprisingly our data are not in concordance with the data presented in a recent study [24] conducted on severe obese subjects undergoing bariatric surgery. The Authors showed data on TL in obese patients prior to and 3, 6, 9 and 12 months after bariatric surgery, showing that TL was significantly shorter than baseline at 3, 6, 9 and 12 months after bariatric surgery. Their results confirm that obese subjects have shorter telomeres compared to non-obese subjects. But more interestingly that TL shows an additional attrition during the immediate post-operative period, probably due to a catabolic state.

In fact in a recent study by Laimer et al. [25], in a 10 years a prospective study, on obese subjects undergoing bariatric surgery, it was observed an significant increase in TL after bariatric surgery in the long term, presumably due to the amelioration of the metabolic traits and inflammatory and catabolic state of the immediate post operative period.

In our study, is even more interesting the finding that the change in TL or TL elongation was inversely correlated with TL at baseline suggesting the lengthening rate was most pronounced in individuals with shorter telomeres at baseline. Shortest TL at baseline presented the greatest increase after weight loss, also this finding is in concordance with other studies in the literature [26–28]. Nordfjall K et al. [26], taking two blood samples from the same subjects, after a 10 years interval, observed that telomere attrition rate was significantly inversely correlated with initial TL indicating that the attrition rate was most pronounced in individuals with long telomeres at baseline. Moreover Aviv et al. [27] in the “Bogalusa study” observed that age-dependent TL shortening was proportional to TL at baseline, underlining the complexity of telomere dynamics in vivo and suggesting that other factors in addition to the “end-replication problem” may influence the telomere shortening in vivo. In the Finnish Diabetes Prevention Study [28] the main finding was that TL increased in about two thirds of the individuals after life

style interventions and that TL increased most in individuals with the shortest TL at the first measurement. It is reasonable to think that the TL maintenance mechanisms are mainly focusing on protecting the shortest telomeres [29–31] and challenging the hypothesis that individual TL can predict possible life span or later tumor development.

The data on fatty liver improvement with weight reduction are not only in accordance with the literature but recently Lee et al. showed that weight loss obtained with BIB placement for 6 months provided a great improvement of nonalcoholic fatty liver disease on liver histology [32].

To the best of our knowledge there are no data on weight loss obtained with BIB placement and telomere length and this, in our opinion, represents an important point of interest of the present findings.

Some limitations of the present study need to be considered. First of all the design of our study, both for logistic and ethical considerations, did not include a control group; nonetheless, analysis of the literature and of the data of our historical controls [15,16,19] seem to be consistent with the present results. Another potential limitation is represented by the relatively limited sample size, due to the peculiar design of the study that is precluded to obtain a larger patient population. Post-hoc power analysis, though, confirmed that such population size should have been adequate to detect a statistical significance of the predicted variation in TL, and this was confirmed by the experimental data.

Finally, in this work we considered a short intervention period, and therefore some individuals may be considered erroneously TL gainers as described in the literature [33]. Our results are consistent with the view that, in morbid obese adult subjects, weight loss intervention with bioenteric intragastric balloon did achieve not only weight loss, but an increase in telomere length, indicating that obesity may hasten the aging process and supporting the evidence of a healthy life style on longevity and health. Further larger longitudinal studies are needed to confirm these results and to better understand the complicated association between telomere complex function and obesity as well as obesity related diseases.

Conflict of interest

The Authors declare no conflict of interest.

Informed consent

Written consent to participate in the study was obtained from each participant. The study protocol was performed in accordance with the ethical standards of the Declaration of Helsinki and the study protocol was approved by the Institutional Ethics Committee.

No specific funding was obtained.

Acknowledgments

The Authors acknowledge the essential collaboration provided by all professionals of the Obesity clinic, Internal Medicine ward and the Endoscopy group of the Nuovo Ospedale Civile S. Agostino-Estense, Modena, Italy.

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