Telomere length elongation after weight loss intervention in obese adults.

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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 vs5.61±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.

Key words: obesity , weight loss , bioenteric intragastric balloon,telomere, telomere length.

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 [8,911], 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 six 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 Bioenteritc 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].

Material and Methods

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 patients 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.

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'-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT -3') and 900 nM Telc primer (5'-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3'); genomic DNA was challenged with 900 nM albu primer (5'- CGGCGGCGGGCGGCGCGGGCTGGGCGGAAA GCTGCACAGAATCCTTG-3') and 900 nM albd primer (5'-GCCCGGCCCGCCGCGCCCGTCC CGCCGGAAAAGCATGGTCGCCTGTT -3') to amplify the single copy albumin gene. Telomere length and albumin copy number were evaluated in a single qPCR reaction and samples (20ng 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. 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.

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 to reject 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 ±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 the G*Power 3.1 software.

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 vs91.00±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 vs20.80±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 figure 1 we observed a positive significant correlation between TL elongation and weight loss suggesting that a greater weight loss was paralleled by a greater telomere elongation (r = 0.44, P=0.007).

Moreover as shown in figure 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=0429,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).

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 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 reasonable to think that the TL maintenance mechanisms are mainly focuses 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. showedthat 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 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.

Acknowledgments

Conflict of interest

The Authors declare no conflict of interest

Informed consent

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

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References

1.Blackburn EH (2001) Switching and signaling at the telomere. Cell 106: 661-673 [PMID: 11572773 DOI: 10.1016/ S0092-8674(01)00492-5]

2.Greider CW, Blackburn EH (1985) Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 43: 405-413 [PMID: 3907856 DOI: 10.1016/0092-86 74(85)90170-9]

3.Shalev I, Entringer S, Wadhwa PD, Wolkowitz OM, Puterman E, Lin J et al (2013) Stress and telomere biology: a lifespan perspective Psychoneuroendocrinology 38(9):1835-42. doi: 10.1016/j.psyneuen.2013.03.010. PMID: 23639252

4. Herbert KE, Mistry Y, Hastings R, Poolman T, Niklason L, Williams . (2008)Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured human vascular smooth muscle cells via telomere-dependent and independent pathways. Circ Res 102:201-8. PMID: 17991883

5. Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T et al (2009) A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med15(9):1082-7. doi: 10.1038/nm.2014.

6. Zhang J, Rane G, Dai X, Shanmugam MK, Arfuso F, Samy RP, Lai MK, Kappei D, Kumar AP, Sethi G. Ageing and the **telomere** connection: An intimate relationship with inflammation. Ageing Res Rev. **2016** Jan;25:55-69. doi: 10.1016/j.arr.2015.11.006

7. Suzuki K, Ito Y, Ochiai J, Kusuhara Y, Hashimoto S, Tokudome S et al (2003) Relationship between obesity and serum markers of oxidative stress and inflammation in Japanese. Asian Pac J Cancer Prev 4: 259–266.

8. Lee M, Martin H, Firpo MA, Demerath EW (2011) Inverse association between adiposity and telomere length: The Fels Longitudinal Study. Am J Hum Biol 23:100-6. doi: 10.1002.PMID: 21080476

9.Njajou OT, Cawthon RM, Blackburn EH, Harris TB, Li R, Sanders JL et al (2012) Shorter telomeres are associated with obesity and weight gain in the elderly. Int J Obes (Lond) 36: 1176–1179.

10.Cui Y, Gao YT, Cai Q, Qu S, Cai H, Li HL et al (2013) Associations of leukocyte telomere length with body anthropometric indices and weight change in Chinese women. Obesity (Silver Spring) doi.10.1002/oby.20321.

11.Garcia-Calzon S, Gea A, Razquin C, Corella D, Lamuela-Raventos RM, Martínez JA et al (2013) Longitudinal association of telomere length and obesity indices in an intervention study with a Mediterranean diet: the PREDIMED-NAVARRA trial. Int J Obes (Lond) doi:10.1038/ijo.2013.68.

12.Bekaert S, De Meyer T, Rietzschel ER, De Buyzere ML, De Bacquer D, Langlois M et al (2007) Telomere length and cardiovascular risk factors in a middle-aged population free of overt cardiovascular disease. Aging Cell 6: 639–647.

13. Diaz VA, Mainous AG, Player MS, Everett CJ (2010) Telomere length and adiposity in a racially diverse sample. Int J Obes (Lond) 34: 261–265.

14. Müezzinler A1, Zaineddin AK, Brenner H. Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis.Obes Rev. 2014 Mar;15(3):192-201. doi: 10.1111/obr.12126. Epub 2013 Oct 25.

15.García-Calzón S, Moleres A, Marcos A, Campoy C, Moreno LA, Azcona-Sanjulián MC et al (2014) EVASYON Study Group. Telomere length as a biomarker for adiposity changes after a multidisciplinary intervention in overweight/obese adolescents: the EVASYON study .PLoS One. 24;9(2):e89828. doi: 10.1371, PMID: 24587065

16.Crous-Bou M, Fung TT, Prescott J, Julin B, Du M, Sun Q et al (2014) Mediterranean diet and telomere length in Nurses' Health Study: population based cohort study. BMJ. Dec 2;349:g6674. doi: 10.1136 PMID: 25467028

17. Dąbrowiecki S, Szczęsny W, Popławski C, Sosnowski D (2011)Intragastric Balloon (BIB system) in the treatment of obesity and preparation of patients for surgery - own experience and literature review. Pol Przegl Chir. 83(4):181-7. doi: 10.2478 PMID: 22166356

18. Ballestri S, Lonardo A, Romagnoli D, Carulli L, Losi L, Day CP et al (2012) Ultrasonographic fatty liver indicator, a novel score which rules out NASH and is correlated with metabolic parameters in NAFLD. Liver Int. 32(8):1242-52. doi: 10.1111, PMID: 22520641

19. Carulli L, Dei Cas A, Nascimbeni F (2012) Synchronous Cryptogenic Liver Cirrhosis and Idiopathic Pulmonary Fibrosis: A Clue to Telomere Involvement. Hepatology 56: 2001-2003 DOI 10.1002/hep.26089

20. Cawthon RM (2002) Telomere measurement by quantitative PCR. Nucleic Acids Res 30: e47.

21. Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, et al. Halcyon study team (2014). Gender and telomere length: systematic review and meta-analysis. Exp Gerontol. 2014 Mar;51:15-27. doi: 10.1016/j.exger.2013.12.004. Epub 2013 Dec 21. Review. PubMed PMID: 24365661; PubMed Central PMCID: PMC4523138.

22. Lin J, Kroenke CH, Epel E, Kenna HA, Wolkowitz OM, Blackburn E, Rasgon NL (2012). Greater endogenous estrogen exposure is associated with longer telomeres in postmenopausal women at risk for cognitive decline. Brain Res. 2011 Mar 16;1379:224-31. doi: 10.1016/j.brainres.2010.10.033. Epub 2010 Oct 18.

23. von Zglinicki T. (2002) Oxidative stress shortens telomeres. Trends Biochem Sci 27(7):339-44. PMID: 12114022

24. Formichi C, Cantare S, Ciuoli C, Neri O, Chiofalo F, Selmi F et al. (2014) weight loss associated with bariatric surgery does not restore short telomere length of severe obese patients after 1 year, Obes sur. (24) 2089-93 doi: 10.10077s11695-014-1300-4

25. Laimer M, Melmer A, Lamina C, Raschenberger J, Adamovski P, Engl J, Ress C, Tschoner A, Gelsinger C, Mair L, Kiechl S, Willeit J, Willeit P, Stettler C, Tilg H, Kronenberg F, Ebenbichler C.Telomere length increase after weight loss induced by bariatric surgery: results from a 10 years prospective study.Int J Obes (Lond). 2015 Nov 26. doi: 10.1038/ijo.2015.238. PMID: 26607038

26.Nordfjall K, Svenson U, Norrback KF, Adolfsson R, Lenner P, Roos Get al (2009) The individual blood cell telomere attrition rate is telomere length dependent. PLoS Genet 5: e1000375.

27. Aviv A, Chen W, Gardner JP, Kimura M, Brimacombe M, Cao X et al. (2009) Leukocyte telomere dynamics: longitudinal findings among young adults in the Bogalusa Heart Study. Am J Epidemiol 169: 323–329.

28. Hovatta I, de Mello VD, Kananen L, Lindstrom J, Eriksson JG, Ilanne-Parikka P et al (2012) Leukocyte telomere length in the Finnish Diabetes Prevention Study. PLoS One 7: e34948.

29. Ouellette MM, Liao M, Herbert BS, Johnson M, Holt SE, Liss HS et al (2000) Subsenescent telomere lengths in fibroblasts immortalized by limiting amounts of telomerase. J Biol Chem 275: 10072–10076.

30.Samper E, Flores JM, Blasco MA (2001) Restoration of telomerase activity rescues chromosomal instability and premature aging in Terc2/2 mice with short telomeres. EMBO Rep 2: 800–807.

31. Teixeira MT, Arneric M, Sperisen P, Lingner J (2004) Telomere length homeostasis is achieved via a switch between telomerase- extendible and - nonextendible states. Cell 117: 323–335.

32. Lee YM, Low HC, Lim LG, Dan YY, Aung MO, Cheng CL, Wee A, Lim SG, Ho KY.(2012) Intragastric balloon significantly improves nonalcoholic fatty liver disease activity score in obese patients with nonalcoholic steatohepatitis: a pilot study. Gastrointest Endosc. 76:756-60. doi: 10.1016/j.gie.2012.05.023. PMID: 22840293

33.Steenstrup T, Hjelmborg JV, Kark JD, Christensen K, Aviv A (2013) The telomere lengthening conundrum–artifact or biology? Nucleic Acids Res 41: e131.

Tables and figures legends

Table 1 General characteristics of study population at baseline and after weight loss with BIB treatment.

Data are expresses as mean±SD. Abbrevaitions. 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.

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.

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.

n=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
Glicaemia	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 (mmHg)	142.32±17.68	127.00±15.65	0.002
DBP (mmHg)	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

Table 1

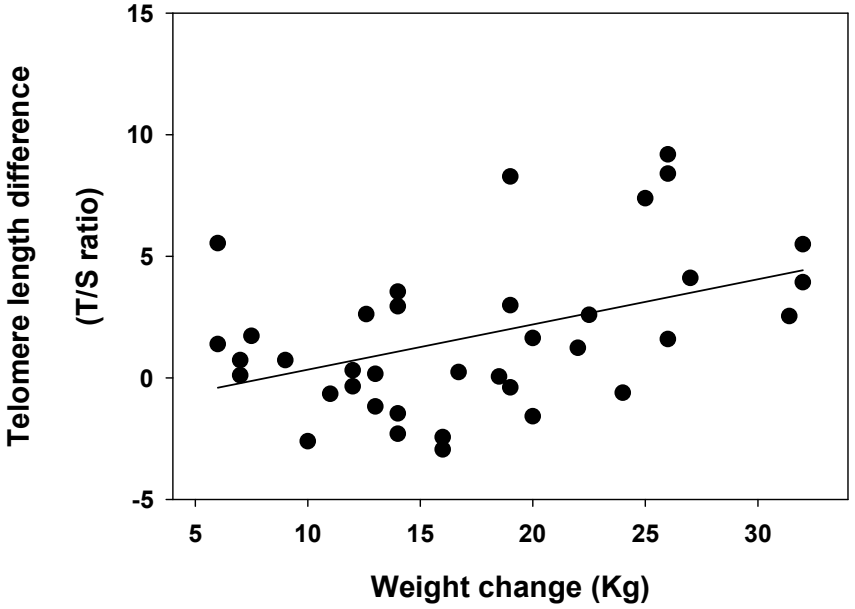
Telomere length difference
(T/S ratio)
15
10
5
0
-5
5
10
15
20
25
30
Weight change (Kg)
r = 0.44
p < 0.01

Fig. 1

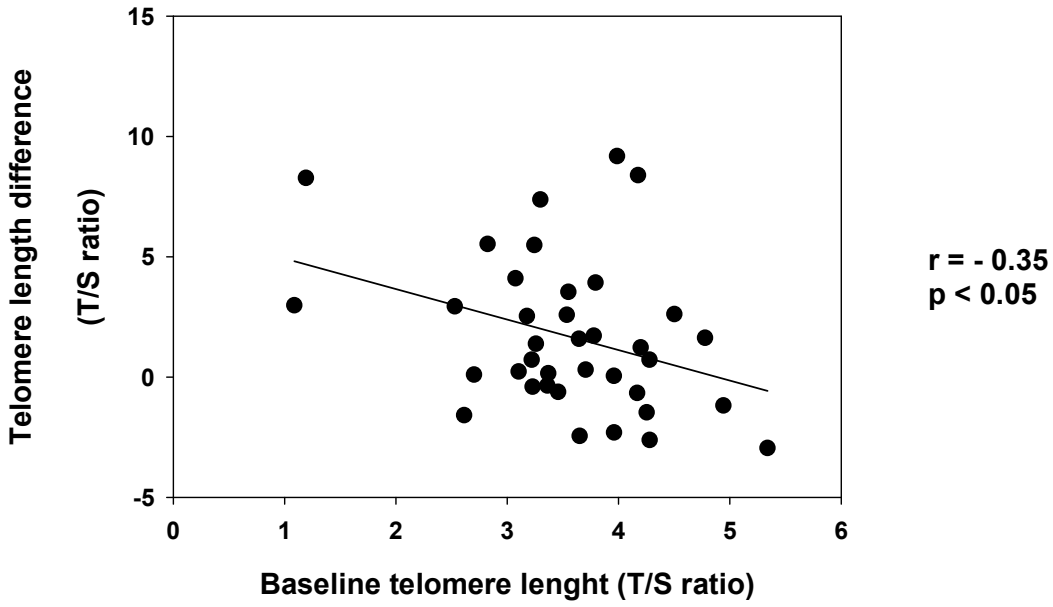
Telomere length difference
(T/S ratio)
15
10
5
0
-5
0
1
2
3
4
5
6
Baseline telomere lenght (T/S ratio)
r = - 0.35
p < 0.05

Fig. 2