Review
EPIDEMIOLOGY/GENETICS

Obesity

Effect of Obesity on Telomere Length: Systematic Review and Meta-Analysis

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Objective: The main objective of this systematic review is to assess the effects of obesity on telomere length.

Methods: The following databases were searched: MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library), LILACS, SPORTdiscus, and Web of Science from inception to August 2014. The search was performed using the following combinations of terms: telomere AND "overweight" OR "obesity" OR "adiposity," without language restriction.

Results: Sixty-three original studies were included in this systematic review, comprising 119,439 subjects. Thirty-nine studies showed either weak or moderate correlation between obesity and telomere length; however, they showed an important heterogeneity.

Conclusions: There is a tendency toward demonstrating negative correlation between obesity and telomere length. The selected studies showed weak to moderate correlation for the main search, and there was an important heterogeneity. For this reason, the causal relationship of obesity and telomere length remains open. Additional controlled longitudinal studies are needed to investigate this issue.

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Introduction

Telomeres are complex structures of ribonucleoprotein at the end of linear eukaryotic chromosomes. They preserve genome information avoiding nucleolytic degradation, recombination, and end-to-end fusion (1). Telomere length (TL) has been postulated as a marker of biological aging, since telomere shortening is a natural aging process that can be accelerated by factors that prematurely induce aging and because it can be attenuated by factors that improve health (2-4). Different health conditions, modulated by oxidative stress (OS), inflammation, and lifestyle variables, have a negative influence on TL, such as cardiovascular diseases (5), smoking emphysema (6) and asthma severity (7). TL, therefore, may be an important biological marker, and the elucidation of the mechanisms involved in the processes of accelerating telomere shortening may help in developing strategies to promote population health.

People suffering with obesity have increased OS (8) and inflammation (9); it has been suggested that obesity might also increase the risk of telomere shortening. The negative health effects of obesity are more relevant in those who have higher central obesity (CO), since it is associated with lower survival rate, even in subjects with normal body mass index (BMI) (10).

The aim of the present systematic review is to critically evaluate and summarize the scientific evidence about the effect of obesity on TL.

Methods

This systematic review follows the MOOSE guidelines (11). The protocol of this review was entered in the PROSPERO database (http://www.crd.york.ac.uk/PROSPERO/) with the record number CRD42014010625.

Search strategy and selection criteria

We searched the following databases: MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library), LILACS, SPORTdiscus, and Web of Science

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from inception to August 2014. We used the strategy of searching the grey literature, contacted leading experts in the field, and reviewing reference lists of other recent systematic reviews.

All articles that assessed the effects of obesity in the TL of humans were included in this review. The search was done combining the following terms: "telomere" AND "overweight" OR "obesity" OR "adiposity," without language restriction. The full search strategy is available in supplementary information and online in: http://www.crd.york.ac.uk/PROSPEROFILES/10625_STRATEGY_20140807.pdf

Eligibility criteria

We included studies that investigated an association between obesity and TL. We defined the following exclusion criteria: (a) obesity, whenever it was used as an adjustment variable; (b) case studies, cases series, experimental models, reviews, responses, and editorial; and (c) duplicated publications or additional studies of already included studies.

Two reviewers, working independently, screened all titles and abstracts to identify studies that could meet the inclusion criteria or that could not be safely excluded without assessing the full text. Divergences were solved by consensus, and when still discordant, a reviewer served as a judge. All eligible papers were abstracted using a data collection form.

Data extraction

The following main variables were extracted from all selected studies: title, first authorship, publication, locale where research was done, year, language, type of study, target population, age of subjects, method of TL evaluation, tissue where TL measurement was done, method for evaluating obesity, effect size, main results, time of follow-up (in cohort studies), sample size, declared limitations, and any other information considered important.

Data presentation and meta-analysis

We performed a random effect meta-analysis of study outcomes when possible and whenever appropriate. Because investigators used heterogeneous methods to assess individual outcomes, a standardized mean difference (SMD) was applied in the analysis. Final values and the standard deviation (SD) of the outcome of interest were extracted to estimate the difference between treatment arms. When meta-analysis was neither possible nor appropriate, studies were synthesized as a narrative.

Three types of meta-analysis were done: SMD, correlations, and odds ratio. In the studies that showed differences of means dividing the subjects into three groups (normal weight—NW, overweight—OW, and obesity—OB), the two extreme groups were used: NW and OB. Metaanalysis was conducted separately for BMI and, to assess the effect of CO, waist-height ratio (WHeR), waist-hip ratio (WHiR), and waist circumference (WC).

Forest plots with point size reflecting study weight were used to graphically represent the results of meta-analysis. We used the Cochrane recommendations for the heterogeneity analysis, which three types of heterogeneity: clinical heterogeneity (variability within participants, interventions and outcomes), methodological heterogeneity (variability in study design and risk of bias), and statistical heterogeneity (variability in intervention effects). Clinical heterogeneity, methodological heterogeneity, or both can cause statistical heterogeneity. In this manuscript, whenever we say heterogeneity we are referring to statistical heterogeneity. The heterogeneity of the combined results was calculated with the I^2 test. Two different software for statistics were used, REVMAN 5.3 and Comprehensive Meta Analyses 2.0.

Subgroup analyses

To reduce heterogeneity, a separate analysis was performed, combining all studies reporting differences in TL, whether through polymerase chain reaction (PCR) or telomere restriction fragment (TRF/Southern blot), the two most frequently used methods to estimate TL; we also analyzed studies that reported data from children and adolescents.

Sensitivity analysis

Meta-analyses were performed once more, removing one study at a time, to check whether heterogeneity was determined by individual studies.

Assessing risk of bias

Different methodological items have been described in the literature as possible sources of bias, and we conferred if the studies complied with them. We considered the following factors: (a) description of the TL evaluation; whenever PCR results were presented, we checked for the use of relative TS ratio or absolute measurements; whenever Southern blot data was present, we checked for measurement of mean or minimum TRF or both; (b) validation of the instrument to estimate obesity; (c) blinding of researcher for TL analysis; (d) data adjustment, at least for age, in TL analysis; (e) sample size, including the calculation; and (f) adequacy of the statistical analysis.

For each item, the following score was arbitrarily attributed: zero (0), if not committed; 0.5 if partially committed; and 1.0 if totally committed. The overall score ranged from 0 to 6.

Results

Figure 1 displays the flow diagram of the search strategy and study selection used in this systematic review. Using this approach, we found 1,197 potential articles in databases and 10 as references in the retrieved studies. After excluding duplicates, 789 studies were selected for further abstract review. Among these, we excluded 716; 23 studies stated that they had used obesity as an adjusting variable in the regression model but did not show numeric results, thus not allowing us to assess the individual effect of obesity on TL; 47 did not look for any association between obesity and telomeres; and in 646, the study design did not meet our inclusion criteria. Sixty-three original studies were finally included in this systematic review, with a total of 119,439 subjects. Table 1 summarizes the major characteristics of the retrieved studies. The method most frequently used for TL assessment was PCR, 47 studies (74%). In the majority of cases, leukocytes were the most assessed cellular response, 51 (80%).

Among the 63 selected studies, 24 (38%), did not find statistically significant variables showing an association between obesity and TL

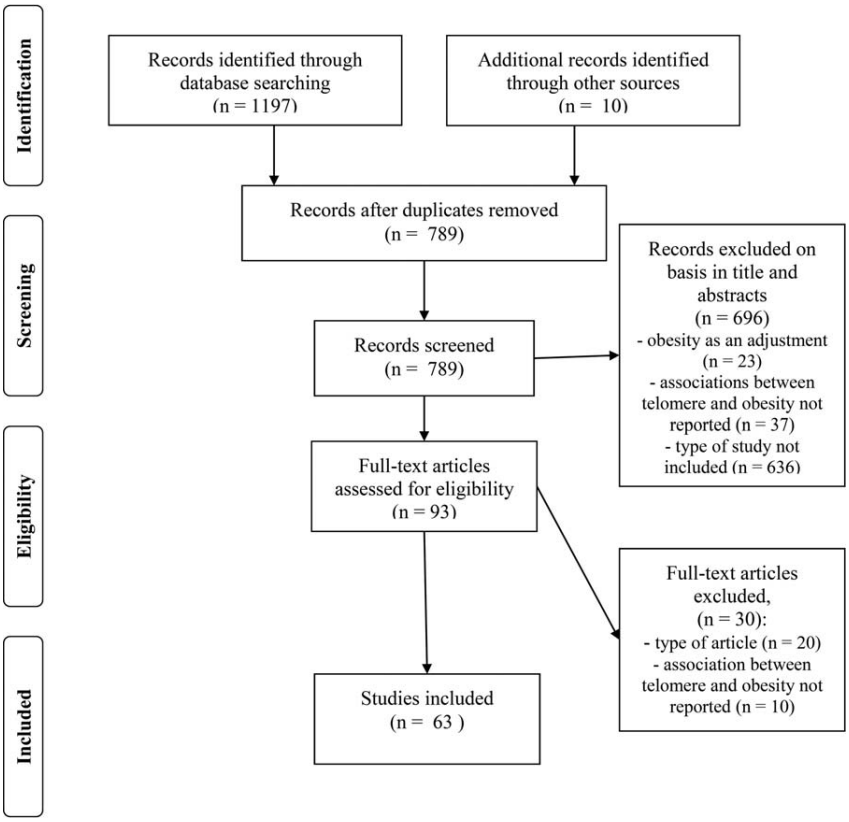

Figure 1 Flow diagram of study selection.

(13,14,31-33,38,42,48-54,58-65,70,73). The remaining 39 studies detected a statistically significant association between obesity and TL, as described in Supporting Information Table S1. Of these, one study found a positive relationship, in women with systemic lupus erythematosus (41). The other 38 studies found an inverse relationship (12,15-30,34-37,39,40,43-47,55-57,66-69,71,72,74). Among these articles, BMI was the most used method to assess obesity; only one study used WC and the adipocytes diameter as a main outcome variable instead of BMI.

Meta-analysis

Standardized mean difference. Figure 2 shows a graphical representation of the meta-analysis including the studies that reported a mean difference in TL between OB and NW (1,947 and 6,063 individuals, respectively), with a SMD of 0.84 (CI 95% 0.22-1.46). The I^2 test depicts an important heterogeneity among studies ($I^2 = 99\%$). The main characteristics of these studies are summarized in Table 2.

As shown in Figure 3 heterogeneity remained high in both subgroups of studies (Southern blot $I^2 = 98\%$ and PCR $I^2 = 99\%$); therefore, type of analysis of TL failed to entirely explain the heterogeneity.

Sensitivity analysis, with the removal of one study at a time did not change the heterogeneity (I^2 was always above 90%, data not shown). We did not find risk of publication bias for the studies reporting difference of means, analyzed by the funnel plot (Supporting Information Figure S1).

Figure 4 graphically represents the meta-analysis of studies that reported mean difference using some index of CO. The SMD is not significant and the heterogeneity is high.

Table 3 summarizes the results of nine articles that reported data for either children or adolescents. Six studies reported inverse associations; two did not present the specific data from children, but an inverse association in the whole sample. In one study, the association was significant only among boys. Three studies did not find statistically significant association between obesity and TL in children. Figure 5A shows the meta-analysis of studies that reported mean difference between children with obesity and normal weight. The SMD between OW and NW is not significant and the heterogeneity among studies was high ($I^2 = 84\%$). Sensitivity analysis (Figure 5B) shows that one study is primarily responsible for heterogeneity (43). The withdrawal of this study, reduced heterogeneity ($I^2 = 30\%$) and the SMD becomes favorable to children of normal weight (SMD = 0.81, CI 95% = 0.55-1.08).

Correlation. The meta-analysis of studies that reported a correlation between BMI and TL is plotted in Supporting Information Figure S2, summarizing data from 48,334 subjects. A trend for a weak inverse correlation was detected (−0.066 CI 95% −0.089

TABLE 1 Main characteristics of the studies included in the systematic review

First author	Country	Method evaluation of telomere	Tissue
Kingma et al. (12)	Netherlands	PCR/TS ratio	Leukocytes
Mason et al. (13)	United States	PCR/TS ratio	Leukocytes
Hovatta et al. (14)	Finland	PCR/TS ratio	Leukocytes
Strandberg et al. (15)	Finland	TRF	Leukocytes
Gardner et al. (16)	United States	TRF	Leukocytes
Huzen et al. (17)	Netherlands	PCR/TS ratio	Leukocytes
Farzaneh-Far et al. (18)	United States	PCR/TS ratio	Leukocytes
O'Callaghan et al. (19)	Australia	PCR/absolute	Rectal mucosa
Njajou et al. (20)	United States	PCR/TS ratio	Leukocytes
O'Bryan et al. (21)	United States	FFISH	CD4+/CD8+
Garcia-Calzon et al. (22)	Spain	PCR/TS ratio	Blood
Buxton et al. (23)	France	PCR/TS ratio	Leukocytes
Cui et al. (24)	China	PCR/TS ratio	Leukocytes
El Bouazzaoui et al. (25)	Netherlands	PCR/TS ratio	AT and ADP
Al-Attas et al. (26)	Saudi Arabia	PCR/TS ratio	Leukocytes
Ma et al. 2013 (27)	China	PCR/TS ratio	Leukocytes
Al-Attas et al. (28)	Saudi Arabia	PCR/TS ratio	Leukocytes
Monickaraj et al. (29)	India	PCR/TS ratio	SAT/VAT
Fernandez-Real et al. (30)	Spain	TRF	SAT
MacEneaney et al. (31)	United States	TRF	EPC
Das et al. (32)	India	PCR/TS ratio	Leukocytes
Sun et al. (33)	United States	PCR/TS ratio EZ	Leukocytes
Cassidy et al. (34)	United States	PCR/TS ratio EZ	Leukocytes
Du et al. (35)	United States	PCR/TS ratio EZ	Leukocytes
Lee et al. (36)	United States	PCR/TS ratio	Leukocytes
O'Donnell et al. (37)	United States	TRF	Leukocytes
Zhu et al. (38)	United States	PCR/TS ratio	Leukocytes
Kim et al. (39)	United States	PCR/TS ratio	Blood
Valdes et al. (40)	United Kingdom	TRF	Blood
Haque et al. (41)	United Kingdom	PCR/TS ratio	Blood
Diaz et al. (42)	United States	PCR/TS ratio	Leukocytes
Zannolli et al. (43)	Italy	TRF	Leukocytes
Al-Attas et al. (44)	Saudi Arabia	PCR/TS ratio	Leukocytes
Nordfjall et al. (45)	Sweden	PCR/TS ratio	Blood
Moreno-Navarrete et al. (46)	France	TRF	SAT
Cherkas et al. (47)	United Kingdom	TRF	Leukocytes
Mirabello et al. (48)	United States	PCR/TS ratio	Leukocytes
Denham et al. (49)	Poland	PCR/TS ratio	Leukocytes
Kim et al. (50)	Korea	PCR/TS ratio	Leukocytes
Bendix et al. (51)	Denmark	TRF	Leukocytes
Song et al. (52)	United States	PCR/TS ratio	Leukocytes
Puterman et al. (53)	United States	PCR/TS ratio	Leukocytes
Tiainen et al. (54)	Finland	PCR/TS ratio	Leukocytes
Cherkas et al. (55)	United Kingdom	TRF	Leukocytes
Prescott et al. (56)	United States	PCR/TS ratio	Leukocytes
Hunt et al. (57)	United States	TRF	Leukocytes
Fitzpatrick et al. (58)	United States	TRF	Leukocytes
Bekaert et al. (59)	Belgium	TRF	Leukocytes
Brouilette et al. (60)	United Kingdom	PCR/TS ratio	Leukocytes
Epel et al. (61)	United States	PCR	Leukocytes
Kiefer et al. (62)	United States	PCR	Leukocytes
Benetos et al. (63)	France	TRF	Leukocytes

TABLE 1. (continued).

First author	Country	Method evaluation of telomere	Tissue
McGrath et al. (64)	United States	PCR/TS ratio	Leukocytes
Bethancourt et al. (65)	Philippines	PCR/TS ratio	Leukocytes
Rode et al. (66)	Denmark	PCR/TS ratio	Leukocytes
Garcia-Calzon et al. (67)	Spain	PCR/TS ratio	Leukocytes
Buxton et al. (68)	Finland	PCR/TS ratio	Leukocytes
Rana et al. (69)	United Kingdom	PCR/TS ratio	Leukocytes
Skilton et al. (70)	?[a]	PCR/TS ratio	Leukocytes
Chen et al. (71)	United States[b]	PCR/TS ratio	Leukocytes
Garcia-Calzon et al. (72)	Spain	PCR/TS ratio	Leukocytes
Weischer et al. (73)	Denmark	PCR/TS ratio	Leukocytes
Formichi et al. (74)	Italy	PCR/TS ratio	Leukocytes

TRF: terminal restriction fragment; PCR: polymerase chain reaction; TS ratio: ratio of telomere repeat copy number (T) to single copy gene copy number (S); EPC: endothelial progenitor cells; AT: adipose tissue; ADP: adipocytes; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; FFISH: flow fluorescence *in situ* hybridization.
[a]Poster abstract.
[b]American Indians.

to −0042). The heterogeneity between studies is large ($I^2 = 73.5\%$) and sensitivity analysis did not change the heterogeneity (I^2 always above 70%, data not shown).

Meta-analysis of the correlation between indices of CO and TL (Supporting Information Figure S3) showed a slightly higher trend toward a negative association (−0.111 CI 95% −0.139 to −0.083). The heterogeneity was high ($I^2 = 81\%$) and the sensitivity analysis did not affect the heterogeneity (data not shown).

To check if a particular method to evaluate CO was significantly correlated with TL, we conducted some sub-analyses. We did not conduct a specific meta-analysis for the assessment of obesity by evaluation of visceral fat because only one study reported this information (69); however, this measure had the highest correlation (−0.402 CI 95% −0.570 to −0.201). The WHeR showed a correlation of −0.149 (CI 95% −0.178 to −0.119, Supporting Information Figure S4A), WHiR −0.096 (CI 95% −0.144 to −0.047, Supporting Information Figure S4) and WC showed a correlation of −0.097 (CI 95% −0.137 to −0.057, Supporting Information Figure S5). Studies of WHeR showed homogeneity ($I^2 = 0.00$), meanwhile studies that correlated WHiR ($I^2 = 82\%$) and WC ($I^2 = 76\%$) showed high heterogeneity.

The funnel plots of the studies that reported a correlation between TL and BMI (Supporting Information Figure S6A) or TL and CO (Supporting Information Figure S6B) showed no evidence of a risk for publication bias.

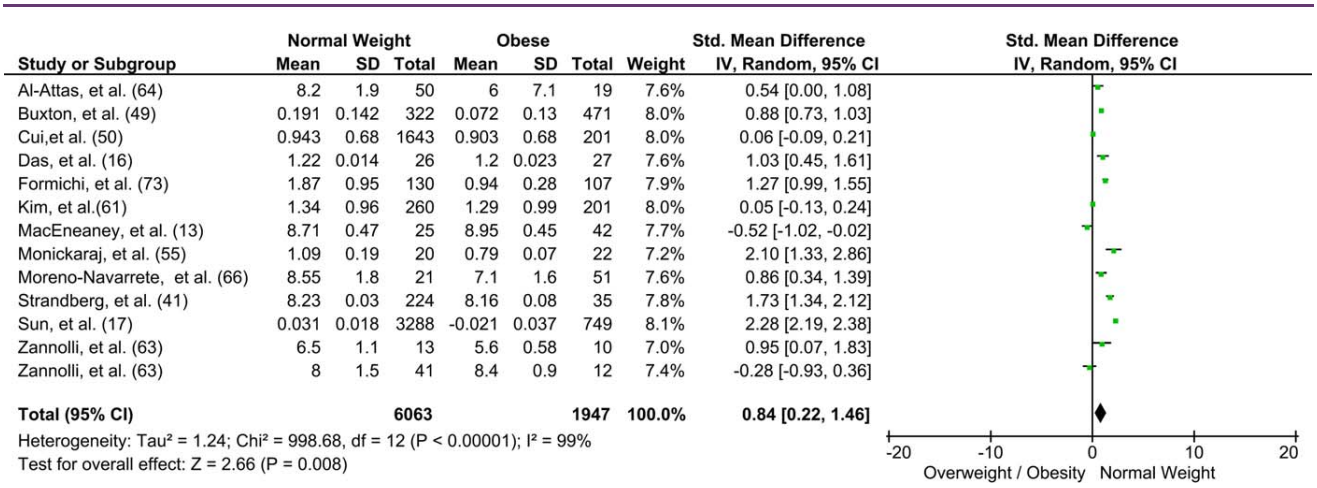

Figure 2 Meta-analysis of studies that reported mean difference in telomere length between individuals with normal weight and obesity. The study Sun et al. (33) shows the *Z* score of the TS ratio. The study Zannolli et al. (43) appears two times because it shows adult and child data seperately. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 2 Studies included in the meta-analysis that compared the means between obesity and normal weight

First author	Method of assessment of telomere, tissue	Normal weight (mean ± SD)	Obese (mean ± SD)	*P* value
Zannolli et al. (43)[a]	TRF, leukocytes	6.56 ± 1.17 Kb	5.68 ± 0.584 Kb	<0.041
Zannolli et al. (43)[b]	TRF, leukocytes	8.0 ± 1.5 Kb	8.4 ± 0.9 Kb	0.402
Buxton et al. (23)	PCR/TS ratio, leukocytes	0.191 ± 0.008	0.072 ± 0.006	<0.0001
Das et al. (32)	PCR/TS ratio, leukocytes	1.22 ± 0.014	1.20 ± 0.023	NS
Al-Attas et al. (44)	PCR/TS ratio, leukocytes	8.2 ± 1.9	6.0 ± 7.1	0.049
Moreno-Navarrete et al. (46)	TRF, leukocytes	8.55 ± 1.8 Kb	7.1 ± 1.6 Kb	0.002
Strandberg et al. (15)	TRF, leukocytes	8.23 ± 0.03 Kb	8.06 ± 0.08 Kb	0.06
MacEneaney et al. (31)	TRF, EPC	8.71 ± 0.47 Kb	8.9 ± 0.45 Kb	NS
Sun et al. (33)	PCR/TS ratio[c], leukocytes	0.031 ± 0.018	−0.021 ± 0.037	NS
Formichi et al. (74)	PCR/TS ratio, leukocytes	1.87 ± 0.95	0.94 ± 0.28	<0.0001
Cui et al. (24)	PCR/TS ratio, leukocytes	0.943 ± 0.68	0.903 ± 0.68	0.005
Kim et al. (39)	PCR/TS ratio, whole blood	1.34 ± 0.96	1.29 ± 0.99	0.03

SD: standard deviation; Kb: kilobase pairs; EPC: endothelial progenitor cells; TRF: terminal restriction fragment; PCR: polymerase chain reaction; TS ratio: ratio of telomere repeat copy number (T) to single copy gene copy number (S).
[a]Data from adults.
[b]Data from children.
[c]*Z* score.

Odds ratio. Only two studies reported odds ratio for having short telomeres related to obesity; a pooled analysis of these articles is graphically shown in Supporting Information Figure S7. The odds ratio for obesity is 1.39 (CI 95% 1.15-1.69). Studies showed homogeneity ($I^2 = 0\%$).

Individual analysis of the quality of selected studies

Initial analysis of the selected studies did not identify possible biases that could compromise the internal validity of the study leading to misinterpretation of the results. The analysis of quality of the

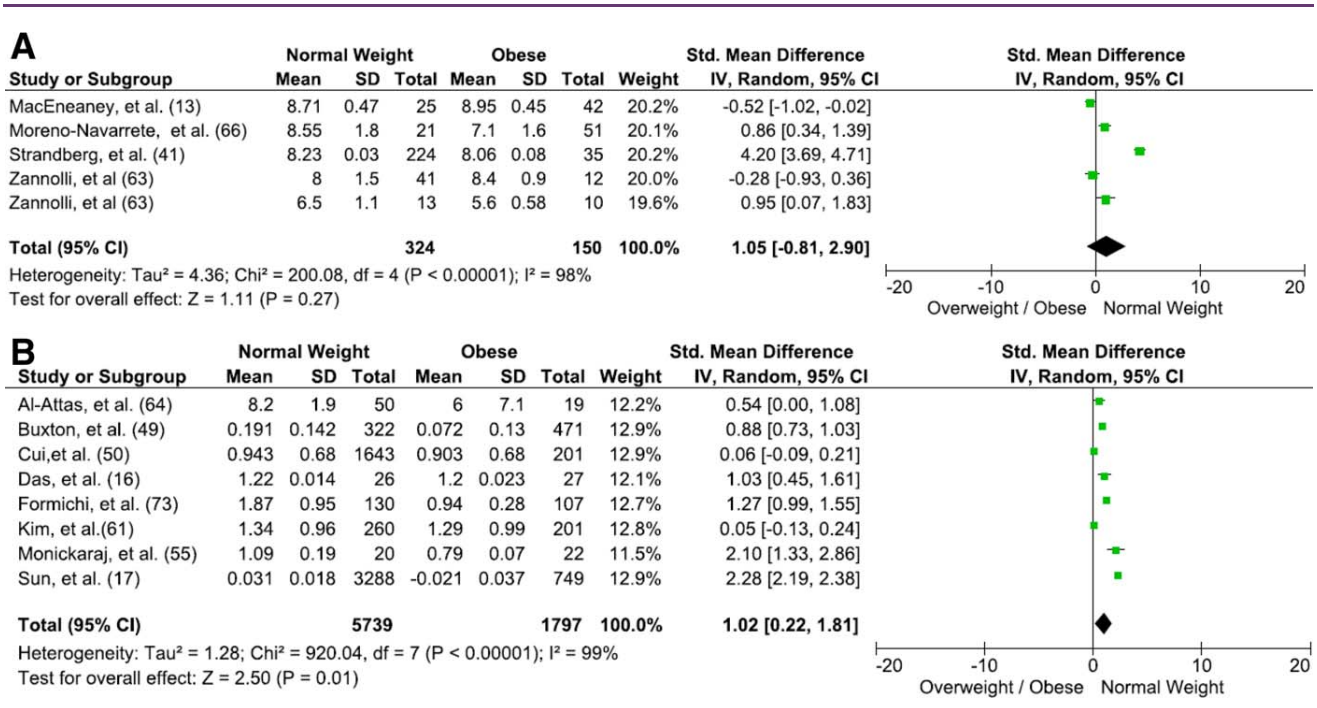

Figure 3 Meta-analysis of articles that measured the length of telomeres via **(A)** Southern blot and **(B)** polymerase chain reaction. The study Zannolli et al. (43) appears two times because it shows adult and child data separately. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

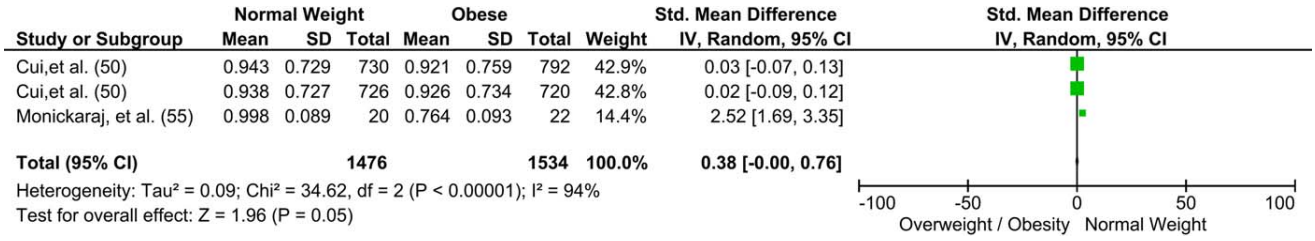

Figure 4 Meta-analysis of studies that reported mean difference in telomere length using indices of central obesity. The study Cui et al. (24) appears two times because it reports data from waist-height ratio and waist-hip ratio. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

selected studies was performed by checking the reports on six different items (Supporting Information Table S2).

The minority of studies reported assessing four out of six items, 27 (42%). Only six studies reported sample calculation, and only nineteen articles reported the blinding of the researchers who analyzed TL in relation to the predictor variables.

Three items were reported by most articles: describing the method of analysis of telomeres, the adjustment of analyses, and statistics. In the statistical analysis, only one article did not score, since data was incorrectly treated as asymmetric, reporting mean and SD.

Articles not included in meta-analysis

Fourteen articles were not included in meta-analysis. Among these, seven did not find statistically significant association, and seven found an inverse association (Supporting Information Table S3).

Discussion

Main findings

The results of our systematic review show a trend toward a negative association between obesity, particularly CO, and TL. However, the pooled analysis showed heterogeneity. The analysis of the funnel plot did not detect risk of publication bias.

TABLE 3 Studies that reported data from children and adolescents (<18 years)

First author	Association in adults	Association in children
Al-Attas et al. (28)	Not specified	Not specified
Al-Attas et al. (44)	Not evaluated	Inverse in boys
Buxton et al. (23)	Not evaluated	Inverse
Lee et al. (36)	Inverse	Inverse
Zannolli et al. (43)	Inverse	NS
Zhu et al. (38)	Not evaluated	NS
Skilton et al. (70)	Not evaluated	NS
Chen et al. (71)	Not specified	Not specified
Garcia-Calzon et al. (72)	Not evaluated	Inverse

NS: no statistically significant.

Although associations between obesity and TL are weak to moderate and many studies did not reach statistical significance, there was a trend toward an inverse correlation between TL and obesity. The only study that reported a positive association (41) was conducted in women with systemic lupus erythematosus, and the authors state that this result might simply reflect a better disease control.

The pooled analysis, exploring SMD, as correlation or odds ratio, confirmed the trend for an inverse association of obesity with TL. When analyzing correlations, CO, especially when measured by WHeR, presented a higher correlation with TL when compared to BMI. We identified two articles that studied the role of visceral fat in TL; one (29) was included in the meta-analysis addressing the SMD with CO and showed a difference of 2.52, favorable to the eutrophic group. The other (69) was included in the meta-analysis addressing association between CO and TL, and it showed the highest correlation. Further studies correlating TL with CO, especially visceral fat, are needed to confirm these findings.

Potential mechanisms of association between telomere shortening and obesity

Among the key factors that may explain the association between telomere shortening and obesity, we emphasize increased OS and the inflammatory processes that accompany obesity (2,3). Increased production of adipokines and OS are likely more related to CO (75). The mechanism by which obesity increases a pro-inflammatory state is probably associated to hyperplasia and hypertrophy of adipocytes, which can be correlated with adipose tissue hypoxia (76); this process likely induces increased production of adipokines, leading to a localized pro-inflammatory state that promotes the development of a systemic pro-inflammatory state (77). The mechanisms that lead to increased OS caused by obesity are not yet fully understood but probably linked to greater production of reactive species and lower intake of antioxidants (78).

Comparison with the literature

Previous narrative reviews have found a negative relationship between levels of obesity and TL; however, the non-systematic nature of such reviews precludes comparing them with our findings, since they lack formal methodological criteria in their

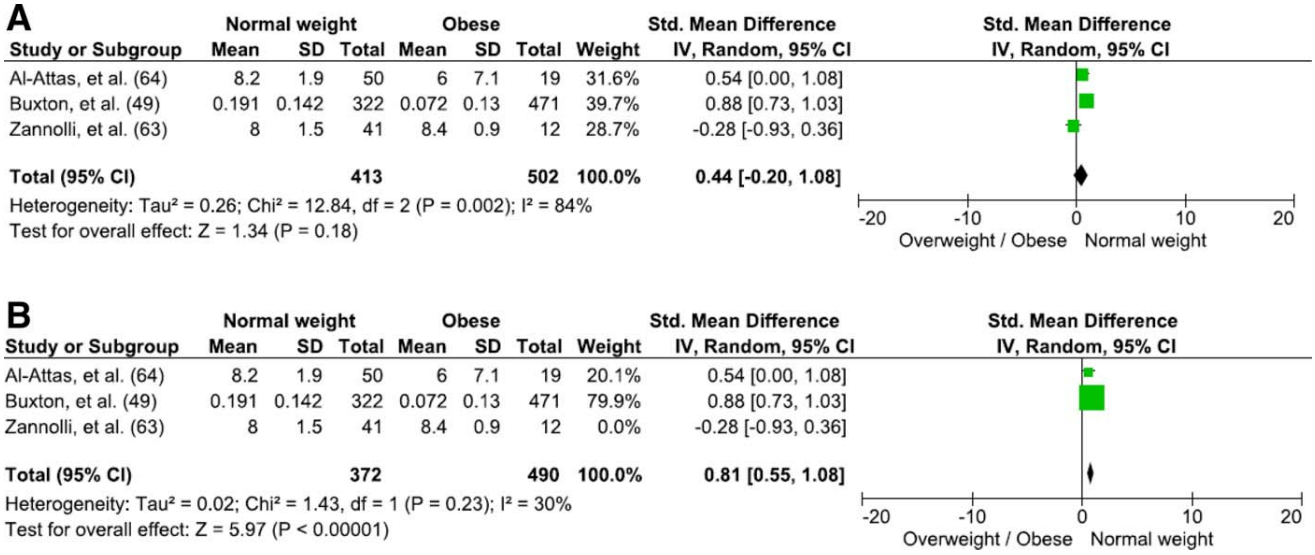

Figure 5 (**A**) Meta-analysis of studies that reported average differences between children with obesity and normal weight. **(B)** Sensitivity analysis: removal of one study at a time. Withdrawal of Zannolli Mohn et al. (43) study significantly reduces the heterogeneity ($I^2 = 30\%$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

selections. Müezzinler et al. (2014) (79) conducted a systematic review addressing the association between obesity and telomeres. This review specifically examined the relationship between BMI and TL of leukocytes and found a trend to a negative correlation but also high variability between studies. Contrarily, our article aims to analyze articles that specifically have reported any method to evaluate obesity type and analyze the role of central fat in this process.

Quality of the studies

In the evaluation of individual quality of the studies, there was no risk of bias that could lead to misinterpretation of results. Taking into account our predefined quality criteria for evaluating TL in obesity, most studies reached minimal acceptability, since they only reported four of six items. It is important to realize that the great majority of the studies evaluated obesity as a secondary outcome. Two important items were poorly reported: sample size calculations (which did not allow us to know whether the researchers were able to find the sample difference sought) and blinding of investigators (which could lead to bias). Adjustment for age (at least) was considered a relevant item for analysis due to the already established relationship of these variables with telomere shortening. Müezzinler et al. (2013) (80) reviewed the relationship between age and TL, concluding that there is an annual loss that could reach 45 base pairs, indicating that the lack of adjustment for age can potentially add bias to the results.

Limitations and strengths of the study

This systematic review and meta-analysis has some limitations; first, the great heterogeneity among the studies that were included warns US to take the results of the pooled analysis data with caution, since they cannot be taken as definitive. Another limitation was the lack of response to contact by the authors of some studies while trying to get additional information. Furthermore, the I^2 test identified a large heterogeneity between studies, reflecting a broad methodological diversity, and therefore, the results of the meta-analysis should be viewed with caution.

Nevertheless, this systematic review also has important strengths. The analysis was not limited by any factor, such as language, type of tissue, or the method of evaluation of TL or obesity. The significant number of databases and the effort to find studies in the grey literature using the database Open Gray (http://www.opengrey.eu/) are efforts that can somehow balance the lack of response to contact by the authors.

This study did not exclude any particular population (i.e., healthy status, age range, race, and gender) which increased study generalizability; nevertheless, this could also contribute to increase the heterogeneity. To verify other sources of heterogeneity (not predicted in the original protocol) we performed the following subgroup analysis: (1) obesity classification, (2) age group (children vs. adults), (3) tissue type (leukocytes vs. adipose tissue), (4) country of origin. However, the analysis did not show any significant change in the results or heterogeneity (data not shown).

Conclusion

There is a tendency toward demonstrating the effect of obesity on TL. Many studies showed weak to moderate statistical significance, and there was an important heterogeneity. For this reason, the question on the causal relationship between obesity and TL remains open. Additional controlled longitudinal studies are needed to investigate this important issue.O

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