# Increased Body Mass Index, Elevated C-reactive Protein, and Short Telomere Length

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**Context:** Obesity is associated with short telomere length. The cause of this association is unknown.

**Objective:** We hypothesized that genetically increased body mass index (BMI) is associated with telomere length shortening and that low-grade inflammation might contribute through elevated C-reactive protein.

**Setting and Design:** We studied 45,069 individuals from the Copenhagen General Population Study with measurements of leukocyte telomere length, BMI, and C-reactive protein in a Mendelian randomization study. Using the three obesity-associated polymorphisms *FTO* rs9939609, *MC4R* rs17782313, and *TMEM18* rs6548238, and the *CRP* promoter polymorphism rs3091244 in instrumental variable analyses, we estimated the associations between genetically increased BMI and telomere length and between genetically increased C-reactive protein and telomere length.

**Results:** In multivariable-adjusted observational analyses, telomere length decreased with seven base pairs (95% confidence interval, −9−−5) per unit increase in BMI, and further adjustment for C-reactive protein attenuated this association to −5 base pairs (−8−−3). In accordance, instrumental variable analysis showed a non-significant telomere length shortening of six base pairs (−37–25) per unit increase in genetically determined BMI. Furthermore, in observational analyses, telomere length decreased with nine base pairs (−16−−2) for a doubling in C-reactive protein, supported by the instrumental variable analyses showing a corresponding genetically determined decrease of 66 base pairs (−124−−7).

**Conclusions:** High BMI is associated with short telomere length observationally. This might possibly be mediated through elevated C-reactive protein, given that genetically elevated C-reactive protein levels are associated with short telomere length. **(*J Clin Endocrinol Metab* 99: E1671–E1675, 2014)**

Increased body mass index (BMI) enhances systemic inflammation and oxidative stress (1). Both processes increase cell turnover and, hence, may accelerate leukocyte telomere shortening (2). Telomeric DNA consists of repetitive tandem repeats of TTAGGG at variable length (3). At each cell division, the telomeres shorten because of limitations in the DNA polymerases. Conditions that increase cell turnover will therefore tend to shorten telomeres (2).

Observationally, increasing BMI is associated with short telomeres (4–6). Confounders such as lifestyle factors, socioeconomic characteristics, and diseases could influence both BMI and telomere length, and the cause of the association is unknown. Entanglement of the causality could widen our understanding of how obesity reduces health. Clarification of the causes of telomere shortening could also suggest how to potentially apply this biomarker in patient care.

Mendelian randomization takes advantage of the random distribution of alleles at meiosis enabling a genetically determined, and thus unconfounded, analysis of the

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Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein.

association between a risk factor such as increased BMI and an outcome such as short telomere length (7). Genotypes associated with BMI (rs9939609 in *FTO*, rs17782313 in *MC4R*, and rs6548238 in *TMEM18*) (8) can be used as instruments in the analyses of the relationship of lifelong exposure to increased BMI on short telomere length, even in the presence of covariates influencing both BMI and telomere length.

The plasma level of C-reactive protein (CRP), an inflammatory marker elevated in overweight and obese individuals (9), is genetically determined by variation in at least eight loci of which the most important is located in the promoter of the *CRP* gene and therefore primarily affects plasma CRP levels (10). This enables a second Mendelian randomization study of the association between genetically elevated CRP levels and short telomere length.

We tested the hypothesis that genetically increased BMI is associated with short telomere length and examined the hypothesis that low-grade inflammation constitutes a pathway between increased BMI and shortened telomeres through elevated CRP.

## Materials and Methods

### Study population

We studied participants from the Copenhagen General Population Study, a Danish general population study which was initiated in 2003 and is still recruiting participants. Details regarding the study population are included in the Supplemental Methods. The study population consisted of 45,069 participants of Danish descent with a telomere length measurement, a BMI greater than18.5 kg/m$^2$ and no more than 50 kg/m$^2$, CRP level measured, and a determined genotype for the three BMI instrument loci, the fat mass and obesity-associated locus (rs9939609, *FTO*), melanocortin 4 receptor locus (rs17782313, *MC4R*), and transmembrane protein 18 (rs6548238, *TMEM18*), as well as the triallelic *CRP* promoter locus rs3091244 (Supplemental Figure 1).

### Telomere length

Telomere length was measured in DNA isolated from blood leukocytes. We used a modified monochrome multiplex quantitative PCR method (11) on a real-time PCR platform. For a complete description of this method, see the Supplemental Methods.

### CRP

High-sensitivity CRP was measured on fresh samples using turbidimetry or nephelometry. The measurement precision was monitored using daily controls, and accuracy was monitored using monthly external controls from a Scandinavian quality control program.

### Genotypes

We used the ABI PRISM 7900HT Sequence Detection System to genotype the *FTO* locus rs9939609, the *MC4R* locus rs17782313, the *TMEM18* locus rs6548238, and the *CRP* locus rs3091244 using TaqMan assays. These polymorphisms were selected a priori as they have been shown to have the strongest association with BMI (8) and CRP (12).

### Statistical analysis

We used Stata version 12.0 (StataCorp). Observational associations were assessed by general linear models. CRP levels were log transformed to approach the normal distribution. For trend tests, individuals were categorized according to BMI deciles coded 1 to 10. The three BMI polymorphisms were combined to an allele score of 0 to 6 constructed as the sum of the number of higher BMI associated alleles.

Associations between genetically increased BMI and telomere length, between genetically increased BMI and CRP levels, and between genetically elevated CRP levels and telomere length were derived from instrumental variable analysis using two-stage least-squares regression. First-stage regression for models using *FTO, MC4R*, and *TMEM18* as instruments for BMI and CRP genotype as instrument for CRP levels, yielded partial R-squareds of 0.0047, and 0.0125, and F-values of 35, and 114. Both F-values are above the conventional limit of 10, and thus, considered to be statistically strong instruments (7).

## Results

Increasing BMI decile was associated with all baseline characteristics examined, except alcohol consumption and cancer (Supplemental Table 1).

The unadjusted telomere length decreased with increasing BMI decile (Figure 1, $P$ trend = $2\times10^{-44}$). The association attenuated after adjustment for age ($P$ trend = $2\times10^{-10}$), but remained after further adjustment for all baseline characteristics except CRP ($P$ trend = $7\times10^{-11}$). When adjustment also included CRP, the association was attenuated further ($P$ trend = $1\times10^{-6}$).

BMI allele score was associated with increased BMI (Supplemental Table 2; $P$ trend = $1\times10^{-46}$) and CRP levels ($P$ trend = $2\times10^{-5}$), but not with any of the potential confounders or with telomere length ($P$ trend = 0.78, Supplemental Figure 2).

Telomere length decreased with 12 base pairs (95% confidence interval (CI) −14–−10) for each unit (in kg/m$^2$) increase in BMI (Figure 2A). Age and multivariable adjustment attenuated this association, and additional adjustment for CRP levels attenuated the association further to −5 base pairs (−8–−3). Instrumental variable analysis yielded a 6-base pair (−37–25) decrease in telomere length per unit genetic increase in BMI. Observational and genetic estimates were not different (Durbin-Wu-Hausman test, $P$ = .60). In women, hormonal levels might influence this association, however, in a stratified analysis, we found

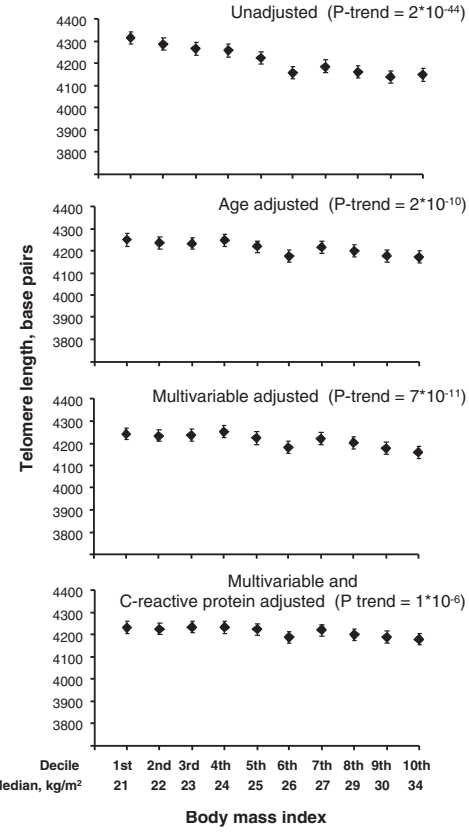


**Figure 1.** Unadjusted, age-adjusted, and multivariable-adjusted telomere length by deciles of BMI. Values are shown as mean and 95% CI of mean.

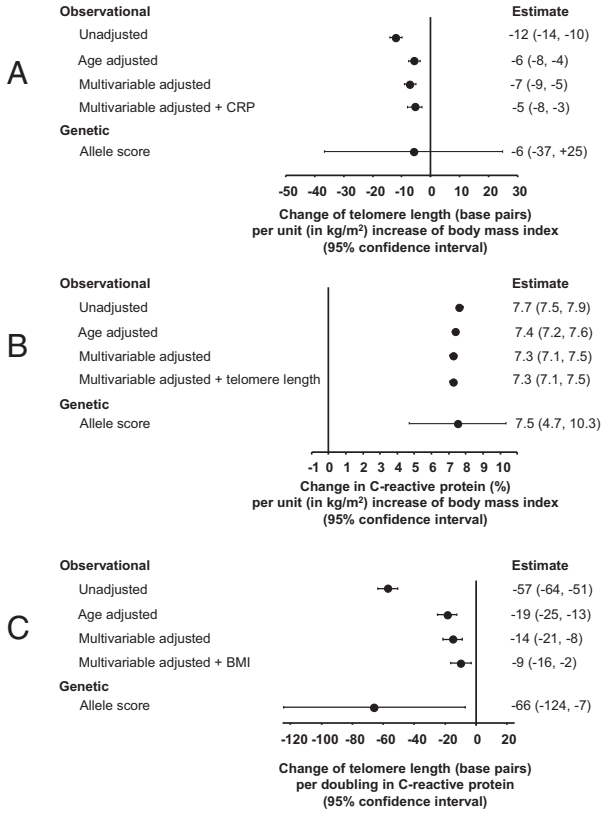


**Figure 2.** Observational and genetic associations of BMI A) with telomere length, B) of BMI with CRP levels, C) and of doubling in CRP levels with telomere length.

similar results in women age below and above 50 years (Supplemental Figure 3).

CRP levels increased with approximately 7.5% per unit increase in BMI in both observational and genetic models (Figure 2B) in accordance with previous findings (13). Therefore, we next conducted a second Mendelian randomization study of the association between genetically elevated CRP and short telomere length.

The triallelic *CRP* promoter polymorphism rs3091244 was associated with CRP levels as expected ($P$ trend = $9\times10^{-113}$), but not with any of the baseline characteristics, including BMI (Supplemental Table 3); however, it was associated with telomere length ($P$ trend = .01, Supplemental Figure 4).

In the unadjusted observational analysis, telomeres were shortened with 57 base pairs (95% CI, −64−−51) for a doubling in CRP levels, but this association attenuated to a 9-base pair shortening (−16−−2) in the multivariable regression model including BMI (Figure 2C). Importantly, instrumental variable analysis showed a shortening of telomeres of 66 base pairs (−124−−7) for a genetic doubling in CRP levels. Observational and genetic estimates were comparable (Durbin-Wu-Hausman test, $P$ = .88).

## Discussion

In this sample of 45,069 individuals high BMI was associated with short telomere length. We hypothesize that this possibly is mediated through elevated CRP, given that genetically elevated CRP levels were associated with short telomere length. This is a novel finding. However, this hypothesis is based on findings in a cross-sectional study and should be tested in longitudinal studies that measure BMI change in relation to telomere shortening before conclusions on causal inferences can be made.

CRP could be part of the biological pathway from increased BMI to short telomere length via low-grade inflammation (14), increased cell turnover in the bone-marrow (15), and possibly subtle changes in the leukocyte distribution (15). CRP acts by binding to damaged cells and by activating the complement system as well as by enhancing phagocytosis by macrophages. CRP also increases generation of oxygen free radicals by neutrophils (16). Given that telomeres are believed to be highly sensitive to damage by oxidative stress (2), it is biologically plausible that CRP, through oxygen free radicals, could damage telomeric DNA and thereby contribute to telomere shortening. Hence, increased BMI and other unhealthy lifestyle factors known to be associated with both increased CRP levels and short telomeres such as smoking,

physical inactivity, and unhealthy diet (2, 6) could each act through this pathway.

Although our observational and genetic estimates of the association between increased BMI and short telomere length pointed in the same direction, our genetic estimate was not statistically significant, and unexpectedly, we could not show an association between increasing BMI allele score and decreasing telomere length. This could be due to lack of statistical power because we would need 2.3 million participants to show this (17). Given that telomere length and BMI are both strongly associated with age and sex, our observational results could be influenced by factors such as hormone levels in women. However, our sensitivity analyses did not suggest differences according to age below and above 50 years in women.

Our observational findings of an association between elevated CRP levels and reduced telomere length are supported by previous studies, where elevated CRP and other inflammatory markers are associated with short telomeres (14). IL-6 and TNF-$\alpha$ stimulate CRP production and might contribute to cell senescence (14). Our results support the hypothesis that elevated CRP level alone might be a cause of telomere shortening, and it is therefore plausible that upstream regulators of CRP would also influence telomere length. CRP could represent a distal factor of several pathways all resulting in shortened telomeres. Unhealthy lifestyles are associated with slightly elevated CRP, which is further increased in many manifest diseases (12, 18). However, Mendelian randomization studies have not found evidence of an association between genetically elevated CRP and these diseases (12, 18, 19). Although it is associated with short telomeres, elevated CRP is also a nonspecific indicator of systemic inflammation and therefore not a likely biomarker for short telomeres.

Biologically, it may seem odd for CRP to be a direct mediator of telomere shortening, and the Mendelian randomization design does indeed entail limitations. One assumption of Mendelian randomization is that the association between the genetic variant and the phenotype is reliably established (7). The genetic variants used in the current study have been selected from previous studies based on their strong associations with BMI and CRP. The rs3091244 polymorphism is located in the promoter region of the *CRP* gene and affects plasma CRP only, without affecting protein structure, and has been found to have the largest effect size among the reported variants (12). However, with a partial R squared of 0.0125, this variant explains only approximately 1% of the variation in CRP levels.

Another assumption of Mendelian randomization is that the genetic variant is independent of confounding factors and that the individual's phenotype is not likely to lead to lifestyle modifications that change the phenotypic response to genotype (7). For the *CRP* polymorphism, there was no association with confounding factors, whereas BMI allele score was borderline associated with cumulative smoking.

A possible limitation of our telomere length measurement is that it only measures an average across chromosomes and not the telomere proportion beyond critical shortening, which might be a better indicator of telomere dysfunction (20). However, as telomere length was strongly associated with age ($P < 1\times10^{-300}$), it does contain biological meaningful information.

In conclusion, high BMI is associated with short telomere length, possibly mediated through elevated CRP, given that genetically elevated CRP levels is associated with short telomere length.

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Author Contributions: L.R., S.E.B., and B.G.N. initiated the study. L.R. did statistical analyses supervised by S.E.B. and B.G.N.. All authors analyzed and interpreted results. B.G.N., M.W., and S.E.B. collected data. L.R. drafted the manuscript, which was scrutinized by the other authors, all of whom accepted the final submitted manuscript.

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