Controlling for baseline telomere length biases estimates of the rate of telomere attrition

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Supplementary Material

Mathematical proof

ΔLTL is estimated from longitudinal datasets in which LTL is measured twice, at baseline ($mLTL_b$) and follow up ($mLTL_{fu}$). The measured ΔLTL (mΔLTL) for the *i*th individual is calculated via the following formula:

$$m\Delta LTL_i = (mLTL_{fu.i} - mLTL_{b.i}) \quad \text{(Equation 1)}$$

Thus, a negative value of mΔLTL indicates telomere attrition and a positive value telomere elongation. An individual's measured LTL can be written as the sum of their true LTL and a measurement error:

$$mLTL_{b.i} = LTL_{b.i} + error_{b.i} \quad \text{(Equation 2)}$$

$$mLTL_{fu.i} = LTL_{fu.i} + error_{fu.i} \quad \text{(Equation 3)}$$

Here, $error_{b.i}$ and $error_{fu.i}$ are the errors introduced by measurement for that individual at baseline and follow-up respectively. We assume that $error_{b.i}$ and $error_{fu.i}$ are drawn from independent distributions. Equation 1 can now be expressed in terms of equations 2 and 3:

$$\begin{aligned} m\Delta LTL_i &= LTL_{fu.i} + error_{fu.i} - (LTL_{b.i} + error_{b.i}) \\ &= \Delta LTL_i + error_{fu.i} - error_{b.i} \end{aligned} \quad \text{(Equation 4)}$$

From equation 4 it is evident that there is an inverse relationship between $m\Delta LTL_i$ and $error_{b.i}$. In other words, a larger positive baseline measurement error for an individual results in a more negative mΔLTL, which implies greater measured telomere attrition, for that individual. This is an example of so-called regression to the mean: baseline values are negatively correlated with measures of change because individuals with high $mLTL_b$ generally have smaller $mLTL_{fu}$ and vice versa.

Supplementary Figures for the simulation model

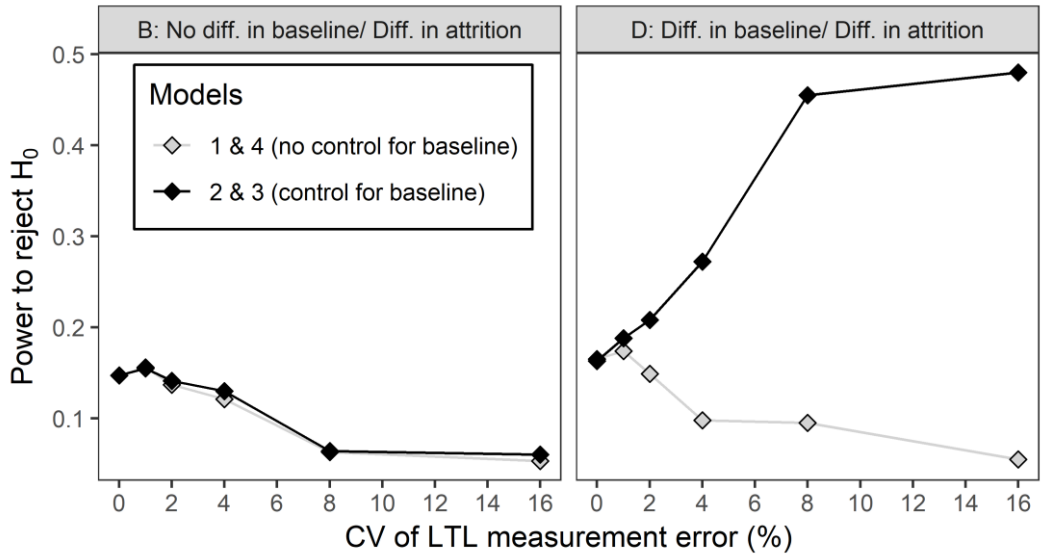

Figure S1. Controlling for LTL_b increases power when there is a difference in LTL_b. Power as a function of measurement error (CV) for the four models under consideration. Data points represent the proportion of simulations yielding a p-value below 0.05 in 1000 replicate simulations. The left and right panels show the power in scenarios B and D respectively. The increase in power with increasing CV in scenario D that occurs with models 2 and 3 reflects the bias in parameter estimates for these models shown in Figure 2D. Power is generally low because of the small true effect size assumed in this simulation of only -2 $bp.year^{-1}$. The difference in LTL_b between smokers and non-smokers in scenario D was LTL_b 141 bp shorter in smokers.

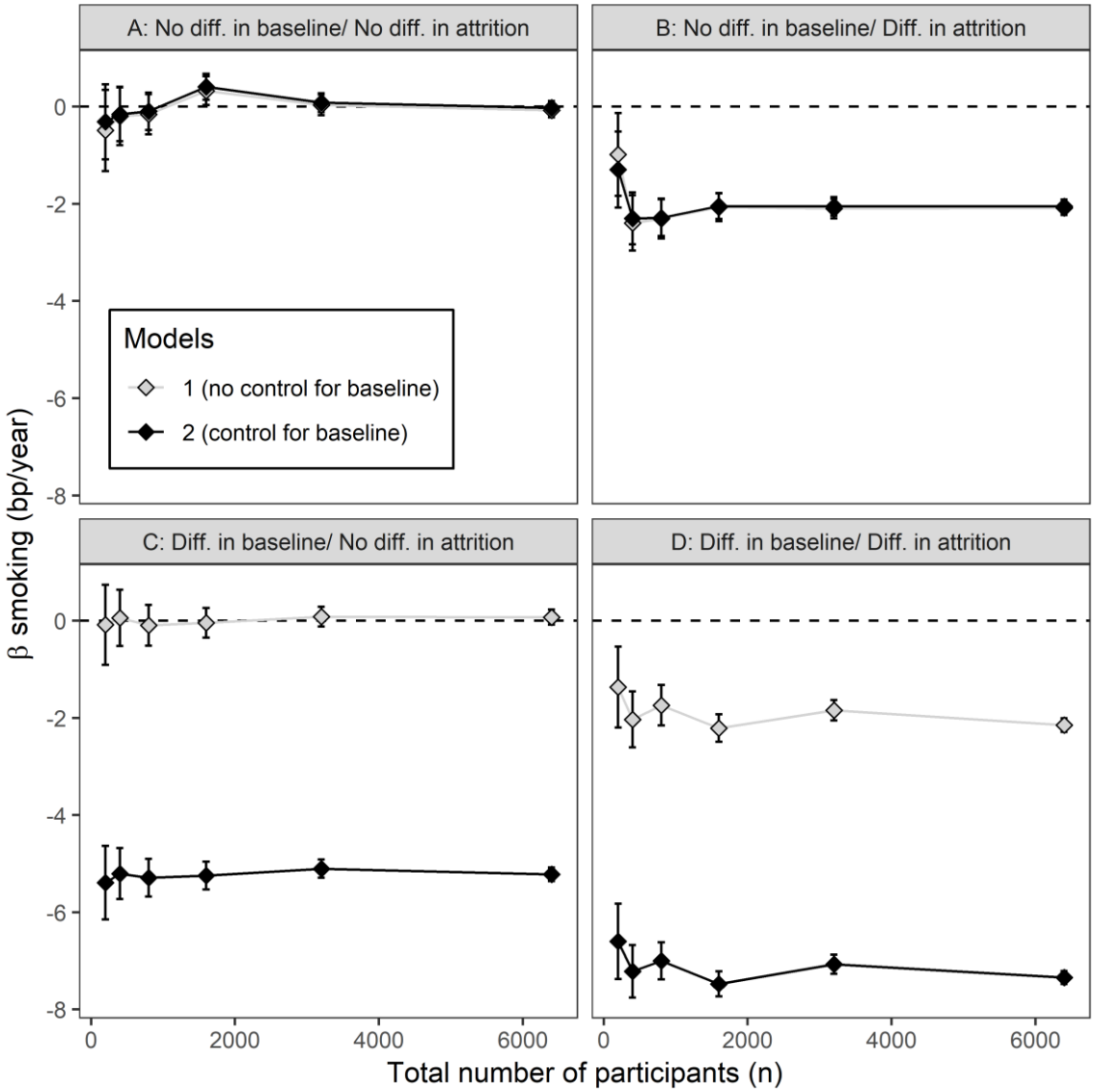

Figure S2. Varying the number of participants in the simulation had no impact on the accuracy of parameter estimates. The estimated difference in mΔLTL between smokers and non-smokers as a function of the number of participants in the simulations. The β estimates were obtained by fitting two alternative models to data simulated given four sets of assumptions regarding the true differences between smokers and non-smokers (scenarios A-D in Table 2). The dashed lines indicate no difference in mΔLTL between smokers and non-smokers. Data points are the mean ± 95% confidence intervals obtained from modelling the data from 1000 replicate simulations. The four scenarios were as follows: (A) no difference in LTL_b and no difference in ΔLTL; (B) no difference in LTL_b but a true difference in ΔLTL; (C) a true difference in LTL_b but no difference in ΔLTL; and (D) A true difference in LTL_b and a true difference in ΔLTL. The true difference in LTL_b between smokers and non-smokers in scenarios C and D was LTL_b 141 bp shorter in smokers. The true difference in ΔLTL between smokers and non-smokers in scenarios B and D was ΔLTL -2 $bp.year^{-1}$ greater in smokers. CV was fixed at 8% for this simulation in order to illustrate the impact of varying participant number.

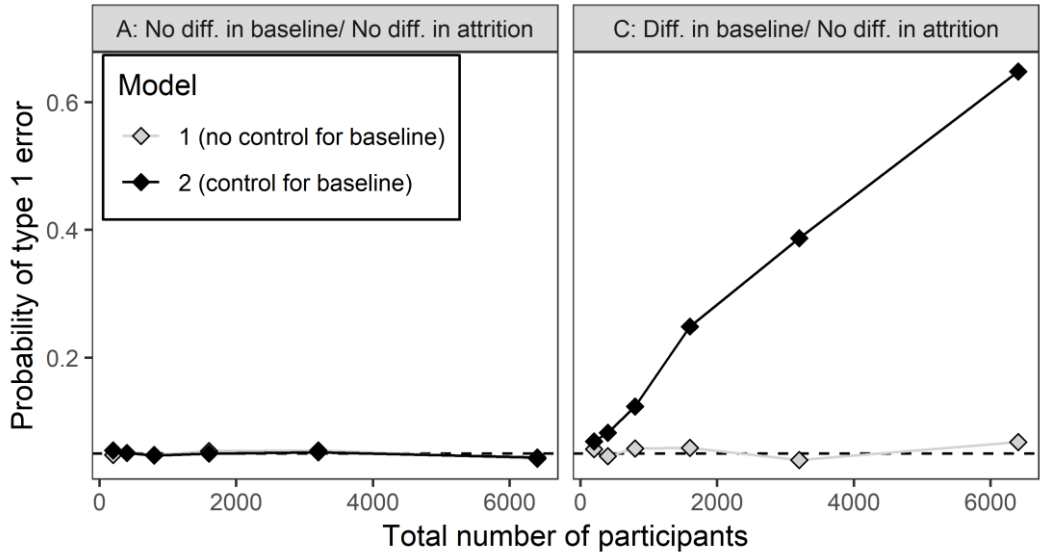

Figure S3. Increasing the number of participants increased the probability of type 1 errors when LTL_b was controlled for in scenario C. Probability of a type 1 error as a function of number of participants for models 1 and 2. Data points represent the proportion of simulations yielding a p-value below 0.05 in 1000 replicate simulations. The left and right panels show the probability of type 1 errors in scenarios A and C respectively. The difference in LTL_b between smokers and non-smokers in scenario C was LTL_b 141 bp shorter in smokers. CV was fixed at 8% for this simulation in order to illustrate the impact of varying participant number.

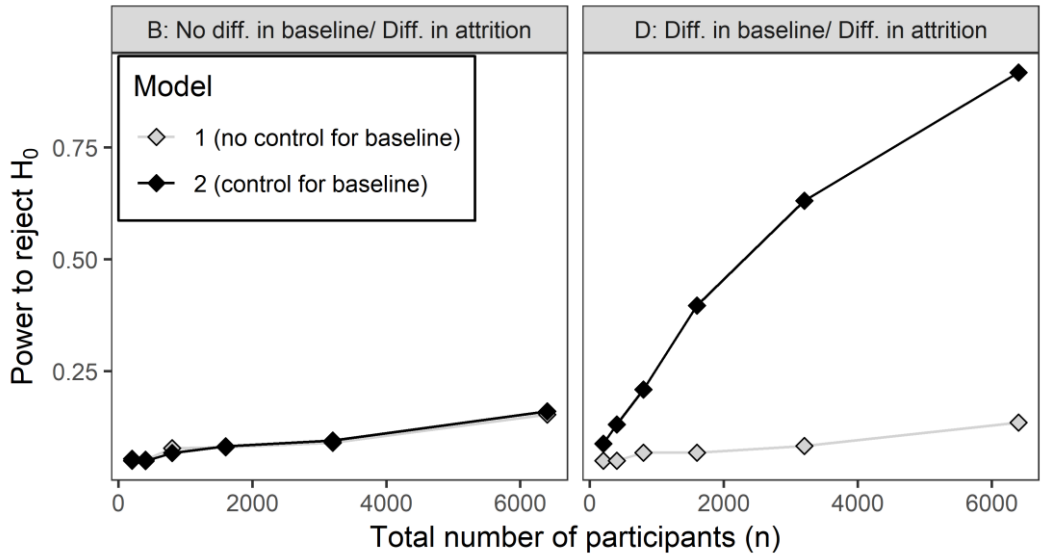

Figure S4. Increasing the number of participants increased the power in all scenarios and this effect was exaggerated by controlling for LTL_b in scenario D. Power as a function of the total number of participants (n) for models 1 and 2. Data points represent the proportion of simulations yielding a p-value below 0.05 in 1000 replicate simulations. The left and right panels show the power in scenarios B and D respectively. The increase in power with increasing CV seen with model 2 in scenario D reflects the bias in parameter estimates shown in Figure S2D. Power is generally low because of the small true effect size assumed in this simulation (-2 $bp.year^{-1}$). The difference in LTL_b between smokers and non-smokers in scenario D was LTL_b 141 bp shorter in smokers. CV was fixed at 8% for this simulation in order to illustrate the impact of varying participant number.

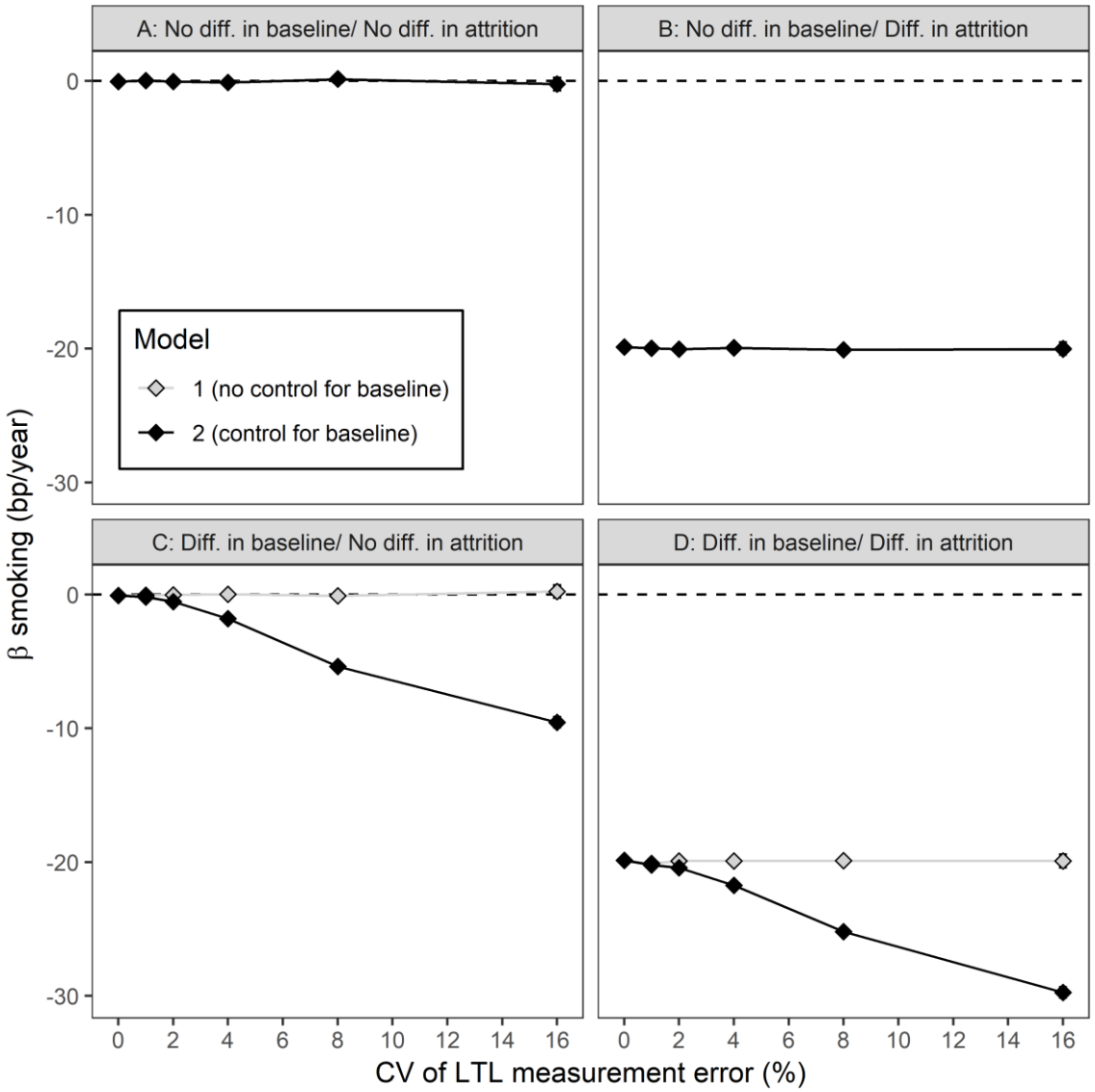

Figure S5. Increasing the true difference in ΔLTL.year^{-1} between smokers and non-smokers had no further impact on the size of the biases compared to Figure 2. The estimated difference in mΔLTL between smokers and non-smokers as a function of measurement error. The β estimates were obtained by fitting two alternative models to data simulated given four sets of assumptions regarding the true differences between smokers and non-smokers. The four scenarios are identical to those given in Table 2, other than that the true difference in ΔLTL between smokers and non-smokers in scenarios B and D was ΔLTL -20 bp.year^{-1} (compared with -2 bp.year^{-1} for the simulation shown in Figure 2). The dashed lines indicate no difference in mΔLTL between smokers and non-smokers. Data points are the mean ± 95% confidence intervals obtained from modelling the data from 1000 replicate simulations.

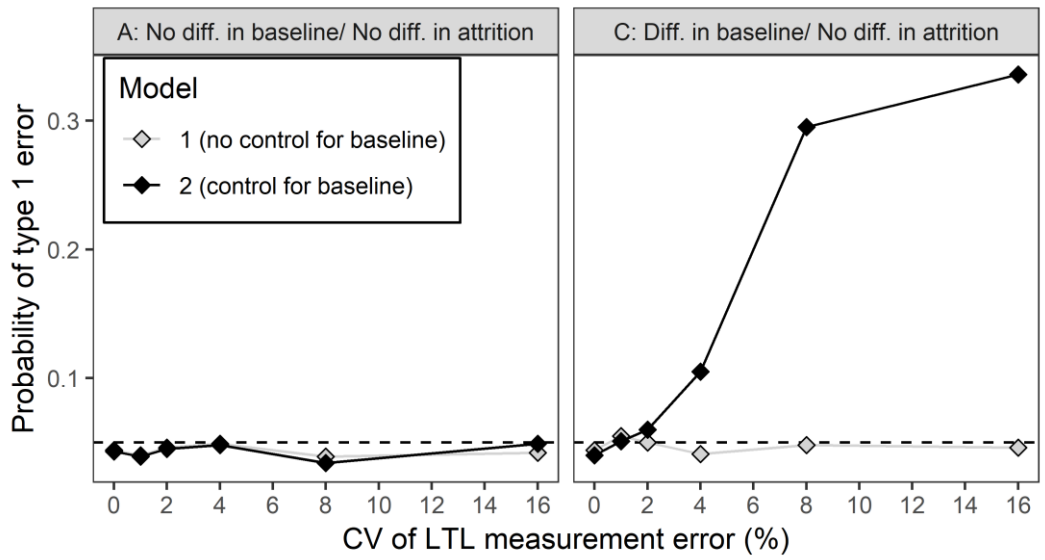

Figure S6. Increasing the true difference in ΔLTL.year^{-1} between smokers and non-smokers had no further impact on type 1 errors compared to Figure 3. Probability of a type 1 error as a function of measurement error (CV) for models 1 and 2. Data points represent the proportion of simulations yielding a p-value below 0.05 in 1000 replicate simulations. The left and right panels show the probability of type 1 errors in scenarios A and C respectively. The difference in LTL_b between smokers and non-smokers in scenario C was LTL_b 141 bp shorter in smokers.

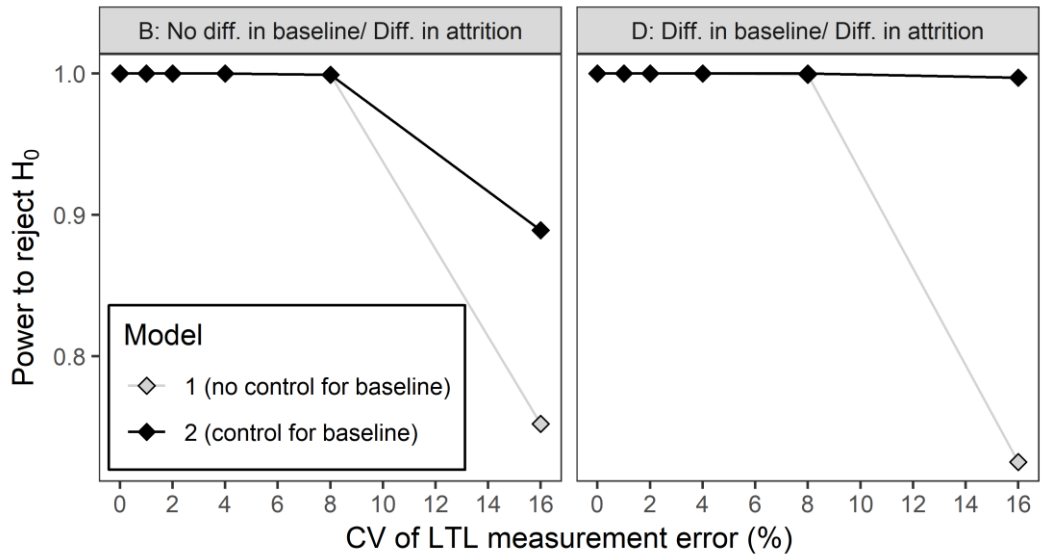

Figure S7. Increasing the true difference in ΔLTL.year^{-1} between smokers and non-smokers increased power compared to Figure S1. Power as a function of measurement error for models 1 and 2. Data points represent the proportion of simulations yielding a p-value below 0.05 in 1000 replicate simulations. The left and right panels show the power in scenarios B and D respectively. The higher power at a CV of 16% seen with model 2 in scenario D reflects the bias in parameter estimates shown in Figure S5D. Power is generally high because of the large true effect size assumed in this simulation (-20 bp.year^{-1}). The difference in LTL_b between smokers and non-smokers in scenario D was LTL_b 141 bp shorter in smokers.

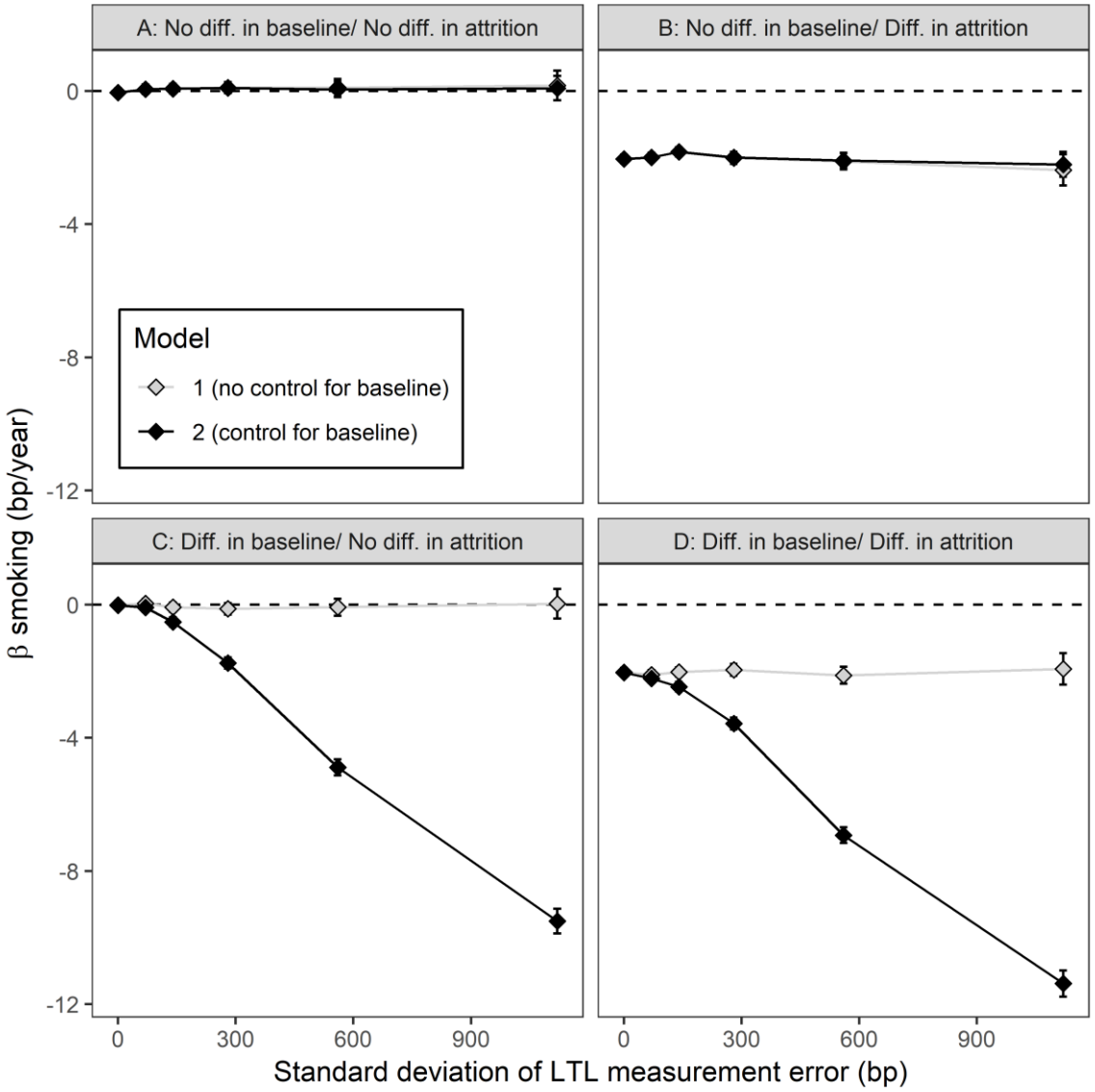

Figure S8. Assuming measurement error to be independent of LTL has no impact on the bias resulting from controlling for LTL_b compared to Figure 2. The estimated difference in mΔLTL between smokers and non-smokers as a function of measurement error here implemented as a fixed standard deviation (as opposed to as a CV in Figure 2). The β estimates were obtained by fitting two alternative models to data simulated given four sets of assumptions regarding the true differences between smokers and non-smokers. Data points are the mean ± 95% confidence intervals obtained from modelling the data from 1000 replicate simulations. The four scenarios are identical to those given in Table 2 and plotted in Figure 2. The dashed lines indicate no difference in mΔLTL between smokers and non-smokers. The true difference in LTL_b between smokers and non-smokers in scenarios C and D was LTL_b 141 bp shorter in smokers. The true difference in ΔLTL between smokers and non-smokers in scenarios B and D was ΔLTL -2 $bp.year^{-1}$ greater in smokers.

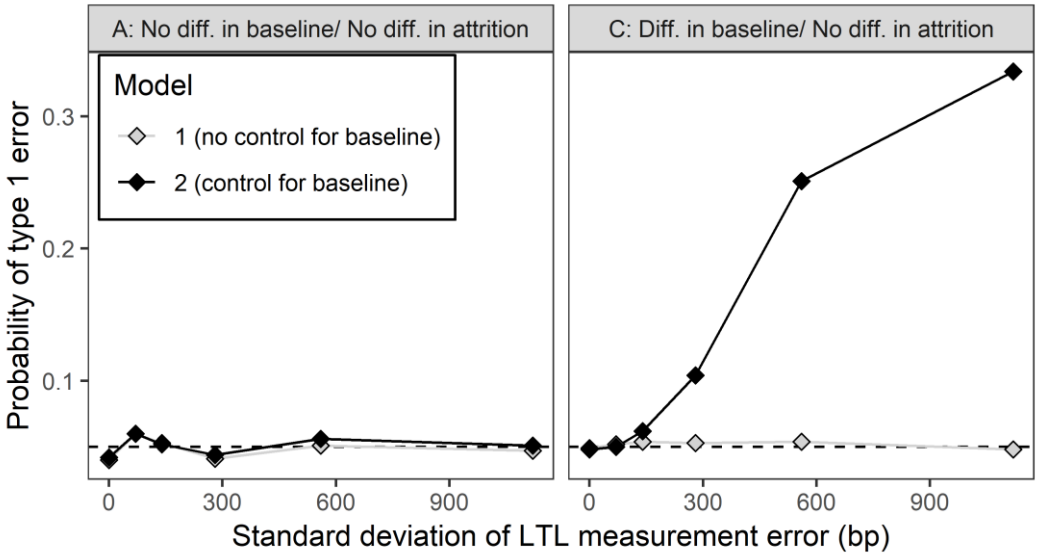

Figure S9. Assuming measurement error to be independent of LTL has no impact on the increased probability of false-positive errors resulting from controlling for LTL_b compared to Figure 3. Probability of a type 1 error as a function of measurement error here implemented as a fixed standard deviation (as opposed to as a CV) for models 1 and 2. Data points represent the proportion of simulations yielding a p-value below 0.05 in 1000 replicate simulations. The left and right panels show the probability of type 1 errors in scenarios A and C respectively. The difference in LTL_b between smokers and non-smokers in scenario C was LTL_b 141 bp shorter in smokers.

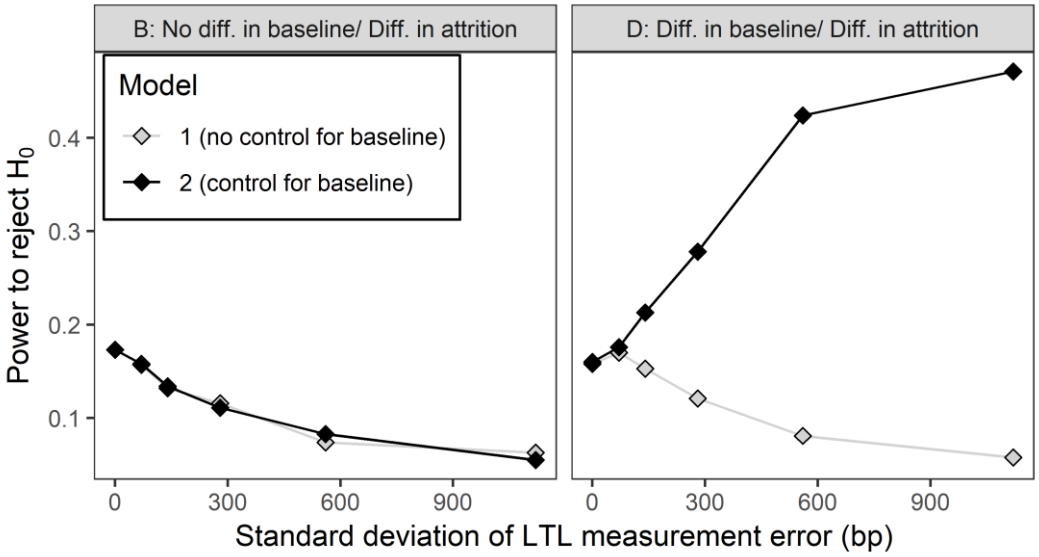

Figure S10. Assuming measurement error to be independent of LTL has no impact on the increased probability of false-positive errors resulting from controlling for LTL_b compared to Figure S1. Power as a function of measurement error here implemented as a fixed standard deviation (as opposed to a CV) for models 1 and 2. Data points represent the proportion of simulations yielding a p-value below 0.05 in 1000 replicate simulations. The left and right panels show the power in scenarios B and D respectively. The increase in power with increasing measurement error in scenario D that occurs with model 2 reflects the bias in parameter estimates for this model shown in Figure S8D. Power is generally low because of the small true effect size assumed in this simulation of only -2 $bp.year^{-1}$. The difference in LTL_b between smokers and non-smokers in scenario D was LTL_b 141 bp shorter in smokers.

Meta-analysis of empirical datasets: generalisation to sex and BMI

Introduction

We asked whether the effects of modelling strategy that we have identified in the main text generalise from smoking behaviour to other putative influences on the rate of telomere attrition, namely sex and body mass index (BMI) (e.g. 1). Meta-analyses show that LTL is shorter in adult males (2) and in individuals with higher BMI (3). We therefore predicted that just as for smoking, estimates of the statistical effects of sex and BMI on mΔLTL will be exaggerated in models controlling for $mLTL_b$ and that the size of this bias should be related to LTL measurement error.

Methods

We analysed data from the same seven cohorts used for the smoking analyses (Table 3 in main text). For the analyses of sex and BMI we used the full dataset for which longitudinal LTL data were available (numbers of participants given in Table S1). As our estimate of BMI, we used the mean of BMI at baseline and follow-up where this was available; and otherwise either BMI at baseline or BMI at follow-up, whichever was available. We were unable to analyse the effect of sex for the Caerphilly Cohort Study (CCS), since this cohort was restricted to male participants.

Table S1. Summary of the datasets analysed for sex and BMI.

Cohort[a]	**Number of participants**		**Correlation between LTL_b and LTL_{fu}**	**Differences between sexes in telomere length/attrition**[b] (standardised β [s.e.])			**Associations between BMI and telomere length/attrition**[c] (standardised β [s.e.])		
				LTL_b	**ΔLTL.year^{-1}**		**LTL_b**	**ΔLTL.year^{-1}**	
	Male	**Female**			**Model 1**	**Model 2**		**Model 1**	**Model 2**
ADE	33	35	0.94	-0.55 [0.23]	-0.18 [0.24]	-0.16 [0.26]	-0.22 [0.12]	0.14 [0.12]	0.16 [0.13]
CCS	756	0	0.05	NA	NA	NA	0.01 [0.04]	0.023 [0.04]	0.012 [0.02]
ERA	108	54	0.96	-0.065 [0.17]	-0.01 [0.17]	0.01 [0.16]	-0.06 [0.08]	-0.05 [0.08]	-0.04 [0.08]
HAS	158	95	0.15	0.09 [0.13]	0.13 [0.13]	0.06 [0.09]	-0.01 [0.06]	0.06 [0.06]	0.07 [0.04]
LBC1921	78	81	0.27	0.37 [0.16]	-0.12 [0.16]	-0.19 [0.16]	-0.01 [0.08]	-0.01 [0.10]	-0.01 [0.08]
LBC1936	444	414	0.49	0.38 [0.07]	0.21 [0.07]	0.09 [0.07]	0.02 [0.03]	0.00 [0.03]	-0.00 [0.03]
NSHD	500	557	0.08	0.19 [0.06]	0.25 [0.06]	0.10 [0.04]	-0.00 [0.03]	-0.01 [0.03]	-0.01 [0.02]

Notes: [a]Acronyms for cohorts as defined in Table 3 of the main text. [b]For sex, positive standardised βs indicate that males have longer LTL_b and greater mΔLTL.year^{-1}. [c]For BMI, positive standardised βs indicate that participants with higher BMI have longer LTL_b and greater mΔLTL.year^{-1}.

Results

The combined dataset available for analysing effects of sex and BMI included data from 3,313 adults, comprising 2,077 males and 1,236 females. Table S1 reports the results of repeating the analysis described in in the main text for smoking, first with sex, and second with BMI. As observed for

smoking, there is a positive relationship between the LTL_b-LTL_{fu} correlation coefficient (a proxy for measurement error) and $\Delta\beta$ (the difference between the estimates derived from models 1 and 2) for both sex and BMI (Figures S1A and B; weighted linear regression for sex: β±se = 0.14±0.06, t = 2.43, p = 0.0722; weighted linear regression for BMI β±se = 0.01±0.01, t = 0.82, p = 0.4480).

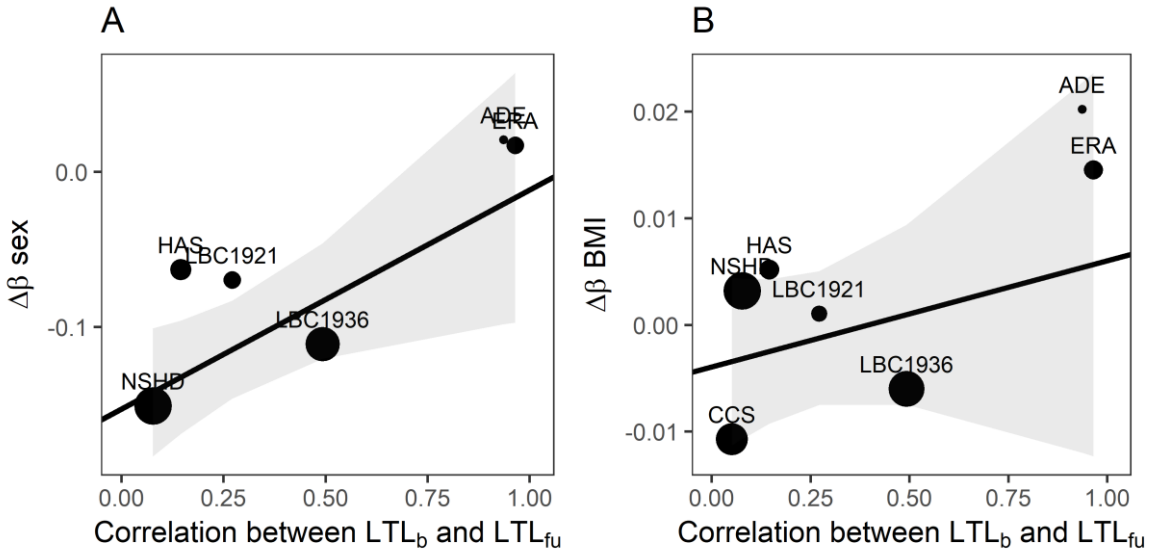

Figure S11. Measurement error predicts biases for sex and BMI. A: The correlation between a signature of LTL measurement error (the correlation between LTL_b and LTL_{fu}) and the difference between the β coefficients for sex derived from models 1 and 2. B: As panel A but the β coefficients are for BMI. In both panels, the solid black line was derived from a linear regression in which the points were weighted by the number of participants in each cohort and the grey ribbon shows the 95% confidence interval for this line.

Discussion

Our results for smoking generalise to two other factors known to be associated with LTL and argued to influence attrition, namely sex and BMI. That is, controlling for baseline telomere length in estimating the effect of BMI or sex on telomere attrition leads to a larger estimated effect compared to not controlling for baseline telomere length. The weaker correlations for sex and particularly BMI, as compared with those observed for smoking (see main paper), are likely to reflect weaker evidence for true associations between sex and BMI and LTL_b compared with the more robust association established for smoking.

References

1. Farzaneh-Far R, Lin J, Epel E, Lapham K, Blackburn E, Whooley MA. Telomere length trajectory and its determinants in persons with coronary artery disease: Longitudinal findings from the heart and soul study. PLoS One. 2010;5(1):e8612.

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