1. Extended Data

Figure #	**Figure title** One sentence only	**Filename** This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: *Smith_ED_Fig1.jpg*	**Figure Legend** If you are citing a reference for the first time in these legends, please include all new references in the main text Methods References section, and carry on the numbering from the main References section of the paper. If your paper does not have a Methods section, include all new references at the end of the main Reference list.
Extended Data Fig. 1	Significant technical parameters affecting LTL measurements based on the stage 1 adjustment.	ExtendedDataFigure1.tiff	Summary boxplots are shown for the 474, 074 LTL measurements for each associated parameter: Enzyme batch (A), PCR machine (B), primer batch (C), operator (D). Individual data points show minimum and maximum measures, the box represents the lower quartile (bottom), upper quartile (top) and median (internal line). The upper and lower whiskers extend to a value no further than 1.5 * IQR from the respective quartile. Linear relationships were seen between LTL and temperature (E) and humidity (F). For both (E) and (F) a fitted regression line is shown with 95% Confidence Intervals (grey shading).
Extended Data Fig. 2	Significant interactions based on the stage 2 adjustment.	ExtendedDataFigure2.tiff	A) LTL by Primer and Operator. B) LTL by primer and PCR machine. PCR machines 5 and 6 were not used at the start of the pilot study (primer batch 1) and machines 7 and 8 were used from the end of the pilot stage (primer batch 3 onwards).
Extended Data Fig. 3	Effect of A260/280 on LTL.	ExtendedDataFigure3.tiff	The distribution of DNA sample A260/280 ratios is illustrated in (A). We observed an increase in LTL with very low and very high A260/280 ratios (B). Data shown is mean LTL (blue0 with 95% Confidence Interval (grey).
Extended Data Fig. 4	Distribution of the coefficients of variation for the repeat samples.	ExtendedDataFigure4.tiff	Distribution of CVs after technical adjustment for both the blinded repeats A) (n=528) and deliberate repeats B) (n=22,615) are shown. The grey dotted line represents the median coefficient of variation with the shaded region representing the interquartile range.
Extended Data Fig. 5	Data on the first and second DNA sample	ExtendedDataFigure5.jpg	A) Histogram showing that the gap between the two sample collections has a mean interval of 5.5 years (range: 2-

	used to estimate regression dilution ratio.		10 years, N=1,312). B) Correlation between the first and second $\log_e$-LTL measure by time, estimated by the difference in years between the two sample collections and shown with 95% confidence intervals. The blue circle reflects the correlation estimate (centre) with size reflecting the number of participants measured each year (exact N shown in brackets). The black line shows the overall pooled correlation for all samples and the red dotted lines indicate the 95% confidence interval for this estimate.
Extended Data Fig. 6	Age and sex relationships for participants used to estimate regression dilution bias.	ExtendedDataFigure6.jpg	The decline of LTL with age is shown for men (blue) and women (plum) for both the first (A) and second (B) LTL measurements. The estimated effect sizes are shown for age (β_Age) and sex (β_Sex) within the figures.
Extended Data Fig. 7	Decline of LTL with age.	ExtendedDataFigure7.jpg	The decline of z-standardized $\log_e$-LTL with age is shown for men (blue) and women (plum) in adjusted data. The y-axis is truncated at -5SD to +5SD with 166 data points (80 women, 86 men) not shown. A small number of participants recruited by UK Biobank fall outside of the stated 40-69 age range.
Extended Data Fig. 8	Decline in LTL with age by sex.	ExtendedDataFigure8.jpg	Using stratified regression for men (blue) and women (plum) for all participants (N=474,074) we considered the non-linear effect of age within each sex. Here we show the predicted shape in a solid line and the observed data in a dashed line with 95% confidence intervals. There is significant non-linearity observed for women, where the rate of LTL decline increases as the population ages.
Extended Data Fig. 9	Telomere lengths within individual ethnic groups	ExtendedDataFigure9.jpg	Data adjusted for both age and sex are shown in purple for individual observations to indicate the range and quantity of data alongside a box-plot to show the median (line) and interquartile range (box) with whiskers extending to a value no further than 1.5 * IQR from the respective quartile. Box plots of data with additional adjustment for BMI, CRP, HbA1c, smoking status,

			alcohol consumption, and measures of physical activity, socioeconomic status and diet are shown in blue. Ethnicity is self-reported and presented as defined by UK Biobank Data-Field 21000. Note that we shorten "Asian or Asian British" to Asian and "Black or Black British" to Black.
Extended Data Fig. 10	LTL by age in different ethnic groups.	ExtendedDataFigure10.jpg	LTL measurements were adjusted for sex, BMI, CRP, HbA1c, smoking status, alcohol consumption, and measures of physical activity, socioeconomic status and diet.

2. Supplementary Information:

A. Flat Files

Item	**Present?**	**Filename** **This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf**	**A brief, numerical description of file contents.** **i.e.: *Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.***
Supplementary Information	Yes	MP1_Supplementary Figures and Tables_AIP.pdf	Supplementary Figures 1-4 and Supplementary Tables 1-4
Reporting Summary	Yes	nr-reporting-summary_NA_Nov21.pdf	
Peer Review Information	No	*OFFICE USE ONLY*	

Measurement and initial characterization of leucocyte telomere length in 474,074 participants in UK Biobank

V. Codd[1,2*], M. Denniff[1], C. Swinfield[1], S.C. Warner[1], M. Papakonstantinou[1], S. Sheth[1], D.E. Nanus[1], C.A. Budgeon[1,2,3], C. Musicha[1,2], V. Bountziouka[1,2], Q. Wang[1,2], R. Bramley[1,2], E. Allara[4,5], S. Kaptoge[4,5,6], S. Stoma[1], T. Jiang[4], A.S. Butterworth[4,5,6,7], A.M. Wood[4,5,6,7,8,9], E. Di Angelantonio[4,5,6,7,10], J.R. Thompson[10], J.N. Danesh[4,5,6,7,12], C.P. Nelson[1,2], N. J. Samani[1,2*].

1. Department of Cardiovascular Sciences, University of Leicester, Leicester, United Kingdom
2. NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, United Kingdom
3. School of Population and Global Health, University of Western Australia, Australia

4. British Heart Foundation Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
5. National Institute for Health Research Blood and Transplant Research Unit in Donor Health and Genomics, University of Cambridge, Cambridge, United Kingdom
6. British Heart Foundation Centre of Research Excellence, University of Cambridge, Cambridge, United Kingdom
7. Health Data Research UK Cambridge, Wellcome Genome Campus and University of Cambridge, Cambridge, United Kingdom
8. Medical Research Council Biostatistics Unit, Cambridge Institute of Public Health, University of Cambridge, Cambridge, United Kingdom
9. The Alan Turing Institute, London, United Kingdom
10. Health Data Science Centre, Human Technopole, Milan, Italy
11. Department of Health Sciences, University of Leicester, United Kingdom
12. Department of Human Genetics, Wellcome Sanger Institute, Hinxton, United Kingdom

*Corresponding authors: Veryan Codd (vc15@leicester.ac.uk) and Nilesh J. Samani (njs@leicester.ac.uk)

Abstract

Leukocyte telomere length (LTL) is a proposed marker of biological age. Here we report the measurement and initial characterization of LTL in 474,074 participants in UK Biobank. We confirm that older age and male sex associate with shorter LTL, with women on average ~7 years younger in "biological age" than men. Compared to white Europeans, LTL is markedly longer in African and Chinese ancestries. Older paternal

age at birth is associated with longer individual LTL. Higher white cell count is associated with shorter LTL, but proportions of white cell subtypes show weaker associations. Age, ethnicity, sex and white cell count explain ~5.5% of LTL variance. Using paired samples from 1,351 participants taken ~5 years apart, we estimate the within-individual variability in LTL and provide a correction factor for this. This resource provides opportunities to investigate determinants and biomedical consequences of variation in LTL.

Introduction

Many cardiovascular, neurodegenerative, neoplastic and other conditions increase in incidence with age. However, as suggested by substantial inter-individual variations in age of onset and disease risk[1], these conditions are not inevitable consequences of aging. We and others have proposed that such variations may, at least in part, reflect variation in biological aging driven by variation in telomere length[2,3]. Telomeres are nucleoprotein complexes at

chromosome ends that maintain genomic stability. They shorten with each cell division and determine cellular lifespan[4]. At a cellular level, mean telomere length (TL) reflects cellular age and replicative history[5]. Because of these and other properties, TL has been proposed as a biomarker of biological age[2].

At a population level, TL has frequently been studied using leukocyte DNA, a practicable measure of TL that correlates well with TL across different tissues within individuals[6]. Leukocyte telomere length (LTL) shows considerable inter-individual variation and is largely genetically determined, with heritability estimates of ~0.70 [7]. Even so, established genetic risk factors explain only a small fraction of the variation in LTL[8,9]. Age, sex, paternal age at birth and ethnicity are associated with LTL, but also account only for a small proportion of the inter-individual variation in LTL[7,10-14]. Even after taking these factors into account, several biological, behavioural and environmental characteristics correlate with, and potentially modify, LTL, including oxidative stress, inflammation, obesity, smoking, physical activity and dietary intake[15-18]. It remains uncertain, however, whether they are correlates or causative determinants. Furthermore, there is uncertainty about LTL's degree of within-individual variation over time[19,20].

Congenital premature aging syndromes arise from extreme shortening of telomeres due to rare mutations in telomere regulatory genes[21]. By contrast, more subtle inter-individual variation in LTL has been linked to risks of several common disorders in middle- and later-life, including certain cancers, coronary artery disease, Alzheimer's disease, osteoarthritis, and lung diseases[22-26]. For many reported LTL-disease associations, however, it remains uncertain whether they chiefly reflect cause-and-effect relationships. For some conditions (e.g., coronary artery disease) causality is supported by associations between genetically-determined variation in LTL and disease risk[8]. However, even when causality is likely, studies have been insufficiently powered to characterize dose-response relationships of LTL

with new-onset ("incident") disease outcomes, even though this is needed to define risk thresholds.

Population biobanks afford significant opportunities to address the key uncertainties outlined above. However, insight into the determinants and biomedical consequences of LTL has been limited by the inability of biobanks to *combine* key study attributes. In particular, studies require robust LTL measurement, long-term follow-up of participants for incident disease outcomes, and exceptional statistical power. Studies also need detailed genomic information on participants, both to characterize the genetic architecture of LTL and to derive genetic "instruments" to enable Mendelian randomization analyses to help judge causality. Importantly, studies also require extensive biomedical phenotyping, including information on behaviours, physiological traits and clinically relevant endpoints. Finally, studies require serial measurements, at least in subsets of participants, to enable quantification and correction for within-individual variation in LTL ("regression-dilution") over time[27,28].

UK Biobank (UKB) is a large population cohort established between 2006 and 2010 of participants aged 40-69 years at recruitment[29]. Participants have been characterised in detail using questionnaires, physical measurements, urinary and plasma biomarker measurements, genomic assays and longitudinal linkage with multiple health record systems[30]. Detailed imaging assessments of the brain, neck, heart, abdomen, bones and joints, and eyes have been conducted in large subsets of participants, as well as repeat blood sampling in several thousands of participants. Here, we report on the creation, quality assurance, and initial interrogation of a resource of LTL measurements in DNA samples of 474,074 participants in UKB. Our analyses highlight the scope and potential of this powerful and detailed resource, which is available to the worldwide research community through application to UKB.

Results

LTL measurements in 488,400 participants

Of the 489,090 DNA samples received by our laboratory from UKB, 488,400 remained after removal of duplicates and samples from participants who had withdrawn from the study (**Methods**; **Figure 1**). Valid LTL measurements were obtained for 474,074 (97.1%) samples. Of the 14,326 (2.9%) participants without a valid LTL measurement, the large majority had insufficient DNA, with only 1,647 repeatedly failing LTL assay QC (**Figure 1**). A small proportion of participants had LTL measured in DNA samples not collected at baseline (**Figure 1**).

As we had performed these measurements over 4 years and required multiple batches of reaction reagents and multiple pieces of equipment, we sought to identify and adjust for potential sources of technical variation within the measurements in a robust manner by recording experimental parameters that may lead to technical noise throughout the entire project. We adopted a three stage approach, adjusting the measurements using the regression coefficients from multivariable regression models at each stage. Full details of each stage are given in the **Methods**. As the assay was run on half-plates, technical parameters influencing the measurements will influence all samples within each half-plate equally. We therefore used the half-plate mean LTL to assess technical variation in stages one and two. In stage one we assessed the contribution of nine technical parameters to LTL variability, of which six had significant associations (**Table 1** and **Extended Data Figure 1**). PCR machine (Rotor-Gene Q), explained the greatest proportion of LTL variation in the multivariable model, followed by enzyme batch, temperature, staff member (operator), primer batch, and humidity. No associations were observed for the time of day of assay runs, pipetting robot (Qiagility), DNA extraction method. In stage two, we then considered all possible pairwise interactions and

identified statistically significant interactions of primer batch with each of operator and PCR machine (**Table 1** and **Extended Data Figure 2**). Both stages therefore highlight parameters that influence the qPCR assay as those that contribute to thermocycler performance (Rotor-Gene Q, temperature) or assay composition (enzyme batch, primer batch, operator). In combination, the significant technical parameters and interactions explained 23.7% of LTL variation in half-plate mean. In stage 3, we estimated sample storage parameters and any influence of DNA sample purity using the A260/280 ratio (a measure of DNA purity) at the individual level. Both time between sample collection to DNA extraction, and DNA extraction to LTL measurement explained <0.01% in the individual level LTL so were not included. However, the A260/280 ratio explained 0.5% of variation in the individual level LTL (**Extended Data Figure 3**) and was therefore included in the technical adjustment.

To assess the impact of adjusting LTL for the relevant technical parameters mentioned above, we considered the mean LTL per week over the four-year assay period (**Figure 2**). As this mean is based on thousands of samples, we expect it to remain relatively stable over time, with biological variation within these means being of very little influence. While the unadjusted LTL measurements showed substantial fluctuations over time (**Figure 2A**), the adjusted LTL measurements were much more consistent across the assay period (**Figure 2B**). Adjustment strengthened the inverse correlation of LTL with age from -0.185 to -0.195 and increased the variance in LTL explained by age and sex from 4.04% to 4.53% (see further analyses below).

<u>Reproducibility of LTL measurements</u>

To assess our assay's reproducibility, we calculated the coefficient of variation (CV) using samples measured on two separate occasions. For the blinded duplicates (n=528) included by

UKB, the distribution of CVs was strongly positively skewed (**Extended Data Figure 4A**), with median CVs of 7.15 (Inter-quartile range (IQR) 3.03-11.69) for the raw LTL measurements and 6.53 (IQR 2.87-11.30) for the adjusted LTL measurements. For a larger set of randomly selected but unblinded repeats (n=22,615), the distribution of CVs was similarly skewed (**Extended Data Figure 4B**) with median CVs of 5.23 (IQR 2.44-6.33) and 5.53 (IQR 2.67-9.68) for the raw and adjusted values respectively.

To quantify within-person variability of LTL values over time, we calculated the regression-dilution ratio (RDR; see **Methods**) using 1,351 available serial measurements of LTL taken at a mean interval of 5.5 years (range: 2-10 years). The RDR for LTL was 0.65 (95% CI: 0.61, 0.68) – similar to that for $\log_e$-transformed LTL (0.68, 95% CI: 0.64, 0.72) – and did not change materially with increasing time between serial measurements or after adjustment for participants' age at sample collection (**Extended Data Figures 5A & 5B**). The well-known correlations of LTL with age, sex and other factors among participants with serial LTL measurements were similar to those in the entire UKB cohort (below and **Extended Data Figure 6**).

Relationship between LTL and selected phenotypes

To give researchers confidence in the dataset we performed a number of selected analyses to reproduce known associations (e.g. LTL with age and sex) or, where there is strong previous evidence but some conflicting reports (ethnicity, paternal age) provide some definitive answers. We also performed further in-depth exploration of some of these, where appropriate. We also sought to explore the potential influence that blood cell composition at sample collection may have on the LTL measurements, as different white blood cell types have been shown to have different TL within an individual.

For these analyses, we focused on participants with LTL measurements on samples collected at UKB's baseline examination, to match the time when the selected phenotypes were assessed (**Figure 1**). We also removed individuals where self-reported sex and genetic sex did not match, leaving 472,174 participants for these analyses. Characteristics of these participants, stratified by quartile of LTL values, are shown in **Table 2**.

As the distribution of the adjusted LTL data was found to be non-normal, we log transformed the data (log_e-LTL, **Supplementary Figure 1**). We further Z-standardised the adjusted, log transformed measurements to allow direct comparison to previous studies where appropriate. Unless otherwise stated all the secondary analyses presented describing the association of LTL with various characteristics use the Z-standardised log_e-LTL.

Age and sex relationships: We confirmed the known relationships between shorter LTL and older age and male sex (**Table 3** and **Extended Data Figure 7**). By comparing these associations, we estimated that being female equated to having longer LTL equivalent to 7.4 years of cross-sectionally estimated LTL shortening with age, which could also be viewed as being "biologically younger". Overall, the inverse association of LTL with older age was steeper in men than women (**Table 3**; $P=8.8x10^{-37}$ for age-sex interaction). Fitting a quadratic term for age within the model to men and women separately showed an almost linear inverse association among men of ($P=0.034$), compared to a shallower non-linear association in younger women that became steeper at older ages ($P=3.80x10^{-16}$, **Extended Data Figure 8**). Further exploration showed that the steepness of the inverse association of LTL with age in women became closer to that in men after the menopause, and was the same between men and women when we restricted the analysis to post-menopausal women aged >55 years which removes potential outliers that may not represent a natural early menopause (**Table 3**).

***Ethnicity*:** Compared to white Europeans, mean LTL was longer in people of Black, Chinese and mixed ancestries (**Extended Data Figure 9**). Adjusting for traits that have previously been associated with LTL and that differ by ethnicity[16,18,31-36] (**Supplementary Table 1**) had minimal effect on the observed ethnic differences in LTL (**Extended Data Figure 9**). Within each ethnic group, we observed similar relationships of shorter LTL with older age and male sex (**Table 4**) to those reported overall, with somewhat steeper associations with age in Black participants (**Table 4**; **Extended Data Figure 10**). Differences in "biological age", defined as the equivalent effect in terms of cross-sectional age-related LTL shortening, between women and men across ethnic groups ranged from 6.17 years for South Asians and other Asians to 9.27 for Chinese.

Paternal and maternal age at birth: Information on paternal and maternal age at birth was available for 97,234 and 170,668 participants, respectively, and on both parents for 70,871 participants. Comparing participants for whom we could derive parental age at birth to those we could not, revealed those participants with this information were more likely to be younger, female, of White ethnicity and have slightly shorter age and sex-adjusted LTL (-0.030 (-0.036, -0.024)). After adjustment for age and sex, having an older father or mother at birth was associated with longer LTL. The positive association per year of older parental age at birth with longer LTL was broadly equivalent to the *inverse* association per year of the participant's age with shorter LTL (**Table 5**). Results were unchanged when restricting analyses for maternal (0.018, 95% CI: 0.016, 0.019) and paternal (0.021, 95% CI: 0.019, 0.022) age at birth only to participants with both parents alive at baseline. Including both maternal and paternal age at birth within the same model greatly attenuated the association of maternal age with LTL (**Table 5**), suggesting paternal age at birth is the principal determinant and that the relationship with maternal age at birth was likely due to correlation between parental ages (r=0.75), despite no evidence of collinearity (variance inflation factor

[VIF]=2.29 and 2.26 for paternal and maternal ages, respectively). When we restricted analysis to participants with parental ages with a difference of between 2-5 years and >5 years in an attempt to break down the correlation between parental ages and avoid collinearity, we found significant positive associations and consistent effect sizes with paternal age at birth but not with maternal age at birth (**Table 5**).

***White blood cells*:** In a model that also included age, sex and ethnicity, we found an inverse association of LTL with total white cell count (WBC) (0.064 SD lower LTL per 1-SD higher white cell count, $P<1x10^{-314}$: **Table 6**). For individual white cell types, there was a positive association of LTL with proportion of neutrophils and inverse associations with proportions of eosinophil and monocytes. There was no association with lymphocyte percentage (**Table 6**).

***Variance in LTL explained*:** In a multivariable model, we estimated the amount of inter-individual variance in LTL explained by the biological factors studied, excluding parental age at birth which was only available for a small fraction of the cohort. Age explained ~3.5%, followed by ethnicity, sex and WBC, explaining 0.84%, 0.68% and 0.37%, respectively (**Table 6**). Allowing for WBC, blood cell proportions individually accounted for very little additional variance (all <0.01%, **Table 6**). In aggregate, these factors explained about 5.5% of the variance in LTL. In this model, where cell composition is also included, we also detected a significant difference in LTL between White participants and the category in UKB called "Asians" (comprising mostly South Asians). However, the difference in LTL was most marked for Black and Chinese ethnicities where the difference in "biological age" compared to White participants was 17.9 years and 15.6 years, respectively (**Table 6**).

Discussion

We have generated relative LTL measurements by qPCR in 474,074 well-characterised participants in UKB, creating an unprecedentedly powerful resource to investigate the determinants and biomedical consequences of naturally-occurring variation in LTL.

Whilst the qPCR method for estimating LTL has been criticized for having higher variability that some other methodologies, such as Southern blotting, it is the only method that is practical to use at this scale. Furthermore, we conducted detailed exploration of potential technical factors that could influence the measurements through careful curation of relevant variables. Removing technical variation from the measurements through statistical adjustment improved measures of inter-assay variation and led to a more stable measurement of LTL over the 4-year measurement period. Despite the unprecedented scale of the project, our assay showed good reproducibility as assessed through inclusion of both blinded as well as deliberate duplicates.

Our confirmation of well-established relationships between shorter LTL and older age and male sex of similar magnitudes to those reported before adds confidence to the validity of our measurements. For example, our estimate that women are younger in "biological age" than men by 7.4 years is very similar to an estimate of 7.0 years based on previous data[37]. Our study's exceptional power allowed us to demonstrate a moderate but significant age-sex interaction in the inverse association of LTL with age, showing shallower associations in younger women compared with men but more similar associations after the menopause or after age 55 years. This observation is consistent with a potential protective effect of oestrogen on LTL attrition[38]. However, our analysis was constrained by the relatively narrow age at recruitment of participants in UKB (40-70 years); other studies have reported steeper associations of shorter LTL with age in younger women[39,40]. Furthermore, the cross-sectional design of both UKB and the other studies that have investigated sex-related associations of

LTL with age, limit the inferences that can be drawn; longitudinal studies are needed to confirm any oestrogen-related associations with LTL.

Our study found that longer LTL is associated with having an older father at the time of birth, again consistent with previous findings[7,10,11]. We acknowledge that we could not calculate parental age for all participants and that this analysis is therefore restricted to those individuals whose parents were alive at the time of recruitment and therefore more likely to be in the younger fraction of participants. Whilst these participants were not fully representative of the populations as a whole, having shorter age- and sex-adjusted LTL, we have no reason to believe that this would influence the relationship between offspring LTL and paternal age at birth. Furthermore, we show consistent findings with other large-scale analyses of this association[7], suggesting that studies which did not show the relationship previously may have been impacted by relatively small sample sizes. Although we also observed an association between longer LTL and having an older mother at birth, additional analysis showed that this was most likely due to correlation of spousal ages and the association is driven predominantly, if not exclusively, through paternal age at birth. It is notable, therefore, that previous studies have reported longer telomeres in the sperm of older men[10].

We also observed substantial ethnic differences in average LTL, confirming previous findings of longer LTL in people of African ancestry[12-14]. Furthermore, compared to people of white European ancestry, we report findings of longer LTL in people of Chinese, South and West Asian and mixed ancestry. Adjusting our analyses for factors where there is some prior evidence of an association of the trait with LTL and a difference in the trait by ethnic group had minimal influence on our findings, suggesting that these are genetic differences between ethnicities and not driven by differences in lifestyle or disease factors that influence LTL. While we cannot completely exclude the possibility that the ethnic differences are due

to other confounders, there is evidence to suggest that ethnic differences in LTL may be driven by polygenic adaptation, with suggestion that shorter LTL Europeans was an adaptation to lower the potential risk of developing melanoma due to loss of skin pigementation[12]. Other potential drivers of LTL adaptation could also be in allowing greater ability of the immune system to respond to bacterial or parasitic infection through longer LTL, despite the potential of increased cancer risk. The exact reasons for the ethnic differences in LTL and any potential biomedical consequences remain to be fully explored.

There has been a long debate about the potential impact of white cell composition on LTL measurements prompted by previous reports of differences in TL between B cells, T cells and monocytes within an individual[41-44]. Here we clarify that, at a population level, total white cell count has a small but significant inverse association with LTL. Accounting for this, the proportions of several white cell types available in UKB additionally explained very little of the inter-individual variance in LTL, suggesting that cell composition has little influence on the LTL measurement. However, our analyses are limited to the major blood cell types measured in UKB that do not include the lymphocyte subsets (T- and B-cells) that have been studied previously[44]. While different cell types have different TL they are also highly correlated within an individual[44], suggesting that LTL is a viable measure of overall TL for epidemiological research.

Using paired samples from 1,351 participants taken on average 5 years apart, we show the regression-dilution ratio for LTL is ~0.65. This degree of within-individual variability is similar to those we observed for systolic blood pressure and total cholesterol, but less than for body mass index in the same UKB participants (**Supplementary Table 2**). A previous study, involving a larger number of paired measurements, reported a somewhat lower regression-dilution ratio (~0.50) for LTL, perhaps because the interval between measurements was more prolonged (9.3 vs 5.5 years), meaning age-related changes in LTL could have contributed

more substantially. The implication from both of these studies is that, despite its high heritability, LTL is a fluctuating factor within individuals in mid-life. Hence, adjusting for RDR should provide a more accurate assessment of any aetiological associations of LTL with disease outcomes and biomedical traits.

As noted earlier, UKB combines several key attributes that make it an exceptionally informative cohort in which to conduct LTL measurements. However, UKB is not a strictly representative sample of the UK general population, as only about 6% of those invited to participate did so[29]. Risk factor levels and mortality rates in UKB are lower than in the general population, likely reflecting a to a "healthy cohort" effect.[45] Furthermore, UKB had a relatively narrow age range at recruitment (45-69 years) and only a small proportion of participants of non-white ethnicity. While studies have shown that risk factor associations in UKB are consistent with those in the general population,[46] these limitations of the cohort should be borne in mind by researchers conducting and interpreting analyses on the LTL data we have added to UKB. Specifically, with respect to the ethnicity associations with LTL, whilst our findings are consistent with previous studies, we cannot exclude the possibility, especially noting the low proportions of non-White participants, that factors that contribute to selection into the UKB also create a collider scenario that produces spurious associations between ethnicity and LTL. More work is needed to clarify the contribution of differential selection into UKB and its potential impact on the observed ethnic differences in LTL.

We have returned our LTL measurements to UK Biobank and the data are available to researchers under the following fields: 22190 (unadjusted LTL), 22191 (technically adjusted LTL) and 22192 (technically adjusted, $\log_e$- and Z-transformed LTL). For researchers performing analyses on all or the majority of participants in UK Biobank, we would advise using Data-Field 22192. Where sub-group analyses are performed, researchers may want to consider using Data-Field 22191 and perform appropriate transformation to achieve a normal

distribution as appropriate for the sub-group. In addition to adjusting analyses for age, sex and ethnicity, we would advise researchers utilising these measurements to consider removing non-baseline samples and adjusting for WBC where appropriate. Importantly, we also provide an estimate of RDR to allow researchers to adjust for inter-individual variation within their analyses[27,28]. To estimate usual LTL we propose an RDR of 0.68 should be used to adjust effect size estimates when using the technically adjusted, $\log_e$ transformed LTL, while untransformed technically adjusted LTL should use an RDR of 0.65.

In summary, we have created a large resource to facilitate investigation of the determinants and biomedical consequences of inter-individual variation in LTL. Here, we provide a detailed description of generation and quality assurance of the measurements. Demonstration of several well-established relationships of LTL should give researchers additional confidence in the use of the resource.

Methods

Measurement of LTL

UK Biobank recruited participants between the ages of 45-69 between 2006 and 2010. Participants were invited to take part by post and identified from National Health Service records, with an aim to provide both socioeconomic and ethnic heterogeneity and cover

individuals living in both urban and rural environments. Full details of recruitment can be found elsewhere (https://www.ukbiobank.ac.uk)[29]. Technicians at UKB extracted DNA from peripheral blood leukocytes as part of a cohort-wide array genotyping project, described in detail elsewhere[47]. DNA was extracted using an automated process for the majority of samples; a small proportion were extracted using a manual method using the same chemistry. UKB transported residual DNA from this project to the University of Leicester LTL assay laboratory in 11 tranches of approximately 50,000 samples. Sample manifests including sample ID and concentration were provided alongside the samples. Prior to assay, samples were first normalised to a concentration of 10ng/ul using automated pipetting robots (Qiagility, Qiagen). Research staff at the University of Leicester conducted LTL measurements blinded to phenotypic information. Measurements were made for LTL on all samples supplied. Samples were only excluded in the event of UKB receiving a request to withdraw from the participant. A total of 122 participants withdrew during the measurement period; samples and data for these participants were destroyed.

Using the multiplex qPCR methodology LTL is measured as the ratio of telomere repeat copy number (T) relative to that of a single copy gene (S, *HBB*, which encodes human hemoglobin subunit beta)[42]. The amounts of both T and S were measured within each reaction and were calculated relative to a calibrator sample (pooled DNA from 20 individuals) which was included on every run. Each measurement run was set up on a 100 well Rotor-Disc (Qiagen) using an automated pipetting robot (Qiagility, Qiagen) and included 47 samples in duplicate, a no template control and the calibrator sample in quadruplicate. Each qPCR reaction contained 1x Sensimix SYBR No-ROX enzyme mix (Bioline), 150nM Tel primers, 45nM of Hgb primers (**Supplementary Table 3**) and 30ng of DNA. The Rotor-Discs were transferred to a Rotor-Gene Q PCR machine for amplification. Cycling conditions for each run were as follows: 95°C 10 min; 95°C for 15 sec, 49°C for 15 sec for 2 cycles; 94°C for 15 sec, 62°C for 10 sec, 72°C for 15 sec with signal acquisition (T), 84°C for 10 sec, 88°C for 10 sec with signal acquisition (S) for 32 cycles. At the end of cycling a dissociation curve was included. Prior to use, each primer batch was assessed for quality by producing a standard curve across the input DNA range of 1200-9.3ng in two-fold dilution (8 points). Primers achieving 90-110% reaction efficiency and an R^2 across the linear range >0.99 were acceptable. Further testing was then performed to reproduce measurements for previously assayed samples with good concordance before further use. The linear range for each primer batch was recorded as a QC metric.

Relative quantities of T and S were calculated for each sample using the Rotor-Gene comparative quantification software (Qiagen). This software calculates the amplification efficiency of each reaction. The relative amount of T and S is calculated using the following equation:

$$\text{Relative concentration} = \text{Average Amplification}^{(\text{calibrator take off} - \text{sample take off})}.$$

Using the calculated average amplification efficiency, rather than assuming 100% efficiency, effectively adjusts the measurements for run-to-run variation. The resulting T/S ratios were calculated for each well, alongside the average T/S and the coefficient of variation for the sample duplicate. We then applied strict, pre-defined QC criteria at both the sample and run levels, as detailed in **Supplementary Table 4**, before accepting the measurements as being valid. Following this, successful data from each run was uploaded into a custom database. All samples that failed QC criteria were re-assayed until valid measurements were achieved, or the sample was deemed to be unsatisfactory or exhausted.

To measure stability and reproducibility of the measurements subsets of samples were deliberately re-run at later dates and the coefficient of variation between the measurements calculated. For this subsets of samples were selected each week and re-measured. These samples were deliberately selected from early tranches so that as the project progressed reproducibility could be assessed over longer time periods. In addition to these deliberate repeats (n=22,516), a small number of duplicate samples (n=528) were included by UKB, spread across the tranches, to which we were initially blinded (blinded duplicates).

Due to the scale of the project, the samples were measured over a 47 month period by 6 members of staff ("operators"), using 5 Qiagility pipetting robots for liquid dispensing and 8 Rotor-Gene PCR machines (**Supplementary Figure 2**). It was necessary to use 19 batches of Sensimix SYBR No-ROX enzyme mix and 7 primer batches for the assays (**Supplementary Figure 3**). Details of these parameters, alongside temperature and humidity (for potential influences on Rotor-Gene and Qiagility performance), were recorded alongside the sample data.

Statistical adjustment of data to minimise technical variation.

Adjustment for T/S experimental/technical variation was performed in three stages using R v3.6.1. First, we sought to identify technical parameters that influenced all measurements

within a qPCR run (half-plate). For this backwards selection using the mean T/S ratios at the half-plate level was used in a linear regression adjusting for enzyme, primer batch, PCR machine, pipetting robot, operator, temperature, humidity, time of day, and extraction method. Only half-plates with at least 20 valid measurements were included. Significant effects were determined using the Bayesian information criterion. The second stage took all significant main effects identified in stage 1 and further tested all possible two-way interactions using the same backwards selection approach as stage 1 for the interaction effects. For both stages we estimate a partial R^2 as the difference between the full model R^2 and the model R^2 leaving a single parameter out. Individual-level T/S ratios were then partially adjusted based on the coefficients from the final model selected in stage 2. A further level of adjustment was then applied at the individual measurement level by fitting a linear regression model on the individual level data adjusting for the 260/280 ratio of the DNA sample (stage 3). Due to an observed non-linear relationship between the T/S and 260/280 ratios both linear and quadratic effects were included. For the purpose of this analysis samples with a missing 260/280 or those that had a measurement within the extremes of the distribution (<1 or >3) were imputed using the mean 260/280 value. We also considered the time between sample collection to DNA extraction, and DNA extraction to LTL measurement using linear regression models to determine if either length of time affected the LTL measurement.

After technical adjustments were applied the LTL measurements (T/S ratios) were $\log_e$-transformed due to non-normality ($\log_e$-LTL). To allow direct comparison of the results of our analyses with previous studies we Z-standardised the $\log_e$-LTL measures.

Estimation of regression dilution bias

DNA was extracted by UKB for 1,884 participants from a second blood sample taken between 2 and 10 years after the original sample, using the same methodology. To remove technical variation between the two measures for estimation of the regression-dilution the original baseline sample was re-plated alongside the second time point sample and 23 pairs of samples were assayed in each qPCR half-plate. As these DNA samples were received towards the end of the project, for many there was insufficient DNA remaining from the baseline sample (which had already undergone measurement) to allow measurements for both of the paired DNAs to be obtained. Quality control parameters were then applied as for the

main dataset. Only samples with valid data for both time points within the same half-plate run were taken forward for analysis (**Supplementary Figure 4**).

We estimated the LTL regression dilution ratio (RDR) coefficient by regressing LTL measured at the second time point on LTL measured at the first time point[27,28]. The RDR is the ratio of the between-individual variance to the total variance (i.e. between-individual variance + within-individual variance); RDR values close to 1 indicate little within-individual variability, whereas values close to 0 imply high levels of within-individual variability. The resulting regression coefficient is the RDR, and the multiplicative regression dilution bias (RDB) correction factor, λ, is simply the inverse of the RDR coefficient i.e.

$$\hat{\lambda} = \hat{\beta}^{-1} = \frac{\sum(w_{i1} - \bar{w}_{.1})^2}{\sum(w_{i1} - \bar{w}_{.1})(w_{i2} - \bar{w}_{.2})}$$

where w_{i1} and w_{i2} are the first and second measurements of LTL respectively for each of the 1,351 participants.

We further adjusted for the difference in ages between the two measurements to consider the impact of time between sample collections on the RDR estimate and after removing the age effect from the first and second measurements by taking the residuals from a linear regression on LTL adjusted for age. We then regressed the age-adjusted second measurement residuals on the age-adjusted first measurement residuals adjusting for baseline age, sex and difference in age between sample collections to estimate the RDR. For non-LTL traits in UKB shown in **Supplementary Table 2**, we used baseline and follow-up visit 1 data and ran the models in the same way to estimate the RDR.

Association of LTL with selected phenotypes in UKB

Before conducting analyses we first removed participants for whom the LTL measurement was made from a non-baseline sample (where baseline visit date was before sample collection date) or where self-reported sex and genetic sex did not match (reflecting potential sample mishandling)[29]. To assess population demographics we estimated means and standard deviations for continuous traits and percentages for categorical traits. To account for familial correlation we randomly excluded one participant from each related pair, where a pair of participants were related if their kinship coefficient was K>0.088 estimated using genetic relatedness. No other exclusions were made other than where individuals had missing data.

We used linear regression models to assess the association of TL with age, sex, parental age at birth, ethnicity and white blood cell traits. Interactions and non-linear effects were considered in the regression model where appropriate. We consider $P<0.05$ as the threshold for nominal statistical significance.

Age and sex relationships were assessed first to identify interactions and non-linear effects in the data to estimate population attrition rates. To further investigate the observed age and sex trends we investigated the role of menopause by matching a male to each female 1:1 on age at baseline running stratified analyses by pre- and post-menopause status. Menopause status was taken from self-reported data (Data-Field 2724), using only "yes" and "no" responses.

We calculated parental age at birth from the reported parental age at baseline minus the age of the participant at baseline. We first modelled parental age at birth adjusting for age and sex and then calculated the difference in paternal and maternal age running analyses stratified by age difference group, 2-5 years and >5 years and run separately. Similarly, for ethnicity, regression models were stratified by ethnic group and run separately to assess the age and sex attrition rates within each ethnic group. We used the UKB defined ethnic groups from self-reported data (Data-Field 21000). Both "British and Black British" and "Asian and British Asian" are shortened to "Black" and "Asian" throughout. The "Asian and British Asian" is largely comprised of South and West Asian ancestries. To assess potential differences in LTL between ethnic groups linear regression models using $\log_e$-transformed technically adjusted LTL measures were run, including age and sex as covariates. The residuals (age and sex adjusted LTL) were subsequently z-transformed. To test whether known factors that associate with LTL and differ between ethnic groups were driving the observed ethnic differences we first assessed whether there were significantly differences in level or proportion across ethnic groups in UKB using ANOVA (continuous traits) or chi-squared (categorical traits) tests. We subsequently further adjusted our LTL-ethnicity analyses for BMI, CRP, HbA1c, physical activity (MET), smoking, alcohol consumption and Townsend deprivation index. We considered collinearity in these models through estimation of the variance inflation factor (VIF) where a value > 5 is considered to indicate collinearity.

Finally, we fitted a multivariable model to assess the contribution of white blood cell traits. All white blood cell traits were winsorized at the 0.5% and 99.5% centile to reduce the impact of extreme values, $\log_e$-transformed if required and Z-standardised. Linear regression models were again used to quantify the association with total white blood cell count on TL.

We also included white blood cell composition in the model with the percentages of neutrophils, monocytes, eosinophils, lymphocytes and basophils. All phenotype analyses were run using Stata v16.0.

Statistics and Reproducibility

We attempted to measure LTL in all participants in UK Biobank for whom a DNA sample was available. Reproducibility of the telomere measurements was assessed subsets of samples were re-run on a second occasion at random and the coefficient of variation calculated as detailed above. In addition, the experiment included 528 duplicate samples to which we were blinded until data had been returned to UK Biobank. LTL measurements were performed blinded to all phenotypic information for participants. No statistical method was used to predetermine sample size for the measurements and all available data was used for phenotypic analyses. Participants were excluded based on relatedness as detailed above and where the LTL measurement was from a non-baseline sample (i.e. did not correspond to the time point at which phenotypic data was collected).

Ethics

The UK Biobank has ethical approval from the North West Centre for Research Ethics Committee (Application 11/NW/0382), which covers the UK. UK Biobank obtained informed consent from all participants. Full details can be found at https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us/ethics. The generation and use of the data presented in this paper was approved by the UK Biobank access committee under UK Biobank application number 6007.

Data availability

Access to samples was made available through the UK Biobank Resource under Application Number 6077. As per the standard terms of UK Biobank, all data for the telomere measurements were returned to UK Biobank to be made available to other researchers. All source data used in this study, including all data related to the telomere measurements are accessible via application to UK Biobank. Further information on registration to access the data can be found at http://www.ukbiobank.ac.uk/register-apply/. Information on the telomere

measurements can be viewed in the data showcase (https://biobank.ndph.ox.ac.uk/showcase/) under the following fields: 22190 (unadjusted), 22191 (adjusted), 22192 (adjusted and z-transformed) and 22194 (both time point measurements for the regression dilution bias experiment).

Code availability

LTL measurement data was added to a custom built database application, the source code for this is viable at https://github.com/LCBRU/telomere. No other custom code was used in this study.

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Author contributions

M.D., C.S, M.P., S.Sh., D.E.N. and V.C. generated the data. S.C.W., C.A.B., R.B., J.R.T., V.C. and C.P.N. curated the data. C.M., V.B., Q.W., A.S.B., J.R.T., V.C. and C.P.N performed statistical analyses. V.C., C.P.N., C.M., Q.W., C.A.B., E.A., S.K., S.St., V.B., T.J., E.D.A., A.M.W., A.S.B., J.R.T., J.N.D. and N.J.S. drafted the manuscript and all authors revised it. V.C., C.P.N., J.R.T., J.N.D. and N.J.S. (Principal investigator) secured funding and oversaw the project.

Competing Interests Statement

The authors declare no competing interests.

Technical parameter	Univariate Model R^2 (%)	Multivariable partial R^2 (%)
Stage 1		
Enzyme	7.87	4.63
PCR machine	6.69	7.43
Primer	4.87	1.04
Operator	2.28	2.51
Temperature	0.73	4.63
Humidity	0.10	0.07
Hours from 6am	0.03	-
Pipetting robot	0.01	-
Extraction method	0.00	-
Stage 2		
Primer* PCR machine	-	2.13
Primer*Operator	-	1.56

Table 1. Estimating the variance explained by each technical parameter. Data during stage 1 and 2 were assessed at the run level with linear regression on half-plate mean LTL. Stage 1: Univariate model R^2 includes only this variable, multivariable partial R^2 is the contribution of the parameter on the total model R^2 (estimated as the difference between the full model R^2 and the model R^2 leaving this parameter out). Stage 2: Estimating the variance explained by the interactions in addition to the full model selected during Stage 1. Stage 2 Model R^2=23.7%.

Trait		LTL Q1		LTL Q2		LTL Q3		LTL Q4	
		N	Mean (SD) / %	N	Mean (SD) / %	N	Mean (SD) / %	N	Mean (SD) / %
Age (years)		118,044	58.6 (7.63)	118,043	57.1 (7.91)	118,044	56.0 (8.07)	118,043	54.4 (8.16)
Sex	Male	61,082	51.8	56,270	47.7	52,143	44.2	46,692	39.6
	Female	56,962	48.2	61,773	52.3	65,901	55.8	71,351	60.4
Ethnicity	Asian	2,116	1.8	2,206	1.9	2,341	2.0	2,494	2.1
	Black	910	0.8	1,302	1.1	1,758	1.5	3,309	2.8
	Chinese	175	0.2	281	0.2	375	0.3	621	0.5
	Mixed	501	0.4	606	0.5	714	0.6	918	0.8
	Other	748	0.6	900	0.8	1,092	0.9	1,473	1.3
	White	113,078	96.2	112,172	95.5	111,234	94.7	108,616	92.5
Menopause	Pre	9,776	16.3	12,893	21.5	16,041	26.7	21,315	35.5
	Post	38,537	24.8	39,273	25.3	39,170	25.3	38,139	24.6
Paternal Age at birth		21,422	27.8 (4.56)	25,250	28.2 (4.68)	28,208	28.7 (4.82)	31,389	29.3 (4.97)
Maternal Age at birth		39,522	26.1 (4.67)	44,497	26.4 (4.75)	48,712	26.9 (4.85)	53,064	27.4 (4.99)
WBC (count)		114,722	7.0 (1.75)	114,587	6.9 (1.72)	114,586	6.8 (1.73)	114,417	6.8 (1.75)
Neutrophil (%)		114,512	60.7 (8.36)	114,388	60.9 (8.16)	114,361	61.0 (8.16)	114,197	61.1 (8.23)
Lymphocyte (%)		114,512	28.9 (7.39)	114,388	28.8 (7.22)	114,361	28.9 (7.22)	114,197	28.9 (7.28)
Basophil (%)		114,512	0.6 (0.43)	114,388	0.6 (0.43)	114,361	0.6 (0.42)	114,197	0.6 (0.43)
Eosinophil (%)		114,512	2.6 (1.75)	114,388	2.6 (1.72)	114,361	2.5 (1.72)	114,197	2.5 (1.72)
Monocyte (%)		114,512	7.2 (2.19)	114,388	7.1 (2.15)	114,361	7.0 (2.13)	114,197	6.9 (2.12)

Table 2. Characteristics of participants with LTL measurements at baseline. Data are shown by LTL quartile with Q1 being shortest LTL and Q4 being longest LTL. N is the available sample size, and the summary statistic is either the mean (standard deviation) for continuous traits or percentage for categorical traits. Ethnicity is self-reported and presented as defined by UKB Data-Field 21000. The Z-standardised values of LTL for each quartile are: Q1, <-0.65; Q2,-0.65≤ to <-0.002; Q3, -0.002 to <0.65; Q4, ≥0.65.

Model	N	Trait	Beta (95% CI)	P value
1 Age and Sex	437,544	Age	-0.024 (-0.025, -0.024)	<1.00E-314
		Sex (Male)	-0.178 (-0.184, -0.172)	<1.00E-314
2 Age and sex interaction	437,544	Age	-0.022 (-0.023, -0.022)	<1.00E-314
		Sex	0.086 (0.045, 0.127)	4.50E-05
		Age*Sex interaction	-0.005 (-0.005, -0.004)	8.80E-37
3 Pre-menopausal age matched	54,560	Male Age	-0.028 (-0.030, -0.026)	2.00E-182
	54,560	Female Age	-0.023 (-0.024, -0.021)	6.00E-116
4 Post-menopausal age matched	141,692	Male Age	-0.027 (-0.028, -0.026)	<1.00E-314
	141,692	Female Age	-0.024 (-0.025, -0.023)	<1.00E-314
5 Pre-menopausal aged ≤55 years age matched	53,407	Male Age	-0.027 (-0.029, -0.025)	9.00E-122
	53,407	Female Age	-0.022 (-0.024, -0.020)	7.20E-79
6 Post-menopausal aged >55 years age matched	111,962	Male Age	-0.029 (-0.031, -0.028)	<1.00E-314
	111,962	Female Age	-0.029 (-0.030, -0.027)	4.00E-302

Table 3: Relationship between LTL and age and sex. All models shown are fit with LTL as the outcome with available sample size N. Model 1 includes age and sex. Model 2 adds an interaction term between age and sex. Models 3 (pre-menopausal), 4 (post-menopausal), 5 (aged ≤55 years) and 6 (aged>55 years) assesses age in sex stratified models where each woman is matched to a man of the same age before stratification. Betas are shown in SDs of $\log_e$LTL with 95% confidence intervals.

Ethnic group	N	Age effect		Sex effect (Male)	
		Beta (95% CI)	P value	Beta (95% CI)	P value
Asian	5,579	-0.024 (-0.027, -0.021)	1.80E-48	-0.148 (-0.203, -0.092)	1.90E-07
Black	3,900	-0.03 (-0.034, -0.026)	3.70E-44	-0.265 (-0.329, -0.201)	4.50E-16
Chinese	1,010	-0.026 (-0.035, -0.017)	6.90E-09	-0.241 (-0.379, -0.104)	5.90E-04
Mixed	1,826	-0.023 (-0.029, -0.017)	1.90E-13	-0.181 (-0.278, -0.085)	2.30E-04
Other ethnic group	2,605	-0.024 (-0.029, -0.019)	1.20E-19	-0.259 (-0.339, -0.179)	3.10E-10
White	301,312	-0.023 (-0.024, -0.023)	<1.00E-300	-0.168 (-0.175, -0.161)	<1.00E-300

Table 4. Age and sex associations within ethnic groups. A linear regression on LTL stratified by ethnicity and adjusting for BMI, CRP, HbA1c, smoking status, alcohol consumption, and measures of physical activity, socioeconomic status, diet and either age or sex. The age association is estimated for a single year increase in age and was also adjusted for sex, and the sex association is the average difference in LTL for men compared to women was also adjusted for age. Ethnicity is self-reported and presented as defined by UKB Data-Field 21000. Only participants with complete phenotypic information were included in this analysis. Betas are shown in SDs of $\log_e$LTL with 95% confidence intervals.

Stage 1						
Trait	**Paternal age only (N=97,234)**		**Maternal age only (N=170,688)**		**Parental age (N=70,871)**	
	Beta (95% CI)	**P-value**	**Beta (95% CI)**	**P-value**	**Beta (95% CI)**	**P-value**
Age	-0.022 (-0.023, -0.021)	1.00E-314	-0.023 (-0.024, -0.022)	1.00E-314	-0.022 (-0.024, -0.021)	3.00E-270
Sex	-0.139 (-0.151, -0.127)	6.00E-111	-0.151 (-0.160, -0.141)	3.00E-223	-0.130 (-0.145, -0.116)	4.00E-72
Paternal age	0.020 (0.019, 0.022)	5.00E-209	-		0.018 (0.015, 0.020)	1.50E-52
Maternal age	-		0.017 (0.016, 0.018)	1.00E-275	0.004 (0.002, 0.007)	5.80E-04

Stage 2				
Trait	**2 - 5 years (N=21,985)**		**>5 years (N=10,759)**	
	Beta (95% CI)	**P-value**	**Beta (95% CI)**	**P-value**
Age	-0.021 (-0.023, -0.019)	7.70E-75	-0.022 (-0.026, -0.019)	4.80E-36
Sex	-0.120 (-0.146, -0.095)	2.80E-20	-0.118 (-0.154, -0.082)	2.20E-10
Paternal age	0.018 (0.012, 0.024)	3.70E-10	0.019 (0.014, 0.024)	6.00E-15
Maternal age	0.004 (-0.001, 0.010)	0.120	0.001 (-0.003, 0.006)	0.550

Table 5. The relationship between parental age at birth and LTL. Stage 1 analyses were performed in the whole cohort where the association with parental age was considered separately for paternal and maternal, before fitting both in the regression model. Stage 2 analyses stratified by the age difference between both parents at birth to allow for the potential impact of the age difference driving the stronger paternal age association. Betas are shown in SDs of $\log_e$LTL with 95% confidence intervals. Sex reflects the effect of male sex.

Trait	Beta (95% CI)	P value	Partial R^2
Age (years)	-0.023 (-0.024 , -0.023)	<1.0E-314	3.52%
Sex (ref: Female)	-0.170 (-0.176 , -0.164)	<1.0E-314	0.68%
WBC	-0.064 (-0.067 , -0.061)	<1.0E-314	0.37%
Neutrophil percentage	0.048 (0.030 , 0.066)	1.92E-07	0.01%
Lymphocyte percentage	0.009 (-0.007 , 0.025)	0.291	0.00%
Basophil percentage	-0.003 (-0.006 , 0.000)	0.075	0.00%
Eosinophil percentage	-0.010 (-0.014 , -0.006)	1.30E-05	0.01%
Monocyte percentage	-0.010 (-0.015 , -0.004)	1.39E-03	0.00%
Ethnicity (ref: White)			0.84%
Mixed	0.126 (0.088 , 0.164)	8.80E-11	
Asian	0.049 (0.028 , 0.071)	5.07E-06	
Black	0.412 (0.387 , 0.436)	1.41E-245	
Chinese	0.359 (0.308 , 0.411)	2.00E-42	
Other	0.185 (0.155 , 0.216)	2.21E-32	

Table 6. Multivariable model on LTL. Partial R^2 is the contribution of the parameter on the total model R^2 (estimated as the difference between the full model R^2 and the model R^2 leaving this parameter out). Total model R^2 is 5.52%. Betas are shown in SDs of $\log_e$LTL with 95% confidence intervals.

Figure Legends

Figure 1. DNA sample workflow to derive the final dataset. After removal of study withdrawals and deliberate duplicate samples there were 488,400 participants for whom we attempted to measure LTL. Either a valid measurement was obtained or the sample was attributed to one of three categories of failure after quality control (QC). For the downstream analyses presented in this paper that related to baseline phenotypes, we removed 1,900 DNA samples whose LTL was measured in a non-baseline DNA sample or where self-reported sex and genetic sex did not match.

Figure 2: Distribution of weekly average LTL across duration of the study. A) The unadjusted LTL trend over time (and 95% Confidence Interval in grey). B) The adjusted LTL trend over time (and 95% Confidence I in grey). Adjustments for enzyme, PCR machine, primer, Operator, temperature, humidity, primer*PCR machine, primer*Operator and A260/280 were made as described in **Methods**. The smoothed curve is based on half plate means, with plotted data points representing overall weekly means. The size of each point indicates the number of runs that week. There were fewer measurements made after week 175, reflecting the period that sample quality control (QC) and re-measurements took precedence following QC checks towards the end of the project.

Extended Data Figure 1. **Significant technical parameters affecting LTL measurements based on the stage 1 adjustment.** Summary boxplots are shown for the 474, 074 LTL measurements for each associated parameter: Enzyme batch (A), PCR machine (B), primer batch (C), operator (D). Individual data points show minimum and maximum measures, the box represents the lower quartile (bottom), upper quartile (top) and median (internal line). The upper and lower whiskers extend to a value no further than 1.5 * IQR from the respective quartile. Linear relationships were seen between LTL and temperature (E) and humidity (F). For both (E) and (F) a fitted regression line is shown with 95% Confidence Intervals (grey shading).

Extended Data Figure 2. Significant interactions based on the stage 2 adjustment. A) LTL by Primer and Operator. B) LTL by primer and PCR machine. PCR machines 5 and 6 were not used at the start of the pilot study (primer batch 1) and machines 7 and 8 were used from the end of the pilot stage (primer batch 3 onwards).

Extended Data Figure 3. Effect of A260/280 on LTL. The distribution of DNA sample A260/280 ratios is illustrated in (A). We observed an increase in LTL with very low and very high A260/280 ratios (B). Data shown is mean LTL (blue0 with 95% Confidence Interval (grey).

Extended Data Figure 4: Distribution of the coefficients of variation for the repeat samples. Distribution of CVs after technical adjustment for both the blinded repeats A) (n=528) and deliberate repeats B) (n=22,615) are shown. The grey dotted line represents the median coefficient of variation with the shaded region representing the interquartile range.

Extended Data Figure 5. Data on the first and second DNA sample used to estimate regression dilution ratio. A) Histogram showing that the gap between the two sample collections has a mean interval of 5.5 years (range: 2-10 years, N=1,312). B) Correlation between the first and second $\log_e$-LTL measure by time, estimated by the difference in years between the two sample collections and shown with 95% confidence intervals. The blue circle reflects the correlation estimate (centre) with size reflecting the number of participants measured each year (exact N shown in brackets). The black line shows the overall pooled

correlation for all samples and the red dotted lines indicate the 95% confidence interval for this estimate.

Extended Data Figure 6: Age and sex relationships for participants used to estimate regression dilution bias. The decline of LTL with age is shown for men (blue) and women (plum) for both the first (A) and second (B) LTL measurements. The estimated effect sizes are shown for age (β_Age) and sex (β_Sex) within the figures.

Extended Data Figure 7. Decline of LTL with age. The decline of z-standardized $\log_e$-LTL with age is shown for men (blue) and women (plum) in adjusted data. The y-axis is truncated at -5SD to +5SD with 166 data points (80 women, 86 men) not shown. A small number of participants recruited by UK Biobank fall outside of the stated 40-69 age range.

Extended Data Figure 8. Decline in LTL with age by sex. Using stratified regression for men (blue) and women (plum) for all participants (N=474,074) we considered the non-linear effect of age within each sex. Here we show the predicted shape in a solid line and the observed data in a dashed line with 95% confidence intervals. There is significant non-linearity observed for women, where the rate of LTL decline increases as the population ages.

Extended Data Figure 9. Telomere lengths within individual ethnic groups. Data adjusted for both age and sex are shown in purple for individual observations to indicate the range and quantity of data alongside a box-plot to show the median (line) and interquartile range (box) with whiskers extending to a value no further than 1.5 * IQR from the respective quartile. Box plots of data with additional adjustment for BMI, CRP, HbA1c, smoking status, alcohol consumption, and measures of physical activity, socioeconomic status and diet are shown in blue. Ethnicity is self-reported and presented as defined by UK Biobank Data-Field 21000. Note that we shorten "Asian or Asian British" to Asian and "Black or Black British" to Black.

Extended Data Figure 10. LTL by age in different ethnic groups. LTL measurements were adjusted for sex, BMI, CRP, HbA1c, smoking status, alcohol consumption, and measures of physical activity, socioeconomic status and diet.

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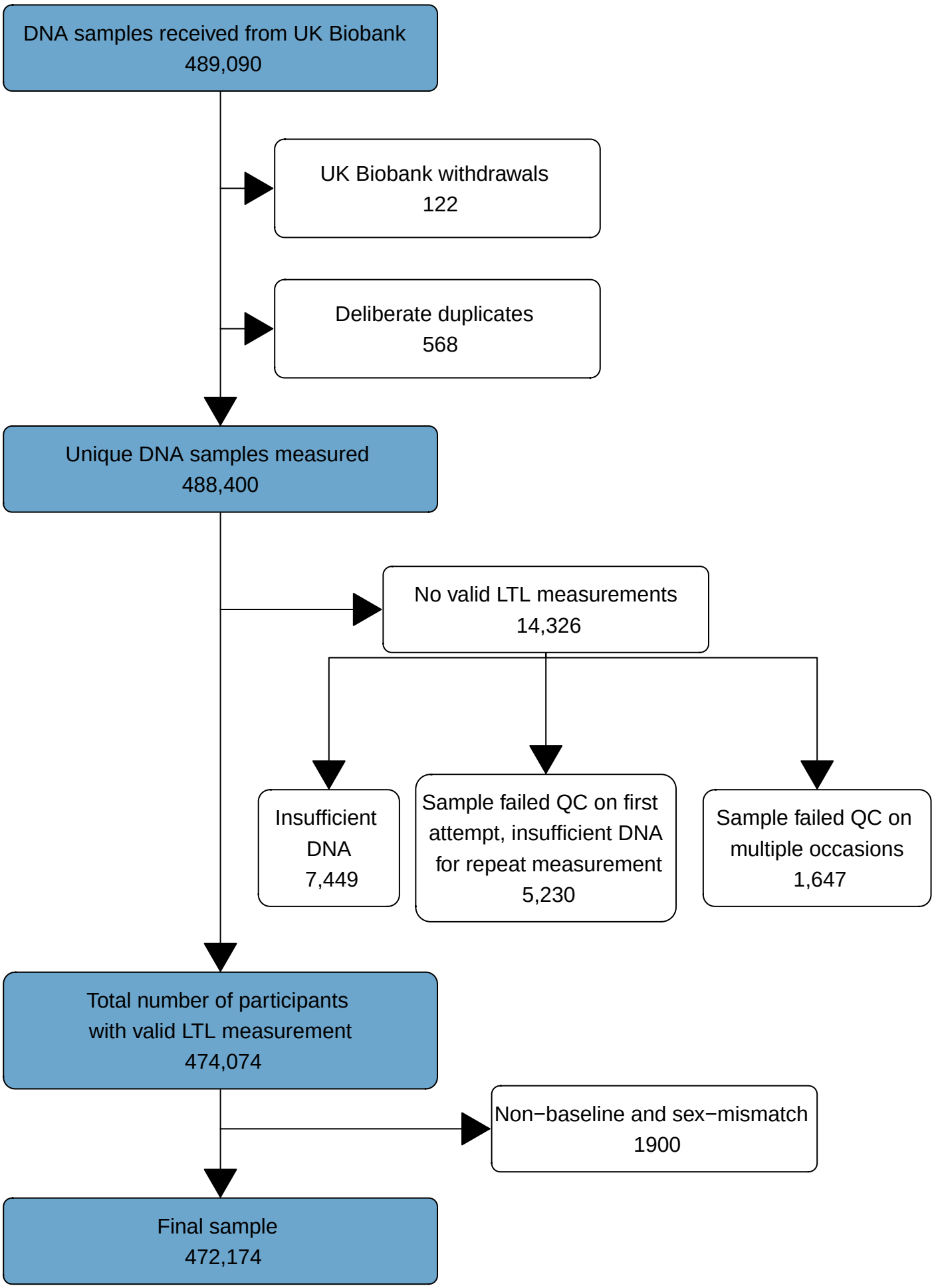
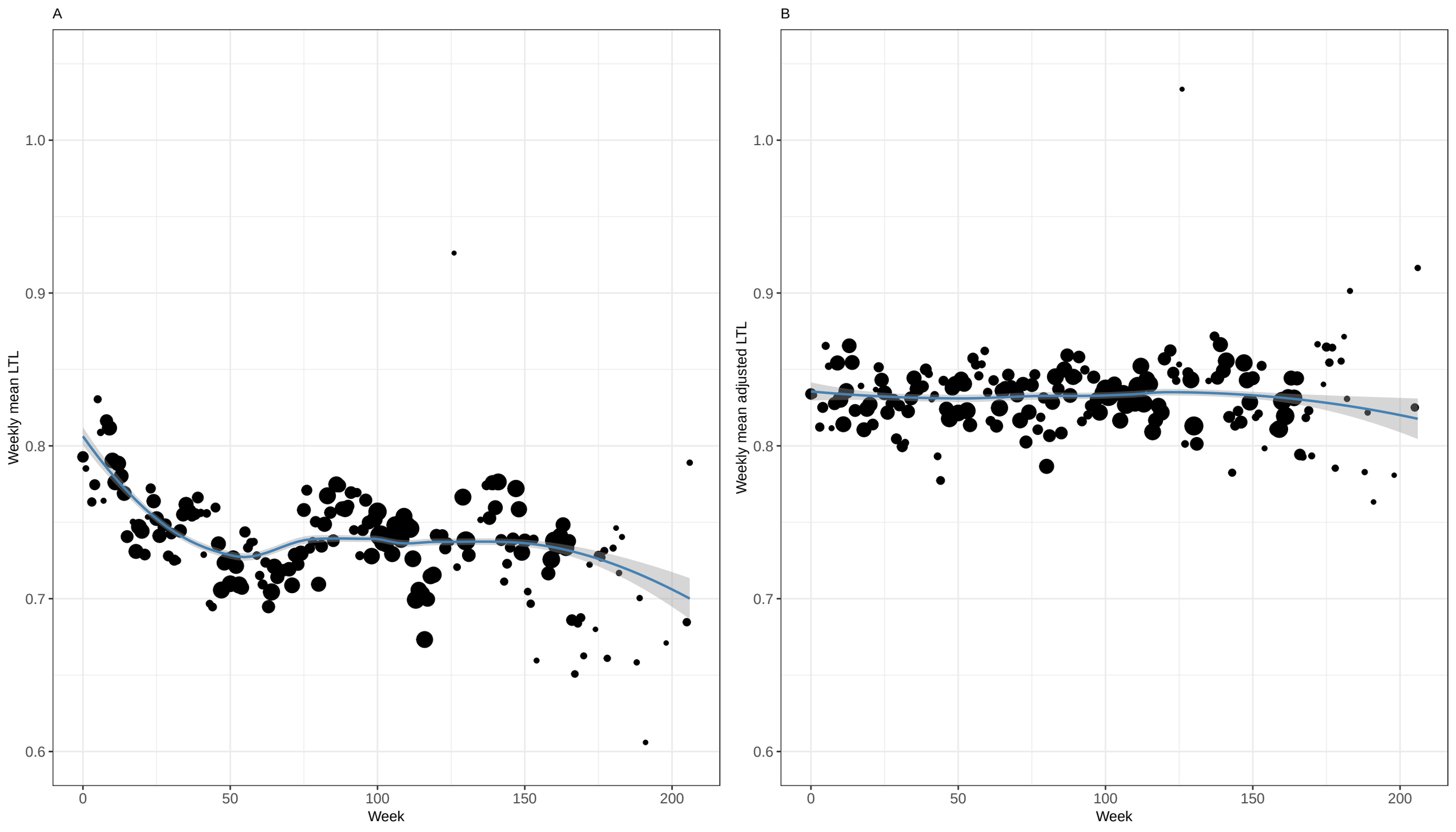
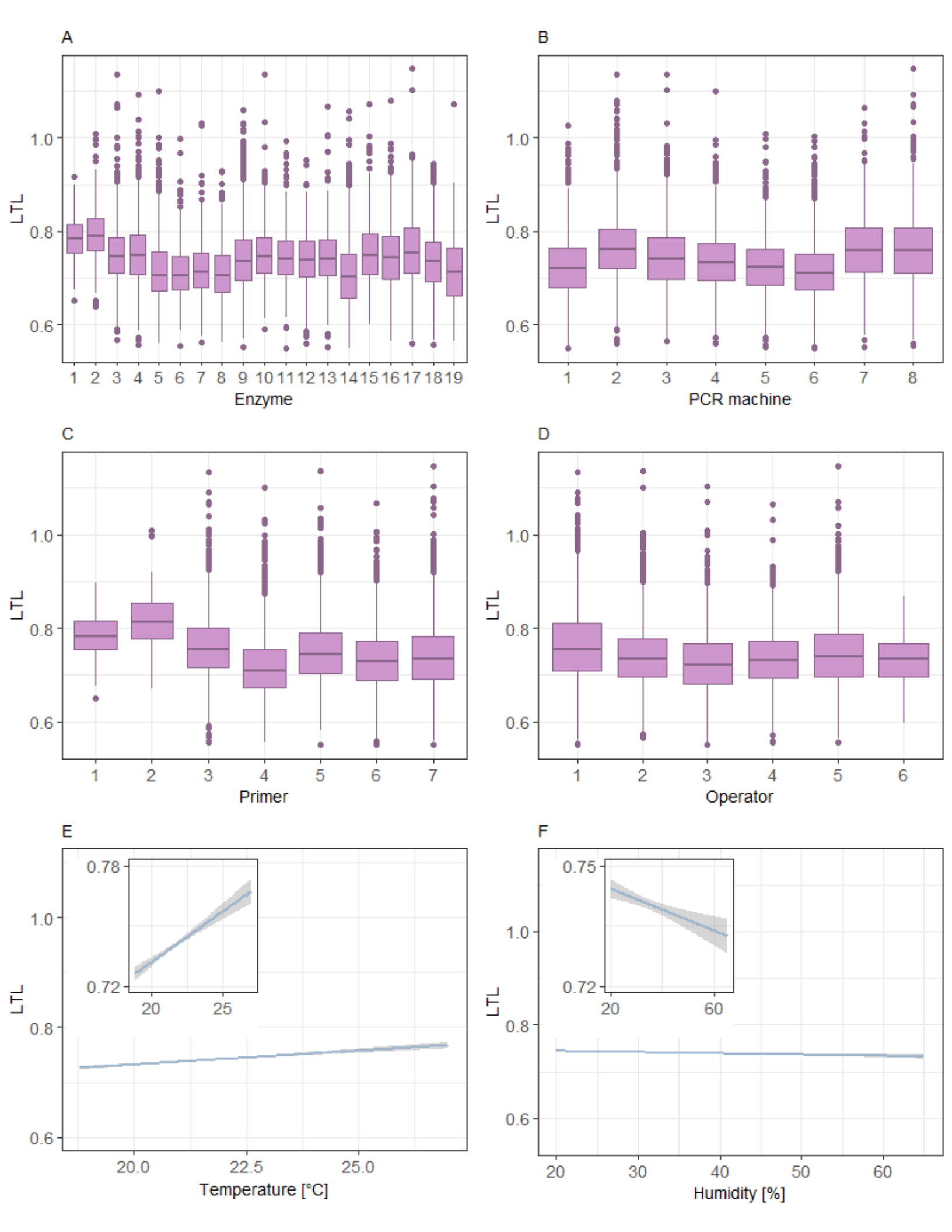
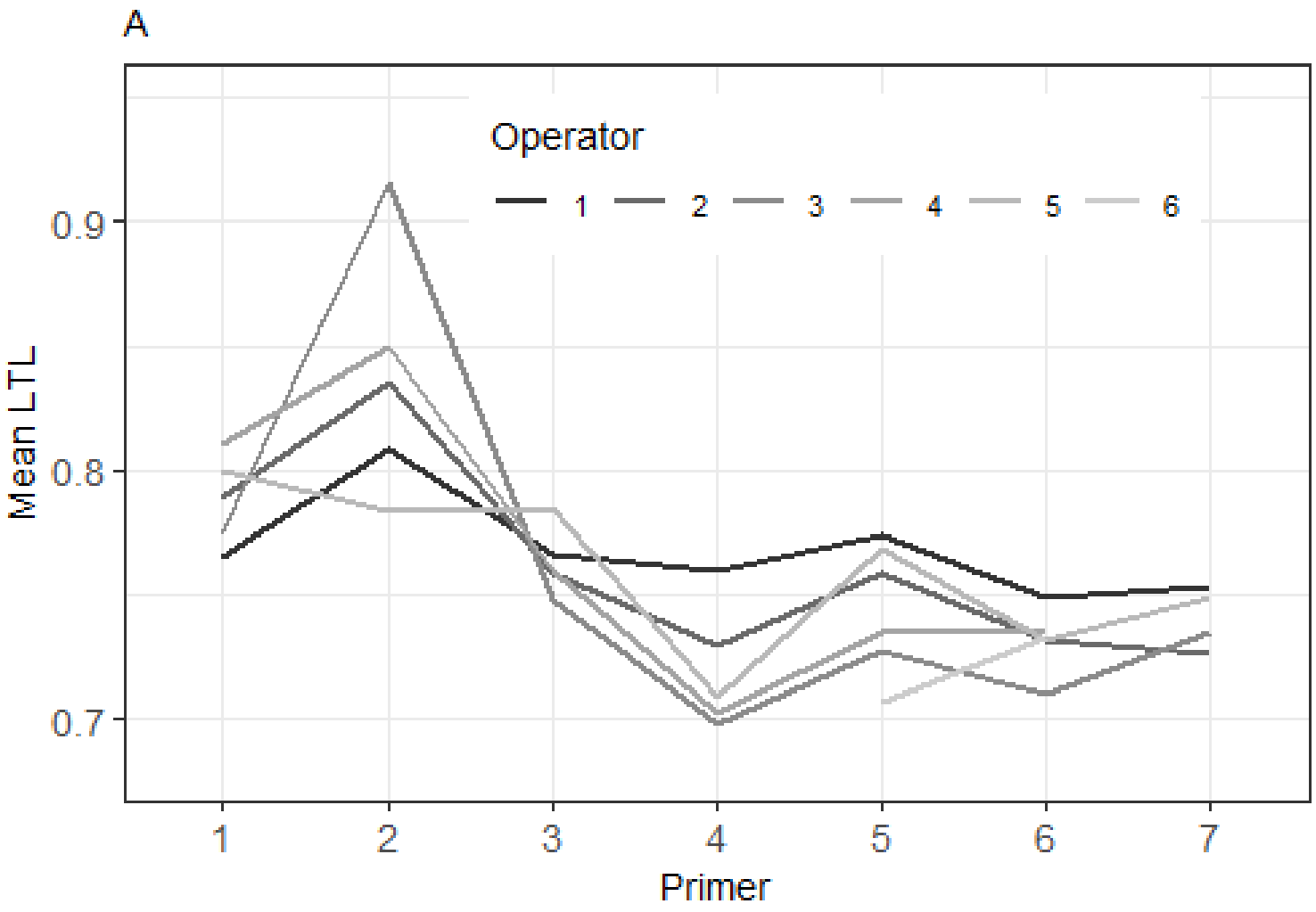
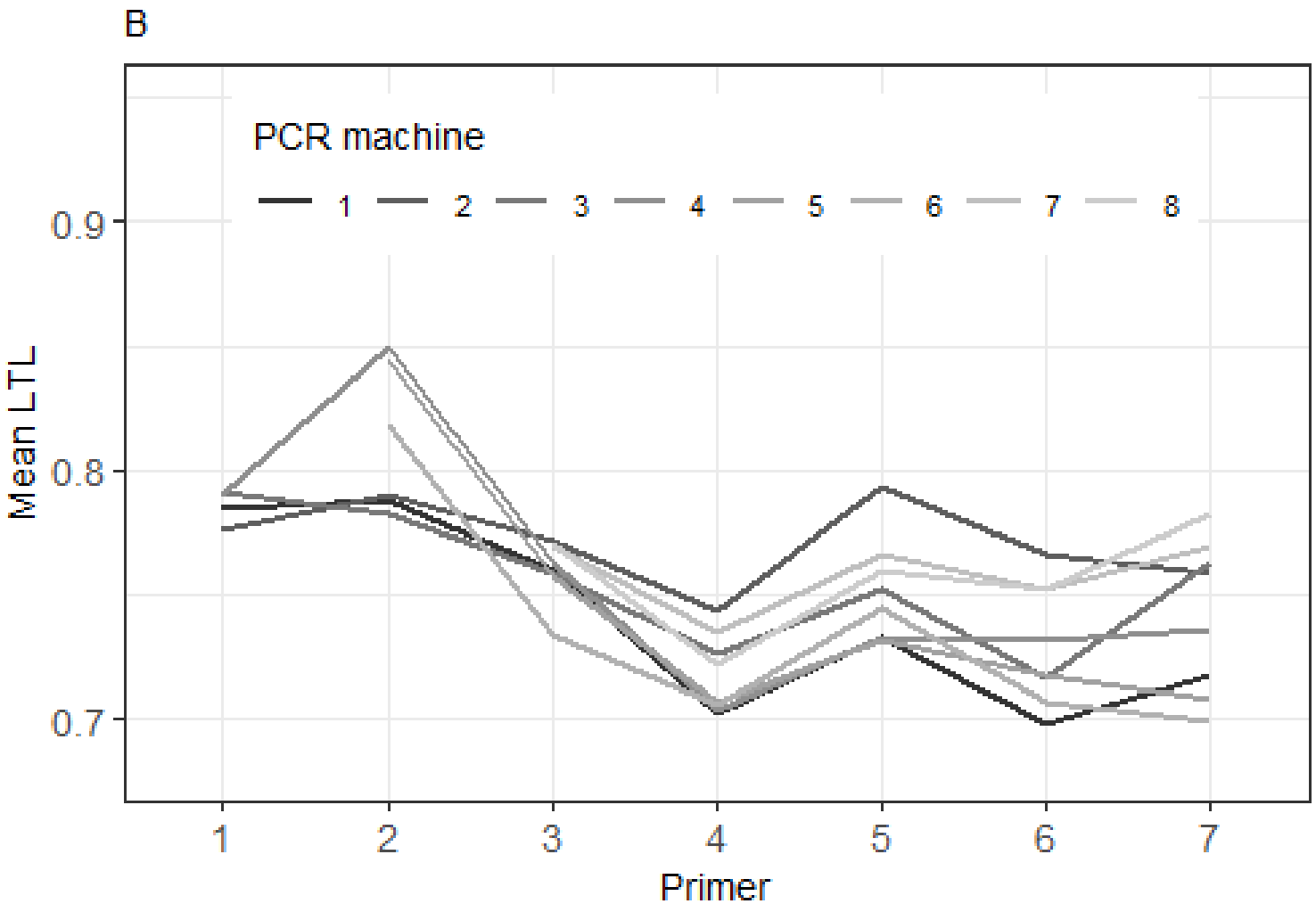
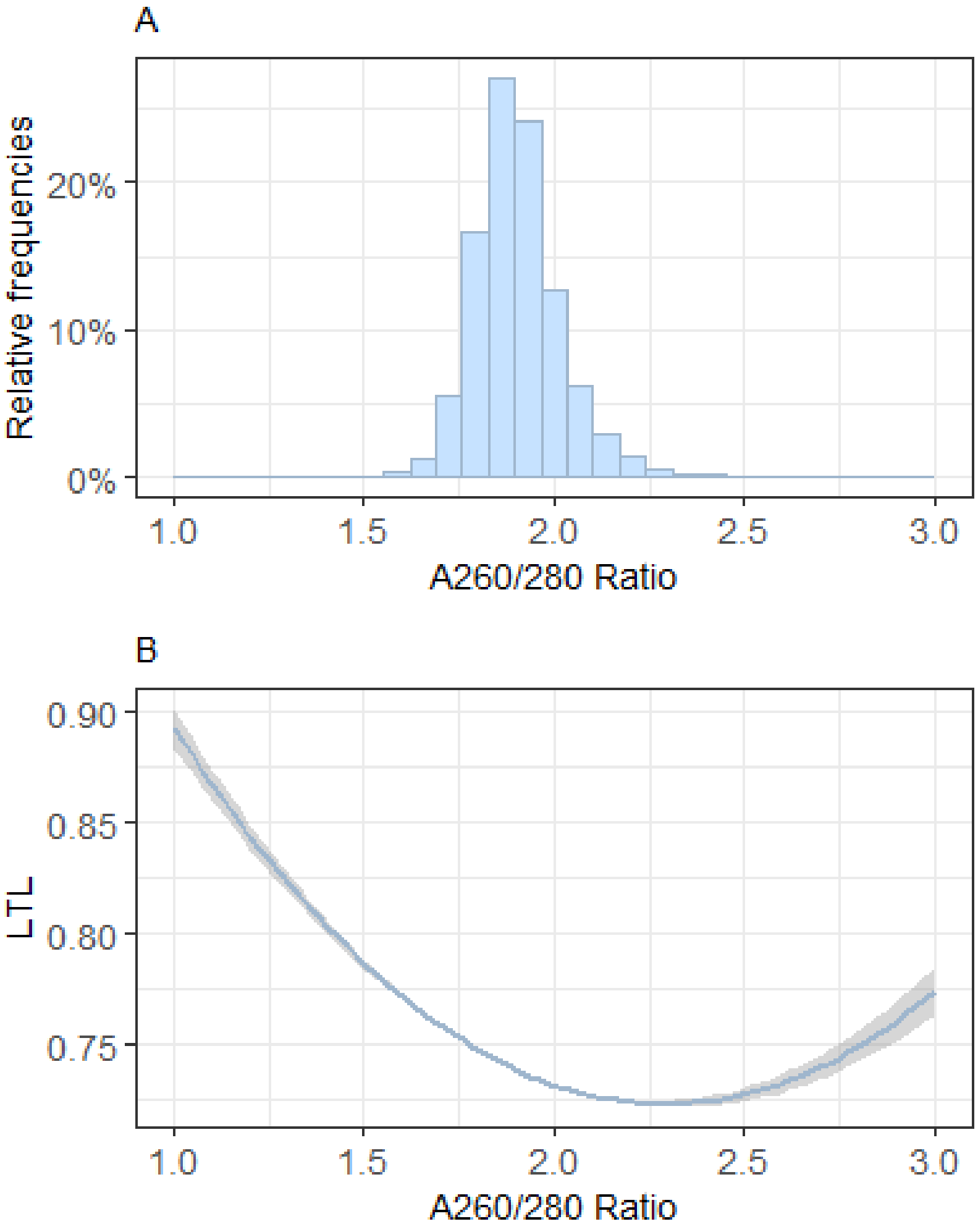

DNA samples received from UK Biobank
489,090
UK Biobank withdrawals
122
Deliberate duplicates
568
Unique DNA samples measured
488,400
No valid LTL measurements
14,326
Insufficient DNA
7,449
Sample failed QC on first attempt, insufficient DNA for repeat measurement
5,230
Sample failed QC on multiple occasions
1,647
Total number of participants with valid LTL measurement
474,074
Non−baseline and sex−mismatch
1900
Final sample
472,174

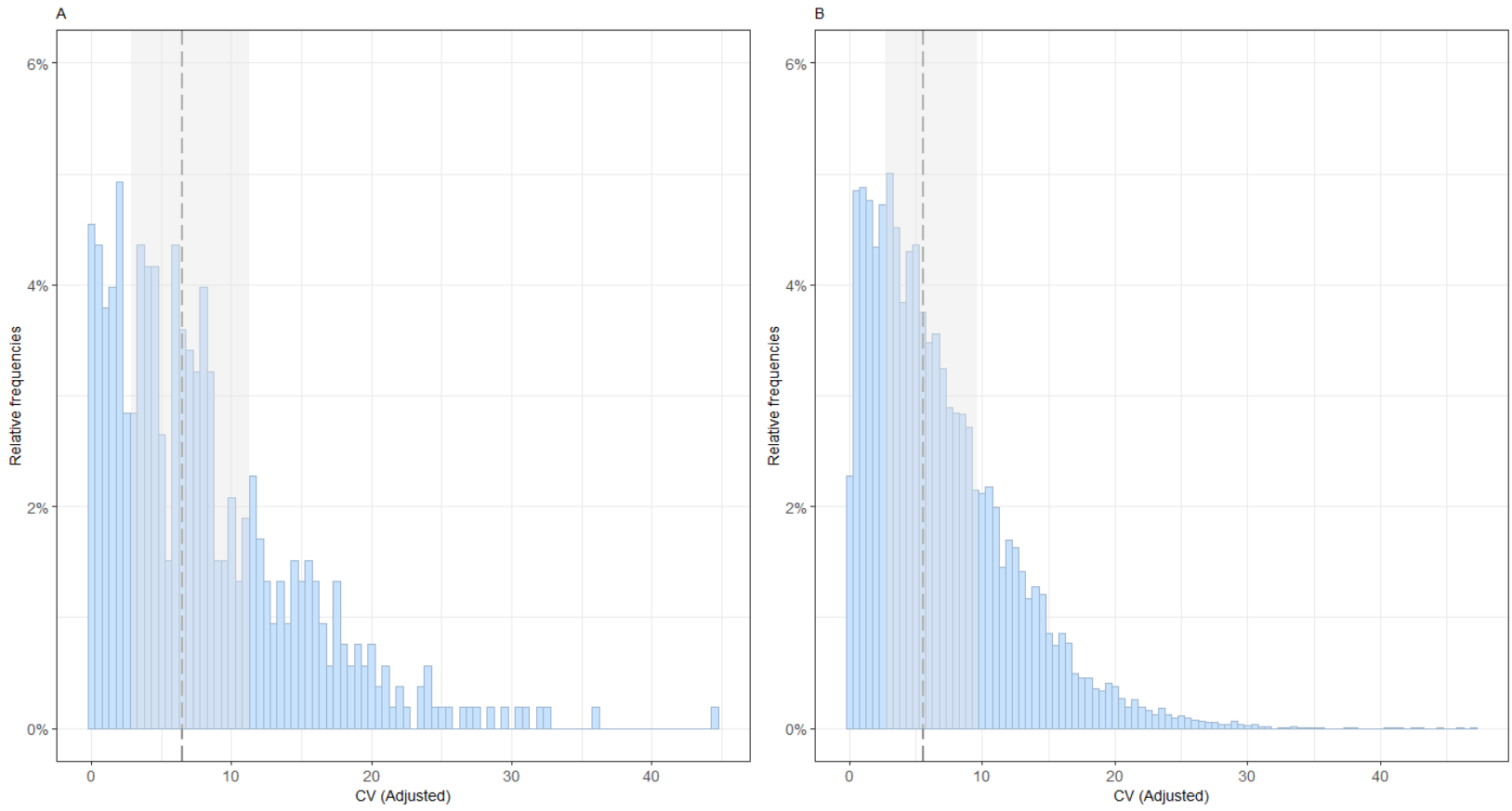

A
B
Weekly mean LTL
Weekly mean adjusted LTL
1.0
0.9
0.8
0.7
0.6
0
50
100
150
200
Week

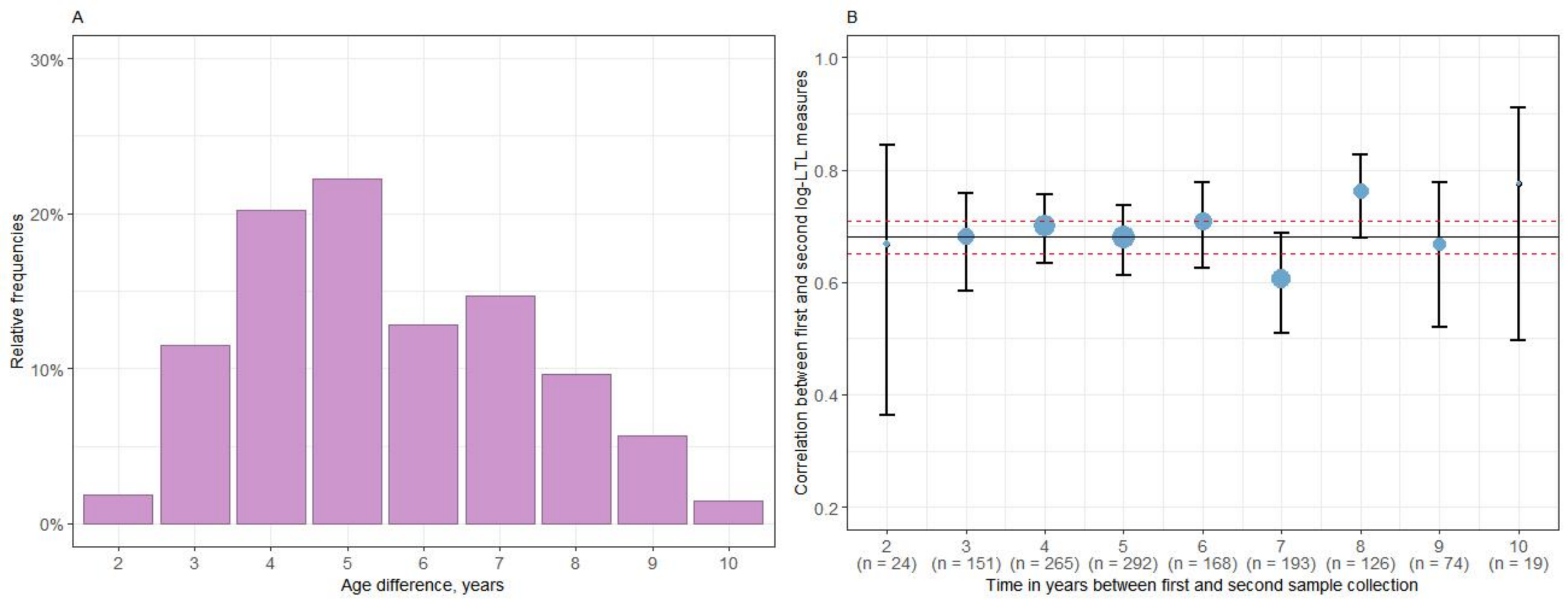

A
LTL
1.0
0.8
0.6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Enzyme
B
LTL
1.0
0.8
0.6
1 2 3 4 5 6 7 8
PCR machine
C
LTL
1.0
0.8
0.6
1 2 3 4 5 6 7
Primer
D
LTL
1.0
0.8
0.6
1 2 3 4 5 6
Operator
E
LTL
1.0
0.8
0.6
0.78
0.72
20 25
20.0 22.5 25.0
Temperature [°C]
F
LTL
1.0
0.8
0.6
0.75
0.72
20 60
20 30 40 50 60
Humidity [%]


A
Operator
1
2
3
4
5
6
0.9
0.8
0.7
Mean LTL
1
2
3
4
5
6
7
Primer


B
PCR machine
1
2
3
4
5
6
7
8
0.9
0.8
0.7
Mean LTL
1
2
3
4
5
6
7
Primer


A
Relative frequencies
20%
10%
0%
1.0
1.5
2.0
2.5
3.0
A260/280 Ratio
B
LTL
0.90
0.85
0.80
0.75
1.0
1.5
2.0
2.5
3.0
A260/280 Ratio


A
B
6%
4%
2%
0%
Relative frequencies
0
10
20
30
40
CV (Adjusted)


A
30%
20%
10%
0%
Relative frequencies
2
3
4
5
6
7
8
9
10
Age difference, years
B
1.0
0.8
0.6
0.4
0.2
Correlation between first and second log-LTL measures
2 (n = 24)
3 (n = 151)
4 (n = 265)
5 (n = 292)
6 (n = 168)
7 (n = 193)
8 (n = 126)
9 (n = 74)
10 (n = 19)
Time in years between first and second sample collection

A

B

$\beta_{Age} = -0.0025$

$\beta_{Sex} = -0.0245$

$\beta_{Age} = -0.0021$

$\beta_{Sex} = -0.0286$

First LTL measurement

Second LTL measurement

2.0

1.5

1.0

0.5

40

50

60

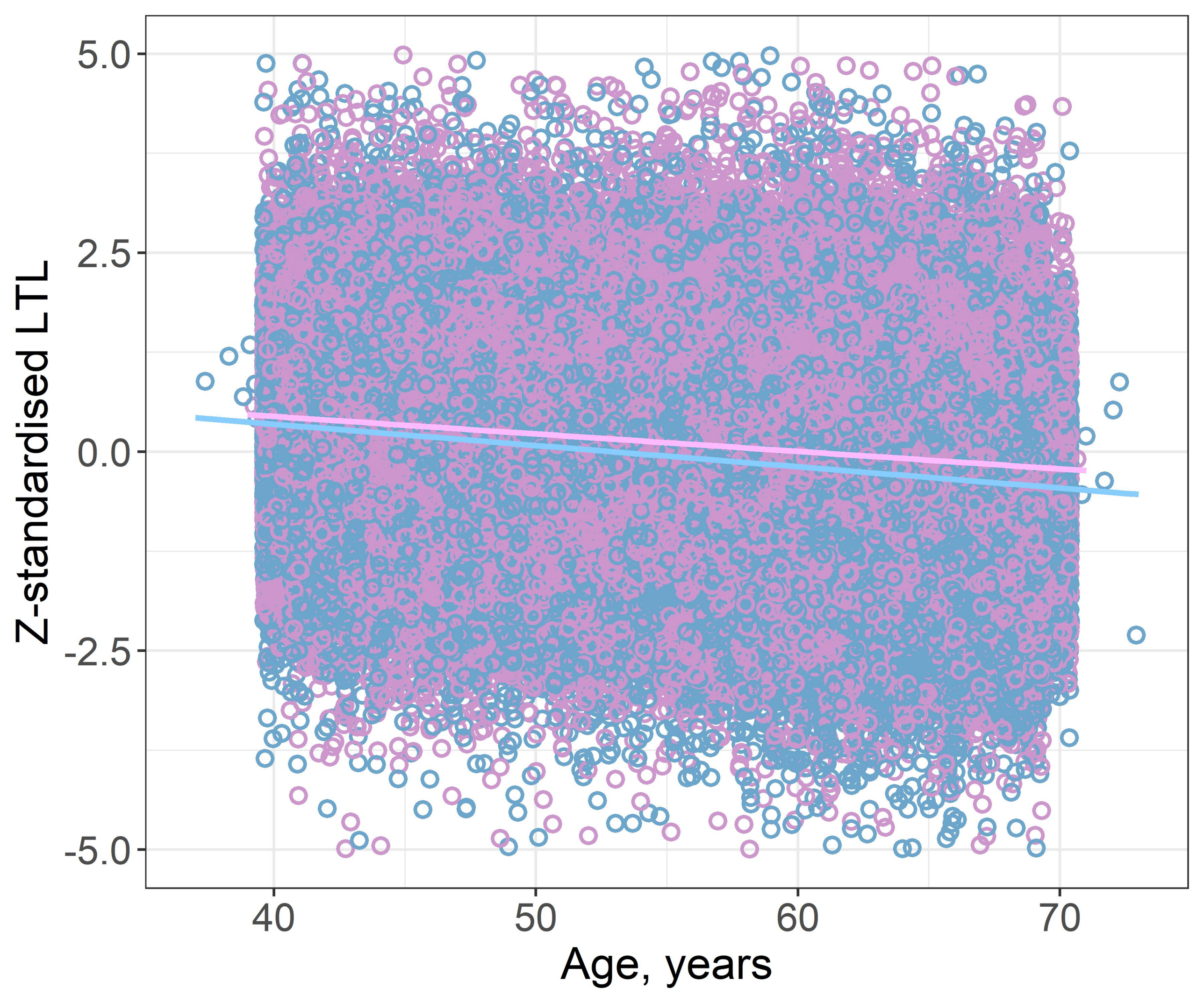
70

80

Age, years

Female

Male

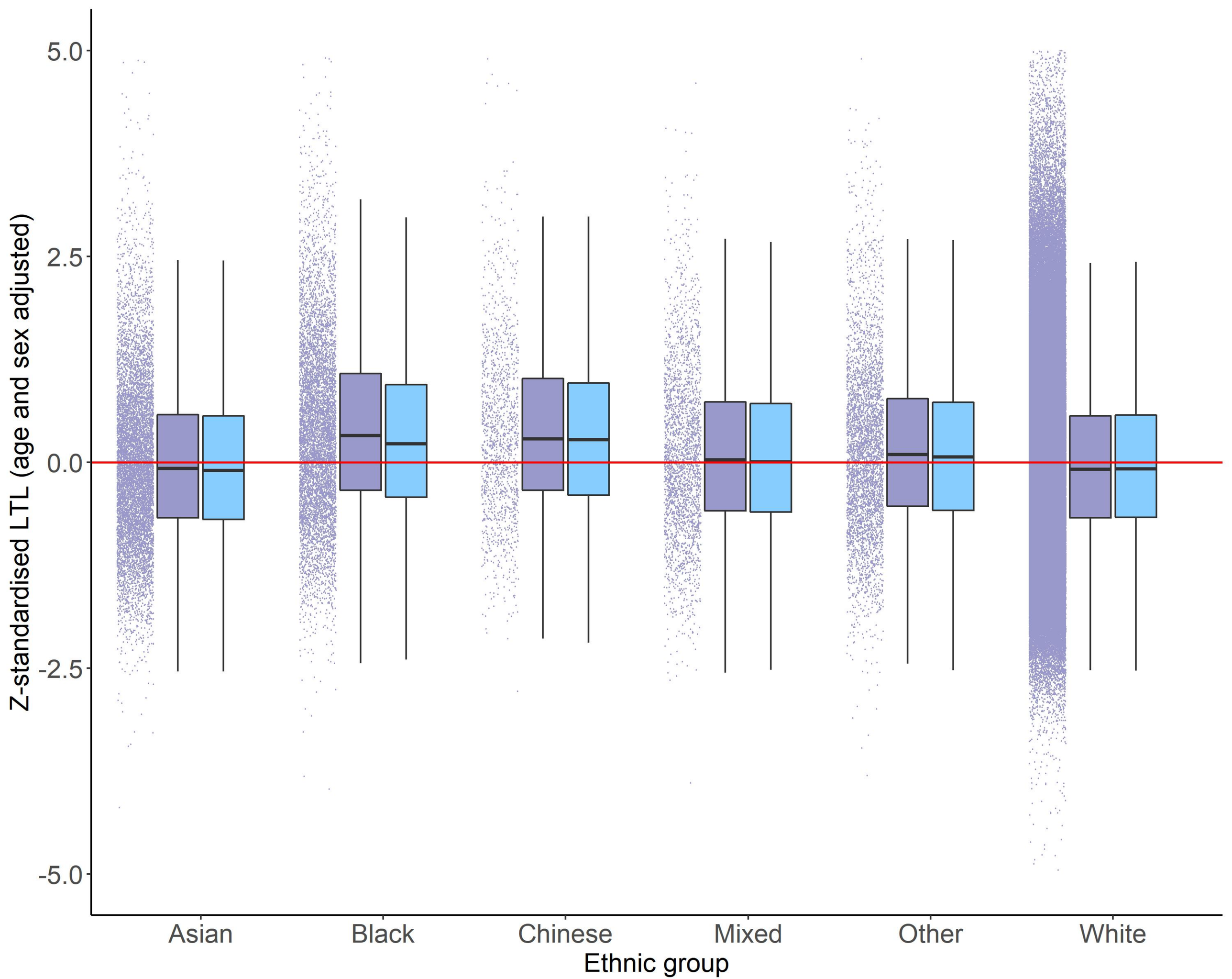

Z-standardised LTL
5.0
2.5
0.0
-2.5
-5.0
40
50
60
70
Age, years

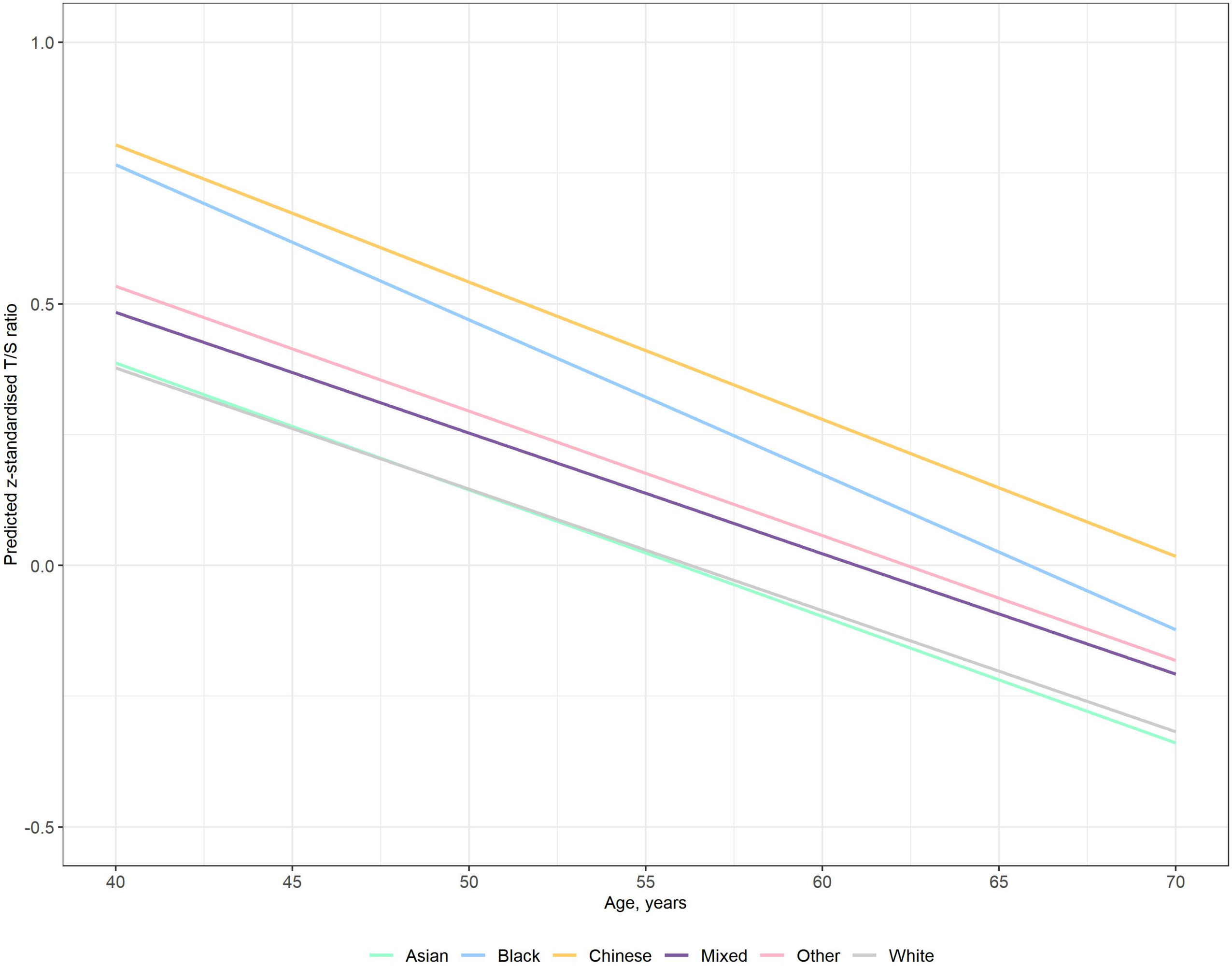
Mean Z-standardised LTL

0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5

40
50
60
70

Age, years


5.0
2.5
0.0
-2.5
-5.0
Z-standardised LTL (age and sex adjusted)
Asian
Black
Chinese
Mixed
Other
White
Ethnic group


Predicted z-standardised T/S ratio
1.0
0.5
0.0
-0.5
40
45
50
55
60
65
70
Age, years
Asian
Black
Chinese
Mixed
Other
White