



Telomere length and lung function in a population-based cohort of children and mid-life adults

Journal:	<i>Pediatric Pulmonology</i>
Manuscript ID	Draft
Wiley - Manuscript type:	Original Article: Epidemiology
Date Submitted by the Author:	n/a
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Keywords:	Airway & Lung Cell Biology, Biomarkers, Epidemiology, Pulmonary Function Testing (PFT), Pulmonology (general)
Other Keywords:	Aging, Cell senescence, Life course, National cohort, Spirometry
Abstract:	<p>Objective: Telomere length is associated with poorer lung health in older adults, possibly from cumulative risk factor exposure, but data are lacking in pediatric and population-based cohorts. We examined associations of telomere length with lung function in children and mid-life adults.</p> <p>Methods: Data were drawn from a population-based cross-sectional study of 11-12 year-olds and mid-life adults. Lung function was assessed by spirometric FEV1, FVC, FEV1/FVC ratio and MMEF25-75. Telomere length was measured by quantitative polymerase chain reaction from blood and expressed as the amount of telomeric genomic DNA to the beta-globin gene (T/S ratio). Associations of telomere length with spirometric parameters were tested by linear and logistic regression models, adjusting for potential confounders of sex, age, body mass index, socioeconomic position, physical activity, inflammation, asthma,</p>

	<p>pubertal status and smoking.</p> <p>Results: Mean T/S ratio was 1.09 (n=1,206, SD 0.55) in children and 0.81 (n=1,343, SD 0.38) in adults. In adults, for every additional unit in T/S ratio, FEV1/FVC and MMEF25-75 z-scores were higher (β 0.21 [95% CI 0.06-0.36] and 0.23 [95% CI 0.08-0.38] respectively), and the likelihood of being in the lowest quartile for FEV1/FVC and MMEF25-75 z-scores was lower (odds ratios 0.59 [95% CI 0.39-0.89] and 0.64 [95% CI 0.41-0.99] respectively). No evidence of association was seen for adult FEV1 or FVC, or any childhood spirometric index after adjustments.</p> <p>Conclusion: Shorter telomere length showed moderate associations with poorer airflow parameters, but not lung size in mid-life adults. However, there was no convincing evidence of associations in children.</p>

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TELOMERE LENGTH AND LUNG FUNCTION IN A POPULATION-BASED COHORT OF CHILDREN AND MID-LIFE ADULTS

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KEYWORDS

Aging; cell senescence; life course; national cohort; spirometry

FUNDING INFORMATION

Australian National Health and Medical Research Council, Grant/Award Numbers: 1041352, 1109355, 1115167, 1045161, 1091124, 1064629, 1046518, 1160906; Royal Children’s Hospital Foundation, Grant/Award Number: 2014-241; Murdoch Children’s Research Institute, Clinician Scientist Award; University of Melbourne; Australian National Heart Foundation, Grant/Award Numbers: 100660, 101239, 100369; Financial Markets Foundation for Children, Grant/Award Numbers: 2014-055, 2016-310; Cure Kids New Zealand

RUNNING TITLE

Telomere Length and Lung Function

ABSTRACT

Objective: Telomere length is associated with poorer lung health in older adults, possibly from cumulative risk factor exposure, but data are lacking in pediatric and population-based cohorts. We examined associations of telomere length with lung function in children and mid-life adults.

Methods: Data were drawn from a population-based cross-sectional study of 11-12 year-olds and mid-life adults. Lung function was assessed by spirometric FEV₁, FVC, FEV₁/FVC ratio and MMEF₂₅₋₇₅. Telomere length was measured by quantitative polymerase chain reaction from blood and expressed as the amount of telomeric genomic DNA to the beta-globin gene (T/S ratio). Associations of telomere length with spirometric parameters were tested by linear and logistic regression models, adjusting for potential confounders of sex, age, body mass index, socioeconomic position, physical activity, inflammation, asthma, pubertal status and smoking.

Results: Mean T/S ratio was 1.09 (n=1,206, SD 0.55) in children and 0.81 (n=1,343, SD 0.38) in adults. In adults, for every additional unit in T/S ratio, FEV₁/FVC and MMEF₂₅₋₇₅ z-scores were higher (β 0.21 [95% CI 0.06-0.36] and 0.23 [95% CI 0.08-0.38] respectively), and the likelihood of being in the lowest quartile for FEV₁/FVC and MMEF₂₅₋₇₅ z-scores was lower (odds ratios 0.59 [95% CI 0.39-0.89] and 0.64 [95% CI 0.41-0.99] respectively). No evidence of association was seen for adult FEV₁ or FVC, or any childhood spirometric index after adjustments.

Conclusion: Shorter telomere length showed moderate associations with poorer airflow parameters, but not lung size in mid-life adults. However, there was no convincing evidence of associations in children.

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INTRODUCTION

Cell senescence may play an important role in the lifecourse pathways of lung health. Incidences of respiratory diseases increase with age, suggesting that age-related processes may be common to these diseases.¹ For example, chronic obstructive pulmonary disease (COPD) is strongly related with frailty as early as mid-life.² Pathophysiological mechanisms thought to underlie respiratory diseases and continuous lung function decline include chronic inflammation, oxidative stress and mitochondrial dysfunction,³ all of which are closely related to cell senescence.⁴ One molecular mechanism strongly implicated in the induction of cellular senescence is telomere shortening.⁵

Telomeres are nucleoprotein structures that preserve the ends of linear chromosomes,⁶ and their shortening is linked to mortality, cancer, and cardiovascular disease.⁷ Short telomeres drive cell senescence, resulting in inhibited tissue repair capacity and function.⁶ The role of telomere shortening in lung health has been examined in adults but studies are lacking in children.

Changes in lung function provide a snapshot of overall lung health developing over the lifecourse. Increasing evidence in adults suggests that shortened telomeres are associated with reduced lung health.^{8,9} For instance, shortened telomeres are found in pulmonary vascular endothelial cells of COPD patients⁸ and in fibrotic areas of adults with pulmonary fibrosis.¹⁰ Further, a study of 46,396 adults aged between 43 and 73 years showed a modest association of shorter telomere length with decreased lung function.¹¹ However, the generalizability of these findings across the lifecourse is uncertain as most studies were conducted in older patients already exhibiting lung disease. Few studies have explored the relationship between telomere length and lung function in younger general populations, despite the fact that pathways to ill health in adulthood begin much earlier in life.¹² Telomere-linked cell senescence could be on the pathway to decreased lung function and increased respiratory disease risk. Understanding this association could enhance our understanding of the pathogenesis of respiratory disease and identify novel mechanisms for intervention.

Exploring the relationships between telomeres and lung function across the lifecourse could elucidate the role of telomere length-driven cell senescence in the development of healthy lungs. In a population-based study of children and mid-life adults, we investigated the associations of telomere length with lung function and specifically telomeres in those with the lowest quartile lung function. Given the progressive nature of the proposed association across the lifecourse, we predicted that the magnitude of associations between telomere length and lung function would be stronger in adults relative to their children.

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MATERIALS AND METHODS

Study design, subjects and procedure

In 2004, the Longitudinal Study of Australian Children (LSAC) recruited a nationally-representative birth cohort (n=5,107), which has since been followed at seven biennial waves. The Child Health CheckPoint (CheckPoint) study was an additional physical health and biomarker module at child age 11-12 years, nested between LSAC's sixth and seventh waves. Ultimately, 1,874 families took part in the CheckPoint. Most non-participation was due to inability to attend or to reschedule a visit during the short period CheckPoint was in each location. Details of the LSAC and CheckPoint study are previously described.¹³⁻¹⁵

CheckPoint's data collection ran from Feb-2015 to Mar-2016. Each child was invited to attend an assessment center with one parent/caregiver. No more than one child from each family participated. The main assessment center operated across Australia in major and regional centers. However, a small number of families attended mini assessments or opted for home visits, and were therefore excluded from the current analysis as these condensed assessments did not include venous blood samples, used to measure telomere length. Children and their attending parent rotated through a series of 15 to 30-minute stations where different aspects of health were assessed and biological samples collected. Child and parent protocols and equipment were identical. The attending parent provided written informed consent for themselves and their child, and the child provided assent.

DNA isolation and telomere length measurement

At the assessment center, medically-trained researchers or phlebotomists collected venous blood at a 15-minute station. Blood was processed within 2 hours at an on-site laboratory and stored at -80 degrees Celsius. For the first two months, this included blood clots from plasma tubes; for logistical reasons, clots were then discontinued and replaced with a whole blood sample for the remaining centers. At the Murdoch Children's Research Institute (MCRI), genomic DNA was isolated from available blood (e.g. whole blood or blood clot)

using the Qiaamp 96 DNA Blood Kit (Qiagen, Venlo, Netherlands). Purity and integrity of DNA were confirmed using spectrophotometry (NanoDrop 2000, NanoDrop Technologies, USA), fluorimetry (Qubit 2.0, Thermo Fisher Scientific, USA) and gel electrophoresis. Telomere length was measured by quantitative polymerase chain reaction, originally described by Cawthon.¹⁶ This method measures the amount of telomeric DNA (T) and a single copy gene beta-globin (S) for each sample. A ratio (T/S ratio) was then calculated by comparing the relative amount of 'T' and 'S' for each participant. The mean intra-assay variability was 1.7 % (standard deviation (SD) 0.3, range 0.9 to 2.6). The inter-assay variability was 1.7 % (SD 1.4, range 0.3 to 6.2). Further details on the telomere procedure and assay are described in the online Supporting Information and epidemiological findings have been published.¹⁷

Lung function assessed by spirometry

Trained researchers conducted spirometry testing at a 30-minute station. Spirometry was performed between 3-8 repeats on each participant using a spirometer (Vyntus Pneumo, Vyntus, USA) running SentrySuite software (Care Fusion, Germany) with a bacterial filter and nose clip, in accordance with international guidelines.¹⁸ Further details of the spirometry method and epidemiology are described elsewhere.^{14,19}

Spirometric indices: Forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), their ratio (FEV₁/FVC) and maximal mid-expiratory flow (MMEF₂₅₋₇₅) were extracted from the SentrySuite platform and converted to z-scores using the Global Lung Initiative equations.²⁰ The quality of flow-volume loops were assessed by trained spirometry experts and were included in analyses if guidelines were met (Supporting Information Table S1).¹⁸

Spirometry trace reliability: Two experienced raters scored the same 21 loops from a random sample of 21 children and the same 19 loops from adults. Cohen's Kappa statistic was used to measure the agreement between waveform classifications. Overall agreement between waveform classifications was substantial (kappa=0.79, p-value<0.001) with a high

percentage of agreement (95%), indicating a high level of agreement by the two raters. Further details are in the online Supporting Information.

Potential confounders

Several variables were considered *a priori* as potential confounders taking published literature into account, including body mass index (BMI), socioeconomic status, puberty, smoking, physical activity and inflammation. Each of these variables has been associated with telomere length,^{4,21-24} as well as being commonly known factors that influence lung growth.²⁵⁻²⁹ BMI was calculated from height (Stadiometer, Invicta IP0955, UK) and weight (InBody230, Biospace, South Korea). For children, an age- and sex-adjusted BMI z-score was calculated using growth reference charts.³⁰ A standardized score that summarizes the social and economic conditions of Australian neighborhoods was calculated using the Socio-Economic Indexes for Areas Index of Relative Disadvantage score (Disadvantage Score); it has a national mean of 1,000 and SD of 100 (higher values represent less disadvantage). For family socioeconomic position we used LSAC's wave 6 composite measure representing parent-reported household income, occupation and education level, standardized nationally to have a mean of 0 and SD of 1 (higher scores represent more advantage). Parental cigarette smoking behavior was collected at LSAC wave 6. Child second-hand smoking was determined when at least one parent was a smoker. Asthma was self-reported in a questionnaire at CheckPoint. The novel inflammation marker of glycoprotein acetyls (GlycA) was measured using the Nightingale nuclear magnetic resonance metabolomics platform (Helsinki, Finland) from blood serum. Physical activity was calculated as the average duration of moderate-to-vigorous physical activity (MVPA) measured using a wrist-worn accelerometer (GENEActiv, Activinsights, Cambridgeshire, UK). Further details of these measures are extensively described elsewhere.¹⁴

Statistical analysis

Stata 14.2 (StataCorp, College Station, TX) was used for all analyses, with children and adults considered separately. Linear regression models were fitted where continuous

1
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3 telomere length was the independent variable and continuous spirometric values were the
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5 dependent variables for each model. Assumptions for linear regression were examined
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7 using histograms and quantile-quantile plots. Spirometric indices in children and adults
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9 followed approximately normal distributions with no discernible outliers. There was minimal
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11 right-skewing for child's and adult's telomere length. Logistic regression models were also
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13 fitted using continuous telomere length as the independent variable to assess the odds of
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15 having poor lung function. The lowest quartile for each spirometric index was compared to
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17 the highest quartile (i.e. the reference group), given that being in the lowest quartile has
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19 been shown to infer future risk of lung disease and increased respiratory morbidity.³¹
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23 For both children and adults, model 1 included adjustments for the potential lifelong
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25 confounders of sex and age, as well as *a priori* potential confounders, including BMI and
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27 socioeconomic position. Adjusted analyses included the covariates as independent
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29 variables. Model 2 additionally included adjustments for the additional potential confounders
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31 physical activity (i.e. MVPA), inflammation (i.e. GlycA), asthma, pubertal status and second-
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33 hand smoking status for children, and smoking status for adults. In addition, sensitivity
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35 analyses were done in adults by adjusting for adults' non-linear age (i.e. centered age
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37 squared and centered age cubed) instead of linear age, as well as, stratifying results by sex.
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39 Further, sensitivity analyses were done by stratifying by original blood sample type (e.g.
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41 blood clot or whole blood) and excluding participants without complete covariate data in
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43 unadjusted regression models to examine the effect of missing data.
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RESULTS

Figure 1 shows the CheckPoint study participant flow. Both telomere length and spirometry data were available for 1,153 children and 1,293 adults.

Sample characteristics

Participant characteristics are displayed in Table 1, and sex stratified results are in Supporting Information Table S2. Mean ages of children and adults were 11 years (SD 0.5) and 44 years (SD 5.1), respectively. Most adults were mothers (87%) as they typically accompanied children to the assessment center, whereas the numbers of boys and girls were similar. Both children and adults' BMI scores and prevalence of asthma were similar to the population for similar ages.³² Adult self-report of diabetes (2.4% vs. 4% for the general population) and being a current smoker (8% vs. 16%) were lower than the general population for similar ages.³² Our sample came from relatively less disadvantaged areas (Disadvantage Score 1,026, SD 61) than the national average (mean 1,000, SD 100). Children had greater T/S ratios than adults (T/S ratio 1.09 vs. 0.81, $p<0.001$). As expected, compared to children, adults had overall greater raw FEV₁, FVC and MMEF₂₅₋₇₅, but lower FEV₁/FVC ratio. Children and adults' spirometric z-scores followed similar distributions and were within normal limits, but with overall FEV₁ and FVC z-scores slightly higher, and FEV₁/FVC and MMEF₂₅₋₇₅ z-scores slightly lower than the international reference populations.²⁰

Association of telomere length with lung function

Linear and logistic regression results are shown in Table 2 and Table 3, respectively. In adults, for every unit increase in T/S ratio, FEV₁/FVC z-score and MMEF₂₅₋₇₅ z-score was 0.21 (95% CI 0.06-0.36, $p=0.008$) and 0.23 (95% CI 0.08-0.38, $p=0.003$) higher, respectively (Table 2, model 2). This equated to z-scores approximately one-fifth of a SD higher (i.e. better) for FEV₁/FVC and MMEF₂₅₋₇₅ z-scores for each one unit T/S increase (equivalent to approximately two SD T/S ratio).

On average, adults with the lowest T/S ratio (0.02) had 0.49 FEV₁/FVC z-score lower and 0.58 MMEF₂₅₋₇₅ z-score lower than those with the highest T/S ratio (2.9). These adjusted associations were only slightly smaller than in the unadjusted linear regression models. A similar pattern was observed when lung function outcomes were considered categorically. For every unit increase in T/S ratio in adults, there was a 0.59 (95% CI 0.39-0.89, p=0.01) and 0.64 (95% CI 0.41-0.99, p=0.04) reduced odds of being in the lowest quartile of FEV₁/FVC and MMEF₂₅₋₇₅ z-scores, respectively (Table 3, model 2). In contrast, telomere length was not associated with FEV₁ or FVC in adults.

Sensitivity analyses showed similar patterns between men and women, and between non-linear age and linear age, although imprecise results were observed for blood clot (Supporting Information Table S3-S5). Moreover, the exclusion of participants without complete covariate data did not alter the unadjusted associations (Supporting Information Table S6).

For children, we saw an unexpected inverse association between higher telomere length and lower FEV₁ and FVC in unadjusted linear regression models. However, this attenuated to null in the adjusted models (Table 2, model 2). When each potential confounder was considered, child's age and BMI were most likely masked confounders in the unadjusted model. Logistic regression models showed little evidence of an association between telomere length and any spirometric index in children (Table 3).

DISCUSSION

Main findings

In a population-based cohort, we found a moderate association between shorter telomeres and reduced lung function in adults relating to airflow limitation (FEV₁/FVC ratio and MMEF₂₅₋₇₅), but not with lung volumes (FEV₁ and FVC). These associations were not seen in children, in whom we saw little evidence to suggest that telomere length was associated with lung function.

Strengths and limitations

Strengths of our study include the objective examination of spirometry, conducted according to international standards, and T/S ratios with low replicate variability. Our study further benefits from the large sample sizes at two generations, children and mid-life adults. For telomere length, we employed the widely used quantitative polymerase chain reaction method which has previously been validated against the gold-standard Southern blot.¹⁶ This method is well-suited for large epidemiological studies but does not quantify absolute telomere length. The informativeness of blood telomere length as a surrogate for telomere length in the lungs remains unknown, but good telomere length correlation between different tissues,³³ and between blood and lung tissue are reported.³⁴ We acknowledge that, relative to the general population, our cohort is underrepresented for adult males, families from disadvantaged neighborhoods and those of Aboriginal/non-Caucasian backgrounds. Lack of these individuals could have weakened the precision of our findings if such children and adults had shorter telomeres and poorer lung outcomes.

Interpretation in light of other studies

In adults, shorter telomeres (lower T/S ratio) was moderately associated with reduced lung function relating to airflow limitation (lower FEV₁/FVC and MMEF₂₅₋₇₅ z-score), but not with FEV₁ and FVC. This suggests that if there are any biological impacts these act on airway relative to lung size, rather than on lung growth itself. This is congruent with the observation

that, after peaking in childhood, lung size in healthy individuals plateaus with little change in FEV₁ and FVC.³⁵ Others have reported stronger associations in individuals with lung pathologies such as COPD, idiopathic pulmonary fibrosis, asthma and lung cancer.^{8,10,36} General population studies in adults have generally found weak to moderate associations between telomere length and lung function.^{11,37} For example, two large studies in healthy adults (aged 28 to 73 years) reported weaker associations between telomere length and lung function (n=44,041 controls), compared to COPD patients (n=934) or asthmatics (n=2,834).⁸ Interestingly, a recent small study (n=280, mean age 62 years) examined several markers of ageing (including sirtuin 1, p16/21 and Ku70/80) and found telomere length to be the only marker consistently associated with lung function in COPD patients.³⁸ Compared to other adult studies, the relatively smaller association we observed may be explained by the fact that our adult cohort was relatively healthier and younger with lower rates of smokers and was slightly less disadvantaged.

To our knowledge, this study is the first to examine the associations of telomere length and lung function in a general cohort of children, where we found no evidence of an association with any spirometric index after adjustments. Possibly, associations become evident later in life when the contributions of cumulative environmental burden have taken effect, which would explain the lack of association in our children but emerging associations in our mid-life adults. Alternatively, it is possible that factors implicated in senescence impact airway and lung health during adult life, rather than childhood. The underlying mechanism is unclear but might include the accumulation of adverse environmental influences that impact both telomeres and lung health,^{3,4} or the loss of stem cell regenerative capacity in association with decreased telomerase activity of the lungs (i.e. pulmonary endothelial cells).³⁹ Ideally, longitudinal studies extending from childhood into adult life are required to investigate this further. Finally, the unexpected inverse association between telomere length and FEV₁ and FVC in children was not present after adjusting for sex, age, BMI and socioeconomic position. This adjustment may not necessarily be appropriate when testing some of the

hypotheses that might explain an association between lung function and telomere length and we are, therefore, cautious about ruling out this relationship.

Clinical implications, unanswered questions and future direction

If replicated in other settings, our findings suggest that telomere length may have some clinical utility in that, at least in adults, these markers of cell senescence appear to correlate with respiratory health. Telomere length may have a role in the pathways to lung diseases. As reported in other studies, our observations are likely to be of greater clinical relevance in association with more pronounced respiratory pathology, such as those with COPD, idiopathic pulmonary fibrosis, asthma and lung cancer.^{8,10,36}

We cannot exclude the possibility that our findings may have arisen by chance, and might be explained by factors beyond the scope of this study. Evidently, there is notable telomere length inter-individual variability at birth and across the lifecourse. Telomere length at any point in life represents the integration of multiple risk factors, both inherited and acquired, including the effects of inherited telomerase genes, telomere length attrition with age and early life environmental contributions. Consequently, telomere’s measure of molecular age might better complement chronological age as a predictor of overall survival and health. This was observed in COPD patients where telomere shortening was associated with all-cause mortality.⁴⁰

Conclusion

In a healthy cohort of mid-life adults, we report a moderate association between shorter telomere length and reduced lung function, specifically with measures of airflow relative to lung volume, FEV₁/FVC ratio and MMEF₂₅₋₇₅. In contrast, no convincing association was observed in children after adjustments. The interaction between telomere dynamics and lung health over the lifecourse is multifaceted with contributions from genetic variation and environmental exposures and warrants further study. Telomere length may have a role in chronic lung conditions but its role in early life in general populations remains unclear. This

represents the first study to investigate these associations in a general cohort of children.

Further studies are needed to reproduce our findings in other settings.

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CONTRIBUTORS

MW, DB, SR and RS conceptualized and developed the CheckPoint study with other investigators. MTN assisted with sample collection, isolated genomic DNA, quantified telomere length, analyzed the data and wrote the first draft of the manuscript. MW is the lead investigator of the Child Health CheckPoint study. RS supervised laboratory work and protocol optimization. SR supervised spirometry testing and quality control of flow-volume loops. All authors commented on the first and subsequent drafts and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

This paper uses unit record data from Growing Up in Australia, the Longitudinal Study of Australian Children. The study is conducted in partnership between the Department of Social Services (DSS), the Australian Institute of Family Studies (AIFS) and the Australian Bureau of Statistics (ABS). The findings and views reported in this paper are those of the author and should not be attributed to DSS, AIFS or the ABS. We thank the LSAC and CheckPoint study participants, staff and students for their contributions.

FUNDING

This work was supported by the Australian National Health and Medical Research Council (NHMRC) (Project Grants 1041352, 1109355), The Royal Children’s Hospital Foundation (2014-241), MCRI, The University of Melbourne, the Australian National Heart Foundation (100660) and Financial Markets Foundation for Children (2014-055, 2016-310). The MCRI administered the grants for the study and provided infrastructural support (IT and biospecimen management) but played no role in the conduct or analysis of the trial. Research at the MCRI is supported by the Victorian Government’s Operational Infrastructure Support Program. MTN was supported by an NHMRC Postgraduate Scholarship (1115167). RS was supported by an NHMRC Senior Research Fellowship (1045161). SR was supported by an MCRI Clinician Scientist Award. KL was supported by an NHMRC Early

Career Fellowship (1091124) and an Australian National Heart Foundation Postdoctoral Fellowship (101239). DB was supported by an NHMRC Fellowship (1064629) and an Honorary Future Leader Fellowship of the Australian National Heart Foundation (100369). MW was supported by an NHMRC Senior Research Fellowship (1046518) and Principal Research Fellowship (1160906) and Cure Kids New Zealand. AG and TD reported no sources of relevant funding. The funding bodies had no role in the design and conduct of the CheckPoint study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

ETHICS APPROVAL

The study protocol was approved by the Royal Children's Hospital Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics Committee (14-26).

COMPETING INTERESTS

None declared.

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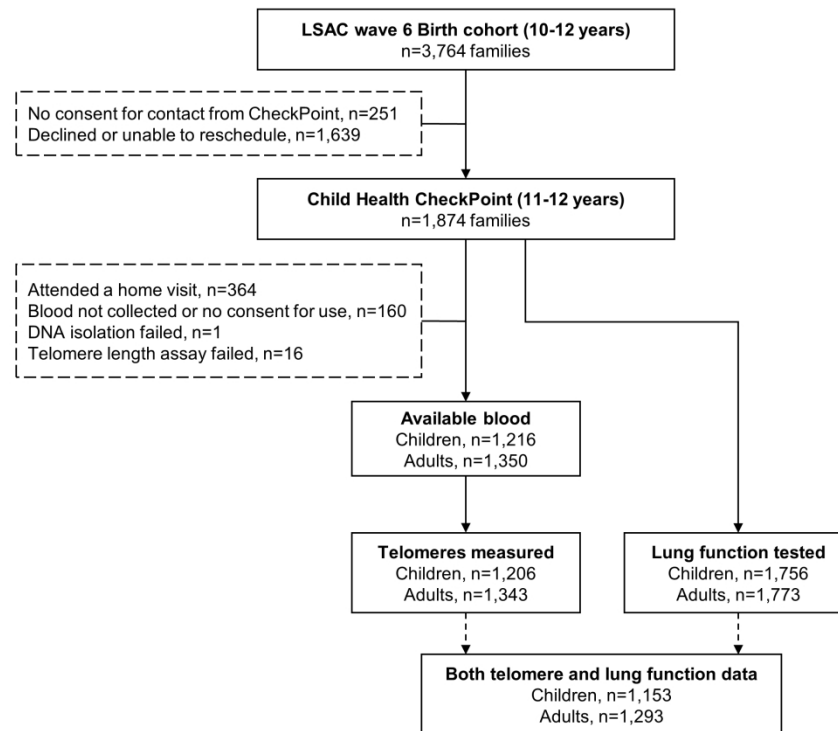


FIGURE 1 Longitudinal Study of Australian Children (LSAC) and Child Health CheckPoint (CheckPoint) participant flow.

219x190mm (300 x 300 DPI)

TABLE 1 Summary characteristics of children and adults

Participant characteristic	Children	Adults
N	1,206	1,343
Age (years)	11.4 (0.5)	43.9 (5.1)
Female, %	51	87
Height (cm)	154 (8)	167 (8)
Body mass index (kg/m ²)	19 (3)	28 (6)
Body mass index z-score	0.3 (1)	-
Current smoking, %	-	8.2
Cigarettes smoked per day	-	2.6 (1.4)
Second-hand smoke, %	13.9	-
Prepubertal, %	10	-
SEIFA Disadvantage Score	1026 (62)	1026 (61)
Socioeconomic position	0.3 (0.9)	0.2 (1.0)
Glycoprotein acetylation (mmol/l)	0.99 (0.13)	1.04 (0.17)
MVPA duration (min)	34 (30)	121 (56)
Asthma, %	12	10
Telomere length (T/S ratio)	1.09 (0.55)	0.81 (0.38)
<i>Spirometric indices raw</i>		
FEV ₁ (liters)	2.5 (0.4)	3.1 (0.6)
FVC (liters)	3.0 (0.5)	4.0 (0.8)
FEV ₁ /FVC ratio (%)	82.9 (7.2)	76.5 (6.7)
MMEF ₂₅₋₇₅ (liters/second)	2.6 (0.7)	2.7 (0.9)
<i>Spirometric indices z-score</i>		
FEV ₁	0.32 (1.0)	0.32 (1.1)
FVC	0.84 (1.1)	0.91 (1.1)
FEV ₁ /FVC ratio	-0.78 (1.1)	-0.90 (1.0)
MMEF ₂₅₋₇₅	-0.51 (1.1)	-0.46 (1.1)

Data are means (standard deviation) except where indicated as %. SEIFA: Socio-Economic Indexes for Areas Index of Relative Socioeconomic; MVPA: moderate-to-vigorous physical activity; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow.

TABLE 2 Changes in each spirometric index z-score per unit increase in T/S ratio in children and adults

Outcome z-score	Children				Adults			
	N	RC (95% CI)	R ²	p-value	N	RC (95% CI)	R ²	p-value
Unadjusted								
FEV ₁	1,153	-0.18 (-0.28 to -0.07)	0.01	0.001	1,293	-0.16 (0.01 to 0.3)	0.004	0.03
FVC	1,153	-0.22 (-0.32 to -0.11)	0.01	0.0001	1,293	-0.05 (-0.09 to 0.2)	0.0004	0.49
FEV ₁ /FVC	1,153	-0.07 (-0.04 to 0.18)	0.002	0.18	1,293	-0.19 (0.05 to 0.33)	0.005	0.008
MMEF ₂₅₋₇₅	1,153	-0.06 (-0.17 to 0.04)	0.001	0.25	1,293	-0.27 (0.12 to 0.41)	0.01	0.0003
Model 1*								
FEV ₁	1,147	-0.06 (-0.17 to 0.04)	0.14	0.21	1,283	0.05 (-0.07 to 0.16)	0.37	0.44
FVC	1,147	-0.10 (-0.19 to 0.004)	0.17	0.06	1,283	-0.05 (-0.17 to 0.06)	0.39	0.37
FEV ₁ /FVC	1,147	0.06 (-0.05 to 0.16)	0.08	0.28	1,283	0.17 (0.03 to 0.31)	0.03	0.02
MMEF ₂₅₋₇₅	1,147	-0.001 (-0.11 to 0.10)	0.08	0.97	1,283	0.20 (0.06 to 0.34)	0.12	0.005
Model 2†								
FEV ₁	788	-0.05 (-0.17 to 0.06)	0.20	0.36	1,005	0.06 (-0.07 to 0.19)	0.39	0.39
FVC	788	-0.08 (-0.20 to 0.04)	0.23	0.19	1,005	-0.06 (-0.19 to 0.07)	0.41	0.36
FEV ₁ /FVC	788	0.04 (-0.08 to 0.17)	0.13	0.49	1,005	0.21 (0.06 to 0.36)	0.08	0.008
MMEF ₂₅₋₇₅	788	0.01 (-0.11 to 0.14)	0.12	0.87	1,005	0.23 (0.08 to 0.38)	0.15	0.003

CI: confidence interval; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow; RC: regression coefficient; R-squared for the linear regression model.

* Model 1 adjusted for sex, age, body mass index, socioeconomic position.

† Model 2 additionally adjusted for physical activity, glycoprotein acetylation, asthma, pubertal status and second-hand smoking status for children, and smoking status for adults.

TABLE 3 Odds ratios for the lowest quartile of spirometric indices per one unit increase in T/S ratio in children and adults

Outcome z-score	Children			Adults		
	N	Odds ratio (95% CI)	p-value	N	Odds ratio (95% CI)	p-value
Unadjusted						
FEV ₁	1,153	1.23 (0.97 to 1.56)	0.08	1,293	0.73 (0.50 to 1.07)	0.11
FVC	1,153	1.27 (1.00 to 1.61)	0.05	1,293	0.85 (0.58 to 1.25)	0.42
FEV ₁ /FVC	1,153	0.91 (0.71 to 1.17)	0.47	1,293	0.59 (0.41 to 0.86)	0.005
MMEF ₂₅₋₇₅	1,153	1.10 (0.86 to 1.41)	0.43	1,293	0.63 (0.43 to 0.92)	0.02
Model 1*						
FEV ₁	1,147	1.02 (0.79 to 1.32)	0.86	1,283	0.73 (0.50 to 1.07)	0.11
FVC	1,147	1.04 (0.80 to 1.35)	0.76	1,283	0.85 (0.58 to 1.25)	0.42
FEV ₁ /FVC	1,147	0.93 (0.71 to 1.21)	0.57	1,283	0.59 (0.41 to 0.86)	0.005
MMEF ₂₅₋₇₅	1,147	1.03 (0.80 to 1.33)	0.82	1,283	0.63 (0.43 to 0.92)	0.02
Model 2†						
FEV ₁	788	1.10 (0.80 to 1.51)	0.58	1,005	0.82 (0.53 to 1.26)	0.36
FVC	788	1.11 (0.79 to 1.54)	0.56	1,005	0.97 (0.63 to 1.49)	0.88
FEV ₁ /FVC	788	1.00 (0.72 to 1.40)	0.98	1,005	0.59 (0.39 to 0.89)	0.01
MMEF ₂₅₋₇₅	788	1.10 (0.79 to 1.52)	0.57	1,005	0.64 (0.41 to 0.99)	0.04

CI: confidence interval; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow.

* Model 1 adjusted for sex, age, body mass index, socioeconomic position.

† Model 2 additionally adjusted for physical activity, glycoprotein acetylation, asthma, pubertal status and second-hand smoking status for children, and smoking status for adults.

TELOMERE LENGTH AND LUNG FUNCTION IN A POPULATION-BASED COHORT OF CHILDREN AND MID-LIFE ADULTS

SUPPORTING INFORMATION

SUPPORTING METHODS

Study design and subjects

In 2004 the Longitudinal Study of Australian Children (LSAC, also known as Growing Up in Australia) recruited a nationally-representative birth cohort (n~5,000) at age 0-1 years and since were followed at seven biennial waves spanning 0-1 to 12-13 years of age. The Child Health CheckPoint (CheckPoint) study was an additional comprehensive physical health and biomarker module at child age 11-12 years, nested between LSAC's sixth and seventh waves. During the LSAC wave 6 assessment in 2014, interviewers obtained written consent from 3,513 families (93% of the 3,764 seen) to be contacted to participate in the upcoming CheckPoint study. Ultimately, 1,874 (50%) adult-child pairs took part. Most non-participation (60%) was due to inability to attend or to reschedule a visit during the short period CheckPoint was in each location. Details of the LSAC and CheckPoint study design and recruitment have been previously described.¹⁻³

Blood collection and genomic DNA isolation

Whole venous blood was collected into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and immediately transported to an on-site laboratory. The blood sample was processed into aliquots within 2 hours into 1.0 mL FluidX tubes (FluidX, Cheshire, UK) and frozen in a -80 °C ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, USA). Samples were transported to the Murdoch Children's Research Institute (MCRI) biobank. Genomic DNA was isolated from available blood (e.g.

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whole blood or blood clot) using the Qiaamp 96 DNA Blood Kit (Qiagen, Venlo, Netherlands). Samples were randomized with child and parent dyads on the same plate to minimize batch effects using a random number generator (Stata 14.2, StataCorp LLC, USA). The sample retrieval, protocol optimization, consumable acquisition, and isolation of genomic DNA spanned April 2016 to January 2017. Purity and integrity of genomic DNA were confirmed using NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Middleton, USA), Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, USA) and gel electrophoresis, prior to storage in a -80 °C ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, USA). Genomic DNA was also isolated from 3 sets of control samples: (1) the K562 leukemic cell line, (2) newborn cord blood and (3) human placental tissue. These control samples have previously been described as having ‘shorter’, ‘average’ and ‘longer’ telomeres relative to peripheral blood samples.⁴⁻⁷ Genomic DNA from each of these control samples was used on all telomere assays to assess day-to-day and plate effects.

Telomere length measurement

Each sample was measured in quadruplicates comprising 4 µl of diluted genomic DNA at 5 ng/µl, 5 µl of SensiFAST SYBR No-ROX Kit master mix (Bioline, Sydney, Australia) and 0.5 µl of each forward and reverse primer at 2 µM. The primer sequences were tel1 (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT), tel2 (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT), bg1 (5'-GCA GGA GCC AGG GCT GGG CAT AAA AGT CA) and bg2 (5'-GGG CCT CAC CAC CAA CTT CAT CCA CGT TC). All ‘T’ and ‘S’ reactions were performed in 384-well plates on a Lightcycler 480 Instrument II (Roche, Melbourne, Australia). The cycling condition began with incubation at 95°C for 10 minutes, followed by 35 cycles of (i) 95°C for 15 seconds and (ii) 62°C for 60 seconds. The final 384-well layout included participant genomic DNA, three sets of control genomic DNA and a no-template control containing RNase-free water instead of a genomic DNA template, each present in quadruplicates. Further details are described in the standard operating procedure on the Growing Up in Australia's CheckPoint website.⁸

Calculation of telomere length

A ratio, known as the T/S ratio, is calculated by comparing the relative amount of 'T' and 'S' for each of these samples to a reference genomic DNA sample (in this case the average T/S ratio of all standard genomic DNA). The final relative telomere length from each sample, based on the T/S ratio, was calculated as the change in Ct of the test sample, normalized to the average T/S ratio of the standard genomic DNA on the corresponding plate. If less than two successful replicates out of the quadruplicates for either 'T' and 'S' were measured then the sample data was omitted (n=16), otherwise, a median was calculated, resulting in a median 'T' and a median 'S' for each sample (n=2549). A cycle threshold (Ct) replicate from 5 to 28 was considered successful as a Ct outside of this range was uncertain.

Telomere length replicate variability

To assess the replicate reliability, the degree of variation between replicates in a qPCR plate, an intra-assay coefficient of variation was calculated. The intra-assay coefficient of variation was calculated as the ratio of the pooled Ct's SD from all samples (each was analyzed in quadruplicate) and the overall Ct mean, and then multiplied by 100. To assess the degree of assay-to-assay and day-to-day consistency an inter-assay coefficient of variation was calculated using the pooled Ct's SD divided by the overall Ct mean of all duplicated samples, and then multiplied by 100. The mean intra-assay coefficient of variation between 'T' and 'S' quadruplicates used in the calculation of the T/S ratio was 2 % (SD 0.3; range 0.9 to 2.6). The inter-assay coefficient of variation between plates was 2 % (SD 1.4; range 0.3 to 6.2).

Spirometry procedure and quality control

Prior to testing, spirometers were calibrated using a 3-litre syringe with adjustments for ambient conditions. At testing, the researcher explained and demonstrated the spirometry test to each participant. In brief, we provided instructions on correct posture with a slightly elevated head, a tight seal around the mouthpiece with no leak, a rapid and complete

inhalation to total lung capacity, followed by a rapid maximal while maintaining an upright posture. Participants performed between three and eight spirometry trials.

Spirometry trace reliability

Spirometry waveforms were obtained for each of three vital capacity breaths and the best wave was chosen using Sentry Suite software. Two experienced raters were presented with 60 spirometry loops. Each rater was presented with the same 21 pre and 20 post Ventolin loops from a random sample of 21 children. The raters were also provided with the same 19 loops from accompanying adults. Presentation of loops to each rater was not random and pre and post-Ventolin loops for individual children were identifiable. We assessed the agreement of the classification of the chosen waveform (0 Unacceptable, 1 Acceptable or 2 Need of review). Cohen’s Kappa statistic was used to measure the agreement between waveform classifications. In addition, assessment was made by examining the percentage of agreement, the proportion of positive agreement and the prevalence-adjusted bias-adjusted Kappa (PABAK). Overall agreement between waveform classifications was substantial (kappa = 0.79, p-value < 0.001) with a high percentage of agreement (95%). The prevalence-adjusted bias-adjusted Kappa (PABAK) was 0.93 and the proportion of observed positive agreement was 0.98, indicating a high level of agreement in classifications by the two raters.

Quality of all flow-volumes loops

The quality of all flow-volumes loops were assessed by trained spirometry experts to determine if the loops met the ATS/ERS criteria (summarized in Table S1).⁹ Briefly, a quality score between 1 and 5 was assigned for each loop:

- 1. Meets all of the ATS/ERS criteria (met acceptability criteria for A, B, C and D).
- 2. Meets all ATS/ERS criteria except for repeatability. Two largest FVC values had a difference of above 150 mL.

3. Meets all ATS/ERS criteria except for repeatability. Two largest FEV₁ values had a difference above 150 mL.
4. Does not meet ATS/ERS guidelines; data excluded from the dataset.
5. Meets all ATS/ERS criteria except for repeatability. Two largest FVC and FEV₁ values had a difference above 150 mL.

Loops with a quality control score of 1, 2, 3 or 5 were included in the analyses. Further details of the spirometry testing method and epidemiology are described elsewhere.^{2,10}

Influence of smoking in adults

One major factor that is known to promote senescence and telomere shortening in addition to its known damaging effects on lung function is smoking.¹¹ In adults, current smoking was associated with overall lower lung function indices, but not with telomere length. Smoking had minor influences on the association between telomere length and each spirometric index (data not shown; available on request). We were unable to fully model these interactions due to the low numbers of smokers in our adult cohort (n=110, 8%).

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For Peer Review

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TELOMERE LENGTH AND LUNG FUNCTION IN A POPULATION-BASED COHORT OF CHILDREN AND MID-LIFE ADULTS

SUPPORTING INFORMATION

SUPPORTING TABLES

Table S1 Spirometry quality of flow-volume loops inclusion criteria based on the American Thoracic Society and European Respiratory Society criteria

A. Start of test	A rapid rise to and clearly defined peak expiratory flow, assessed by visual inspection of the flow-volume trace.
B. Within manoeuvre	Free from artefact, cough within the first second, glottic closure, or obvious leak, assessed by visual inspection of the flow-volume trace.
C. End of test	Clear end-expiratory plateau with no sharp drop or cessation of flow, assessed by visual inspection of the volume-time trace. There was no specification for a minimal forced expiratory time.
D. Repeatability	Two largest FEV ₁ and FVC values were within 150 mL.

FEV₁: forced expiratory volume in one second; FVC: forced vital capacity.

Table S2 Summary characteristics of children and adults stratified by sex

Participant characteristic	Boys	Girls	Men	Women
N	590	616	175	1169
Age (years)	11.4 (0.5)	11.5 (0.5)	46.3 (6.3)	43.5 (4.8)
Height (cm)	153 (8.1)	154 (7.7)	179 (7.0)	165 (6.1)
Body mass index (kg/m ²)	19 (3.2)	19 (3.4)	29 (4.8)	28 (6.3)
Body mass index z-score	0.3 (1)	0.3 (1)	-	-
Prepubertal, %	15	5	-	-
Current smoking, %	-	-	5.1	8.6
Cigarettes smoked per day	-	-	2.7 (1.4)	2.6 (1.4)
Second-hand smoke, %	13.4	14.4	-	-
SEIFA Disadvantage Score	1028 (62)	1025 (62)	1023 (71)	1027 (59)
Socioeconomic Position	0.3 (0.9)	0.3 (0.9)	0.4 (1.1)	0.2 (0.9)
Glycoprotein acetylation (mg/dl)	0.98 (0.12)	0.99 (0.13)	1.10 (0.20)	1.03 (0.16)
MVPA duration (min)	40 (33)	27 (27)	124 (50)	121 (57)
Asthma, %	14	11	4.6	11.4
Telomere length (T/S ratio)	1.08 (0.53)	1.10 (0.55)	0.86 (0.47)	0.80 (0.36)
<i>Spirometric indices raw</i>				
FEV ₁ (liters)	2.4 (0.4)	2.4 (0.4)	3.9 (0.6)	2.9 (0.5)
FVC (liters)	3.0 (0.5)	2.9 (0.5)	5.1 (0.8)	3.8 (0.6)
FEV ₁ /FVC ratio (%)	81 (6.9)	85 (6.9)	75 (7.0)	77 (6.6)
MMEF ₂₅₋₇₅ (liters/second)	2.4 (0.7)	2.7 (0.7)	3.3 (1.0)	2.6 (0.8)
<i>Spirometric indices z-score</i>				
FEV ₁	0.23 (1.0)	0.40 (1.0)	0.11 (1.0)	0.35 (1.1)
FVC	0.83 (1.1)	0.86 (1.2)	0.66 (1.0)	0.95 (1.1)
FEV ₁ /FVC ratio	-0.87 (1.1)	-0.69 (1.1)	-0.84 (1.1)	-0.91 (1.0)
MMEF ₂₅₋₇₅	-0.59 (1.1)	-0.43 (1.1)	-0.39 (1.0)	-0.47 (1.1)

Data are means (standard deviation) except where indicated as %. SEIFA: Socio-Economic Indexes for Areas Index of Relative Socioeconomic; MVPA: moderate-to-vigorous physical activity; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow.

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Table S3 Changes in each spirometric index z-score per unit increase in T/S ratio in adults, adjusted for centered age squared and centered age cubed instead of linear age

Outcome z-score	Centered age squared				Centered age cubed			
	N	RC* (95% CI)	R ²	p-value	N	RC* (95% CI)	R ²	p-value
FEV ₁	1,005	0.08 (-0.06 to 0.21)	0.33	0.27	1,005	0.06 (-0.07 to 0.19)	0.37	0.37
FVC	1,005	-0.05 (-0.18 to 0.08)	0.38	0.45	1,005	-0.06 (-0.19 to 0.07)	0.41	0.34
FEV ₁ /FVC ratio	1,005	0.23 (0.08 to 0.39)	0.06	0.004	1,005	0.23 (0.07 to 0.38)	0.06	0.005
MMEF ₂₅₋₇₅	1,005	0.25 (0.10 to 0.41)	0.09	0.001	1,005	0.24 (0.09 to 0.40)	0.11	0.002

CI: confidence interval; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow; RC: regression coefficient; R-squared for the linear regression model.

* Adjusted for sex, age, body mass index, socioeconomic position, physical activity, glycoprotein acetylation, and additionally, asthma, pubertal status and second-hand smoking status for children, and smoking status for adults.

Table S4 Changes in each spirometric index z-score per unit increase in T/S ratio in adults, stratified by sex

Outcome z-score	Males				Females			
	N	RC* (95% CI)	R ²	p-value	N	RC* (95% CI)	R ²	p-value
FEV ₁	131	0.02 (-0.36 to 0.40)	0.41	0.92	874	0.05 (-0.09 to 0.19)	0.12	0.50
FVC	131	-0.25 (-0.62 to 0.11)	0.42	0.17	874	-0.04 (-0.17 to 0.10)	0.06	0.61
FEV ₁ /FVC ratio	131	0.46 (0.02 to 0.89)	0.07	0.04	874	0.16 (-0.01 to 0.33)	0.09	0.06
MMEF ₂₅₋₇₅	131	0.33 (-0.13 to 0.79)	0.12	0.16	874	0.21 (0.04 to 0.37)	0.12	0.01

CI: confidence interval; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow; RC: regression coefficient; R²: r-squared for the linear regression model.

* Adjusted for sex, age, body mass index, socioeconomic position, physical activity, glycoprotein acetylation, asthma, and additionally, pubertal status and second-hand smoking status for children, and smoking status for adults.

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Table S5 Changes in each spirometric index z-score per unit increase in T/S ratio stratified for blood sample (whole blood vs blood clot)

Outcome z-score	Children				Adults			
	N	RC* (95% CI)	R ²	p-value	N	RC* (95% CI)	R ²	p-value
<i>Whole blood</i>								
FEV ₁	598	-0.004 (-0.20 to 0.19)	0.18	0.96	731	0.17 (-0.05 to 0.39)	0.40	0.13
FVC	598	-0.007 (-0.21 to 0.19)	0.20	0.94	731	0.02 (-0.20 to 0.23)	0.41	0.89
FEV ₁ /FVC ratio	598	0.01 (-0.20 to 0.22)	0.11	0.92	731	0.30 (0.03 to 0.56)	0.08	0.03
MMEF ₂₅₋₇₅	598	0.008 (-0.21 to 0.22)	0.11	0.94	731	0.27 (0.02 to 0.53)	0.16	0.03
<i>Blood clot</i>								
FEV ₁	229	-0.01 (-0.18 to 0.16)	0.22	0.89	274	-0.03 (-0.22 to 0.16)	0.40	0.77
FVC	229	0.13 (-0.14 to 0.17)	0.27	0.87	274	0.002 (-0.18 to 0.18)	0.43	0.98
FEV ₁ /FVC ratio	229	-0.06 (-0.24 to 0.11)	0.28	0.49	274	-0.10 (-0.32 to 0.11)	0.12	0.35
MMEF ₂₅₋₇₅	229	-0.02 (-0.18 to 0.15)	0.20	0.84	274	-0.04 (-0.27 to 0.18)	0.17	0.72

CI: confidence interval; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow; RC: regression coefficient; R-squared for the linear regression model.

* Adjusted for sex, age, body mass index, socioeconomic position, physical activity, glycoprotein acetylation, asthma, and additionally, pubertal status and second-hand smoking status for children, and smoking status for adults.

Table S6 Unadjusted changes in each spirometric index z-score per unit increase in T/S ratio excluding participants without complete covariate data*

Outcome z-score	Children				Adults			
	N	RC (95% CI)	R ²	p-value	N	RC (95% CI)	R ²	p-value
FEV ₁	788	-0.21 (-0.33 to -0.08)	0.01	0.001	1,005	0.11 (-0.06 to 0.28)	0.002	0.19
FVC	788	-0.24 (-0.37 to -0.12)	0.02	0.0002	1,005	-0.004 (-0.17 to 0.16)	0.0004	0.95
FEV ₁ /FVC ratio	788	0.07 (-0.06 to 0.20)	0.002	0.27	1,005	0.21 (0.04 to 0.37)	0.006	0.01
MMEF ₂₅₋₇₅	788	-0.07 (-0.19 to 0.06)	0.001	0.30	1,005	0.26 (0.09 to 0.42)	0.01	0.002

CI: confidence interval; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; MMEF₂₅₋₇₅: maximal mid-expiratory flow; RC: regression coefficient. Complete covariate data included sex, age, body mass index, socioeconomic position, physical activity, glycoprotein acetylation, asthma, and additionally, pubertal status and second-hand smoking status for children, and smoking status for adults.