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Nathalie Michels: Conceptualization, Investigation, Methodology, Writing - Review & Editing, Supervision

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A multi-exposure approach to study telomere dynamics in childhood: a role for residential green space and waist circumference.

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Abstract

Background: Telomeres are vulnerable to various environmental exposures and lifestyle factors, encompassed in the exposome. Recent research shows that telomere length is substantially determined early in life and that exposures in childhood may have important consequences in setting later life telomere length.

Objectives: We explore in a child population the associations of 17 exposures with telomere length and longitudinal telomere change.

Methods: Children (2.8-10.3y at baseline, 51.3% boys) were followed-up for five to seven years. Relative telomere length was measured at baseline and follow-up using quantitative real-time PCR. Exposures and lifestyle factors included: body composition (body mass index and waist circumference), dietary habits (sugar- and fat-rich food intake, vegetables and fruit intake), psychosocial stress (events, emotions, behaviour), sleep duration, physical activity, and residential environmental quality (longterm black carbon, particulate matter exposure, and residential green space). Cross-sectional (n=182) and longitudinal (n=150) analyses were assessed using linear regression models, adjusting for age, sex, socioeconomic status and multiple testing.

Results: Our longitudinal analyses showed that higher residential green space at baseline was associated with (β=0.261, p=0.002) lower telomere attrition and that children with a higher waist circumference at baseline showed a higher telomere attrition (β=-0.287, p=0.001). These two predictors were confirmed via LASSO variable selection and correction for multiple testing. In addition, children with more unhealthy exposures at baseline had a significantly higher telomere attrition over the follow-up period compared to children with more healthy exposures (β=-0.200, p=0.017).

Discussion: Waist circumference and residential green space were identified as predictors associated with telomere attrition in childhood. These results further support the advantages of a healthy lifestyle from early age onwards and the importance of a green environment to promote molecular longevity from childhood onwards.

Keywords: telomeres; exposome; child; longitudinal; lifestyle; diet; residential green space

Funding: This study was supported by the Research Foundation-Flanders, Belgium (project number G073315N).

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital. Written informed consent was obtained from all parents and from all children aged 12 years and older. Children younger than 12 years gave verbal consent.

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1. Introduction

Telomeres are nucleoprotein structures that function as the protective end caps of human chromosomes [1]. They are composed of TTAGGG repeat complexes and protect the genomic DNA against end-to-end fusion and degradation. DNA loses telomeric repeats during each cell division because DNA polymerase is unable to replicate the 3' end of the DNA strand completely [2]. This end replication problem results in telomere attrition over time with cellular age, eventually leading to loss of protection and chromosomal instability [3]. Telomere length is considered as a biomarker of biological ageing and a risk factor for several age-related diseases, including cardiovascular disease, diabetes mellitus, and cancer [4-6].

Although telomere length is heritable [7], environmental and lifestyle exposures may influence its dynamics. Several health deteriorating exposures including unhealthy diet, sedentary lifestyle, stress, and air pollution have been associated with shorter telomere lengths [8-14], while beneficial exposures such as, physical activity, sleep duration, vegetable and fruit intake have been linked to longer telomere lengths [15-17]. However, the majority of the aforementioned studies focused on one specific exposure as predictor of telomere dynamics. Whereas epidemiological research in the past was confined to individual exposures, nowadays, there is a spiked interest towards combining multiple exposures as this provides a more holistic approach to broaden our understanding of age-related diseases [18, 19]. Some recent studies combined a limited number of lifestyle and/or stress exposures as a predictor for telomere length [20-24], although a multi-exposure approach that combines stress and lifestyle factors with more environmental exposures such as air pollution and residential green space remains unexplored. The integration of multiple exposures in a multi-exposure factor might lead to a better understanding of disease aetiology. Unfortunately, the majority of the studies addressing multiple exposures use cross-sectional data, while longitudinal studies examined only stress/depression without considering other lifestyle or environmental factors [21, 23-26].

There is increasing evidence that telomere length tracks during adulthood, and that adult telomere length is largely determined by telomere length at birth and telomere attrition in early life [27, 28]. Therefore, age-related diseases that have been associated with adult telomere length might find their origin in the fetal and childhood period of life. Hence, investigating exposures in childhood might provide better insights in the developmental origins of disease. Therefore, this study explored the cross-sectional and longitudinal association of multiple intrinsic and extrinsic factors with telomere length and longitudinal telomere change in children and adolescents.

2. Methods

This study examines separate and combined effects of child body composition, sleep duration, physical activity, psychosocial stress, dietary habits, and residential environmental quality in relation to childhood telomere length and telomere dynamics. First, the individual effects of this wide variety of childhood exposures towards telomere length and longitudinal telomere change were tested using single-exposure regressions. In secondary analyses, this study tested the cumulative effect of all exposures, using a multi-exposure factor, combining all exposures, as a predictor for telomere length and longitudinal telomere change. To our knowledge, this combination of exposures as predictor for telomere length is new in a population of children.

2.1. Participants

Data was used from the longitudinal ChiBS study (Children's Body composition and Stress) [29]. Baseline participants were children between 2.8 and 10.3 years old, who were approached through school and kindergarten settings in the municipality of Aalter (Belgium) and its surroundings and were followed-up for seven years. The ChiBS cohort consists of multiple waves, although the current study only included 3 out of 6 waves as these were the only ones with telomere data; the baseline survey in 2008 (n=513), the first follow-up survey in 2010 (n=505) and the fifth follow-up survey in 2015 (n=242). For the current study, participants were included based on participation in the study wave of 2015 (N=242), and the availability of telomere length data in 2015 (N=184). Children from African ethnic origin (n=2) were excluded, as ethnicity may influence telomere length/attrition [30], resulting in a cross-sectional sample size of 182 participants in the wave of 2015. Of these remaining 182 participants, 142 participants had also telomere length data in 2010, while 91 of these 182 participants had telomere length data in 2008.

To optimize the sample size, and to use the longest follow-up period as possible we decided to merge the baseline data as follows. For the 91 participants having telomere length data in 2008, we used the data of 2008 as baseline. For the 59 participants who had no telomere length data in 2008 but did have telomere length data in 2010, we used the wave of 2010 as baseline. An overview of the study design is shown in Figure 1.

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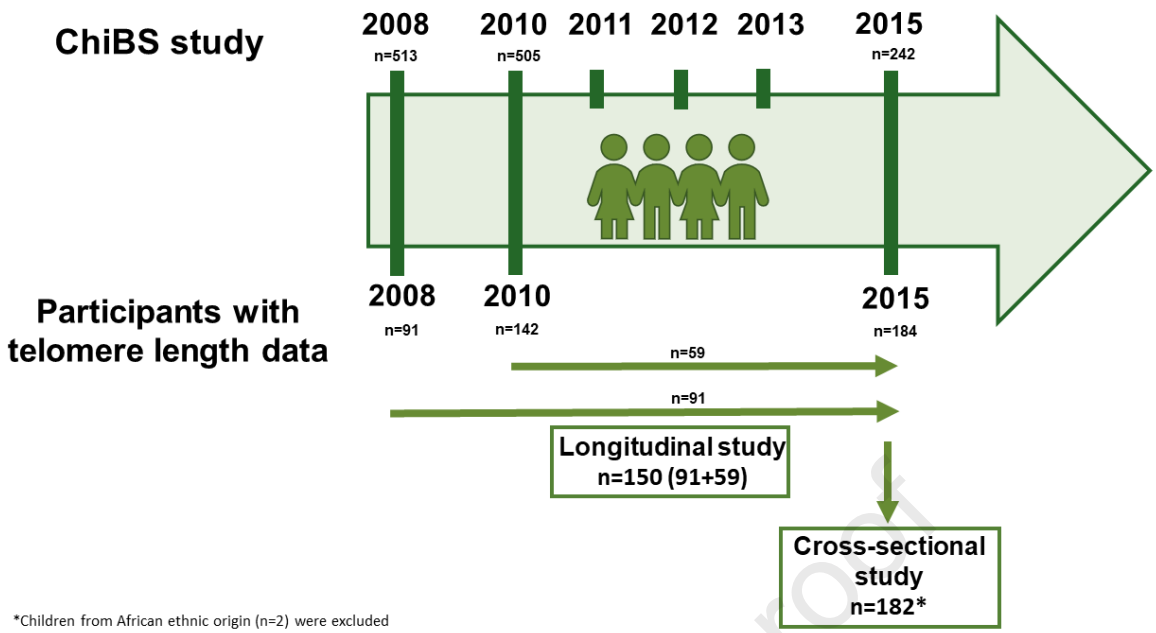

Figure 1 Overview of the study design.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital. Written informed consent was obtained from all parents and from all children aged 12 years and older. Children younger than 12 years gave verbal consent. The examination day included biological sampling, anthropometric measurements and questionnaires for parents and children.

2.2. Relative average telomere length measurement

Peripheral blood was obtained from the children via venipuncture in Ethylenediaminetetraacetic acid (EDTA) tubes. After centrifugation (10 min, 2000g), DNA was extracted from the buffy coat using QIAamp DNA Mini Kit (Qiagen, Inc., Venlo, the Netherlands) and stored at -80°C. Next, DNA quantity and purity were assessed using a Nanodrop 1000 spectrophotometer (Isogen, Life Science, Belgium) and DNA integrity was evaluated using gel-electrophoresis. The average relative leukocyte telomere length was measured by a modified quantitative real-time PCR (qPCR) protocol as described previously [30, 31]. Telomeres were measured in triplicate on a 7900HT Fast Real-Time PCR System (Applied Biosystems) in a 384-well format. On each plate, a six point serial dilution of pooled buffy coat DNA was run to assess PCR efficiency, which ranged between 95 and 105%. The relative average telomere length was calculated using qBase software (Biogazelle, Zwijnaarde, Belgium), expressed as the ratio of telomere copy number to single-copy gene number (T/S) and normalized to the average T/S ratio of the entire sample set. The reliability of our assay was assessed by calculating the intraclass correlation coefficient (ICC) [32] with 95%

confidence interval (CI) of triplicate measures. The ICC (95% CI) of T/S ratios, telomere runs, single-copy gene runs were 0.859 (0.836 to 0.881), 0.969 (0.963 to 0.974) and 0.956 (0.948 to 0.962), respectively. Based on the six inter-run calibrators, the inter-assay ICC (95% CI) was 0.882 (0.489 to 0.959).

2.3 Exposure measurements

Based on the available data, we assessed the following 17 exposures: Body Mass Index (BMI), waist circumference, sleep duration, physical activity, feelings of happiness, sadness, angriness and anxiety, prosocial behaviour, peer relationship problems, conduct problems, emotional problems, negative events in the last 12 months, sugar- and fat-rich food intake, vegetable and fruit intake, black carbon (BC) exposure, particulate matter exposure and residential green space. These 17 exposures were considered as sub-exposures which were divided into 6 groups of main exposures: body composition, physical activity, sleep, psychosocial stress, dietary habits, and environmental quality. Based on this classification a multi-exposure factor was calculated to integrate all 17 exposures. An overview of this classification and the composition of the multi-exposure factor is shown in Figure 2.

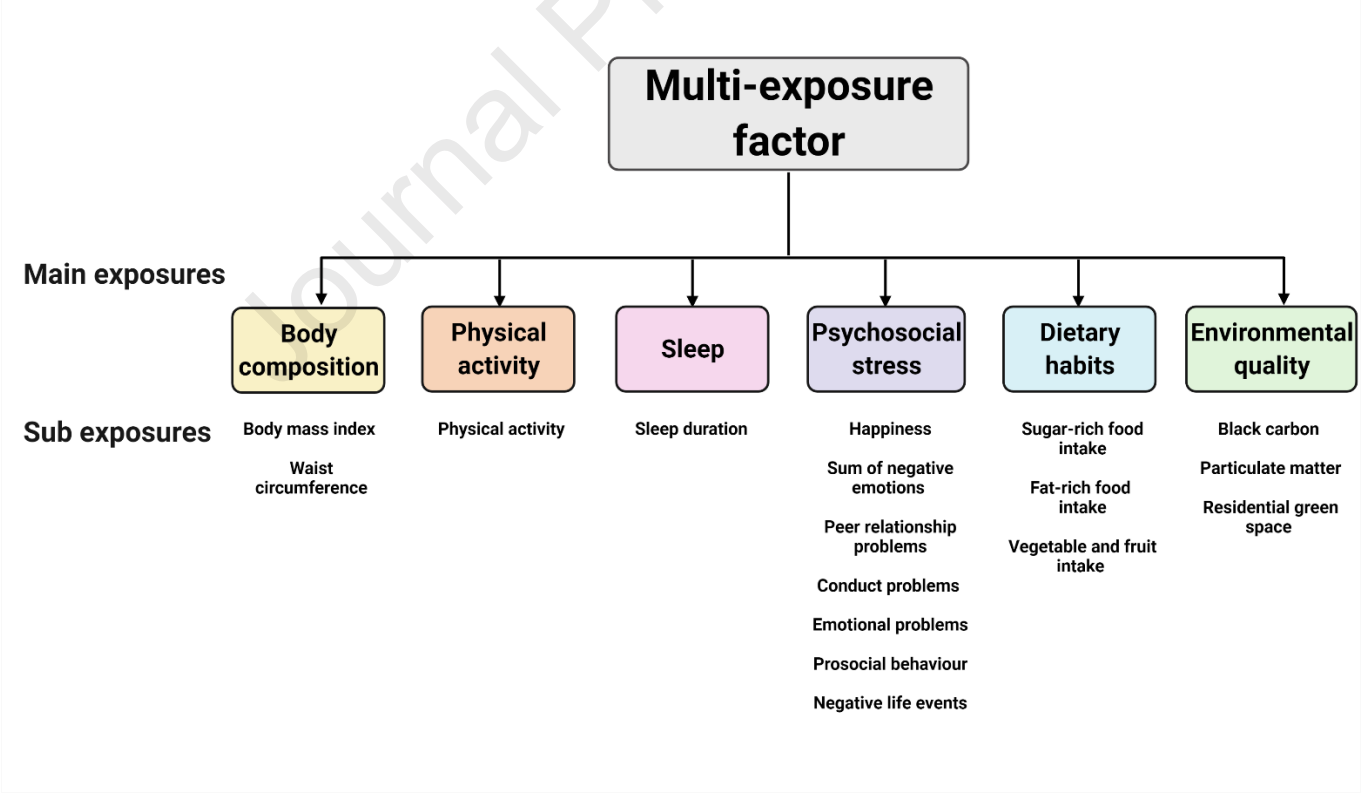

Figure 2: *Overview of the composition of the multiple-exposure factor.*

Body composition was assessed by measuring the BMI (dividing children's weight by height squared, kg/m^2) and waist circumference (measured as the mid-point between the top of the iliac crest and the lower coastal edge). Overweight was determined using the International Obesity Task Force (IOTF) classification [33].

Physical activity was assessed using a parental report [34], which questioned how much time per weekday/during the weekend children spent on physical activity (sports, physical activity at school, playing outside, ...). For analyses, a weighted average of the time spent on physical activity per week was calculated via the following formula: (5*time spent on physical activity on weekdays + 2*time spent on physical activity on weekend days)/7.

Children's sleep duration was reported by the parents using the sleep duration question from the School Sleep Habits Survey (two-week test–retest r=0.68) [35]. The average sleep duration per day was calculated, using the following formula: (5*hours sleep on weekdays + 2*hours sleep on weekend days)/7.

Psychosocial stress was measured using different questionnaires covering different aspects of stress related to emotions, negative life events and emotional and behavioural problems. To describe emotions, children reported recent feelings of happiness and negative emotions (sadness, angriness and anxiety) on a 0-10 Likert scale. Validation of this questionnaire was tested in the wave of 2012: the negative emotions score (sum of three negative emotions) showed a Spearman correlation of r= 0.48 ($p<0.001$), with the negative effect score of the validated Positive and Negative Effect Schedule questionnaire [36] in a subsample of 153 children. Via the Coddington Life Events Scale (CLES), children reported the frequency and timing of negative events in the last 12 months, such as familiar issues (e.g. divorce), school issues (e.g. failing a grade), social issues (e.g. moving), criminal issues, economic issues (e.g. job loss of a parent) and illness/death of child, family, friend or pet [37]. Child emotional and behavioural problems over the past six months were assessed by the Strengths and Difficulties Questionnaire (SDQ), filled out by the parents, via four subscales with each five items: prosocial behaviour, conduct problems, emotional problems, and peer relationship problems (Cronbach's alpha= 0.63-0.74) [38].

Dietary habits were assessed using a qualitative food frequency questionnaire (FFQ) filled in by the parents, from which the sugar- and fat-rich food intake were calculated as described by Lanfer et al [39], and the fruit and vegetable intake frequency (portions fruit and vegetables/per week) was calculated based on the intake frequencies of several fruit and vegetable groups.

The environmental quality at the child's residence was estimated, taking into account three exposures: black carbon, particulate matter, and green space. Daily residential exposure levels ($\mu g/m^3$) for airborne black carbon (BC) and particulate matter particles with a diameter less than or equal to 2.5 µm ($PM_{2.5}$) were estimated using a high resolution spatial temporal interpolation method (kriging) [40] combined with a dispersion model [41, 42]. This model calculates the daily interpolated exposure concentrations in a high-resolution receptor grid based on information from the Belgian telemetric air-quality networks, point sources, and line

sources. The overall model performance was evaluated by leave-one-out cross-validation and was based on 14 monitoring points for black carbon and 34 for $PM_{2.5}$. Validation statistics of the interpolation tool gave a spatial temporal explained variance (R^2) of more than 0.80 for $PM_{2.5}$ and 0.74 for BC. The model shows that modelled BC and PM air pollution at residence correlated with internal exposure to nano-sized black carbon particles measured in urine [43]. Annual averages of the daily residential exposure levels for BC and $PM_{2.5}$ were used in the current analyses. In addition to these ambient air pollution data, the residential green space was determined. The participant's residential address at follow-up was geocoded to estimate green space exposure in seven buffers around the residential address (50 m, 100 m, 300 m, 500 m, 1000 m, 2000 m and 3000 m). The estimates at the 2000 m buffer were used in the current analyses, but sensitivity analyses checked the similarity with the other buffers. Residential green space was estimated using the Green Map of Flanders 2012 from the Agency for Geographic Information Flanders (AGIV) which provides high-resolution (1x1m) information on natural elements, identified as all non-agricultural vegetation. This non-agricultural vegetation was categorized into three measures: high green (vegetation height higher than 3 m), low green (vegetation height lower than 3m) and a total vegetation cover (sum of both, high and low green). More information on the Green Map of Flanders is provided in Dockx et al., 2022 [44]. All analyses were carried out using Geographic Information System (GIS) functions using ArcGIS 10 software (Esri Inc., US).

2.4. Multi-exposure factor

To assess the overall effect of the exposures, a multi-exposure factor, integrating all 17 explored exposures (Figure 2) , was calculated using an approach modified from Mirabello et al. [45]. First, all sub-exposures were ranked from healthy to unhealthy using percentiles. The unhealthiest quartiles received a 'risk score' of 1 while the other 3 quartiles received a risk score of 0. Second, these risk scores were summed up per main exposure group and divided by the number of sub-exposures in that main exposure group. Finally, the sum was taken of the average risk scores of all main exposures to obtain the multi-exposure factor per child, where a higher multi-exposure factor reflects more unhealthy exposures ranging from zero to six as theoretical maximum.

2.5. Covariates

We accounted for a priori selected covariates that include known determinants of children's telomere length and variables with a potential link with the exposures, such as age, sex and parental socioeconomic status (SES) [46-51]. Parental SES was estimated based on the highest achieved education (maximum of both parents), according to the International Standard Classification of Education (ISCED) [52]. Due to the low number of participants in

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category zero to four, the ISCED-categories were combined into two levels (levels 0–4 as low: no post-secondary education; and levels 5–6 as high: at least one parent with post-secondary education).

2.6. Statistical analysis

Statistical analyses were performed using the statistical software SPSS 27 (SPSS Inc, Chicago, IL) and R version 4.0.5, all p-values <0.05 were considered significant. Independent t-test and Mann-Whitney U test were conducted to estimate sex and SES differences in exposures and telomere length measurements and to estimate differences between included and excluded participants. Paired t-test and Wilcoxon signed rank test were used to estimate differences between baseline and follow-up. Spearman correlations were calculated for all baseline exposures.

In this study, both cross-sectional and longitudinal analysis were performed. Information on the cross-sectional analysis and cross-sectional results is given in the supplemental material.

For the longitudinal analyses, ideally, the exposures of 2008 were used as baseline, resulting in a seven-year follow-up (2008-2015). For participants of whom no data was available in 2008 (n=59), exposures of 2010 were used as baseline, resulting in a five-year follow-up. Telomere attrition was calculated according to Verhulst et al. [53] to account for the phenomenon of regression towards the mean and was corrected for five/seven years follow-up period. According to Little's test (p=0.003) missing data was not MCAR in the longitudinal dataset. Therefore, a multiple imputation was performed using SPSS.

2.6.1. Linear regression models

First, the longitudinal relation between all 17 baseline exposures and telomere attrition over seven or five years was checked using individual linear regression models per exposure, corrected for age, sex and SES. Correction for multiple testing was performed using threshold of effective tests (TEF) [54]. Second, the multi-exposure factor instead of the individual exposures was used as predictor. Again, linear regression analysis was executed correcting for the same covariates. Standardized regression coefficients (β) were calculated by multiplying the unstandardized regression coefficient by the standard deviation of the predictor variable and dividing by the standard deviation of the outcome variable.

2.6.2. LASSO regression

The standard linear regression model (or the ordinary least squares method) performs poorly in a situation where you have many potential candidate predictor variables, as is the case in this study [55, 56]. Penalized regression methods, provide a better alternative in this kind of

situations [57]. Therefore, the variable selection method LASSO regression was used as confirmatory analysis [57, 58]. LASSO regression was performed using the *glmnet R* package.

2.6.3. Mediation analysis

Since our significant predictors, waist circumference and residential green space can theoretically be linked to each other [59-61] and to improve insights, an explorative mediation analysis was performed. Covariates, exposure and mediator were all measured at baseline. Mediation analysis was performed using the macro 'Process' in SPSS.

2.6.4. Sensitivity analysis

Besides the education level of the parents, the neighbourhood median income can also be used as proxy for the socioeconomic status. Due to small variation in this variable in our population (all participants' residences were in the municipality of Aalter (Belgium) and its surroundings), we did not include the neighbourhood median income as confounder in our main analyses. However, we tested the robustness of our significant models by correcting them for neighbourhood median income. Therefore, neighbourhood median income was added as covariate to the longitudinal linear regression models of waist circumference and residential green space and to the LASSO regression analysis.

3. Results

3.1. Subject characteristics

Differences in characteristics between included and excluded participants in the wave of 2015 are shown in Supplemental Table 1. In Table 1, the characteristics of the study population at baseline and at follow-up are shown. The telomere length changed significantly over the five and seven year time period ($p<0.0001$). Spearman correlations showed that relative telomere length could be tracked across the different follow-up surveys ($r= 0.69$ to 0.73, $p<0.0001$). Relatively few children were overweight in this study population (6.04% in 2015). Telomere length was significantly higher in girls compared to boys ($p=0.003$). Additionally, sex differences were seen for some exposures in 2015: BMI ($p=0.039$), waist circumference ($p<0.001$), and emotional problems ($p=0.022$) were higher in girls, whereas physical activity ($p=0.018$) was higher in boys. SES was lower for girls ($p=0.048$). No telomere length differences were observed for SES, although emotional problems ($p=0.015$) were higher in low SES and residential green space (total green in buffer 2000 m) was lower in low SES ($p=0.006$). Differences in characteristics between both baseline waves (2008 and 2010) are presented in Supplemental Table 2. Spearman correlations were calculated for all baseline exposures (Supplemental Table 3).

Table 1. Characteristics of the study population

	Baseline (2008/2010)	**Follow-up 2015**	**p-value**
	(N=150)	(N=182)	
Age (year)	5.95 (4.90-7.10)	12.58 (11.50-13.79)	<0.01[A]
Sex, boys (n,%)	77 (51.3%)	95 (52.20%)	
High SES[C] (n,%)	116 (77.3%)	147 (80.80%)	
Body composition			
BMI (kg/m²)	15.20 (14.50-16.10)	17.27 (15.69-19.19)	<0.01[A]
Waist circumference (cm)	51.90 (49.48-55.38)	62.23 (58.22-67.06)	<0.01[A]
Sleep			
Sleep duration (hours/night)		9.50 (9.00-10.00)	<0.01[A]
Physical activity			
Physical activity (hours/week)	11.92 (8.00-15.00)	8.00 (4.50-13.00)	<0.01[B]
Stress			
Emotions			
Happy (0-10)	8 (6-9)	8 (7-9)	0.20[B]
Sad (0-10)	2 (0-5)	1 (0-3)	0.01[B]
Anxious (0-10)	0 (0-3)	0 (0-1)	0.09[B]
Angry (0-10)	2 (0-4)	2 (1-3)	0.80[B]
Negative emotions (0-30)	6 (2-11)	4(2-7)	0.06[B]
Behaviour (SDQ)			
Conduct problems (0-10)	2 (0-3)	1 (0-2)	<0.01[B]
Emotional problems (0-10)	2 (1-3)	2 (1-3)	0.72[B]
Peer problems (0-10)	1 (0-2)	1 (0-2)	0.18[B]
Prosocial behaviour (0-10)	8 (7-10)	9 (8-10)	0.08[B]
CLES			
Negative event score last 12 months	39 (10-64)	56 (16-117)	<0.01[B]
Dietary quality			
Sugar-rich food intake (% of total intake)	29.62 (20.23-37.54)	22.92 (16.00-31.00)	<0.01[A]
Fat-rich food intake (% of total intake)	31.19 (25.27-41.88)	29.58 (24.37-36.63)	0.01[A]
Vegetable and fruit intake (portions/week)	14 (11-19)	10 (6-16)	0.46[A]
Residential environmental quality			
PM2.5 (µg/m³)		12.13 (11.87-12.58)	
Black carbon (µg/m³)		0.92 (0.89-0.98)	
Residential green space , total green in buffer 2000 m (%)		29.77 (22.32-36.66)	
Telomere length			
Relative Telomere length (T/S ratio)	1.08 (0.92-1.21)	0.94 (0.82-1.09)	<0.01[A]

Abbreviations: CLES, Coddington Life Events Scale; SDQ, Strengths and Difficulties Questionnaire; PM2.5, Particulate matter (<2.5µm); SES, parental socioeconomic status; BMI, Body Mass Index.

Values are expressed as medians (P25-P75).

[A] Paired t-test *p-value* for individual change over the longest follow-up time.

[B] Wilcoxon signed rank test (no normal distribution) for individual change over the longest follow-up time.

[C] At least one parent with post-secondary education

3.2. Association between individual exposures and telomere attrition

Analyses of the baseline individual exposures in association with telomere attrition in childhood are shown in Supplemental Table 5 and a volcano plot is shown in Figure 4. Firstly, a higher waist circumference was associated with a higher telomere attrition (β=-0.287; 95% CI [-0.462;-0.112]; p=0.001). Secondly, a higher percentage of residential green space was associated with a lower telomere attrition rate (β=0.261; 95% CI [0.099;0.424]; p=0.002). After correction for multiple testing (TEF=0.004), these associations remained significant.

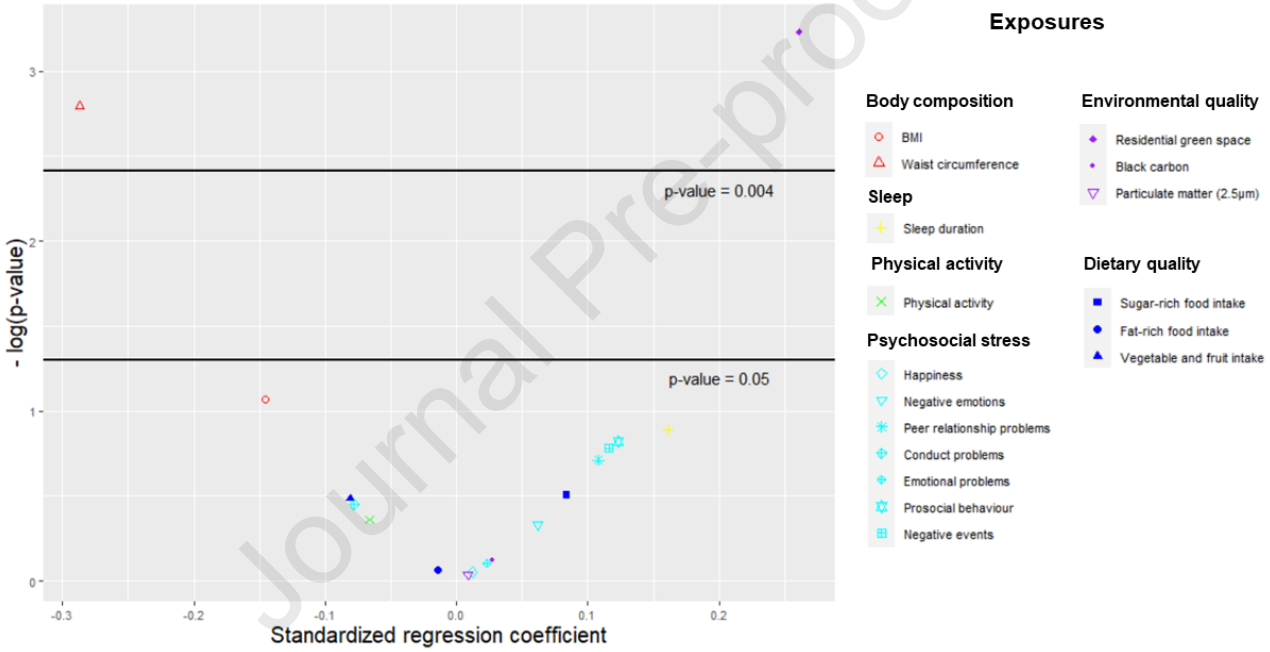

Figure 4: *Volcano plot presenting the significance of the associations between baseline exposures and telomere attrition in function of the standardized regression coefficient. Lowest black horizontal line represents the p-value = 0.05. Highest black horizontal line represents p= 0.004, correction p-value for multiple testing, performed using Threshold for effective tests (TEF)). Both significant exposures at the 0.05 significance level remain significant after correction for multiple testing using TEF. Models were adjusted for sex, age and socio-economic status.*

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For residential green space, several buffers for high green, low green, and total green were tested. All buffers of high green and total green were associated with a lower telomere attrition. After correction for multiple testing (TEF=0.008), only high green in a 300, 500 and 2000 m buffer, and total green in a 50, 100, 500, 1000 and 2000m buffer remained significant. Results are presented in Figure 5. As total green in a buffer of 2000 m showed the strongest association, this buffer was selected for the calculations of the multi-exposure scores.

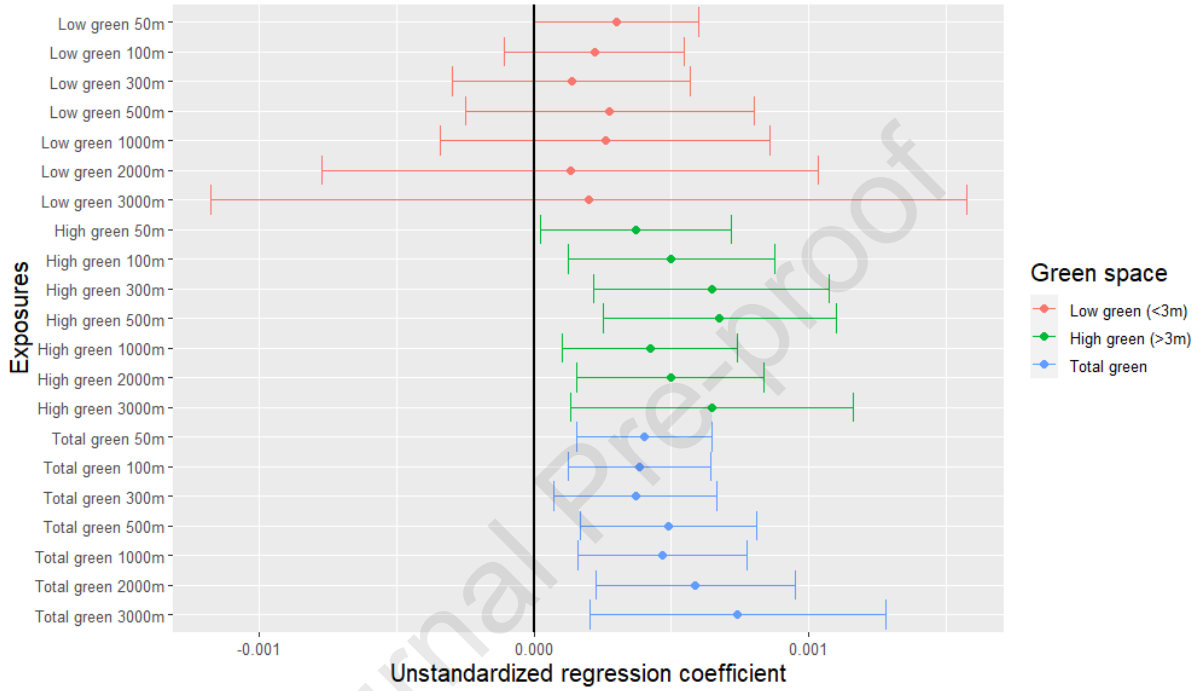

Figure 5: *Overview of the unstandardized effect green space on telomere attrition obtained by multiple linear regression for the different buffers of low, high and total green space. The bars represent the 95% confidence interval.*

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3.3. Variable selection using LASSO regression as confirmatory analysis

Variable selection was performed using LASSO regression. λ=0.0236 was determined as optimal λ after 10 fold cross-validation and two predictors were retained: waist circumference (β= -0.003) and residential green space (β= 0.003). This supports our previous longitudinal results obtained via linear regression.

3.4.Multi-exposure factor

The maximum multi-exposure factor was 3.76 at baseline. No association was found between the multi-exposure factor and telomere attrition (β=-0.133, p=0.117). However, when the multi-exposure was categorised in two groups: a 'healthy exposure group' (multi exposure factor 0-2, n=81) and an 'unhealthy exposure group' (multi-exposure factor 2-4, n=69) a significant difference in telomere attrition was found between these two groups (β=-0.200, p=0.017), see Figure 6.

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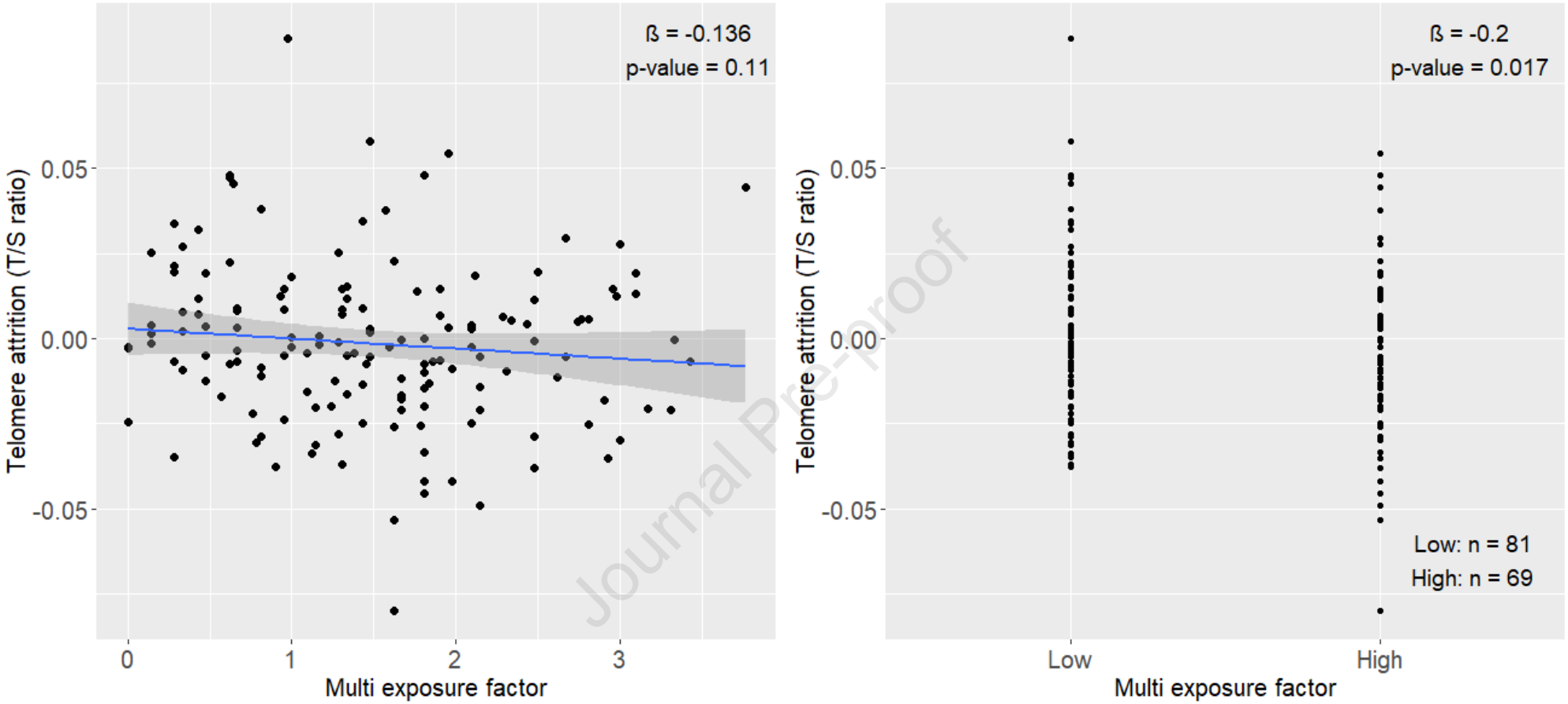

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393 ***Figure 6: Scatterplots presenting the relationship between longitudinal telomere attrition and the multi-exposure factor.*** *Original multi-exposure*
394 *factor calculated from baseline data (Left). Multi-exposure factor from baseline data was categorised in two groups: 'Low' (multi exposure factor 0-2, n=81) and*
395 *'High' (multi-exposure factor 2-4, n=69) (Right)*

3.5. Mediation analysis

To improve insights in the determinants of telomere attrition, an explorative mediation analysis was performed which revealed that the relationship between residential green space and telomere attrition was partially (16.66%) mediated by waist circumference (total effect= 0.0006; indirect effect= 0.0001). The results of this mediation analysis are presented in Figure 7.

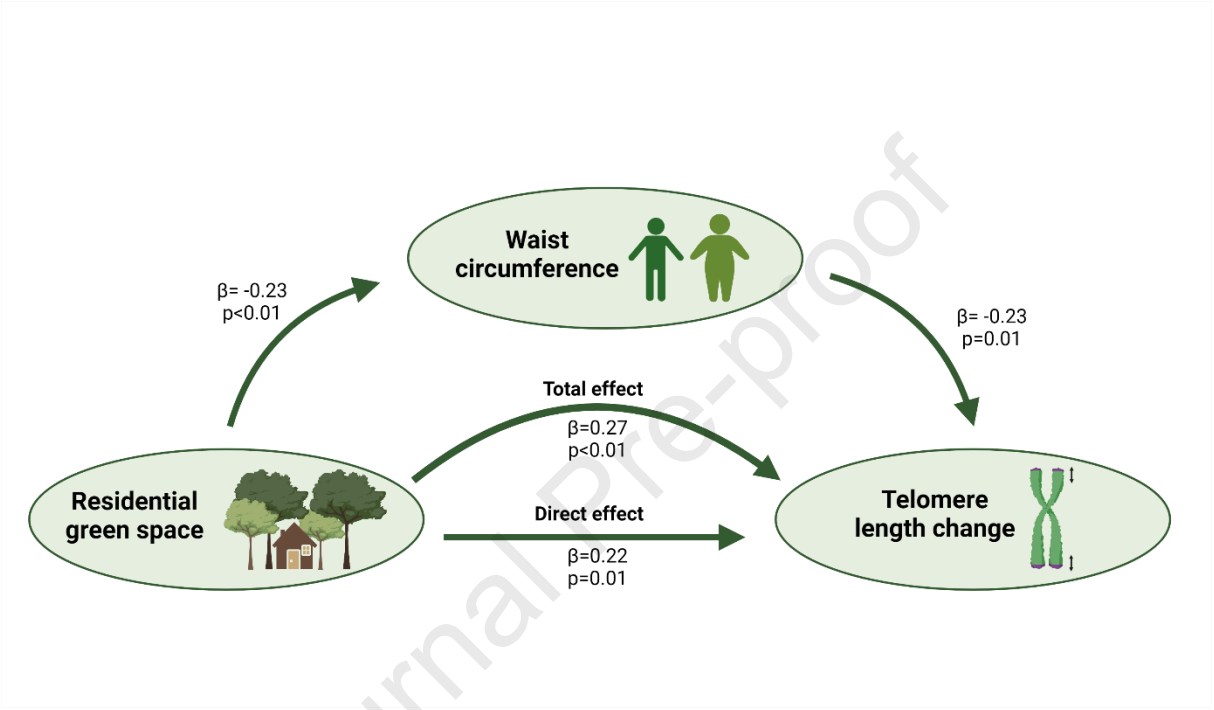

Figure 7: *Schematic diagram of mediation analysis results. Differences in waist circumference significantly mediated the effect of residential green space on longitudinal telomere change. Path values are standardized regression coefficients and p-values. (created with Biorender.com).*

3.6. Sensitivity analysis

Neighbourhood median income was positively correlated with residential green space (Spearman r=0.44, p<0.001), while no significant correlation was found with waist circumference. After adding neighbourhood median income as covariate, the effect of both exposures, waist circumference and residential green space, towards telomere attrition remained significant according to the 0.05 significance level in our linear regression models (β=-0.259 p=0.004; β=0.202 p=0.02, respectively). However, after correction for multiple testing using TEF (p=0.004) only waist circumference remained significant.

In the LASSO regression with the additional confounder neighbourhood median income, an optimal λ=0.002307 was determined after 10 fold cross-validation and the same two predictors were retained: waist circumference (β= -0.00238) and residential green space (β= 0.00134).

4. Discussion

Understanding telomere dynamics in childhood is important as early life telomere length is an important predictor of telomere length in adulthood [11] and it might thus predict overall life-span health [62]. In this longitudinal study, we investigated change in telomere length from childhood to adolescence in association with multiple exposures using a multi-exposure approach. Child waist circumference and residential green space were associated with telomere attrition. Moreover, the effect of green space on telomere attrition was partly mediated through waist-circumference. Using a multi-exposure factor, more unhealthy exposures (which reflect an unhealthier lifestyle) were associated with higher telomere attrition.

To our knowledge, this is the first longitudinal study utilizing a combination of exposures in a child cohort and additionally the first study to explore the longitudinal association between telomere change and residential green space in children.

4.1. Individual exposures and telomere dynamics

In previous studies, telomere length and/or telomere attrition have been associated with body composition [9], sleep duration [15], physical activity [63], psychosocial stress [64, 65], dietary habits [66], air-pollution [12] and residential green space [67]. However, this study found only significant associations for sugar-rich food (in girls) (see Supplementary Material), waist circumference and residential green space.

4.1.1. Body composition

Evidence using adult populations, regarding the relationship between overweight or obesity and telomere length is inconclusive, although there is a tendency towards a negative relation [9]. In children, some studies report an inverse relation between overweight and telomere length [68-70], while others report a non-significant relationship [71, 72]. To the best of our knowledge, all these childhood studies were cross-sectional. In the current longitudinal study, a higher waist circumference was associated with a significantly higher telomere attrition whereas a higher BMI showed no significant association with telomere attrition. Waist circumference might act as a better proxy of central adiposity than BMI and is more related to cardiovascular health [73]. Indeed, telomere length and cardiovascular health are strongly related and important risk factors regarding cardiovascular health are associated with early life telomere length [74]. In the current ChiBS cohort, we already showed that telomere dynamics are associated with some cardiovascular risk factors including triglycerides [75].

4.1.2. Dietary habits

A higher sugar-rich food intake was cross-sectionally associated with shorter telomeres in girls (see Supplementary Material), although fat-rich food intake or fruit and vegetable intake were

not significant predictors. However, in literature significant associations between these dietary factors and telomere length/attrition were observed [17, 76-78]. These studies had a larger sample size than the current study and utilised more detailed FFQ's which allowed precise quantification of food intake (in grams/day).

Besides looking at single dietary factors it might be more interesting to investigate the diet as a whole using dietary indices. Nevertheless, this requires more information on portion sizes. A study in children and adolescents showed that the dietary antioxidant capacity is associated with telomere length, emphasizing the importance of oxidative stress as cause of telomere shortening [79].

4.1.3. Residential environmental quality

In contrast to our results, increased ambient air pollution (including black carbon, $PM_{2.5}$, and a shorter distance to major roads) has been associated with shorter telomeres in different age-groups [11, 12, 80-84]. However, residential green space in the surrounding of a child's home was associated with a slower rate of telomere attrition in our study. This is in line with the limited current evidence, that supports the idea that residential green space is associated with longer telomeres [67, 85, 86]. Our results suggest that mainly high green (vegetation >3m) might be a crucial factor in this association. The sensitivity analysis indicated that the effect of residential green space on telomere attrition decreased by adding neighbourhood median income to the linear regression model, which we can attribute to the strong positive correlation between residential green space and neighbourhood median income. Surprisingly, residential green space remained significant to the 0.05 level even when correcting for this confounder, but not anymore after correcting for multiple testing.

Several potential mediators of the relationship between residential green space and telomere length have been proposed, including air pollution mitigation [87, 88], stress reduction [89-91] and physical activity promotion [92, 93]. As these exposures were not related to telomere attrition in our population, we could not evaluate their potential mediating effects. However, our data shows that waist circumference, a proxy for abdominal obesity, is associated with telomere attrition. Since other studies have already shown an association between green space and obesity [59, 60], we conducted an explorative mediation analysis, by which waist circumference could be identified as a (partial) mediator in the relationship between residential green space and telomere attrition. Despite the fact that we found no association between physical activity or psychosocial stress and longitudinal telomere change, our significant pathway from green space to waist circumference might theoretically still be explained by stress reduction and/or physical activity promotion. Indeed, exposure to green space can promote physical activity and thus extra energy expenditure, which can in turn lead to a reduction in waist circumference [92]. Moreover, residential green space has been associated

with lower stress-levels in this cohort [94]. The stress hormone cortisol has been associated with BMI or fat-mass index [95] and stress can also induce emotional eating behaviour [96]. This mediation could not be evaluated in ChiBS as measures of cortisol were only available for a subpopulation.

4.2. Multi-exposure factor

In this study, a tendency towards a negative association between telomere attrition and the multi-exposure score was observed. After dichotomisation, we found a significant higher telomere attrition in the group with unhealthier exposures. This is consistent with the findings of Sun *et al*. [20] who found that none of the individual lifestyle factors were associated with telomere length in adult women, although a combined effect of lifestyle practices was. Such findings could be explained by some exposures being correlated and amplifying each other's effect on the outcome. Additional literature on the accumulated effect of multiple environmental and lifestyle exposures on the telomere biology system is scarce, especially in a longitudinal setting. Further research is required to confirm our finding that an integrative assessment of multiple exposures has an influence on telomere length.

4.3. Underlying biological mechanisms

Because the telomere sequence consists of guanosine-rich parts (TTAGGG), which are highly sensitive for DNA damage, telomeres are a vulnerable target for oxidative stress and inflammation [97] induced by environmental and lifestyle exposures. Most – if not all - exposures in this study are known to be to some extent involved in processes leading to increased oxidative and inflammatory burden on the human body. Reactive oxygen species or low grade inflammation, due to e.g. particulate matter, unhealthy diet, stress, and obesity might induce single-strand breaks in the G-rich telomeres [98-101]. Oxidative stress might also explain the beneficial effect of residential green space on telomere attrition, as previous studies showed that higher residential green space was associated with reduced oxidative stress in children [102-104]. In addition, residential green space might theoretically promote physical activity [102], which can in turn lead to a lower waist circumference. This mechanism could explain the mediation effect of waist circumference on the association between residential green space and telomere length. Indeed, waist circumference as a widely used proxy for central adiposity has also been associated with increasing inflammation and oxidative stress [105-107], two mechanisms that are also very likely to be involved in the shortening of telomeres.

4.4. Health relevance of the effect of residential green space and waist circumference

To better interpret the observed effect of residential green space and waist circumference on telomere attrition, an annual telomere change percentage was calculated. For this purpose, the difference of telomere length between the follow-up and the baseline was first divided by the time of the follow-up period (in years) to obtain the telomere length change per year. Subsequently, the annual telomere change per year was divided by the baseline telomere length and multiplied by 100 to obtain a percentage of yearly telomere change for each participant.

The mean annual telomere change in our longitudinal population was -1.6%. An IQR increase in waist circumference (5.9 cm) is associated with -1.0% (95% CI; -1.57% to -0.44%) yearly telomere length change while an IQR increase in residential green space (11.6%) is associated with +0.64% (95% CI: 0.22% to 1.06%) yearly telomere length change. This highlights the health relevance of the observed effect of residential green space and waist circumference on telomere change.

4.5. Strengths and limitations

Strengths of our study include the long-term measurements of multiple exposures and telomere length over a five to seven-year period in children, which is unique in literature. Still, our study has some limitations. First, telomere length is variable from birth onwards and prenatal factors contribute to the initial telomere length setting, which may in turn contribute to later-life telomere length. This study does not take into account the contribution of these prenatal factors. Second, some exposures (sleep duration, physical activity, dietary habits and psychosocial stress) could benefit from a methodological improvement. Third, although we included several important health-related exposures, even a more extensive integration of additional exposures could provide more evidence on the environmental contribution to telomere length and telomere change over the early life course. Fourth, our study population consisted of Caucasian children, limiting its generalizability as ethnicity might be a predictor of telomere length. Fifth, we did not account for change of exposures (e.g. Moving of residence, changes in diet, sleep,…) during the follow-up period of 5-7 years in our longitudinal analysis, while it is probable that some exposures changed for the subjects within this timeframe. Finally, our sample size is rather small, resulting in a reduced power of our analysis that may explain the limited significant findings in this study.

5. Conclusions

This study showed that from a list of 17 exposures, mainly waist circumference seemed a clinical important negative factor while residential green space seemed a protective factor

558 towards longitudinal telomere change in children. These linear regression results were
559 confirmed by LASSO and after correction for multiple testing. Furthermore, the relationship
560 between residential green space and telomere attrition was partially mediated by waist
561 circumference. Cross-sectionally, a higher sugar-rich food intake in girls was associated with
562 shorter telomere lengths. By combining all exposures in one multi-exposure factor, tendency
563 towards a positive relation between telomere attrition and a higher multi-exposure factor was
564 visible. Unhealthy exposures are associated with a faster telomere attrition. Therefore,
565 promoting a healthy lifestyle from early age onwards remains important for the molecular
566 longevity, as exposures can track from childhood to adulthood.

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Highlights:

- Telomere attrition over 5-7y and 17 exposures were measured in 4-7y old children
- More unhealthy exposures at baseline resulted in higher telomere attrition
- Residential green space at baseline was associated with lower telomere attrition
- Waist circumference partially mediated this association
- Lifestyle and green space during childhood seems important for molecular longevity

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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