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Thaïs De Ruyter: Writing- Original Draft, Formal Analysis, Data Curation

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Tim S. Nawrot: Conceptualization, Writing - Review & Editing, Supervision

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

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

4

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29

30 Abstract

31

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

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

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

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

54

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

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

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

66

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

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96 1. Introduction

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

106

107 Although telomere length is heritable [7], environmental and lifestyle exposures may influence
108 its dynamics. Several health deteriorating exposures including unhealthy diet, sedentary
109 lifestyle, stress, and air pollution have been associated with shorter telomere lengths [8-14],
110 while beneficial exposures such as, physical activity, sleep duration, vegetable and fruit intake
111 have been linked to longer telomere lengths [15-17]. However, the majority of the
112 aforementioned studies focused on one specific exposure as predictor of telomere dynamics.
113 Whereas epidemiological research in the past was confined to individual exposures,
114 nowadays, there is a spiked interest towards combining multiple exposures as this provides a
115 more holistic approach to broaden our understanding of age-related diseases [18, 19]. Some
116 recent studies combined a limited number of lifestyle and/or stress exposures as a predictor
117 for telomere length [20-24], although a multi-exposure approach that combines stress and
118 lifestyle factors with more environmental exposures such as air pollution and residential green
119 space remains unexplored. The integration of multiple exposures in a multi-exposure factor
120 might lead to a better understanding of disease aetiology. Unfortunately, the majority of the
121 studies addressing multiple exposures use cross-sectional data, while longitudinal studies
122 examined only stress/depression without considering other lifestyle or environmental factors
123 [21, 23-26].

124

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

133 2. Methods

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

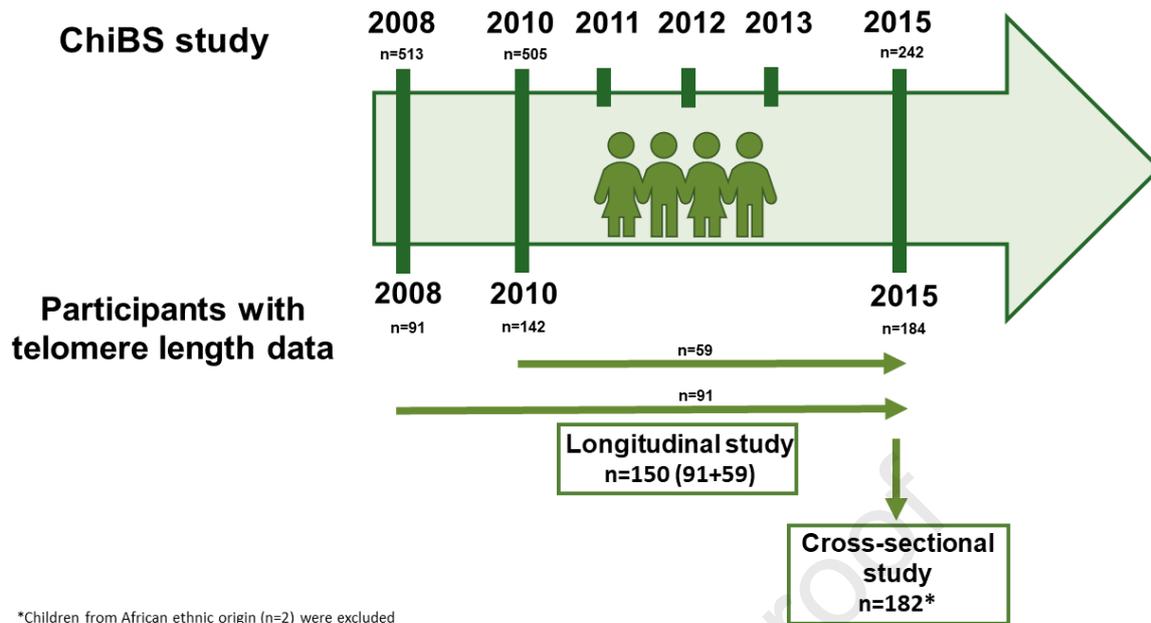
142

143 2.1. Participants

144 Data was used from the longitudinal ChiBS study (Children's Body composition and Stress)
145 [29]. Baseline participants were children between 2.8 and 10.3 years old, who were
146 approached through school and kindergarten settings in the municipality of Aalter (Belgium)
147 and its surroundings and were followed-up for seven years. The ChiBS cohort consists of
148 multiple waves, although the current study only included 3 out of 6 waves as these were the
149 only ones with telomere data; the baseline survey in 2008 (n=513), the first follow-up survey
150 in 2010 (n=505) and the fifth follow-up survey in 2015 (n=242). For the current study,
151 participants were included based on participation in the study wave of 2015 (N=242), and the
152 availability of telomere length data in 2015 (N=184). Children from African ethnic origin (n=2)
153 were excluded, as ethnicity may influence telomere length/attrition [30], resulting in a cross-
154 sectional sample size of 182 participants in the wave of 2015. Of these remaining 182
155 participants, 142 participants had also telomere length data in 2010, while 91 of these 182
156 participants had telomere length data in 2008.

157

158 To optimize the sample size, and to use the longest follow-up period as possible we decided
159 to merge the baseline data as follows. For the 91 participants having telomere length data in
160 2008, we used the data of 2008 as baseline. For the 59 participants who had no telomere
161 length data in 2008 but did have telomere length data in 2010, we used the wave of 2010 as
162 baseline. An overview of the study design is shown in Figure 1.



163

164 **Figure 1 Overview of the study design.**

165 The study was conducted according to the guidelines laid down in the Declaration of Helsinki
 166 and the project protocol was approved by the Ethics Committee of the Ghent University
 167 Hospital. Written informed consent was obtained from all parents and from all children aged
 168 12 years and older. Children younger than 12 years gave verbal consent. The examination day
 169 included biological sampling, anthropometric measurements and questionnaires for parents
 170 and children.

171

172 2.2. Relative average telomere length measurement

173 Peripheral blood was obtained from the children via venipuncture in
 174 Ethylenediaminetetraacetic acid (EDTA) tubes. After centrifugation (10 min, 2000g), DNA was
 175 extracted from the buffy coat using QIAamp DNA Mini Kit (Qiagen, Inc., Venlo, the
 176 Netherlands) and stored at -80°C. Next, DNA quantity and purity were assessed using a
 177 Nanodrop 1000 spectrophotometer (Isogen, Life Science, Belgium) and DNA integrity was
 178 evaluated using gel-electrophoresis. The average relative leukocyte telomere length was
 179 measured by a modified quantitative real-time PCR (qPCR) protocol as described previously
 180 [30, 31]. Telomeres were measured in triplicate on a 7900HT Fast Real-Time PCR System
 181 (Applied Biosystems) in a 384-well format. On each plate, a six point serial dilution of pooled
 182 buffy coat DNA was run to assess PCR efficiency, which ranged between 95 and 105%. The
 183 relative average telomere length was calculated using qBase software (Biogazelle, Zwijnaarde,
 184 Belgium), expressed as the ratio of telomere copy number to single-copy gene number (T/S)
 185 and normalized to the average T/S ratio of the entire sample set. The reliability of our assay
 186 was assessed by calculating the intraclass correlation coefficient (ICC) [32] with 95%

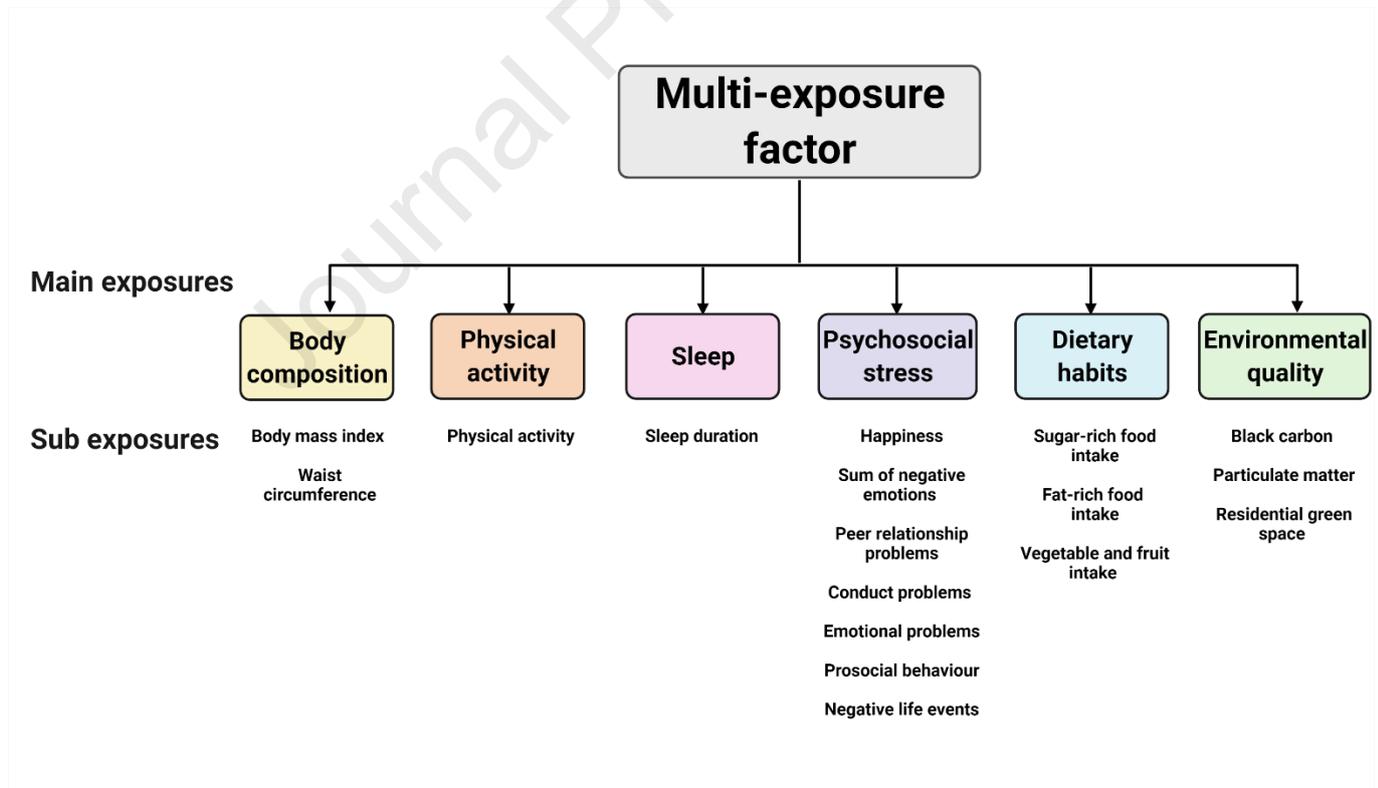
187 confidence interval (CI) of triplicate measures. The ICC (95% CI) of T/S ratios, telomere runs,
 188 single-copy gene runs were 0.859 (0.836 to 0.881), 0.969 (0.963 to 0.974) and 0.956 (0.948
 189 to 0.962), respectively. Based on the six inter-run calibrators, the inter-assay ICC (95% CI)
 190 was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

191

192 2.3 Exposure measurements

193

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



204

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

206 **Body composition** was assessed by measuring the BMI (dividing children's weight by height
 207 squared, kg/m²) and waist circumference (measured as the mid-point between the top of the
 208 iliac crest and the lower coastal edge). Overweight was determined using the International
 209 Obesity Task Force (IOTF) classification [33].

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

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

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

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

238
239 **The environmental quality** at the child's residence was estimated, taking into account three
240 exposures: black carbon, particulate matter, and green space. Daily residential exposure levels
241 ($\mu\text{g}/\text{m}^3$) for airborne black carbon (BC) and particulate matter particles with a diameter less
242 than or equal to $2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) were estimated using a high resolution spatial temporal
243 interpolation method (kriging) [40] combined with a dispersion model [41, 42]. This model
244 calculates the daily interpolated exposure concentrations in a high-resolution receptor grid
245 based on information from the Belgian telemetric air-quality networks, point sources, and line

246 sources. The overall model performance was evaluated by leave-one-out cross-validation and
247 was based on 14 monitoring points for black carbon and 34 for PM_{2.5}. Validation statistics of
248 the interpolation tool gave a spatial temporal explained variance (R^2) of more than 0.80 for
249 PM_{2.5} and 0.74 for BC. The model shows that modelled BC and PM air pollution at residence
250 correlated with internal exposure to nano-sized black carbon particles measured in urine [43].
251 Annual averages of the daily residential exposure levels for BC and PM_{2.5} were used in the
252 current analyses. In addition to these ambient air pollution data, the residential green space
253 was determined. The participant's residential address at follow-up was geocoded to estimate
254 green space exposure in seven buffers around the residential address (50 m, 100 m, 300 m,
255 500 m, 1000 m, 2000 m and 3000 m). The estimates at the 2000 m buffer were used in the
256 current analyses, but sensitivity analyses checked the similarity with the other buffers.
257 Residential green space was estimated using the Green Map of Flanders 2012 from the
258 Agency for Geographic Information Flanders (AGIV) which provides high-resolution (1x1m)
259 information on natural elements, identified as all non-agricultural vegetation. This non-
260 agricultural vegetation was categorized into three measures: high green (vegetation height
261 higher than 3 m), low green (vegetation height lower than 3m) and a total vegetation cover
262 (sum of both, high and low green). More information on the Green Map of Flanders is provided
263 in Dockx et al., 2022 [44]. All analyses were carried out using Geographic Information System
264 (GIS) functions using ArcGIS 10 software (Esri Inc., US).

265

266 *2.4. Multi-exposure factor*

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

276

277 *2.5. Covariates*

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

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

286

287 *2.6. Statistical analysis*

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

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

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

304 *2.6.1. Linear regression models*

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

313 *2.6.2. LASSO regression*

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

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

319 2.6.3. Mediation analysis

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

324 2.6.4. Sensitivity analysis

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

333 3. Results

334

335 3.1. Subject characteristics

336 Differences in characteristics between included and excluded participants in the wave of 2015
337 are shown in Supplemental Table 1. In Table 1, the characteristics of the study population at
338 baseline and at follow-up are shown. The telomere length changed significantly over the five
339 and seven year time period ($p < 0.0001$). Spearman correlations showed that relative telomere
340 length could be tracked across the different follow-up surveys ($r = 0.69$ to 0.73 , $p < 0.0001$).
341 Relatively few children were overweight in this study population (6.04% in 2015). Telomere
342 length was significantly higher in girls compared to boys ($p = 0.003$). Additionally, sex
343 differences were seen for some exposures in 2015: BMI ($p = 0.039$), waist circumference
344 ($p < 0.001$), and emotional problems ($p = 0.022$) were higher in girls, whereas physical activity
345 ($p = 0.018$) was higher in boys. SES was lower for girls ($p = 0.048$). No telomere length
346 differences were observed for SES, although emotional problems ($p = 0.015$) were higher in low
347 SES and residential green space (total green in buffer 2000 m) was lower in low SES
348 ($p = 0.006$). Differences in characteristics between both baseline waves (2008 and 2010) are
349 presented in Supplemental Table 2. Spearman correlations were calculated for all baseline
350 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/m ²)	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			
<u>Emotions</u>			
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
<u>Behaviour (SDQ)</u>			
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
<u>CLES</u>			
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/m ³)		12.13 (11.87-12.58)	
Black carbon (µg/m ³)		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.

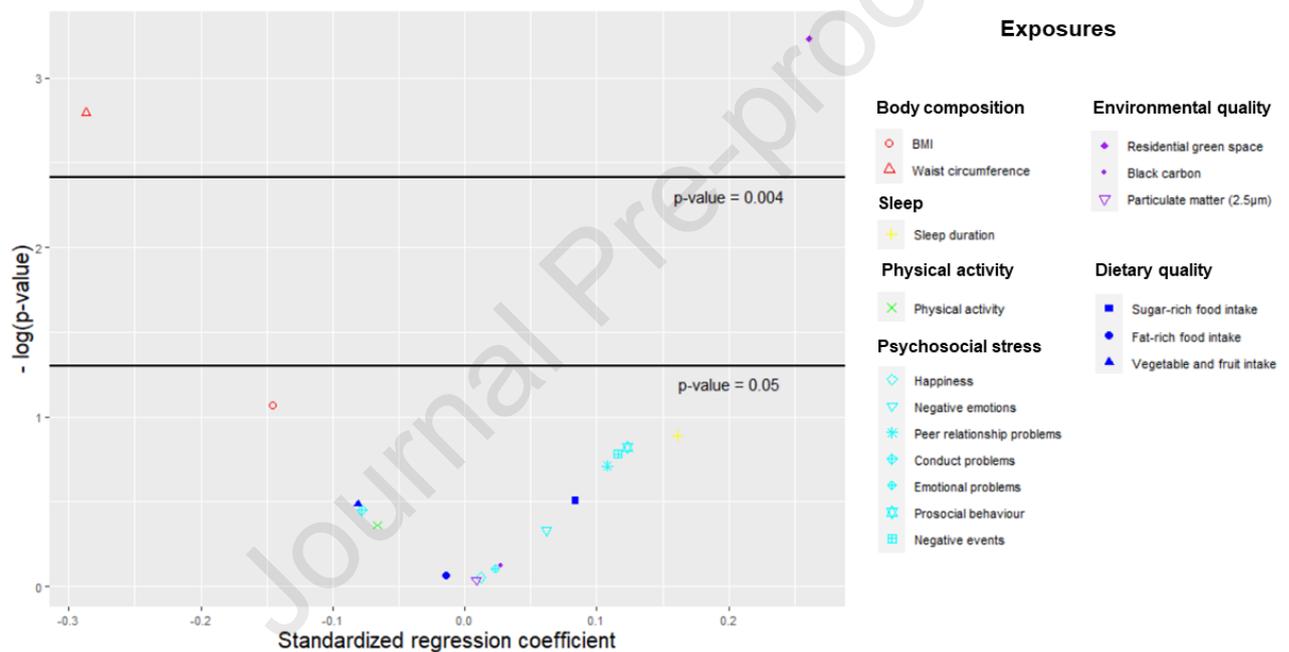
^C At least one parent with post-secondary education

351

352

353 3.2. Association between individual exposures and telomere attrition

354 Analyses of the baseline individual exposures in association with telomere attrition in childhood
 355 are shown in Supplemental Table 5 and a volcano plot is shown in Figure 4. Firstly, a higher
 356 waist circumference was associated with a higher telomere attrition ($\beta=-0.287$; 95% CI [-0.462;-
 357 0.112]; $p=0.001$). Secondly, a higher percentage of residential green space was associated
 358 with a lower telomere attrition rate ($\beta=0.261$; 95% CI [0.099;0.424]; $p=0.002$). After correction
 359 for multiple testing (TEF=0.004), these associations remained significant.

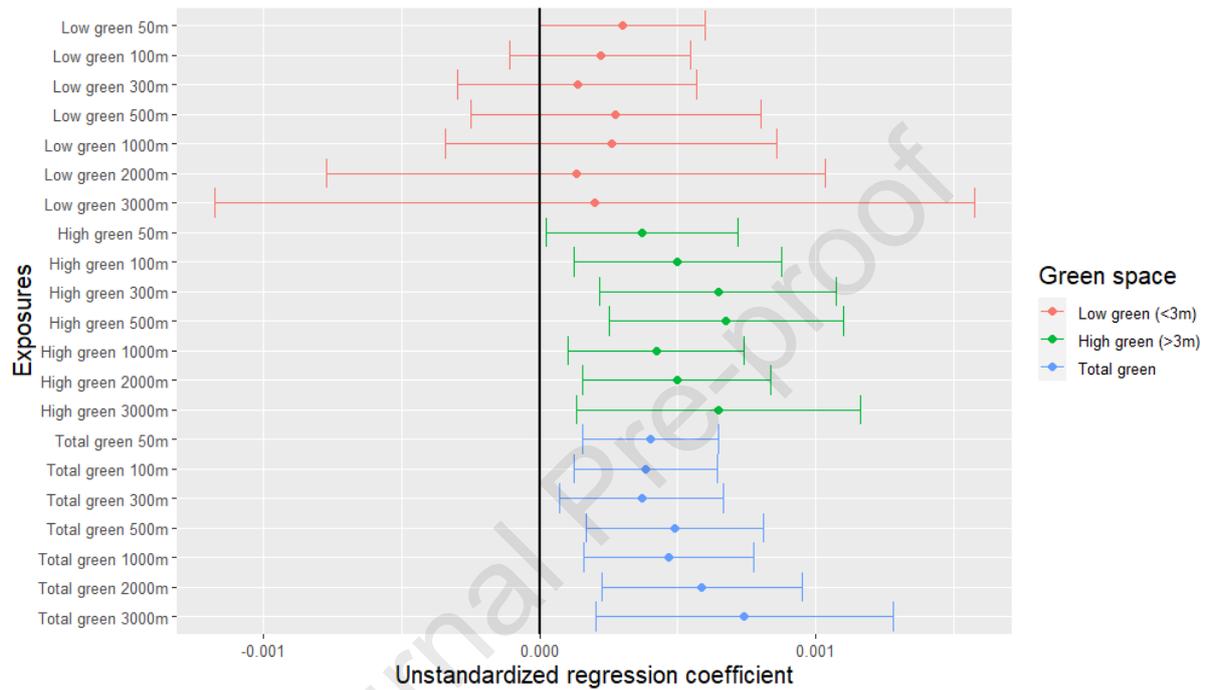


360

361

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

368 For residential green space, several buffers for high green, low green, and total green were
 369 tested. All buffers of high green and total green were associated with a lower telomere attrition.
 370 After correction for multiple testing (TEF=0.008), only high green in a 300, 500 and 2000 m
 371 buffer, and total green in a 50, 100, 500, 1000 and 2000m buffer remained significant. Results
 372 are presented in Figure 5. As total green in a buffer of 2000 m showed the strongest
 373 association, this buffer was selected for the calculations of the multi-exposure scores.
 374



375

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

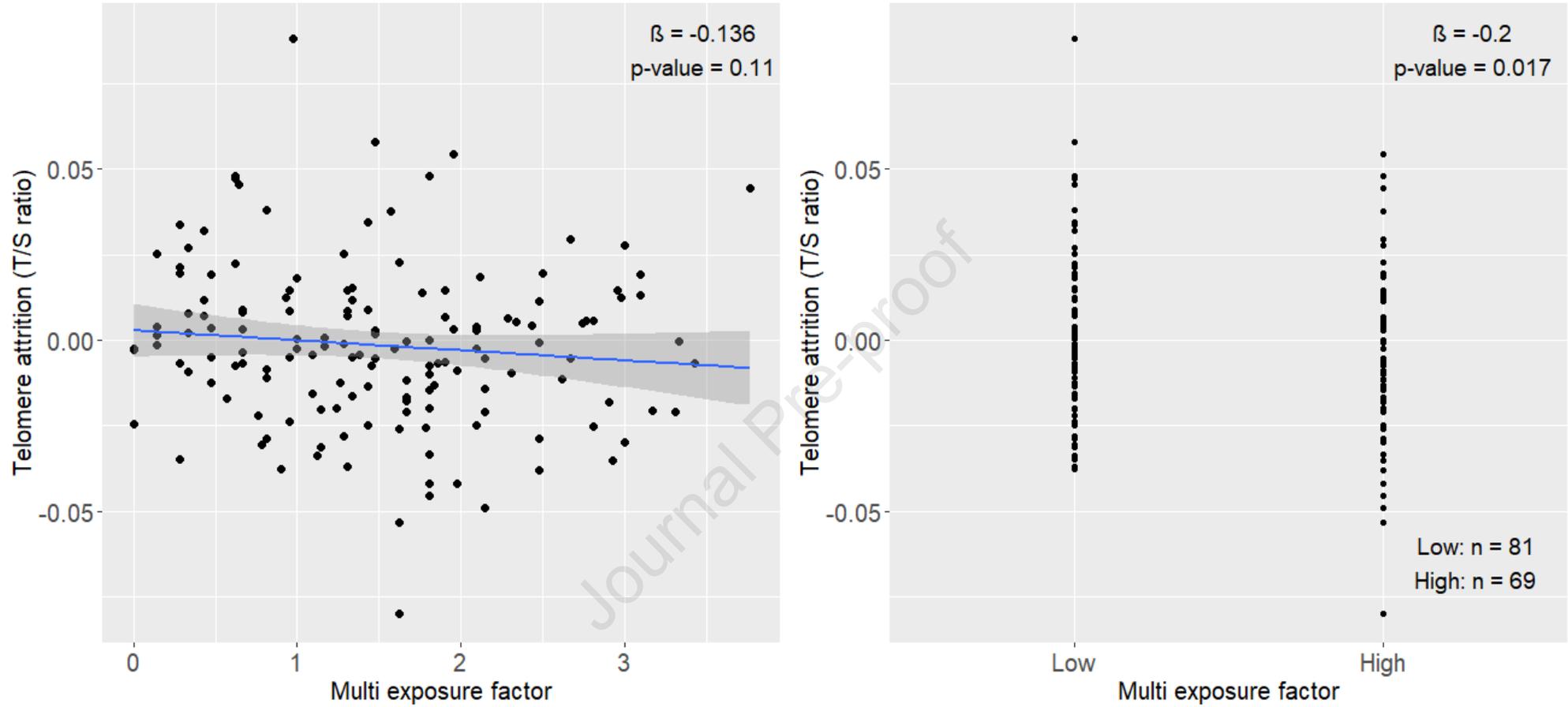
379 *3.3. Variable selection using LASSO regression as confirmatory analysis*

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

384

385 *3.4. Multi-exposure factor*

386 The maximum multi-exposure factor was 3.76 at baseline. No association was found between
387 the multi-exposure factor and telomere attrition ($\beta=-0.133$, $p=0.117$). However, when the multi-
388 exposure was categorised in two groups: a 'healthy exposure group' (multi exposure factor 0-
389 2, $n=81$) and an 'unhealthy exposure group' (multi-exposure factor 2-4, $n=69$) a significant
390 difference in telomere attrition was found between these two groups ($\beta=-0.200$, $p=0.017$), see
391 Figure 6.



392

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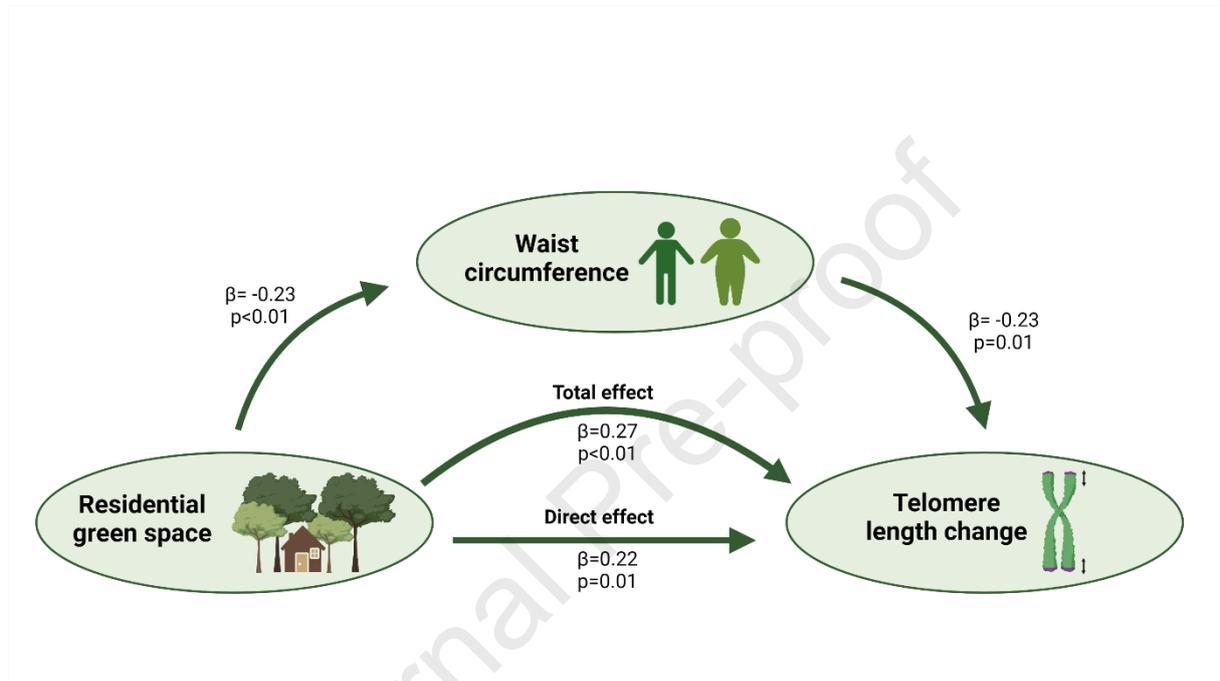
Figure 6: Scatterplots presenting the relationship between longitudinal telomere attrition and the multi-exposure factor. Original multi-exposure factor calculated from baseline data (Left). Multi-exposure factor from baseline data was categorised in two groups: 'Low' (multi exposure factor 0-2, n=81)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

396

397 **3.5. Mediation analysis**

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

402



403

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

407 **3.6. Sensitivity analysis**

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

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

418

419 4. Discussion

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

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

431 4.1. Individual exposures and telomere dynamics

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

437 4.1.1. Body composition

438 Evidence using adult populations, regarding the relationship between overweight or obesity
439 and telomere length is inconclusive, although there is a tendency towards a negative relation
440 [9]. In children, some studies report an inverse relation between overweight and telomere
441 length [68-70], while others report a non-significant relationship [71, 72]. To the best of our
442 knowledge, all these childhood studies were cross-sectional. In the current longitudinal study,
443 a higher waist circumference was associated with a significantly higher telomere attrition
444 whereas a higher BMI showed no significant association with telomere attrition. Waist
445 circumference might act as a better proxy of central adiposity than BMI and is more related to
446 cardiovascular health [73]. Indeed, telomere length and cardiovascular health are strongly
447 related and important risk factors regarding cardiovascular health are associated with early life
448 telomere length [74]. In the current ChiBS cohort, we already showed that telomere dynamics
449 are associated with some cardiovascular risk factors including triglycerides [75].

450 4.1.2. Dietary habits

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

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

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

462 **4.1.3. Residential environmental quality**

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

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

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

493

494 *4.2. Multi-exposure factor*

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

505 *4.3. Underlying biological mechanisms*

506 Because the telomere sequence consists of guanosine-rich parts (TTAGGG), which are highly
507 sensitive for DNA damage, telomeres are a vulnerable target for oxidative stress and
508 inflammation [97] induced by environmental and lifestyle exposures. Most – if not all -
509 exposures in this study are known to be to some extent involved in processes leading to
510 increased oxidative and inflammatory burden on the human body. Reactive oxygen species or
511 low grade inflammation, due to e.g. particulate matter, unhealthy diet, stress, and obesity might
512 induce single-strand breaks in the G-rich telomeres [98-101]. Oxidative stress might also
513 explain the beneficial effect of residential green space on telomere attrition, as previous studies
514 showed that higher residential green space was associated with reduced oxidative stress in
515 children [102-104]. In addition, residential green space might theoretically promote physical
516 activity [102], which can in turn lead to a lower waist circumference. This mechanism could
517 explain the mediation effect of waist circumference on the association between residential
518 green space and telomere length. Indeed, waist circumference as a widely used proxy for
519 central adiposity has also been associated with increasing inflammation and oxidative stress
520 [105-107], two mechanisms that are also very likely to be involved in the shortening of
521 telomeres.

522

523

524 *4.4. Health relevance of the effect of residential green space and waist circumference*

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

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

538 *4.5. Strengths and limitations*

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

555 *5. Conclusions*

556 This study showed that from a list of 17 exposures, mainly waist circumference seemed a
557 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.

Journal Pre-proof

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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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