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Association of Greenspace Exposure with Telomere Length in Preschool Children

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Abbreviations: Particulate Matter 2.5 with diameter less than 2.5 micrometer (PM2.5), Telomere Length (TL), Leukocyte Telomere Length (LTL), Normalized Difference Vegetation Index (NDVI), Quantitative Polymerase Chain Reaction (qPCR), Telomere amplification product (T), Single-copy gene (S), Telomere / Single copy gene ratio (T/S), Reactive Oxygen Species (ROS), Interquartile Range (IQR), Confidence Interval (CI).

Abstract

Exposure to greenspace has been associated with a wide range of health benefits; however, the available evidence on the association of this exposure with telomere length (TL), an early marker of ageing, is still scarce. We investigated the association of greenspace exposure with TL in a sample of 200 preschool children (aged 5-7 years) residing in Sabzevar, Iran (2017). We comprehensively characterized different aspects of greenspace exposure encompassing residential, kindergarten, and total (including both residential and kindergarten) surrounding greenspace (using satellite-derived Normalized Difference Vegetation Index), residential and kindergarten distance to green spaces, time spent in private gardens and public green spaces, and the number of plant pots at home. Relative leukocyte TL (LTL) in blood samples of the study participants was measured using quantitative polymerase chain reaction (qPCR). We applied mixed effects linear regression models with kindergarten and qPCR plate as random effects, to estimate the association of indicators of greenspace exposure (one at a time) with LTL, controlled for relevant covariates. We observed an inverse association between distance from home and kindergarten to green spaces larger than 5000m^2 and LTL. Moreover, higher total surrounding greenspace at 300m and 500m buffers and higher surrounding greenspace at 300m buffer around kindergarten and home were associated with longer LTL. Furthermore, longer time spent (h/week) in the public green spaces was associated with longer LTL. Our findings for residential and kindergarten distance to any green space (regardless of the size), residential surrounding greenspace at 100m and 500m buffers, kindergarten surrounding greenspace at 100m buffer, time spent in private gardens (h/week) and the number of plant pots at home were not conclusive. Our findings were generally suggestive for a positive

association between greenspace exposure and LTL in preschool children. More studies are needed to confirm these findings in other settings with different climates and populations.

Keywords: Urbanization; Natural Environments; Ageing; Development; LMICs; Pediatric

1. Introduction

The on-going urbanization worldwide has led an increasing number of the global population, including children, living in urban areas where residents often have less access to natural environments, including green spaces (UN-HABITAT 2016). Further to being more urbanized, the global population is also ageing, particularly in urban areas. According to a recent United Nations report, between 2000 and 2015, the number of people aged 60 years or over increased by 69% in urban areas compared to 25% in rural areas (United Nations 2015). As a result, healthy ageing is becoming a top public health priority worldwide. Early life, including pre- and postnatal periods and early childhood, is considered as an important window of susceptibility for environmental exposures when the aging process has already started (Bijnens et al. 2017, Dadvand et al. 2013). According to the Developmental Origins of Health and Disease (DOHaD) concept, exposures during this window may permanently change the body's physiology, structure, and metabolism and hence promote health or diseases (e.g., non-communicable diseases (NCDs)) in later stages of life (Barker 1997, Dietert et al. 2010, Gluckman and Hanson 2004). For example, exposure to a hostile environment during this critical period of development and growth, has been shown to induce a number of short- and long-term adaptation responses such as changes in endocrine or metabolic function, or in gene expression or differentiation that, in turn, could lead to adverse health outcomes such as obesity, ischemic heart disease, hypertension, or diabetes during the adult life (Mandy and Nyirenda, 2018). Accordingly, reducing exposure to environmental risk factors and/or enhancing exposure to beneficial environmental factors during early life have been proposed to be one of the means to promote healthy ageing (United Nations 2015, Kuh et al. 2014, Martens and Nawrot 2018). Considering its beneficial health effects (Nieuwenhuijsen et al. 2014, WHO Regional Office for Europe 2016), exposure to greenspace during early life could therefore have important implications for promoting healthy ageing and in the metropolitan area of Tehran (Iran) has already been considered as an alternative strategy for it (Ahmadi et al., 2017).

Telomeres are specialized structures located at the ends of human chromosomes to protect their integrity, block its degradation, provide genome stability, and avoid loss of genetic information (Lu et al. 2013). Telomere length (TL) is considered as a cellular marker of aging and its shortening has been associated with a higher risk of developing different adverse health outcomes such as cardiovascular diseases (Haycock et al., 2014), including myocardial infarction (Brouilette et al., 2003), hypertension (Benetos et al., 2001), atherosclerosis (Samani et al., 2001) or stroke (Ding et al., 2012), and type 2 diabetes mellitus (Willeit et al., 2014). Various environmental and lifestyle factors such as stress (Entringer, Epel, Kumsta, Lin, Hellhammer, Blackburn, Wüst, et al. 2011, Mathur et al. 2016), physical inactivity (Mundstock et al. 2015, Arsenis et al. 2017, Tucker 2017), social environment (Needham et al. 2012), air pollution (Miri et al. 2018), and noise have been associated with shorter TL (Martens and Nawrot 2018). In this context, exposure to greenspace, could potentially result in less shortening of TL through reducing stress (Entringer, Epel, Kumsta, Lin, Hellhammer, Blackburn, Wust, et al. 2011, Stigsdotter et al. 2010, Roe et al. 2013), fostering social interaction and social cohesion (Mitchell et al. 2014, Gascon et al. 2015), increasing physical activity (Arsenis et al. 2017, Mundstock et al. 2015, Fanshawe et al. 2007), and reducing exposure to urban-related environmental hazards such as air pollution (Dadvand et al. 2012, Dadvand et al. 2015a) and noise (Meillere et al. 2015, Dzhambov and Dimitrova 2014). However, the evidence available on a potential impact of exposure to greenspace on TL is still very scarce (Bijnens et al. 2015), with no study available on such an impact in children. Moreover, to date, most of the available studies on health effects of greenspace exposure have been conducted in high-income countries and there is a paucity of such studies for low- and middle-income countries. This study aimed to evaluate the association of exposure to greenspace with TL in preschool children in Iran.

2. Methods

2.1. Study setting

This study was conducted in Sabzevar, a town with a population of ~240,000 residents (2016) located in the northeast of Iran (coordinates: 36°12′ N 57°35′, elevation: 977 m). Approximately 21,000 of the population are between 5 to 9 years old. Sabzevar has an arid climate with four distinct seasons and an annual average rainfall of 176.8 mm. The highest precipitation is during winter and most vegetation can be seen in spring. The annual mean relative humidity is 43%, and the annual mean, minimum and maximum temperature in the city are 18, -2 and 45 ˚C respectively (Khorasan Razavi Weather Center, http://www.razavimet.ir/fa/node/38). About 27% (7.1 km^2) of Sabzevar city is covered by green space resulting in a 29.4 m^2 of green space per capita.

2.2. Study population

The enrolment of the study participants (i.e., preschool children) was conducted through kindergartens during March 2017 to June 2017. Of over 80 kindergartens in Sabzevar, 60 kindergartens with about 900 children aged between 5 to 7 years were selected randomly, who accepted participation in our study. Briefing sessions were held with the children's parents in every kindergarten, and the research aims, steps and inclusion/exclusion criteria were fully explained to them. Finally, 200 participants from 27 kindergartens (seven children on average from each kindergarten) who met our inclusion criteria were enrolled in our study (participation rate of 22%) (Figure 1). We included healthy children who did not constantly use supplements/vitamins and drugs, had no genetic disease, and were in kindergarten for at least one year and did not change their home since birth. Before the enrollment into the study, the children's parents/legal guardians signed the consent form approved by the Clinical Research Ethical Committee (IR.SSU.SPH.REC.1395.66) of the Shahid Sadoughi University of Medical Science, Yazd, Iran.

2.3. Exposure assessment

We carried out a comprehensive assessment of exposure by characterizing different aspects of exposure to greenspace including: a) residential as well as kindergarten distance from green spaces (any size as well as those larger than 5000 m^2) as an indicator of *access to green spaces* (Ludlow, Mitchell, and Webster 2003), b) surrounding greenspace at residential as well as kindergarten address, c) time spent in private green spaces as well as public gardens (measured as hours per week), and d) the number of plant pots at participant's home. This strategy resulted in generating 16 exposure variables for each participant, as detailed below.

2.3.1. Access to green space

Distance from home address as well as kindergartens of participants to nearest green space (any green space) and distance to green space with area more than 5000 m^2 (major green space) (Ludlow, Mitchell, and Webster 2003) were calculated as surrogates of access to green space (Ludlow, Mitchell, and Webster 2003). Data needed for this purpose were calculated using land use map of the study area (2015) prepared by the municipality of Sabzevar in ArcGIS v10.5 software.

2.3.2. Surrounding greenspace

We applied satellite-derived Normalized Difference Vegetation Index (NDVI) to characterize surrounding greenspace separately for residential and kindergarten addresses. NDVI is commonly used as a numerical indicator of greenspace (i.e., photosynthetically active vegetation), which our previous study showed to be a valid measure to characterize green spaces (Gascon et al., 2016). The index depends on the visible and near-infrared light reflected by land surface and is calculated as (Weier and Herring 2011):

$$NDVI = (NIR — VIS)/ (NIR + VIS)$$

where NIR and VIR are respectively land surface reflectance of near-infrared and red (visible) parts of spectrum. It ranges from -1 to + 1, where 0 indicates no vegetation and + 1 means the highest possible density of green leaves (Dadvand et al. 2017, Gascon et al. 2016). The NDVI for the study area was calculated based on the Landsat images at 30m x 30m resolution obtained on June 15, 2017 (Figure 1).

Greenspace surrounding residential and kindergarten address(es) of each participant was abstracted as the average of NDVI in buffers of 100m, 300m and 500m around his/her geocoded home address and kindergarten (Dadvand, de Nazelle, Figueras, et al. 2012).

Total surrounding greenspace index was calculated for each buffer (100, 300 and 500m) by averaging NDVI values around home address and kindergartens separately for each buffer, weighted by the daytime, which children spent at home (18 h) and kindergartens (6 h) (Dadvand et al. 2015b).

2.3.3. Time spent in green spaces

Data on the amount of time spent in different types of green space was obtained through questionnaires answered by the parents (Dadvand, Poursafa, et al. 2018, Dadvand, Hariri, et al. 2018). The time spent in green space was asked separately for the time spent in private gardens such as yard garden, patio, etc (h/week), and the time spent in the public green spaces such as park, forest, etc (h/week)

2.3.4. Number of plant pots at home

The data on the number of plant pots at participant's home was collected through a questionnaire filled by parents.

2.4. Measurement of leukocyte telomere length

Two mL of blood were taken from each child in the morning at the reference lab of Sabzevar and transferred to laboratory in Vacutainer® Plus Plastic K2EDTA Tubes (USA) within less than two h and kept in -80 ˚C until analyses. The DNA was extracted from blood samples using DNA extraction kit (GeNet Bio, Korea) according to the manufacturer's instructions. The purity ratio (A260/230) and yield (A260/280) (ng/μL) of extracted DNA samples were determined using Nanodrop spectrophotometer (BIO INTELLECTICA Nano100, Canada). All DNA samples were stored at -80 °C until the time of analyses.

To measure LTL, we performed quantitative real-time polymerase chain reactions (qRT-PCR) based on the method by Cawthon which determines telomere copy number relative to single-gene (human beta-globin) copy number (T/S ratio) (Cawthon 2009, Cawthon 2002, Pieters, Janssen, and Dewitte 2016). This ratio is known as relative LTL. Q-RT-PCR was carried out using SYBR green PCR master mix 2x (Amplicon, Denmark) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA) with the following primers: telomere forward 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′, telomere reverse 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′, single-copy gene forward 5′-GCTTCTGACACAACTGTGTTCACTAGC-3′, and single-copy gene reverse 5′-CACCAACTTCATCCACGTTCACC-3′. Telomere and single-copy numbers were evaluated in triplicate (3 measured per individual) in a total of 10 plates. Each reaction for telomere evaluation contained 5 μL of SYBR green PCR master mix 2x, 0.1 μL of forward primer (10 pmol/μL), 0.9 μL of reverse primer (10 pmol/μL) and 1 μL (25 ng/μL) of DNA. Single-copy gene (SCG) (SCG) was performed as the telomere reaction, with the exception that we added 0.3 μL (10 pmol/μL) of forward primer and 0.7 μL (10 pmol/μL) of reverse primer. The cycling thermal profile was the same for telomere and single-copy gene including one cycle at 95°C for 15 min, followed by 50 cycles of 95°C for 15 seconds, 60 °C for 20 seconds, and 72°C for 20 seconds. Each plate contained 5 serial concentrations of a standard human genomic DNA sample (150 ng, 50 ng, 16.7 ng, 5.55 ng and 1.85 ng per well), giving amplification efficiencies ranging from 95 to 105%. Melting curve analyses were used at the end of each cycle to confirm amplification specificity and absence of primer dimers. Finally, the relative T/S ratio was calculated as follow (Cawthon 2009, Cawthon 2002):

$$\textit{Relative T/S} = \frac{2^{-(Ct_{TL}-Ct_{SCG})sample}}{2^{-(Ct_{TL}-Ct_{SCG})control}}$$

Where $Ct_{TL}$and Ct_{SCG} were the average Ct of each sample and single copy gene, respectively. In this study, the average value of T/S ratio of samples (the numerator) was used as control (the denominator).

To provide reproducibility, inter-assay variability was measured in three different days. The % of coefficient of variation (%CV) was 0.74% for LTL and 47% for single-copy gene. LTL is expressed in relative units as the ratio of telomere copy number proportional to single-copy gene number (T/S) relative to the average telomere length in the entire sample set (T/S ratio).

2.5. Statistical analyses

2.5.1. Main analyses

Considering the multilevel nature of our data, we developed linear mixed effect models with relative LTL as the outcome, indicators of greenspace exposure (one at a time) as fixed effect predictor and the kindergartens and qPCR plates as random effects. The models were further adjusted for *a priori* selected set of variables: age (continuous), sex (girl/boy), body mass index (BMI, continuous) (Gielen et al. 2018), exposure to environmental tobacco smoke at home (yes/no) (Theall et al. 2013) and two indicators of household socioeconomic status (SES) as well as two indicators of the neighborhood SES (Needham et al. 2012). Parental education as the highest education level between paternal and maternal education of the participant (no education/ primary school, secondary school, or university) and income (<15 million and >15 million Rials) were used as indicators of household SES, while the percentages of illiterate adults as well as unemployment in the census tract based on the last census in Iran (2016) were applied as indicators of the neighborhood SES. The associations were reported for each interquartile range (IQR) increase in NDVI, distance from green space areas, time spent in the green space area, and number of flowerpots based on all study participants. Consistent with previous studies of the environmental determinants of TL (Miri et al., 2019; Zhao et al., 2018), relative LTL was transformed using its natural logarithm to assure the normal distribution of the residuals of our models. Consequently, to enhance the interpretation of the regression models we expressed all β coefficients and 95% confidence intervals (CIs) as the percentage increase (or decrease) in LTL for each IQR increase in the exposure variables. The percent change in LTL was calculated as $(e^{(\beta)}-1) \times 100\%$, and 95% CI were calculated as $(e^{[(\beta \pm 1.96 \times SE)]}-1) \times 100\%$, in which SE is the estimated standard error and β is the estimated regression coefficient. Stata 14 (StataCorp L.P., College Station, TX) has been used for all statistical analyses.

2.5.2. Sensitivity analyses

The models were further adjusted for age squared (continuous), car ownership (yes/no) and home ownership (yes/no), and also for environmental tobacco smoke in other places than the home (yes/no).

2.5.3. Stratified analyses

Main analyses were stratified by sex (girl/boy), parental education (no education/primary school, secondary school, or university) and percentage of unemployment in the census tract in tertiles. The associations were reported per IQR increase in each greenspace indicator based on participants in each stratum.

2.5.4. Mediation analyses

We explored the mediation role of air pollution in the association between total surrounding greenspace (including both residential and kindergarten surrounding greenspace) and telomere length, for which we could hypothesize such a mediation. Our previous study based on the same sample of schoolchildren demonstrated a negative association between exposure to $PM_{2.5}$ and LTL (Moslem et al., 2020). Estimated $PM_{2.5}$ levels (for detailed information regarding exposure assessment, see the Supporting information in page 41 Supplemental Materials) at home address and kindergarten were used to develop total $PM_{2.5}$ exposure as the annual average of $PM_{2.5}$ levels at home and kindergarten, weighted by the time the child would spend at home (18 h) and kindergarten (6 h). Following the approach of Baron and Kenny (Baron and Kenny 1986) for mediation analyses, we fitted separately a model for the outcome given the mediator and the exposure, and a model for the mediator given the exposure to compute the percentage of mediation. Kindergartens and qPCR plates were considered as random effects in the model for the outcome. Bootstrap was used to obtain percentile-based 95% confidence intervals for the different estimates.

3. Results

3.1. Study population

Descriptive statistics of the sociodemographic characteristics and exposure variables of the study participants are presented in Table 1. From 200 participants included in the study, 89 (44.5%) were girls and 111 (55.5%) were boys. The median (IQR) age of participants was 7 (1) years. Most of the participants had either the father or the mother with high school education (57%) and 13% of children had exposure to environmental tobacco smoke at home (Table 1). The median (IQR) T/S ratio of children was 0.83 (0.7) with larger T/S ratio for girls compared to boys (0.89 vs. 0.79) (Supplemental materials, Table S1). A graphical display of the correlation matrix between greenspace indicators was developed (Figure 2). In our study NDVI values of different buffers around home and kindergarten as well as total surrounding greenspace were positively correlated. As expected, distances to any green spaces and larger than 5000 m^2 were negatively correlated with NDVI indicators and also with time spent mainly in public green spaces.

3.2. Main analyses

Percent changes (95% CIs) in LTL in relation to the indicators of greenspace exposure are presented in Table 2, in which the IQR for each exposure variable is also displayed. In fully adjusted models, we observed that an IQR increase in distance from home and as well as kindergarten to green spaces larger than 5000 m^2 was associated with a 20.5% (95% C = -29.9%, -9.7%) and 21.8% (95% CI = -31.4%, -10.8%) decrease in LTL (Table 2). For the distance to any green space regardless of its size we did not observe any association with LTL. As presented in Table 2, we observed that an IQR increase in greenspace around home and kindergarten as well as total surrounding greenspace in a 300m buffer, was associated with a 8.3% (95% CI = 1.2%, 15.9%), 13.3% (95% CI = 2.2%, 25.7%) and a 12.0% (95% CI = 3.1%, 21.7%) increase in LTL, respectively. We also observed that an IQR increase in greenspace around kindergarten and total surrounding greenspace in a 500m buffer was related with a 14.5% (95% CI = 0.4%, 28.4%) and an 8.4% (95% CI = 0.3%, 17.1%) increase in LTL, respectively. Similarly, we observed positive associations for surrounding greenspace in a 100m buffer around home and kindergarten and in a 500m buffer around home with LTL. Spending more time in the public green spaces (park, forest, etc.) was associated with a 39.0% (95% CI = 22.8%, 57.3%) increase in LTL. Longer time spent in private gardens and more plant pots at home did not show any association with LTL (Table 2)."

3.3. Sensitivity analyses

The results of the models further adjusted for age squared, car ownership and home ownership were generally similar to the main analyses in terms of strength of the association and direction (Supplemental materials, Table S2). Moreover, after further adjustment of analyses for the exposure to environmental tobacco smoke in other places than the home, we did not observe any notable change in our findings either in terms of direction and strength of the associations.

3.4. Stratified analyses

The results of the stratified analyses by sex and SES are shown in table S3 and S4 in Supplemental materials. In the sex-stratified analyses for the residential (100m buffer), kindergarten (300m buffer) and total surrounding (100m buffer) greenspace, we observed positive associations ranging from a 17% to a 26% increase in LTL among girls and null for boys. For the other indicators of greenspace exposure, the associations for boys were also null (Table S3) except the time spent in public greenspace and residential distance to greenspaces for which we observed similar associations for boys and girls.

In the stratified analyses based on socioeconomic status (see Supplemental Material, Table S4), we observed some suggestions for a potential trend across the strata of parental education with stronger associations for children with higher parental education. Considering the indicator of neighborhood SES (% of unemployment in the census tract), the stratified analyses in tertiles suggested that stronger associations could be shown in the 1st tertile, which corresponds to the lower percentage of unemployment. In addition, interaction terms suggested a stronger association in the 1st tertile of % unemployment for surrounding greenspace around kindergarten (300m), and for public green space they suggested a higher and positive association in children whose parents had higher education level; however, all p-values for the interaction terms were more than 0.01.

3.5. Mediation Analyses

Our mediation analysis showed that 30.0% (95% CIs: -1.71, 3.12) of the association between total surrounding (300m buffer) telomere length could be mediated through exposure to $PM_{2.5}$.

4. Discussion

To our knowledge, this is the first study on the association between greenspace exposure and TL in children. Moreover, our study provides new evidence regarding the health effects of green spaces in children from low and middle-income countries. Our study benefitted from a comprehensive assessment of greenspace exposure, including residential and kindergarten surrounding greenspace and distance to green spaces as well as time spent in private and public green spaces and the number of plant pots at home. We found that more greenspace surrounding home (300m buffer), kindergarten (300m and 500m buffer), and combination of these two (i.e., total surrounding greenspace at 300m and 500m buffer), less distance to green spaces larger than 5000 m^2 from home and kindergarten, and longer time spent in public green spaces were positively associated with LTL. For the residential and kindergarten surrounding greenspace in other buffers, distance to any green space (regardless of its size) and the number of plant pots at home, we did not observe any association. We observed some suggestions for stronger associations for girls, for those participants with parents having higher educational levels and living in neighborhoods with lower percentage of unemployment; however, these patterns were not consistent for all indicators of greenspace exposure. Moreover, our stratified analyses were likely under-powered, and this issue needs to be considered when interpreting our findings for these analyses. We also found that about one-third of our observed association between total surrounding greenspace and LTL could be explained by reduction in exposure to air pollution ($PM_{2.5}$).

4.1. Interpretation of the findings

We are not aware of any study on the association between greenspace exposure and TL in children; therefore, it is not possible to compare our findings with those of others. However, our findings are in line with some previous observations and they might suggest that exposure to greenspace can potentially result in less shortening of LTL, which could promote healthy aging by reducing the risk of developing different adverse health outcomes that have been already associated with shorter telomeres. So far, only two studies have evaluated the association between greenspace exposure and TL (Woo et al. 2009, Bijnens et al. 2015). Bijnens *et al.* assessed TL from placental tissues of 211 twins and showed that maternal residential surrounding greenspace (measured using NDVI) during pregnancy was positively associated with TL (Bijnens et al. 2015). In a cohort study of 976 men aged 65 and over, Woo *et al.* found shorter TL in participants living in zones with lower green spaces presence (Woo et al. 2009). It is worthy to mention that in our study, the greenspace surrounding home and kindergarten for each participant were not strongly correlated, suggesting that our observed associations between these indicators and TL were not dependent on each other.

We observed an inverse association between the distance to a green space larger than 5000m^2 and LTL, but not for any green space regardless of its size. Previous studies have reported that larger green spaces are more likely to be used for physical activity (McCormack et al. 2010) and mitigate air pollution, which in turn, have been associated with longer LTL (Arsenis et al. 2017, Martens and Nawrot 2018). In this context, we also found some suggestions for stronger associations for measures of surrounding greenspace in larger buffer sizes. This is consistent with previous evidence in which the associations between green spaces indicators in larger buffers and health outcomes were stronger (Su et al. 2019) and more relevant to physical activity levels(McMorris et al. 2015; Borwning and Lee 2017). Previous studies have reported that more time spent in green spaces is associated with lower stress, better mental health, and improved social contacts (Beyer et al. 2014, Amoly et al. 2014, Dadvand, Hariri, et al. 2018), which, in turn, could be associated with longer LTL (Mathur et al. 2016, Starkweather et al. 2014, Costa et al. 2015). These findings are in line with our results in which, more time spent in public green spaces was associated with longer LTL. Even though our study is the first to evaluate the sex- and SES-stratified associations between greenspace exposure and LTL, previous literature has shown that health effects of green spaces could differ between sex and SES. Our stratified analyses did not show any effect modifications by sex or SES, but the results were suggestive for stronger associations in girls compared to boys and in higher SES. A number of studies have observed that the relationships between green spaces exposure and health outcomes could be stronger in girls (Annerstedt et al. 2012, Roe et al. 2013), which goes in line with our findings. However, the evidence is not consistent as some studies are not supportive for these associations or showed an inverse relationship with TL, stronger in boys (Ruijsbroek et al. 2017, Richardson et al. 2017). Regarding socioeconomic status, different studies have evaluated the modification of the health benefits of greenspace exposure by SES, however is still unclear its role. In some cases, stronger relationships have been found for participants with lower SES (Maas et al. 2006, McEachan et al. 2016), and in other studies non-statistically significant associations were observed (Casey et al. 2016). Therefore, it might be essential to continue considering the effect modification analyses by sex and SES when evaluating the health effects of greenspace exposure, as it seems that the available evidence is still inconsistent.

4.2. Biological plausibility

The mechanisms underlying health effects of green spaces are yet to be established. Some of the possible mechanisms suggested are promoting social contacts, inducing psychological restoration/stress reduction, improving functioning of the immune system due to microbial input, promoting physical activity and reducing obesity, and mitigating urban-related environmental hazards including air pollution, noise, and heat (Nieuwenhuijsen et al. 2014, WHO Regional Office for Europe 2016). Some of these mechanisms could also be relevant for TL. Higher levels of physical activity and lower levels of obesity have been associated with reduced inflammation and oxidative stress and enhanced restoration of TL (Mundstock et al. 2015). Conversely, psychological and life stress (Law et al. 2016, Epel et al. 2004), noise exposure (Meillere et al. 2015), and air pollution (Martens and Nawrot 2018, Miri et al. 2018) have been associated with shorter TL. The results of the mediation analyses showed that air pollution could explain about one-third of our observed association between total surrounding greenspace and LTL. This finding is in line with previous evidence that linked the existence of green spaces with a reduction of PM2.5 concentrations (Chen et al. 2016, Dadvand, de Nazelle, Triguero-Mas, et al. 2012, Dadvand et al. 2015b). At the same time, a recent systematic review and meta-analysis of the available evidence showed that higher long-term exposure to PM2.5 was associated with shortened telomeres (Miri et al. 2018). Such an exposure has been hypothesized to induce TL shortening through reactive oxygen species (ROS) mediated telomeric DNA damage and inflammation (Kordinas, Ioannidis, and Chatzipanagiotou 2016, Miri et al. 2018). Additionally, it has been reported that accelerated telomere shortening could be associated with different clinical outcomes such as cancer, cardiovascular diseases or diabetes (Haycock et al., 2014; Willeit et al., 2014), which are also associated with exposure to high levels of PM2.5 over a long period of time (Cao et al., 2018; Hayes et al., 2020; Lao et al., 2019).

4.3. Limitations

Our study should be interpreted in the context of its limitations. Firstly, our measures of proximity to green spaces did not address the quality of these spaces, while quality aspects such as safety, aesthetics, biodiversity, facilities and amenities could have had implications for our analyses. Additionally, to develop the total surrounding greenspace, we assumed that the children on average spent 6 hours at kindergarten and 18 hours at home. Such an assumption was based on the average of working hours of kindergartens in our study area and not collected data from our participants. Moreover, by only including greenspace surrounding home and kindergarten to develop the total surrounding greenspace, we have effectively overlooked exposures that could have happened in other potentially important microenvironments such as homes of friends and relatives, commuting routes to and from home, or public places that could have been visited frequently by participants and their families. Furthermore, our exposure assessment through NDVI data did not differentiate the type of vegetation or land cover, which could have influenced our observed associations for the greenspace surrounding homes and kindergartens. Additionally, our satellite data for the greenspace indicators around home and kindergarten was at 30m x 30m resolution and by using images with higher resolution, we might have been able to obtain a more precise assessment of exposure; however, such a higher resolution was less likely to result in a notable change in our observed associations (Su et al., 2019). Moreover, our data on time spent in green spaces was obtained through questionnaires, which could have resulted in exposure measurement error. Moreover, our measure of exposure to indoor plant was merely based on the number of plant pots home, while lacking information on important determinants of this exposure such as visibility of the plants for the children (given their height), species of the plants (which could have been relevant, among others, to their ability to reduce air pollution), or the placement of plants at home (e.g. whether they were located in places where the child spent much of her/his time). The unavailability of such detailed information might have been the reason for our null findings for the indoor plants. Additionally, in our study we did not cover greenspace exposure during prenatal period as we did not have data on the maternal residential address during this period or their potential mobility during pregnancy. The potentially important impact of prenatal greenspace exposure on TL remains as an open question for the future studies. Lastly, our study did not cover exposure to other air pollutants such as ozone, that could have a key mediator role in the pathway between greenspace exposure and LTL as the observed with PM2.5. In addition, we did not collect data on physical activity, which, could act as another mediator, given that green spaces have been reported, although inconsistently, to enhance physical activity (McCrorie et al., 2014) and, at the same time, physical activity has been suggested to induce potential protection against the shortening of TL (Arsenis et al., 2017).”

5. Conclusions

We observed a positive association of residential/kindergarten greenspace, as well as time spent in public green spaces and inverse association of distance to major green spaces with LTL in a sample of Iranian preschool children. Given the rapid urbanization coupled with ageing population worldwide, healthy ageing has become a top public health priority worldwide. Our findings, if confirmed by future studies, could provide policymakers with evidence base to implement targeted interventions to decelerate ageing process early in life. Further research is warranted to evaluate these associations using longitudinal design based on repeated measurements of TL in other settings with different climates and genetic backgrounds. These studies are also recommended to disentangle the effects of pre- and postnatal greenspace exposures and the role of air pollution.

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Figure and Table captions

Figure 1. Map of Sabzevar, Normalized Difference Vegetation Index (NDVI,) and location of the kindergartens.

Figure 2. Graphical representation of the spearman's correlations between indicators of greenspace exposure.

Table 1. Baseline and exposure characteristics of the study population.

Table 2. Adjusted mixed effects regression models of the association between Log LTL and measures of green spaces. Percentage changes in LTL are presented per one interquartile range (IQR) change in green space indicator.

Table 1. Baseline and exposure characteristics of the study population.

Baseline and exposure characteristics	Sample (N=200)
LTL, T:S ratio	0.83 (0.7)
Age, years	7 (1)
Missings, n	1
Sex, %	
Male	89 (44.5)
Female	111 (55.5)
Missings, n	0
Environmental tobacco smoke at home, %	
No	169 (84.5)
Yes	26 (13.0)
Missings, n	5
BMI, kg/m2	14.72 (2.6)
Missings, n	17
Parental education in 3 cat, %	
Elementary	52 (26.0)
High School	114 (57.0)
University	32 (16.0)
Missings, n	2
Income, %	
≤15 million Rials	173 (86.5)
>15 million Rials	22 (11.0)
Missings, n	5
Illiterate adults, % per census tract	25.2 (28.0)
Missings, n	0
Unemployment, % per census tract	7 (7.9)
Missings, n	0
Distance to any green spaces (in meters)	
Home	106.8 (118.5)
Kindergarten	88.1 (109.2)
Distance to green space ≥ 5000 m2 (in meters)	
Home	269.7 (335.4)
Kindergarten	328.5 (497.6)
Surrounding green space index (NDVI)	
Home – 100m	0.067 (0.015)
Home – 300m	0.072 (0.012)
Home – 500m	0.073 (0.012)
Kindergarten – 100m	0.068 (0.011)
Kindergarten – 300m	0.075 (0.019)
Kindergarten – 500m	0.075 (0.016)
Total surrounding green space index (NDVI)	
Total surrounding green space -100m	0.069 (0.015)
Total surrounding green space -300m	0.073 (0.013)
Total surrounding green space -500m	0.075 (0.011)
Time spent in the green space	
Private garden (h/week)	6 (12)
Public green space (h/week)	18.7 (21.6)
Plant pots at home (number)	3 (6)

Values are mean (SD) for continuous normal distributed variables, median (IQR=interquartile range) for continuous non-normal distributed variables, and frequency (percentage) for categorical variables. LTL = Leukocyte Telomere Length; BMI = Body Mass Index; NDVI = Normalized Difference Vegetation Index.

Table 2. Adjusted mixed effects regression models of the association between Log LTL and measures of green spaces. Percentage changes in LTL are presented per one interquartile range (IQR) change in green space indicator.

	Percentage change (95% CI)	p-value[1]
Distance to any green space (in meters)		
Home (IQR=118.5)	0.02 (-9.3, 10.0)	0.996
Kindergarten (IQR=109.23)	8.6 (-4.6, 23.6)	0.213
Distance to green space ≥ 5000 m^2 (in meters)		
Home (IQR=335.42)	-20.5 (-29.9, -9.7)	**<0.001**
Kindergarten (IQR=497.64)	-21.8 (-31.4, -10.8)	**<0.001**
Surrounding greenspace		
Home-100m (IQR=0.016)	5.4 (-2.6, 14.0)	0.191
Home-300m (IQR=0.012)	8.3 (1.2, 15.9)	**0.021**
Home-500m (IQR=0.012)	6.4 (-0.9, 14.2)	0.085
Kindergarten-100m (IQR=0.011)	3.5 (-2.9, 10.4)	0.289
Kindergarten-300m (IQR=0.019)	13.3 (2.2, 25.7)	**0.018**
Kindergarten-500m(IQR=0.016)	14.5 (0.4, 28.4)	**0.043**
Total surrounding greenspace index		
Total surrounding greenspace-100 m (IQR=0.011)	7.6 (-2.0, 18.1)	0.125
Total surrounding greenspace-300 m (IQR=0.019)	12.0 (3.1, 21.7)	**0.007**
Total surrounding greenspace-500m (IQR=0.016)	8.4 (0.3, 17.1)	**0.041**
Time spent in the green space (h/week)		
Private garden (IQR=12)	4.9 (-9.1, 21.1)	0.511
Public green space (IQR=21.62)	39.0 (22.8, 57.3)	**<0.001**
Plant pots at home (IQR=6)	4.1 (-4.0, 13.0)	0.330

Each cell represents an independent model: 95% CI = 95% confidence interval; LTL = Leukocyte Telomere Length. [1]p: wald test p values. Mixed effects regression models for greenspace exposure were adjusted for age, sex, body mass index (BMI), parental education, income, environmental tobacco smoke at home, illiterate percent per census tract and unemployed percent per census tract. Note: Kindergartens and qRT-PCR plates id were used as random effects in all models. The percentage changes (95% CIs) reported based on 1 IQR increase in distance from home and kindergartens to the closest green space, 1 IQR increase in NDVI, 1 IQR increase for spent time in the public or private green spaces and 1 IQR increase in indoor plant pots at home.