Gene-environment interaction in the association of residential greenness and 25(OH) vitamin D

Results from the GINIplus & LISA birth cohorts

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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.

Keywords: Residential greenness, NDVI, Vitamin D, 25(OH)D, adolescents, epidemiology

Abstract

There is increasing awareness for beneficial health effects of green space surrounding the home, but the underlying mechanisms are not yet fully understood and challenging to study given the correlation with other exposures. Here, the association of residential greenness and vitamin D including a gene-environment interaction is investigated. 25-hydroxyvitamin D (25(OH)D) was measured by electrochemiluminescence at ages 10 and 15 years in participants of two German birth cohorts GINIplus and LISA. Greenness was measured using the Landsat-derived Normalized Difference Vegetation Index (NDVI) in a 500m buffer surrounding the home. Linear and logistic regression models were applied at both time points adjusted for several covariates (N_{10Y}=2,504, N_{15Y}=2,613). In additional analyses vitamin D-related genes, physical activity, time spent outdoors, supplements, and measurement season were investigated as potential confounders or effect modifiers. A 1.5-SD increase in NDVI was significantly associated with increased 25(OH)D values at ages 10 and 15 years (β_{10y}=2.41nmol/l, p=<0.01; β_{15y} =2.03nmol/l, p=0.02). In stratified analyses, the associations were not seen in participants spending more than 5h/day outside in summer, having a high physical activity level, taking supplements, or being examined during the winter season. In a subset (n=1,732) with genetic data, a significant gene-environment interaction of NDVI with *CYP2R1*, an upstream gene in 25(OH)D synthesis, was observed at age 10 years. When investigating 25(OH)D sufficiency, defined as values above 50nmol/l, a 1.5-SD increase in NDVI was

associated with significantly higher odds of having sufficient 25 (OH)D levels at age 10 years (OR=1.48, 1.19-1.83). In conclusion, robust associations between residential greenness and 25(OH)D levels were observed in children and adolescents independent of other confounders and additionally supported by the presence of a gene-environment interaction. Effects of NDVI were stronger in those having lower vitamin D levels at age 10 years due to their covariate profile or genetically lower 25(OH)D synthesis.

Introduction

Obtaining adequate vitamin D levels is essential for human health. The best known and most important function of vitamin D is the stimulation of intestinal calcium absorption and regulation of calcium and phosphate metabolism being essential of skeletal health in adults and children (Bikle, 2021). However, as vitamin D receptors are present in most human cells and tissues (Maestro et al. 2016), additional extra-skeletal effects of vitamin D are indicated. Consequently, vitamin D deficiency (usually defined as 25(OH)D <30nmol/L) (Giustina et al. 2020) and/or insufficiency (25(OH)D < 50nmol/L) have been linked to cardiovascular disease (Gil et al. 2018), obesity (Peireira-Santos et al. 2015), modification of the immune system (Charoenngam and Holick 2020), carcinogenesis and tumor progression (Feldman et al. 2014), and mortality (Gaksch et al. 2017). Most of the available evidence originates from studies in adults, however there are similar health implications for children and adolescence (Mailhot and White 2020, Huh and Gordon 2008).

Up to 90% of vitamin D in the human body is obtained by the cutaneous formation of vitamin D after Ultraviolet B sunlight (290–315 nm) exposure, it is then converted mainly by CYP2R1 to 25-hydroxyvitamin D (25(OH)D) which is the major circulating and storage form of vitamin D with a half-life of about 2-3 weeks and thereby reflecting the current vitamin D status (Bikle, 2021) . Since a considerable part of the inhabited land lies above 35° degree latitude where only very little vitamin D is produced during the winter season from November through March (Webb et al. 1988), vitamin D deficiency is common also in children and adolescents (González-Gross et al. 2012). In the German National Health Interview and Examination Survey for Children and Adolescents (KiGGS) for example 69% of the children in the age group 7-13 years had 25(OH)D concentrations below 50nmol/l (Bergmann et al. 2015). Supplements are often recommended highlighting the public health relevance of improvement of vitamin D status, however, it is difficult to disentangle direct health effects of vitamin D in the body and confounding due to its association with a healthier lifestyle including higher physical activity (Wanner et al. 2015; da Silva et al. 2019) or time spent outdoors, which are further determinants of overall vitamin D status despite latitude, pigmentation, use of sunscreen, and clothing coverage (Holick, 2007). To overcome potential confounding, Mendelian Randomization studies using genetic variants as proxies for the exposure have been performed (Zheng et al. 2017). In the UK Biobank, for example, the association between genetic variants related to 25(OH)D levels and CVD risk was investigated and L-shaped associations were observed, where CVD risk as well as blood pressure decreased with higher genetically predicted 25(OH)D and levelled off at about 50 nmol/l suggesting a causal non-linear association between vitamin D and CVD (Zhou et al. 2022). Green spaces are common settings where much vitamin D might be accrued. In 2019, 81.7 % of the population of high-income countries lived in urban areas (United Nations 2018). People in cities tend to have limited access to green space due to competing demands on land-use and processes of urban sprawl and inner city densification often leading to the destruction of vegetation. At the same time, awareness of the potential health effects of green vegetation (i.e. 'greenness', 'green space') has increased dramatically in recent years. Vitamin D has been hypothesized as a potential mechanism linking green space and both cardiometabolic and cognitive health (Astell-Burt and Feng 2020). This is likely to closely intersect with a vast and growing body of evidence documenting associations between higher greenness and improvements in physical activity (McMorris et al. 2015; Villeneuve et al. 2018), overweight (Luo et al. 2020), birth weight (Hu et al. 2021), and lower mortality rates (Rojas-Rueda et al. 2019). Furthermore, positive associations of urban green space with mental health including depression, cognition (de Keijzer et al. 2016), child well-being (Feng and Astell-Burt 2017; McCormick, 2017), and perceived stress (Pun et al. 2018) have been suggested. People with higher greenness in their neighborhood might benefit in several ways including promotion of an active lifestyle, recreation, social contact, and cohesion or therapeutic effect on mental health (Markevych et al. 2017). Additionally, vegetation might mitigate harmful environmental exposures such as air pollution (Franchini and Mannucci 2018; Kumar et al 2019), noise (Dzhambov and Dimitrova 2014) and excessive ultraviolet radiation or heat (Knight et al. 2021), especially in tropical areas.

Recently, a longitudinal cohort study among older adults suggested positive associations between residential greenness and 25(OH)D non-deficiency and authors hypothesized that green space might promote exposure to sunlight (Zhu et al. 2020). This could hint to a further pathway linking greenness to health. Further indirect evidence of such a pathway is also demonstrated by reported harms in a tropical population with excessive exposure to UV light, indicating higher skin cancer risk in Australian adults with more green space nearby (Astell-Burt et al. 2014).

Therefore, the aim of this study is to investigate this association in adolescents participating in two German birth cohort studies and to study gene-environment interactions utilizing genes in the 25(OH)D synthesis and metabolism. It was hypothesized that especially participants with genetically lower 25(OH)D levels due to reduced biosynthesis activity might benefit from higher greenness. So far, no study has investigated the gene-environment interaction between vitamin D related genes and NDVI on measured 25(OH)D levels and therefore results supporting this hypothesis could further strengthen the evidence that NDVI might improve vitamin D by promoting sunlight exposure. Additionally, the potential effect modification of time spent outdoors and physical activity are further elucidated.

Material and methods

Study population

The study population consists of participants from the two ongoing German birth cohorts, the German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy (GINIplus) and the Lifestyle-related factors on the Immune System and the development of Allergies in childhood (LISA) study (von Berg et al. 2010; Zutavern et al. 2006). Briefly, in the GINIplus study healthy full-term neonates were recruited in selected maternity wards of the cities of Munich (n= 2,949) and Wesel (n=3,042) between 1995-1998. GINIplus was initiated as a two-armed study with one arm being a prospective, double-blinded, randomised intervention trial with three hypoallergenic formulae compared to standard cow's milk formula as control, while the second arm is observational. In the LISA study, also healthy full-term neonates were recruited between 1997-1999 in Munich (n= 1,467),Wesel (n= 348), Leipzig (n=976), and Bad Honnef (n=306). LISA was designed as a population-based observational study. Parents and later the study participants themselves gave written informed consent. The studies complied with the Ethical Principles of the World Medical Association Declaration of Helsinki and were approved by the regional ethics committees. Participants of both birth cohorts were regularly followed up with questionnaires and examinations. Here we used data of the 10- and 15-year follow-up where blood samples were taken during a clinical examination.

The present analysis is restricted to children living in the Munich study area (including two administrative regions of Bavaria: Upper Bavaria and Swabia), in the Leipzig study area (including two administrative regions of Saxony: Nordsachen and Leipzig) and area of Wesel (including two administrative regions of North-Rhein Westphalia: Münster and Düsseldorf). For further details see Markevych et al. (2019). For the study area in Bad Honnef, addresses were only partially available (n=229 at age 15 years), therefore GIS exposures have not been assigned to them. Ultimately, the study population comprises 2,504 children at the age of 10 years, and 2,613 adolescents at the age of 15 years, who had 25(OH)D measured and available information on residential greenness, of whom 1,797 participated at both time points (Supplementary Figure 1).

Vitamin D

Either fasting or non-fasting blood samples were drawn at age of 10 years between November 2006 and May 2009 and at the age 15 years between April 2011 and May 2014, centrifuged after collection, and stored frozen at -80° until measurement. Total 25(OH)D in serum was measured on the fully automated Modular system (E170, Roche Diagnostics, Mannheim, Germany). Technical details can be found in Thiering et al. (2015). In brief, the Roche Diagnostics Vitamin D total assay is a electrochemiluminescence protein binding assay for the measurement of total 25(OH)D in human serum and plasma. It utilizes a capture protein, which binds to both 25(OH) D_3 and 25-(OH) D_2.

Residential greenness and related variables

Details of the assessment of residential greenness can be found in Markevych et al. (2014) and Thiering et al. 2016. In brief, the Normalized Difference Vegetation Index (NDVI) was derived from Landsat 5 Thematic Mapper and Landsat 8 Operational Land Imager satellite images on cloud-free days (see Supplementary Table 1 for more details). NDVI is an indicator of green vegetation with values ranging from +1 indicating a high density of green leaves, to –1 representing water features, and values close to zero referring to barren areas of rock, sand, or snow (Weier and Herring 2000). NDVI maps were calculated based on two vegetation-informative bands [near infrared (NIR) and visible red (RED)], available at a resolution of 30 m × 30 m, according to the formula: NDVI = (NIR – RED)/(NIR + RED). Residential greenness was defined as the mean of NDVI values in circular 300m, 500m, 1,000m or 3000m buffers around each participant's home addresses at the beginning of the 10-year and the end of the 15-year examination periods, in years 2006 and 2015, respectively. NDVI represents the total vegetation, therefore it includes green spaces such as parks and forests that people can enter and spend time within, but also farmlands or other areas that might be green, but not open to people's visits such as private gardens. Results for NDVI are presented in a 500m buffer in the manuscript, as this is a distance walkable in 5-10 minutes and often used in greenspace research (Labib et al. 2020), sensitivity analyses including different buffer sizes are shown in the supplement. Additionally, for sensitivity analyses the May to August mean NDVI for the years 2005 to 2009 (referring to 10-year follow-up) and 2011 to 2014 (15-year follow-up) were assessed from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite images in resolution of 250 m (https://search.earthdata.nasa.gov/).

Covariates

All covariates were selected *a priori*. Parental education was defined as the highest educational level of either mother or father at birth and categorized based on the number of years of school education (low. ≤ 9 years; medium: 10 years, high: >10 years). Height and weight were measured during the clinical examinations in the study centers. In questionnaires, participants' parents were asked about the weekly hours of moderate and vigorous physical activity (PA). PA categories were defined based on physical activity recommendations for school-aged children and youth in Janssen (2007) as "low" if the sum of moderate and vigorous PA was <7h/wk; as "medium" if the sum of PA was at least 7 h/wk; and as "high" if participants performed moderate/vigorous PA at least for 10.5 h/wk and if at least 3.5 h/wk thereof were vigorous PA. The number of hours per day spent outside during summer and winter was categorized in 'low': ≤3 h/d, 'medium':3-5h/d and 'high" >5h/d for summer and in 'low': ≤1 h/d, 'medium':1-2h/d and 'high" >2h/d for winter. Vitamin and mineral dietary supplementation was assessed with a food frequency questionnaire (filled in by the parents at age 10 years and by participants at age 15 years) as a whole, there were no specific questions for vitamin D supplementation.

Therefore, it was not used for adjustment, instead, a stratified analysis by supplementation status was performed.

Gene-environment interaction

In a subset (n=1,732) of the present study population from the study centers Munich (n=1,143) and Wesel (n=589), genetic data was available. Details of the genotyping can be found in Kilanowski et al. 2022. In brief, participants of GINIplus and LISA were genotyped on the Affymetrix Chip 5.0 or 6.0 (Thermo Fisher, USA) in Munich and on the Infinium Global Screening Array GSA v2 MD in Wesel. All data were imputed using the HRC version 1.1 (McCarthy et al. 2016) on the Michigan Imputation Server (Das et al. 2016).

To study potential gene-environment interactions and their biological plausibility, one upstream variant (rs12794714 in *CYP2R1*) in 25(OH)D synthesis and one downstream variant (rs2282679 in *GC*) in 25(OH)D metabolism were selected. *CYP2R1* gene on chromosome 11 encodes the principal vitamin D 25-hydroxylase which converts cholecalciferol and ergocalciferol (vitamin D2 and D3) into 25(OH) vitamin D2 and D3. The minor allele A of rs12794714 leads to reduced 25(OH)D. The *GC* gene on chromosome 4 encodes the vitamin D binding protein which transports vitamin D metabolites in the body. Although the minor allele G of rs2282679 is related to lower 25(OH)D levels (Wang et al. 2010), it is not directly involved in 25 (OH)D synthesis (Berry et al. 2012) and therefore not subject to interaction with external factors. Therefore, it serves as a negative control for the interaction with NDVI here.

Statistical methods

All analyses were performed in R 4.1.3 (R Core Team 2022). In order to correct the seasonal variation of 25(OH)D levels, all regression models were adjusted for the month of examination/blood draw .N=26 of the 25(OH)D measurements at age 10 years and N=16 at age 15 years showed a high distance to the remaining data points (exceeding the median plus 2.5 times the interquartile range (IQR) of the respective month's measurements). As those are considered valid measurements, but might act as leverage points in the regression they were replaced with median plus 2.5 IQR. For NDVI no such imputations or corrections have been made. Generalized additive models (GAM) as implemented in the 'mgcv" R packages (Wood 2011) were applied to inspect the linearity of the associations between NDVI variables and outcome (Supplementary Figure 2). As there were no deviations from linearity, linear models were used.

Associations between covariates were tested using ANOVA, Chi-squared test, or Kruskal-Wallis test, depending on the distribution and data type (metric/categorical) of the variables. To assess the association of NDVI and 25(OH)D, linear regression models were conducted for both time points separately. Although 1,797 individuals participated at both time points, longitudinal modelling was only applied as sensitivity analys, as

25(OH)D levels fluctuate quite a bit in the body[1] with a half-life of 2-3 weeks and therefore, the effects are expected to be rather short term (weeks) than long-term (years). Additionally, for the presented analyses the spatial contrasts of NDVI between participants are more important than the temporal ones which might be influenced by annual climate (temperature, humidity, etc.) and month of assessment. For comparison with previous publications,[35] vitamin D sufficiency was defined as 25(OH)D ≥ 50 nmol/l and analyzed using logistic regression. All estimates are displayed per a 0.15 increase in NDVI: beta estimates for linear models and odds ratios (OR) for logistic regression. This increment was selected, because it represents 1.5 times the standard deviation (SD) of NDVI for most buffer sizes and is also close to the interquartile range, therefore it is possible to compare results between exposures. Different *a priori* selected sets of adjustment variables were used: The basic model included month of blood draw, sex, study area, study, parental education, and BMI. Further adjustment of models included physical activity or time spent outdoors in summer and winter, or all of the above. In addition, stratified analyses and analyses including two-way interaction terms were performed to study the association in subgroups and gene-environment interactions. Sensitivity analyses were performed with additional adjustment for tree cover percentage or population density. For the analysis of gene-environment interaction effects, the results for the Munich and Wesel study centers were meta-analyzed as genotype data originated from different genotyping platforms. The inverse variance method provided by the "meta" package in R (Balduzzi et al. 2019) was applied, assuming common effects, as I^2 was below 60% and tests for heterogeneity non-significant in all models.

Results

A description of the study population is displayed in Table 1. Most study participants had parents with high educational levels (more than 10 years of school education), were living in the Munich study area, and were participants of the GINIplus study. The distribution of sex was nearly balanced. Levels of physical activity and time spent outdoors were lower at the 15-year follow-up compared to age 10 years. Although the proportion of measurements taken during the summer season (May to October) was higher at age 15 years compared to age 10 years, 25(OH)D levels were lower at the 15-year follow-up (Supplementary Figure 3, Table 1). The proportion of participants taking vitamin or mineral supplements was comparable between the 10 and 15-year follow-ups.

Participants living in greener areas spent more time outdoors, especially in the summer (Supplementary Table 2, p-value for summer at ages of 10 and 15 years <0.001, p-value for winter age 15 years <0.001). While higher greenness was associated with higher physical activity at age 15 years (p=0.019), for age 10 years only a non-significant tendency was observed (p=0.122, Supplementary Table 2).

Adjustment variables in the basic model without NDVI explained 32.1% of the variance 25(OH)D values at age 10 years, and 31.7% at age 15 years. For both time-points, month of blood draw was the strongest predictor of 25(OH)D values with the highest values in July, August, and September (Supplementary Table 3). Higher BMI was associated with lower 25(OH)D at both ages. Males had higher 25(OH)D levels than females at age 10 years, but no sex difference was observed at age 15 years. When adding the further adjustment variables sequentially, time spent outdoors in summer did substantially increase 25(OH)D values ($\beta_{10years}$= 2.85 (p=0.008) and $\beta_{15years}$= 6.52 (p<0.001) for 3-5 h/d, $\beta_{10years}$= 5.84 (p<0.001) and $\beta_{15years}$=11.47 (p<0.001) for more than 5h/d), especially at age 15 years. Physical activity was also positively associated with 25(OH)D values ($\beta_{10years}$= 3.91 (p=0.001) and $\beta_{15years}$= 2.30 (p=0.079) for medium PA compared to low, $\beta_{10years}$= 4.98 (p<0.001) and $\beta_{15years}$=6.47 (p<0.001) for high PA compared to low).

Table 2 shows the results of the exposures of interest from the linear and logistic regression models associating NDVI in a 500m buffer with 25(OH)D levels and 25(OH)D sufficiency at ages 10 and 15 years. Higher NDVI was consistently associated with increased 25(OH)D levels. An increment of 0.15 in NDVI (1.5-SD increase) was associated with a 2.41 nmol/l (p=0.001) increase in 25(OH)D in the basic adjustment model at age 10 years and with a 2.03 nmol/l (p=0.020) increase at age 15 years. The magnitude of the effect estimate was generally higher at age 10 years compared to age 15 years and effect estimates were consistent with further adjustment for time spent outdoors and physical activity. When using different buffer sizes of 300m, 1000m and 3000m, there was for continuous 25(OH)D data a clear trend with weakest associations for 300m and 3000m buffer, intermediate for 500m buffer, and strongest associations for 1000m buffer (Supplementary Table 4). For mean May to August NDVI consistent associations and higher effect estimates were observed (Supplementary Table 4). In stratified analyses (Figure 1), no significant association of NDVI with 25 (OH)D were observed in participants who were spending more than 5h/day outside in summer, having high physical activity, or taking dietary supplements. Additionally, no association between NDVI and 25(OH)D was seen in participants of whom measurements were taken between November and April. Higher effect estimates within the more urbanized Munich area were found compared to the more rural Wesel area (Supplementary Table 5 & 6) with a significant interaction at age 15 years (p=0.040). For the Leipzig area there were not enough participants to allow meaningful stratification. Regarding sex-specific effects, higher effect estimates were observed for females at age 10 years and at age 15 higher ones for males in stratified analyses (Supplementary Table 5, however there was no significant interaction of greenness with sex ($p_{(interaction)10\ years}=0.447$, $p_{(interaction)15\ years}=0.242$). Longitudinal association analyses yielded qualitatively comparable results (Supplementary Table 7) and additional adjustment for population density attenuated the effect estimates for NDVI, while those remained stable after adjustment for tree cover (Supplementary Table 8).

The analysis was stratified by two genetic variants, one related to 25(OH)D synthesis (*CYP2R1*) and one related to 25(OH)D metabolism (*GC*) (Figure 2, Supplementary Table

9). For participants carrying at least one minor allele in CYP2R1 (rs12794714, AA or AG genotype) and therefore genetically lower 25(OH)D levels, larger estimates for NDVI effects ($\beta_{AA/GA,10years}$=3.49, p=0.003; $\beta_{AA/GA,15years}$=1.67, p=0.319) were observed compared to homozygous major allele carriers (GG genotype) $\beta_{GG,15years}$=-1.04, p=0.606; $\beta_{GG,15years}$=-1.32, p=0.108). This gene-environment interaction was significant at the age of 10 years ($p_{(interaction)}$=0.039). For the downstream variant in *GC* related to 25(OH)D metabolism, there was no gene-environment interaction present ($p_{(interaction)10years}$=0.500, $p_{(interaction)15years}$=0.709) and similar associations in participants carrying at least one minor allele (rs2282679, GG or GT genotype).

Discussion

In this manuscript, the association of residential greenness, as measured by NDVI, and 25(OH)D concentrations in serum in two German birth cohorts was analyzed taking into account potential confounders and modifiers. In line with the findings of Zhu et al. (2020),[35] who observed in the Chinese Longitudinal Healthy Longevity Survey that each 0.1 unit increase in NDVI was significantly associated with 13% higher odds for 25(OH)D non-deficiency in 1,336 elderly adults, this was shown here in a younger age group. Additionally, in the better powered linear regression model the association between NDVI and 25(OH)D levels was robust, and the plausibility of stratified analyses and gene-environment interaction supported the hypothesis that the vitamin D status of participants living in greener neighborhoods is improved. This study adds to the notion that increased 25(OH)D levels in people living in greener environments might be a plausible mechanism linking greenness and its health effects in children. However, it has to be noted that NDVI represents a measurement for total vegetation which includes all types of greenery such as grass, trees, farmlands, etc. Therefore, it is not specific for the potential to influence sunlight exposure and additionally subject to geographical differences that were also observed in the present study. Regarding different types of greenery, one might hypothesize that while grass cover could be positively associated with sunlight exposure, dense tree stands could reduce exposure. The in-depth investigation of different vegetation types specific for study areas is beyond the scope of this study, although negative associations between tree cover and 25(OH)D have been observed in sensitivity analyses.

The analyses of Zhu et al. (2020)[35] did not include data on time spent outdoors and only a very rough physical activity measure ("Do you exercise: yes vs. no"), while it was possible with the present data to study the potential influence of physical activity in more detail. Routine as well as recreational physical activity has been investigated previously in relation to greenness. In a US cohort of 50,884 women, participants in the highest tertile of residential greenness were more likely to be more physically active and had lower risk of being obese.[22] While mediation analysis showed that the association between greenness and obesity was strongly influenced by physical activity in this study, inclusion of physical activity did only marginally attenuate the association of NDVI and 25(OH)D in our cohorts. Of note, especially in adults, higher physical activity may additionally be a proxy for a healthier lifestyle, such as keeping a healthy diet, sleeping longer hours, etc.

In the Healthy PLACES study in California, authors demonstrated through wearable GPS units and accelerometers that higher greenness increased the odds for contemporary physical activity in 208 children aged 8‐14 years (Almanza et al. 2012). In the present study, no GPS data were available; therefore, it is impossible to determine whether physical activity was conducted indoors, outdoors, or in greener locations. Participants/parents were asked how many hours per week they spent outdoors.

Although this variable was positively associated with both NDVI and 25(OH)D, it is still subjectively determined and did not fully explain the association of NDVI and 25(OH)D. Stratified analyses showed, that higher greenness was especially beneficial for the vitamin D status of participants who reported less time spent outdoors. This suggests that higher NDVI in the neighborhood might promote particularly shorter time windows or less conscious sunlight exposure which is still sufficient to increase 25(OH)D synthesis. As the cutaneous biosynthesis of vitamin D is subject to a feedback loop, it is not possible to "overdose" vitamin D by sunlight exposure alone. Therefore, it seems plausible that no additional effect of NDVI on 25(OH)D is observed in groups with higher levels due to other factors such as genetic predisposition, dietary supplementation, or high physical activity that might be performed at least partly outdoors.

Although robust associations were observed, results did partly differ between 10 and 15 years of age with more pronounced associations at age 10 years. This could either be due to the fact that not exactly the same individuals participated at both time points or that the lifestyle of participants changed during puberty which is likely given the observation that physical activity and time spent outdoors were lower at age 15 years. This observed lifestyle change is in line with previous research in adolescents that showed with increasing age reduced time spend outdoors (Gao et al. 2022), reduced physical activity (Dumith et al 2011), increased screen time, less healthy eating and a higher proportion of individuals consuming alcohol or smoking (Marques et al. 2020). Potentially, adolescents might also spent more time with academic- related activities such as homework or studying than children and might have a higher mobility to visit places outside their near neighborhood. Additionally the lower variation in 25(OH)D levels at age 10 years might have increased the power to detect associations.

Another potential confounder that needs to be considered is BMI, which has been previously related to 25(OH)D (Ruiz-Ojeda et al. 2018). In the UK Biobank, NDVI was significantly associated with lower BMI, waist circumference, and whole-body fat, additionally higher odds of activity-travel mode and doing >30min walking were observed for participants living in homes with higher NDVI (Sarkar, 2017). In the present analyses, all models were adjusted for BMI and also physical activity was investigated as confounding factor, but it did not substantially attenuate the association between greenness and 25(OH)D, although effects of NDVI were attenuated in the more physically active groups. The study by Sarkar (2017) in the UK Biobank showed additionally an effect modification by urbanicity, stronger effect estimates of greenness were observed for participants living in areas with higher urbanicity. In our study, effect estimates were also higher for the more urbanized Munich area compared to the more rural Wesel area highlighting the importance of considering greenness in urban planning, but at the same time emphasizing that NDVI might not be completely comparable between different geographical locations as it does not account for different types of vegetation.

There is an ongoing discussion on the appropriate buffer sizes (Browning and Lee 2017). It was *a priori*decided to use 500m as buffer size for the calculation of greenness as this distance can be walked within approximately 5-10 minutes and has been often used in green space research (Labib et al. 2020). Although this represents nearby greenness, it does not necessarily represent visible or accessible green spaces (e.g. could be a golf course behind a high wall). Sensitivity analyses showed even stronger associations for 1000m buffer, which could imply that area-level characteristics drive the observed association rather than greenery in the vicinity of the residential address.

While the genetics of 25(OH)D levels are well characterized (Manousaki et al. 2020) and Mendelian Randomization studies have been used to investigate the causal effects of 25(OH)D on human health (Afzal et al. 2014, Zhou and Hyppönen 2022), the inclusion of a gene-environment interaction between NDVI and 25(OH)D related genetic variants in our study represents a novelty. The results indicate that participants with higher CYP2R1 activity (rs12794714) are potentially less sensitive to the effect of greenness on 25(OH)D and support the congruence hypothesis, wherein the intersection of personal and place-based differences define pathway-potency and overall levels of benefit from urban greening interventions (Astell-Burt et aö- 2022). The need for this kind of investigation was also emphasized in a recent review on gene-environment interactions in relation to vitamin D (Shraim et al. 2022), that stated that the available evidence suggests the presence of gene-environment interactions, but that those are under-researched. Six out of seven studies included in the review yielded significant interaction of vitamin D related genes with exposures related to sunlight. Four studies hereof reported on season of blood draw, three used personal reports of sunlight exposure e.g. hours sitting at work and one included UV exposure data, but no study used an environmental variable such as NDVI which might represent a link to sun exposure and is targetable from a public health perspective. Therefore, futures studies on green spaces might consider vitamin D as potential pathway to health effects and could further strengthen the evidence for interactions between greenness and genetic susceptibility.

Strengths & Limitations

Limiting points that need to be mentioned are that there is no information available whether physical activity was performed indoors or outdoors and that the time spent outdoors was based on parent report instead of objectively measured data. In an ideal setting, GPS data would have allowed to objectively measure how long and where participants stayed outdoors, however collection of this kind of data was not possibly due to feasibility. Additionally, Wanner et al. (2015) observed in data from the US National Health and Nutrition Examination Survey (NHANES) that both indoor and outdoor physical activity contribute to increased 25(OH)D levels justifying the inclusion of total physical activity in the presented models. Additionally, NDVI as measurement for greenness does not consider if green areas are accessible, however it covers in contrast to land-use data also smaller green areas. Another limitation is related to potential measurement bias with regard to NDVI. It was not possible to obtain average vegetation over all seasons or measurements at the exact same time in all study regions as Landsat satellite revisit the same place only about twice a month which makes image acquisition difficult especially in cloudy mountainous areas such as Munich. When using average May to August greenness exposure derived at a lower resolution using MODIS images, association results were consistent. Further, there was no information on the type of housing available, therefore it cannot be studied here if those residences differ systematically in NDVI. However, sensitivity analyses have been performed with additional adjustment for population density showing attenuated effect estimates for NDVI, which was expected given the high correlation of NDVI with urbanicity (Supplementary Table 8).On the other hand, major strengths of the present study are the extensive characterization of the two birth cohorts, which allowed us to cautiously adjust the models for potential confounders and analyze the associations at two time points and stratified by potential effect modifiers. As the present study was conducted in children and adolescents, results might be less confounded by other factors such as pre-existing illness or occupation of the participants. It might be assumed, that children and adolescents represent an excellent study population in this context as they spent more time outdoors compared to adults (Beyer et al. 2018; Chaput et al. 2018) especially in proximity to their home and are more physically active. In contrast to Zhu et al. (2020), who only used a dichotomized outcome, linear regression models were applied here that provide higher statistical power. Although the study population consisted of participants from three heterogeneous areas in Germany, significant associations were observed in the pooled population. Furthermore, the availability of genetic data allowed us to study the biological mechanism.

Conclusions

Robust associations between greenness at the home address measured by NDVI and 25(OH)D levels in children and adolescents were observed, which remained significant after adjusting for physical activity or self-reported time spent outdoors and were additionally supported by the presence of gene-environment interaction. Effects of NDVI were stronger in those having lower vitamin D levels due to their covariate profile or genetically lower 25(OH)D synthesis.

Funding

This Australian–German collaboration was supported by the Deutscher Akademischer Austausch Dienst (DAAD) PPP Australian 2019, Zuwendungsvertrag: 57446380, funds on which were provided by the Federal Ministry of Education and Research (BMBF) (German partners) and by the University of Wollongong (Australian partners).

Iana Markevych is currently supported from the “NeuroSmog: Determining the impact of air pollution on the developing brain” (Nr. POIR.04.04.00-1763/18-00) which is implemented as part of the TEAM-NET programme of the Foundation for Polish Science, co-financed from EU resources, obtained from the European Regional Development Fund under the Smart Growth Operational Programme.

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Tables

Table 1: Study population characteristics at ages 10 and 15 years.

	10 Years (n=2504)			15 Years (n=2613)		
Study area, n (%)	Munich	1418	(56.6)		1375	(52.6)
	Leipzig	266	(10.6)		271	(10.4)
	Wesel	820	(32.7)		967	(37.0)
Study, n (%)	GINIplus	1650	(65.9)		1787	(68.4)
	LISA	854	(34.1)		826	(31.6)
Parental Education, n (%)	low	234	(9.8)		246	(9.9)
	medium	918	(38.4)		960	(38.6)
	high	1240	(51.8)		1284	(51.6)
	missing	112			123	
Sex, n (%)	female	1214	(48.5)		1292	(49.4)
	male	1290	(51.5)		1321	(50.6)
BMI [kg/m^{-2}], n, mean (SD)	2490	17.4	(2.4)	2488	20.8	(3.1)
Physical Activity, n (%)	low	559	(26.7)		767	(36.6)
	medium	685	(32.7)		677	(32.3)
	high	851	(40.6)		652	(31.1)
	missing	409			517	
Season, n (%)	Nov-April	1173	(46.8)		1027	(39.3)
	May- Oct	1331	(53.2)		1586	(60.7)
Time spent outdoors in winter [h], n, median (IQR)	2464	2.0	(2.0)	2497	2.0	(1.0)
Time spent outdoors in summer [h], n, median (IQR)	2453	4.0	(2.0)	2500	3.5	(3.0)
Supplements (vitamins/minerals)	no	1574	(84.4)		1576	(83.8)
	yes	292	(15.6)		305	(16.2)
	missing	638			732	
25(OH)D sufficiency, n (%)	yes (≥50nmol/l)	2117	(84.5)		1809	(69.2)
	no (<50nmol/l)	387	(15.5)		804	(30.8)
25(OH)D [nmol/l], n, mean (SD)	2504	74.6	(24.2)	2613	66.7	(28.6)
NDVI, n, mean (SD)	2504	0.48	(0.09)	2613	0.55	(0.09)

Table 2: Associations of NDVI in a 500m buffer with 25(OH)D values (linear model) and sufficiency (logistic regression model), estimates per 1.5-SD increase in NDVI.

		Basic model[a]			**Basic model + physical activity**			**Basic model + time spent outdoors**			**All**		
Linear model		**Beta**	**CI**	**p**	**Beta**	**CI**	**p**	**Beta**	**CI**	**p**	**Beta**	**CI**	**p**
	10 years												
	NDVI	2.41	(1.03,3.79)	0.001	2.59	(1.08,4.11)	0.001	2.25	(0.85,3.65)	0.002	2.41	(0.88,3.94)	0.002
	15 years												
	NDVI	2.03	(0.32,3.73)	0.020	2.41	(0.53,4.29)	0.012	1.78	(0.05,3.50)	0.044	2.27	(0.37,4.17)	0.019
Logistic model		**OR**	**CI**	**p**	**OR**	**CI**	**p**	**OR**	**CI**	**p**	**OR**	**CI**	**p**
	10 years												
	NDVI	1.48	1.19-1.83	<0.001	1.47	1.16-1.85	0.001	1.42	1.14-1.77	0.001	1.40	1.11-1.77	0.005
	15 years												
	NDVI	1.08	0.90-1.30	0.394	1.08	0.88-1.33	0.467	1.08	0.89-1.30	0.434	1.09	0.88-1.35	0.42

[a] Basic model adjusted for: month of blood draw, sex, study area, study, parental education, and BMI

Figures

Figure 1: Associations between NDVI and 25(OH)D stratified by time spent outdoors in summer, physical activity level, vitamin supplementation and season of blood draw. Results from linear model, adjusted for month of blood draw, sex, study area, study, parental education, and BMI, beta estimates per 1.5-SD increase in NDVI.

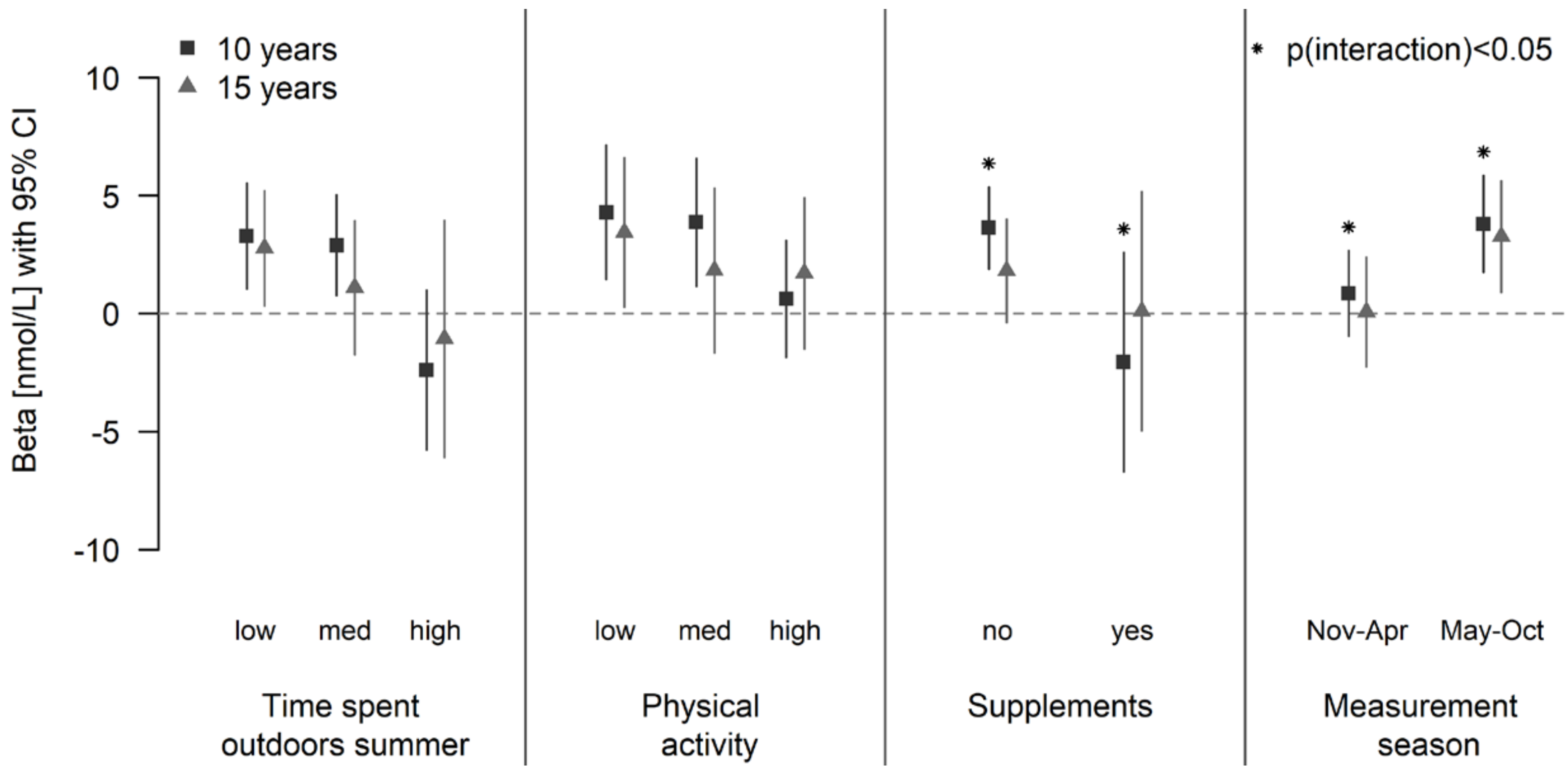

Figure 2: Association of NDVI with 25(OH)D in a subset of participants from Munich and Wesel with available genetic information (N=1,481 age 10, N=1,325 age 15) stratified by rs12794714 of *CYP2R1* and rs62282679 of *GC*. Linear model adjusted for month ofblood draw, sex, study, parental education, and BMI beta estimates per 1.5-SD increase in NDVI.