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EXPLORING THE IMPACT OF LIFESTYLE AND ENVIRONMENTAL EXPOSURES ON APPETITE HORMONE LEVELS IN CHILDREN AND ADOLESCENTS: AN OBSERVATIONAL STUDY

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Abstract

Background: Appetite hormones are considered a promising target in fighting obesity as impaired appetite hormone levels have already been associated with obesity. However, further insights in the drivers of appetite hormone levels are needed.

Objectives: In this study, we investigated the associations of fasting appetite hormone levels with lifestyle and environmental exposures in children and adolescents.

Methods: A total of 534 fasting blood samples were collected from children and adolescents (4-16y ,50% boys) and appetite hormone levels (glucagon-like peptide-1 (GLP-1), peptide YY (PYY), pancreatic polypeptide (PP), leptin and ghrelin) were measured. Exposures included dietary quality (fiber-rich food intake, sugar propensity, fat propensity), psychosocial stress (happiness, negative emotions, negative life events and emotional problems), sleep duration, physical activity and environmental quality (long term black carbon (BC), particulate matter <2.5 µM ($PM_{2.5}$), nitrogen dioxide (NO_2) exposure, and green space in a 100 m and 2000 m radius around the residence). A multi-exposure score was calculated to combine all the exposures at study in one measure. Associations of individual exposures and multi-exposure score with appetite hormone levels were evaluated using linear mixed regression models adjusting for sex, age, socioeconomic status, waist-to-height ratio and multiple testing.

Results: GLP-1 was associated with air pollution exposure (NO_2 β*=-0.13, BC β*=-0.15, $PM_{2.5}$ β*=-0.16, all p<0.001). Leptin was associated with green space in a 100 m radius around the residence (β*=-0.11; p=0.002). Ghrelin was associated with negative emotions (active ghrelin β*=-0.16; p=0.04, total ghrelin β*=-0.23; p=0.0051) and happiness (active ghrelin β*=0.25; p<0.001, total ghrelin β*=0.26; p<0.001). Furthermore, total ghrelin levels were associated with the multi-exposure score, reflecting unhealthy exposures and lifestyle (β*=-0.22; p=0.036).

Discussion: Our findings provide new insights into the associations of exposures with appetite hormone levels, which are of high interest for preventive obesity research. Further research is crucial to reveal the underlying mechanisms of the observed associations.

Key words: Appetite hormones, Children, GLP-1, Ghrelin, Leptin, Residential green space, Lifestyle , Air pollution

Funding sources

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Approval Ethics Committee

This study complies with the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital (No. BC-11166). Written informed consent was obtained from all parents and from all children aged 12 years and older. Children younger than 12 years gave verbal consent.

Conflict of interest declaration

The authors declare that they have no conflicts of interest

1.Introduction

The prevalence of obesity has surged in recent decades, reaching pandemic levels. Besides being considered as a disease itself, obesity predisposes to other non-communicable diseases (NCD's), thereby reducing both life expectancy and quality (Blüher, 2019). This pressing global health concern has sparked substantial interest in appetite research, particularly focusing on appetite hormones as promising targets to combat the obesity pandemic. Appetite hormones have a pivotal function in the regulation of food intake, energy levels, and glucose homeostasis. Furthermore, alterations in these hormone levels are closely tied to the challenge of regaining weight after calorie-restricted diets and have been linked to significant weight loss and metabolic enhancements observed following bariatric surgery (Huang et al., 2023; Mok et al., 2019).

Several appetite-regulating hormones have yet been identified. **Figure 1** gives an overview of the most well-known ones, including GLP-1, PYY, PP, ghrelin and leptin, and how they impact appetite. Numerous studies have found associations between obesity and alterations in the aforementioned circulating appetite hormone levels, although the exact mechanisms remain unclear (Montégut et al., 2021). Thus, further insight is crucial in the external factors, such as lifestyle and environmental exposures, that may impact the dynamics of these hormones.

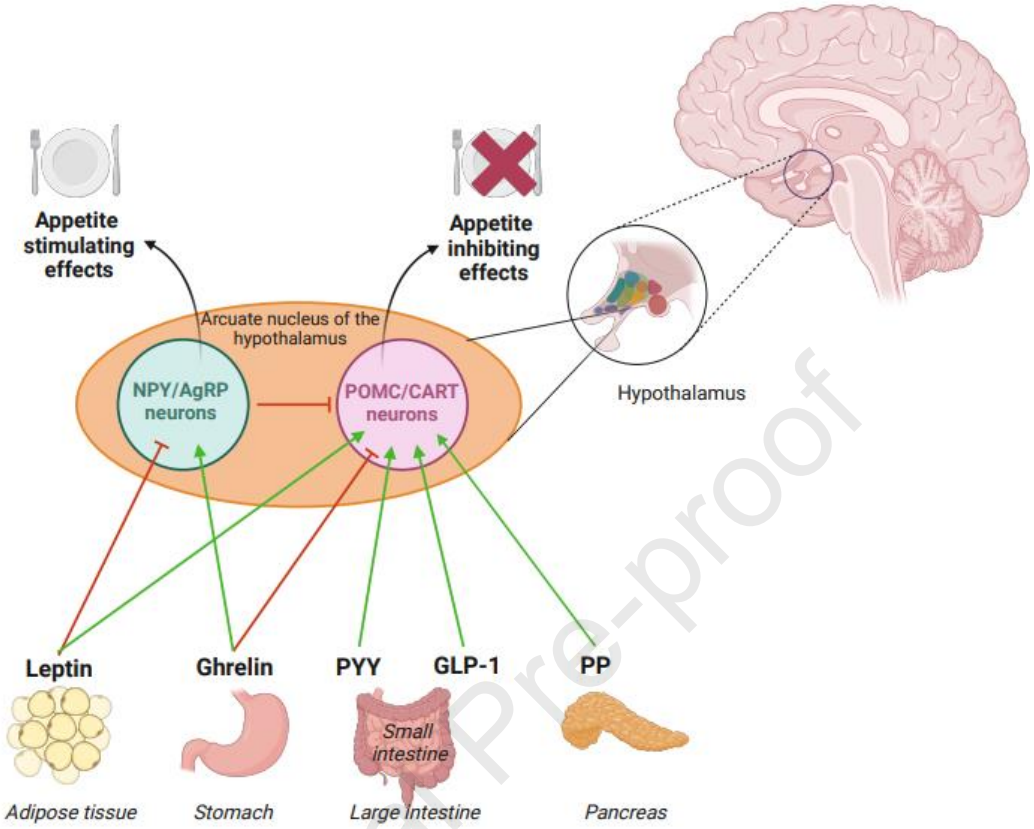

Figure 1: Overview of the appetite regulating hormones considered in this study and their mechanisms of action in the homeostatic control of food intake. Appetite hormones are peripherally secreted in several organs and act on the arcuate nucleus of the hypothalamus, the key appetite regulating center (Schwartz et al., 2000). The arcuate nucleus consists of two distinct, antagonistic neuron populations. On the one hand, orexigenic neurons, with appetite stimulating effects, express NPY and AgRP,(Hahn et al., 1998) on the other hand anorexigenic neurons, with satiety promoting effects, express POMC and CART(Hill, 2010). Besides the homeostatic control system, the hedonic appetite control plays also an important role in the (hypothalamic) regulation(Lutter & Nestler, 2009) of food intake but is not covered in this figure. **Abbreviations**: GLP-1, Glucagon-like-peptide-1; PYY, Peptide tyrosine tyrosine; PP, Pancreatic polypeptide; NPY, Neuropeptide Y; AgRP, agouti-related protein; POMC, pro-opiomelanocortin; CART, cocaine and amphetamine-regulated transcript.

The acute effects of nutrient intake on distinct appetite hormone levels have been well investigated (Misra et al., 2009; St-Pierre et al., 2009; Van Der Klaauw et al., 2013; Yildiz et al., 2004), but the chronic health related effects remain largely unexplored. Alongside food intake, other crucial lifestyle factors like sleep duration, physical activity and psychosocial stress have been associated with appetite hormones (Dorling et al., 2018; Lin et al., 2020; Tomiyama, 2019). For instance, chronic sleep deprivation in adults has been associated with higher ghrelin levels, indicating increased appetite, and higher leptin levels, potentially

indicating leptin resistance (Lin et al., 2020). However, inconsistent results were found (Hagen et al., 2015). Additional ambiguity arises regarding the impact of chronic physical activity: some studies suggest reduced fasting levels of leptin and elevated levels of active ghrelin, GLP-1, PYY, and PP, while others only indicate significant differences in leptin levels (Dorling et al., 2018). The relationship between stress and obesity has been well-established, particularly with ghrelin and leptin as potential mediators (Abizaid, 2019; Adam & Epel, 2007; Tomiyama, 2019). More recently, GLP-1, PYY and PP levels also have been linked to stress via the gut microbiome (Lach et al., 2018). In addition to all aforementioned lifestyle factors, emerging evidence suggests that environmental exposures, such as air pollution, are associated with appetite hormone levels (Molfino et al., 2020; Wang et al., 2014; Wei et al., 2016a). A study in Mexican children reported negative associations of air pollution with ghrelin and GLP-1 serum levels, and positive associations with leptin levels (Calderón-Garcidueñas et al., 2015). To our knowledge, no study investigated a link between a green living environment and appetite hormone levels, although an exploratory study suggested a link between living in a green environment and lower frequency and intensity of cravings for a range of substances, including food (Martin et al., 2019). These findings emphasize the importance of further investigating both environmental exposures and lifestyle as potential predictors of alterations in appetite hormone levels. Existing studies mainly focus on only one or a few specific exposures or hormones, which prevents comparison between exposures and between hormones. Moreover, studies targeting a child or adolescent population are scarce, despite the potential harm that exposures at this age may pose for future health.

Therefore, we present a study exploring the associations of lifestyle and environmental exposures with fasting appetite hormone levels in Flemish children and adolescents. This cross-sectional study examined the associations of five distinct blood appetite hormones (GLP-1, PYY, PP, leptin and ghrelin) with 14 predictors, covering dietary habits, psychosocial stress, physical activity, sleep duration, air pollution, and residential green space. In a second step, we combined all the exposures into one multi-exposure score to test whether an overall unhealthy exposure is associated with appetite hormone levels. This exploratory study strives to contribute to the growing body of knowledge concerning the drivers of appetite hormone levels in obesity research.

2. Methods

2.1 Study population

The hypotheses of this study were tested using secondary data obtained from a large longitudinal study. The baseline survey was conducted in spring 2008, with follow-up surveys in spring 2010, 2011, 2012, 2013, 2015 and 2018 as part of the European IDEFICS project (Identification and prevention of dietary- and lifestyle-induced health effects in children and infants) and the Flemish ChiBS (Children's Body composition and Stress) study (Ahrens et al., 2011; Michels et al., 2012).

This study complies with the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital (No. BC-11166). Written informed consent was obtained from all parents and from all children aged 12 years and older. Children younger than 12 years gave verbal consent.

The examination visits included venous blood sampling, anthropometric measurements and questionnaires for parents and children to gather data on demographic characteristics, socioeconomic status, lifestyle behaviors, diseases etc. Waist circumference was measured in upright position with relaxed abdomen and feet together, as the mid-point between the top of the iliac crest and the lower coastal edge to the nearest 0.1 cm. Parental education was estimated based on the highest achieved education (maximum of both parents) and divided into two levels (low: no post-secondary education; high: at least one parent with post-secondary education). Based on the home address of the children, neighborhood median income, was defined using Belgian census area data, as previously reported,(Martens et al., 2020) and distance to major road (m) and noise exposure (dB) were calculated via ArcGIS software based on a region-wide noise map based on the environmental reporting (MIRA) from the Flanders Environment Agency (VMM).

For the current analysis, data from the study waves of 2010 and 2015 was used because of the availability of fasting blood samples. The participants were Flemish children and adolescents (aged 4-16 years) from the municipality of Aalter and its surroundings. A flow chart of the included participants for the current study is outlined in **Figure 2** with exclusion criteria. From the 765 children in the study waves of 2010 (n=523) and 2015 (n=242), a blood sample was available for 587 children. We excluded children that were not fasting prior to blood withdrawal (n=42), and children with missing information on socioeconomic status (SES) (n=9) or waist-to-height ratio (n=2). Hence, the current analyses include 534 participants, of which

133 children participated in both study waves. None of the participants reported an endocrine disorder.

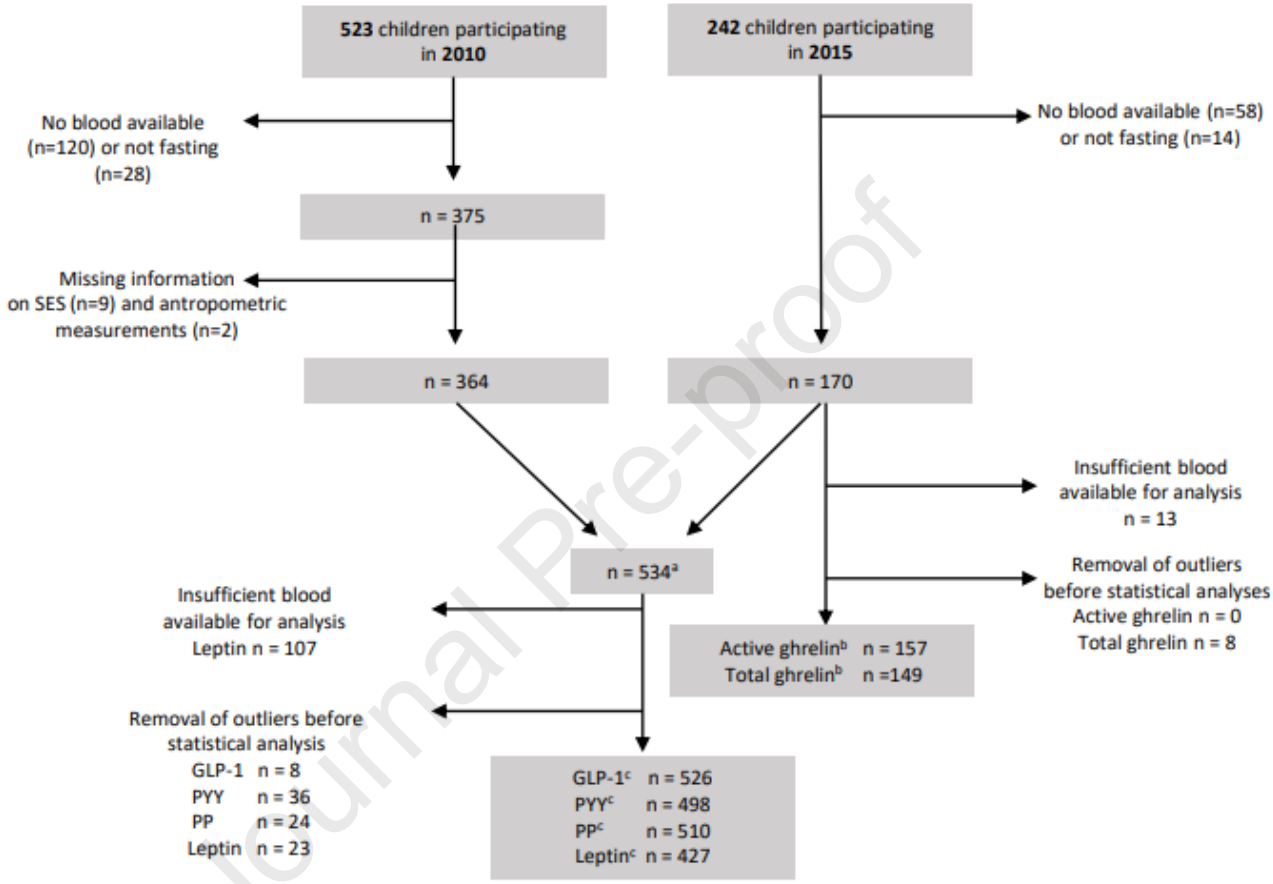

Figure 2: Participant flow chart describing exclusion criteria

[a] Of these 534 participants, 133 participants had data in both timepoints and were therefore included twice.

[b] Ghrelin was only measured in the study wave of 2015

[c] Based on missing information of exposures different sample sizes were used for exposure –outcome combinations. An overview of the exact sample sizes per exposure is provided in Table 1

Abbreviations: GLP-1, Glucagon-like-peptide-1; PYY, Peptide tyrosine tyrosine; PP, Pancreatic polypeptide; SES, socioeconomic status.

2.2 Fasting blood samples

Fasting blood was obtained at both study waves by venipuncture. To obtain serum, blood samples were kept at room temperature for 30 min to allow clotting, and were centrifuged at 2500× g for 10 min. To obtain plasma, EDTA-tubes were gently inverted 10 times to allow the blood to mix with the anti-coagulant, and were centrifuged for 15 min at 2500x g. Plasma was separated and divided into two aliquots. For active ghrelin measurements,

phenylmethylsulfonyl fluoride (PMSF) and HCL were added to prevent degradation from acyl to de-acyl ghrelin. Serum and plasma were stored at -80°C until further analysis.

2.3 Appetite hormone measurements

2.3.1 GLP-1, PYY and PP measurements

For all serum samples total GLP-1, PYY and PP levels were measured using EMD Millipore MILLIPLEX Human Metabolic Hormone Magnetic Bead Panel, according to the manufacturer's instructions (HMH3-34K, Millipore, Merck, Darmstadt, Germany). The inter-assay coefficients of variation (CV) were 16.95%, 24.09% and 19.29% and intra-assay CV were 4.60% ,7.97% and 8.79% for GLP-1, PYY and PP measurements respectively. Measurements were performed using the Bio-Plex MAGPIX multiplex reader, and data were analyzed using the Bio-Plex Manager 6.1 software (Bio-Rad).

2.3.2 Leptin measurements

In the wave of 2010, leptin serum concentrations were measured using a Meso Scale Discovery sandwich electrochemiluminescence immunoassay (inter-assay CV 2.4% for low, 3.9% for high controls; intra-assay CV 2.7% for low, 5.1% for high controls). In 2015, leptin serum concentrations were measured using the Millipore radioimmunoassay kit for human leptin (HL-81K kit, Millipore, Merck, Darmstadt, Germany). The inter- and intra-assay CV were 3.0 - 6.2% and 3.4% - 8.3%, respectively.

2.3.3 Ghrelin measurements

For the samples of wave 2010, no ghrelin was measured. In the wave of 2015, active and total ghrelin levels were measured in EDTA-plasma using human total ghrelin radioimmunoassay kits (GHRT-89HK, Millipore, Merck, Darmstadt, Germany). Inter- and intra- assay CV were 9.6–16.2 and 6.5–9.5%; 14.7–17.8% and 3.3–10.0%, for active and total ghrelin, respectively.

2.4 Exposure assessment

Based on the available data in this cohort, we focused on the following exposures in relation to appetite hormone levels: dietary quality, psychosocial stress, sleep duration, physical activity, and environmental quality.

Dietary quality was evaluated using the qualitative Children's Eating Habits Questionnaire (CEHQ), a parent-reported food frequency questionnaire (FFQ) that was developed and

validated within the IDEFICS project (Huybrechts et al., 2011; Lanfer et al., 2011; Suling et al., 2011). This 43 food-item-containing instrument was used to calculate the sugar and fat propensity, and fiber-rich food intake. Sugar and fat propensity food intake were calculated as previously described (Lanfer et al., 2011). Fiber-rich food intake frequency per week was calculated as the sum of the per week intake frequency of the following foods: raw and cooked vegetables; fresh fruits; whole meal bread, dark roll, dark crispbread; nuts and seeds.

Psychosocial stress was measured using three questionnaires covering stress related to emotions, negative life events and emotional problems. Firstly, children reported recent feelings of happiness and negative emotions (sadness, angriness and anxiety) on a 0-10 Likert scale to describe their emotions. This questionnaire was validated in the ChiBS wave of 2012: the negative emotions score (sum of three negative emotions) showed a Spearman correlation of $r = 0.48$ ($p<0.001$), with the negative affect score of the validated Positive and Negative Effect Schedule questionnaire (Laurent et al., 1999) in a subsample of 153 children. Secondly, via the previously validated Coddington Life Events Scale (CLES) children reported the frequency and timing of negative events in the last 12 months, such as familiar issues (e.g. divorce), school issues (e.g. failing a grade), social issues (e.g. moving), criminal issues, economic issues (e.g. parental job loss) and illness/death of child, family, friend or pet (Coddington, 1972). Lastly, the child's emotional problems were assessed by the Strengths and Difficulties Questionnaire (SDQ), which was filled out by the parents (Goodman, 1997).

Children's sleep duration and **physical activity** were both reported by the parents using the sleep duration question from the School Sleep Habits Survey (two-week test–retest r=0.68) (Shahid et al., 2011) and a physical activity questionnaire, previously validated in prescholers (Burdette et al., 2004). The average sleep duration per day was calculated based on the following formula: (5*weekdays + 2*weekend days)/7. Average time spent on physical activity per week was estimated based on the questionnaire as follows: (5*weekdays + 2*weekend days)/7.

Environmental quality was assessed by modelled ambient air pollution and residential green space measurements. Ambient air pollution was estimated using a high-resolution spatial-temporal interpolation method that estimated daily levels of outdoor exposure levels at the child's residential address ($\mu g/m^3$) for nitrogen dioxide (NO_2), particulate matter particles with a diameter less than or equal to 2.5 µm ($PM_{2.5}$), and its most toxic constituent, black carbon (BC) (Janssen et al., 2008). The model interpolates air pollution data from fixed monitoring stations, by combining land cover data, obtained from satellite images of the CORINE land

cover dataset and a dispersion model, that uses emission from point sources like industries, and line sources like highways (Lefebvre et al., 2011, 2013). The interpolated exposure concentrations are obtained in a high-resolution receptor grid (25x25 m). The interpolation model yields daily estimated levels of air pollution levels based on which the annual exposure averages were calculated that were used in the statistical analyses of this study. In the Flemish region of Belgium, more than 80%, 78% and 74% of the temporal and spatial variability was explained by this interpolation tool for $PM_{2.5}$, NO_2 and BC, respectively (Lefebvre et al., 2011; Mahieu et al., 2012). The model was validated by linking the modelled residential $PM_{2.5}$ and BC with nanosized black carbon particles in urine and placental tissue (Bové et al., 2019; Saenen et al., 2017).

Residential green space of the participants was estimated using the Green Map of Flanders 2012 from the Agency for Geographic Information Flanders (AGIV) which provides high-resolution (1×1 m) information on green space, which was defined as all non-agricultural vegetation. This green space was categorized into three measures based on height: high green (vegetation height higher than 3m), low green (vegetation height lower than 3m) and a total green space (sum of both, high and low green). More detailed information on the Green Map of Flanders has been published before (Dockx et al., 2021). The participant's residential addresses were geocoded and the percentage of green space was calculated in six buffers around the residence (50 m, 100 m, 300 m, 500 m, 1000 m and 2000 m). In primary analysis, total green space close to the residence (100 m) and a wider environment (2000 m) were used. Sensitivity analyses checked the similarity with the other buffers and stratified based on vegetation height (low *vs.* high green). All analyses were performed using Geographic Information System functions ArcGIS 10 software (Esri Inc., US).

2.5 Multi-exposure score and lifestyle score

To assess the overall effect of the exposures, a multi-exposure score, integrating all explored exposures was calculated. For this purpose, the five main exposures (dietary quality, psychosocial stress, sleep duration, physical activity and environmental quality) were divided into groups of sub-exposures, as visualized in **Figure 3**. Next, the multi-exposure score was calculated using the following procedure that was executed separately for the observations of each study wave (2010 and 2015). First, Z-scores were calculated for all sub-exposures. The Z-scores calculated for fiber-rich food intake, happiness, residential green space, sleep duration and physical activity were reversed. Hence, negative Z-scores reflected more healthy

exposures while positive Z- scores reflected more unhealthy exposures for all sub-exposures at study. Next, the mean Z score for all main exposures was calculated as follows: Z-scores from all sub-exposures in one main exposure group were summed up and divided by the number of sub-exposures in that main exposure group. Finally, the sum was taken of all five main exposure scores to obtain the multi-exposure score per child, where a higher multi-exposure score reflects more unhealthy exposures or unhealthy lifestyle. In addition to the multi-exposure score, a lifestyle score was also calculated, in exactly the same manner, although without the "environmental quality" exposures and thus only containing four main exposures (**Figure 3**). Due to the high correlations between the air pollution markers, only $PM_{2.5}$ was considered in the multi-exposure and lifestyle score.

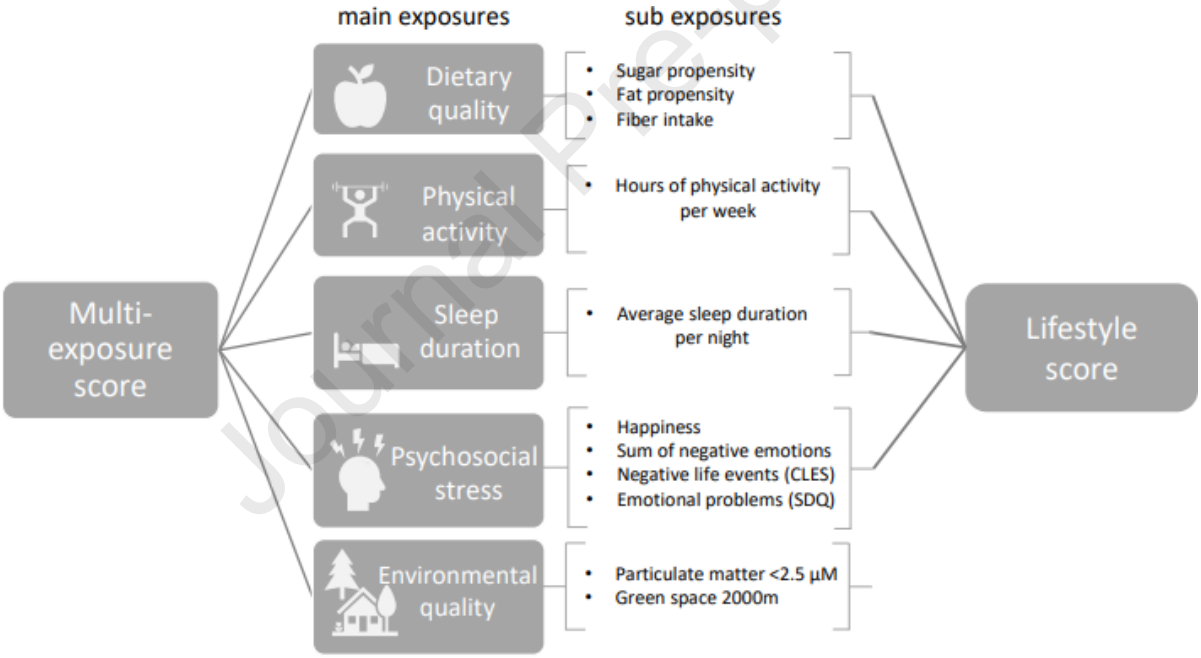

Figure 3: Overview of the composition of the multi-exposure score and lifestyle score.
Abbreviations: CLES, Coddington Life Events Scale; SDQ, Strengths and Difficulties Questionnaire.

2.6 Statistical analysis

Participants' characteristics were presented as medians ± interquartile range (IQR) for continuous variables and frequency (n) and percentage (%) for categorical variables. Correlations between the appetite hormones were evaluated using Spearman rank correlation test. To account for batch effects, the appetite hormone levels were normalized across batches, and these normalized values were used in all following statistical analyses. All

appetite hormone levels were natural log transformed to improve normality of the distributions. Hormone level outliers were removed following the 1.5* IQR rule (see **Figure 2**).

The associations between all individual exposures, the multi-exposure score and GLP-1, PYY, PP and leptin were evaluated using multivariable linear mixed regression models. Linear mixed models allow to account for the clustered data due to some participants having participated in both study waves, by adding a random intercept for study ID. For active and total ghrelin, only data from the 2015 study wave was available. Therefore, multivariable linear regression models were performed to evaluate the associations with the individual exposures and multi-exposure score. All models were adjusted for a priori selected potential confounders and covariates that include known determinants of children's appetite hormone levels or the exposures: sex (male or female), age (continuous), waist-to-height ratio (continuous) as proxy for body composition, SES (low or high parental education) (Makovey et al., 2007; Wilasco et al., 2012). In the mixed model analyses, we also adjusted for the study wave (2010 or 2015) in which the participant participated.

Next, several sensitivity analyses were conducted. First, the regression analyses to test associations between individual exposures and appetite hormone levels were performed separately on both study waves, to test the robustness of the results in both study waves. Second, waist-to-height ratio could possibly act as a collider and hence could induce distorted associations. Therefore, all analyses were evaluated by excluding waist-to-height ratio as covariate. Third, for residential green space, associations were evaluated using all buffers (50m, 100m, 300m, 500m, 1000m and 2000m) which additionally can be divided into high-growing green (>3m) and low-growing green (<3m). Fourth, as association of green space and appetite hormones might be directly or indirectly by noise or air-pollution, the effect of noise and air pollution on the relationship between residential green space and appetite hormones was assessed for the significant associations by adding these terms separately in the linear mixed models. Fifth, models on residential green space and air pollution were additionally adjusted for median neighborhood income as an additional index of SES. Lastly, moderation by lifestyle in the association between air pollution variables and appetite hormones was formally tested as previous studies have suggested a moderating effect of lifestyle on the association between air pollution markers and health outcomes (Li et al., 2020; Wu et al., 2022). Concretely, the regression model now included an interaction term between the air-

pollution marker and lifestyle score (pollution*lifestyle), next to the main effects of air-pollution, lifestyle score and confounders. The significance of that interaction term was evaluated.

The effect size of all multiple linear (mixed) regression models is presented as a standardized regression coefficient (*β**), calculated via the following formula: $\beta^* = \beta \frac{S_X}{S_Y}$ with β, the unstandardized regression coefficient, S_X and S_Y, the standard deviations of the exposure and outcome, respectively. A slight modification in this calculation was made for the modelled air pollution data. Since the mean was higher in 2010 than in 2015 for all air pollution markers at study, a SD of the entire population resulted in an overestimation of the true dispersion in these data (**Figure S1**). Therefore, an average of the SD's of the air pollution values from both waves was calculated, and this average SD of both waves was then used in the formula above. Percentage change in dependent variable for each IQR increase in independent variable was calculated using the following formula: $\%\ change\ in\ dependent\ variable = \left(exp\left(\beta * IQR(independent\ variable)\right) - 1\right) * 100$ (UCLA: Statistical Consulting Group., 2021). All statistical analyses were conducted in R version 4.2.2 and all p-values < 0.05 for two-sided tests were considered statistical significant. In the regression models, multiple testing was corrected by restricting the false discovery rate as lower than q-values < 0.05 (Benjamini & Hochberg, 1995).

3. Results

3.1 Characteristics of the study population

Table 1: Characteristics of the study participants

Characteristic	N	Overall[a] N = 534	2010[a] N = 364	2015[a] N = 170	p-value[c]
Sex	534				0.11
Male		267 (50%)	173 (48%)	94 (55%)	
Female		267 (50%)	191 (52%)	76 (45%)	
Socio-economic status (parental education)	534				0.010
Low		144 (27%)	111 (30%)	33 (19%)	
High		390 (73%)	253 (70%)	137 (81%)	
BMI[b]	534				0.17
Underweight		75 (14.0%)	44 (12.1%)	31 (18%)	
Normal weight		427 (80.0%)	299 (82%)	128 (75%)	
Overweight		26 (4.9%)	16 (4.4%)	10 (5.9%)	

Characteristic	N	Overall[a] N = 534	2010[a] N = 364	2015[a] N = 170	p-value[c]
Obese		6 (1.1%)	5 (1.4%)	1 (0.6%)	
Age, years	534	9.00 ± 3.84	7.95 ± 2.10	12.58 ± 2.24	<0.001
Waist-height ratio	534	0.42 ± 0.05	0.43 ± 0.04	0.40 ± 0.03	<0.001
GLP-1, pg/mL	534	202.56 ± 117.61	234.32 ± 97.63	123.96 ± 59.21	<0.001
PP, pg/mL	534	65.23 ± 81.39	90.84 ± 79.35	29.63 ± 33.26	<0.001
PYY, pg/mL	534	100.43 ± 49.90	106.46 ± 44.91	81.26 ± 42.01	<0.001
Leptin, ng/mL	427	2.34 ± 3.98	1.42 ± 1.69	4.72 ± 5.37	<0.001
Active ghrelin, pg/mL	157	93.28 ± 92.10	-	93.28 ± 92.10	
Total ghrelin, pg/mL	156	1,356.15 ± 785.00	-	1,356.15 ± 785.00	
Sugar propensity, % of total intake	496	26.03 ± 14.57	28.79 ± 14.38	23.20 ± 14.21	<0.001
Fat propensity, % of total intake	496	29.84 ± 12.47	30.43 ± 13.21	28.92 ± 12.24	0.10
Fiber intake per week, portions per week	461	22.00 ± 14.00	21.00 ± 12.00	23.00 ± 15.50	0.14
Sleep duration, hours/night	416	10.50 ± 1.44	11.00 ± 0.79	9.50 ± 1.00	<0.001
Physical activity, hours/week	529	12.50 ± 10.00	14.50 ± 9.00	8.00 ± 8.50	<0.001
Negative life events last 12 months (CLES)	459	47.00 ± 73.00	40.00 ± 71.00	62.00 ± 102.25	<0.001
Feelings of angriness, anxiety or sadness (0-30)	454	5.00 ± 8.00	6.00 ± 8.00	4.00 ± 5.00	<0.001
Feelings of happiness (0-10)	455	8.00 ± 2.00	8.00 ± 3.00	8.00 ± 2.00	0.5
Emotional problems (SDQ) (0-10)	528	2.00 ± 2.00	2.00 ± 2.00	2.00 ± 2.00	0.3
Green space 100m around residence, %	534	43.42 ± 19.17	42.70 ± 19.24	44.06 ± 18.37	>0.9
Green space 2000m around residence, %	534	29.22 ± 12.03	28.53 ± 12.18	29.84 ± 11.78	0.6
Residential BC exposure, µg/m³	534	1.31 ± 0.41	1.35 ± 0.17	0.92 ± 0.08	<0.001
Residential NO_2 exposure, µg/m³	534	15.30 ± 4.66	16.72 ± 3.92	11.77 ± 3.30	<0.001
Residential $PM_{2.5}$ exposure, µg/m³	534	15.66 ± 3.71	16.08 ± 0.90	12.16 ± 0.72	<0.001
Multi-exposure score	292	0.13 ± 2.37	0.25 ± 2.18	-0.20 ± 2.58	0.14
Lifestyle score	292	0.16 ± 2.08	0.44 ± 1.97	-0.16 ± 2.05	0.024

[a] Characteristics are presented as medians ± interquartile range (IQR) for continuous variables and frequency (n) and percentage (%) for categorical variables.
[b] Classification based on BMI z-scores obtained by calculating the BMI (weight (kg)/height (m)2) and adjusted for age and sex using British 1990 growth reference data(Cole et al., 1998). Classifications were made by the International Obesity Task Force classification(Cole & Lobstein, 2012)
[c] Pearson's Chi-squared test for categorical variables; Wilcoxon rank sum test for continuous variables.
Abbreviations: GLP-1, Glucagon-like-peptide-1; PYY, Peptide tyrosine tyrosine; PP, Pancreatic polypeptide; CLES, Coddington Life Events Scale; SDQ, Strengths and Difficulties Questionnaire; BC, Black carbon; NO_2, Nitrogen dioxide, $PM_{2.5}$, Particulate matter <2.5 µM.

Table 1 shows the characteristics of the participants of the study population. Participating children (50.0% girls) had a median age of 9.00±3.84 years and 6% was overweight or obese (defined by IOTF classification (Cole & Lobstein, 2012)). **Figure 4** depicts the Spearman correlations between the distinct appetite hormone levels in this study. Negative correlations were observed for leptin levels with GLP-1 (ρ=-0.36; p<0.001), PYY (ρ =-0.11; p=0.03) and PP levels (ρ =-0.39; p=<0.001). Positive correlations were observed for GLP-1 levels with PYY (ρ =0.33; p<0.001) and PP levels (ρ =0.49; p<0.001); and for PYY with PP levels (ρ =0.34; p<0.001). In 2015, active and total ghrelin were strongly correlated (ρ =0.69; p<0.001) and negative correlations were found for leptin and GLP-1 with active (ρ=-0.29; p<0.001,

ρ=0.2;p=0.01) and total ghrelin (ρ=-0.31; p<0.001, ρ=-0.2, p=0.01). For all hormones at study, significant correlations were found over both study visits.

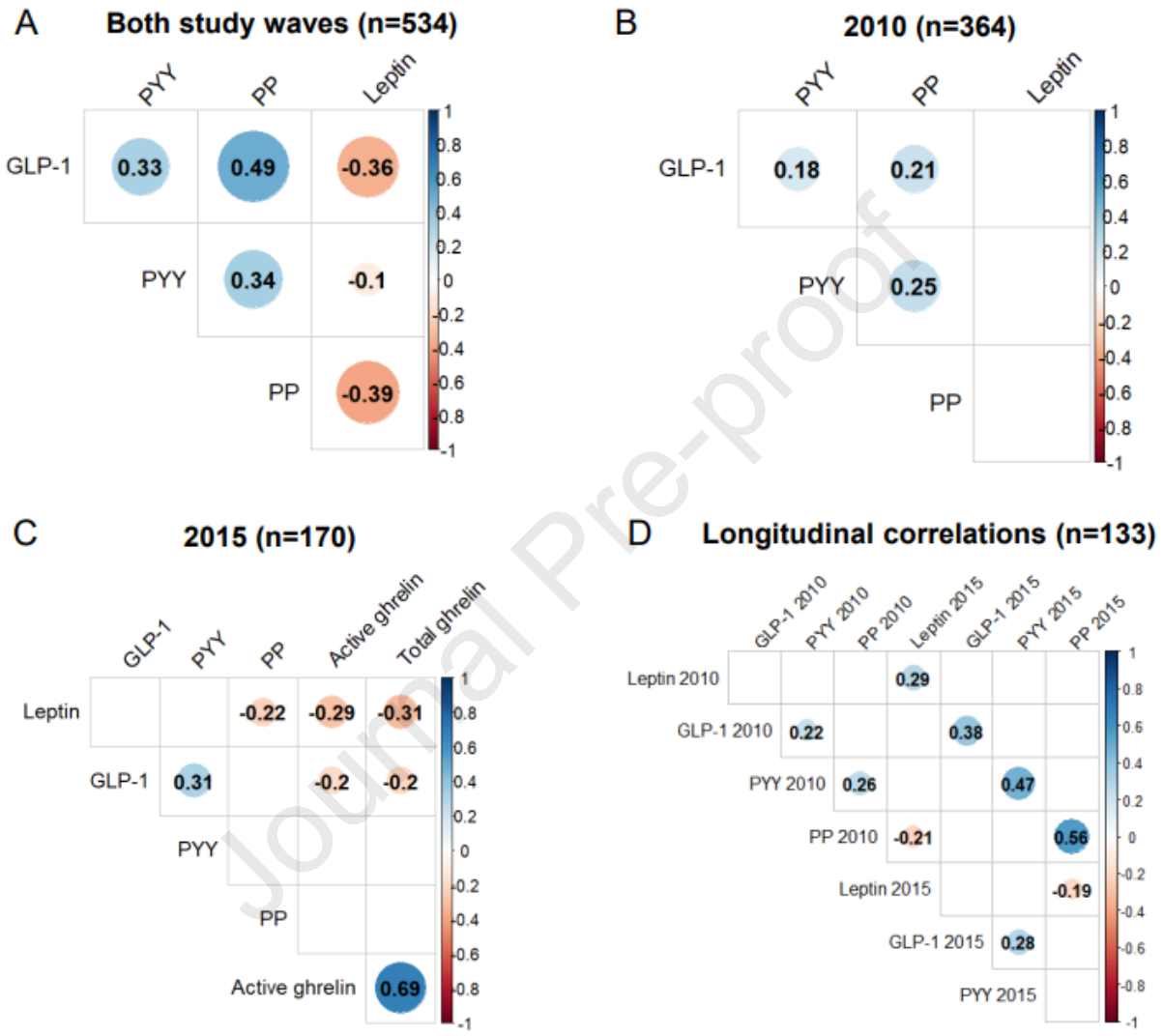

Figure 4: Spearman correlation coefficients between the fasting hormone levels in participants recruited in (A) 2010, (B) 2015, (C) both study waves and (D) longitudinal correlations. In (D) the longitudinal correlations for participants participating in both study waves are shown. Spearman correlations with a *p*-value >0.05 are not shown. **Abbreviations**: GLP-1, Glucagon-like-peptide-1; PYY, Peptide tyrosine tyrosine; PP, Pancreatic polypeptide.

3.2 Individual exposures and fasting appetite hormone levels

Figure 5 and **Table S1** give an overview of the associations between individual exposures and appetite hormones. After correction for multiple testing, several associations between appetite

hormones and exposures related to environmental quality and psychosocial stress were observed. Concerning environmental exposures, negative associations were observed for GLP-1 levels with all air pollution markers at study (NO_2 β*=-0.13; 95% CI [- 0.21;- 0.04]; *q* = 0.023 , BC β*=-0.15; 95% CI [-0.23;-0.07]; *q*=0.0033 , $PM_{2.5}$ β*=-0.16, 95% CI [-0.25;- 0.07]; *q*=0.0033). In line with this association, a trend was observed between higher GLP-1 levels and more green space in a radius of 2000 m around the residence. Lastly, lower leptin levels were associated with more green space in a small buffer (100 m) around the residence of the children (β*=-0.11; 95% CI [-0.18;-0.04]; *q*=0.024). Regarding psychosocial stress, both active and total ghrelin showed positive associations with feelings of happiness (active ghrelin β*=0.25; 95% CI [0.11;0.39]; *q*=0.010, total ghrelin β*=0.26; 95% CI [0.11;0.41]; *q*=0.011). Additionally, an inverse association was observed between total ghrelin and negative emotions (β*=-0.23; 95% CI [-0.39;- 0.07]; *q*=0.035), a similar non-significant trend was visible between active ghrelin and negative emotions. None of the exposures was significantly associated with PP or PYY.

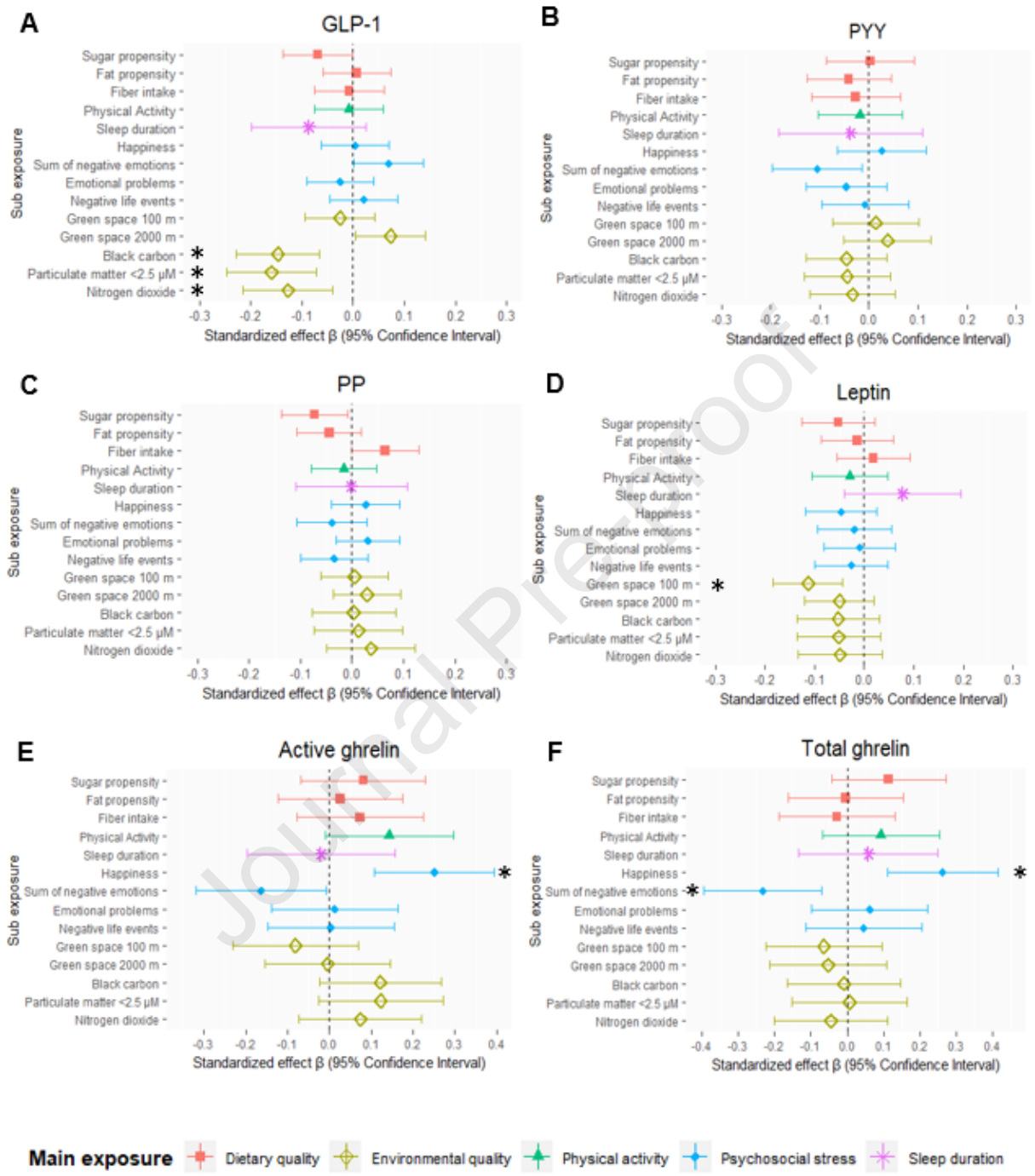

Figure 5: Associations between the individual exposures and appetite hormones levels for (A) GLP-1, (B) PYY, (C) PP, (D) Leptin, (E) Active ghrelin and (F) Total Ghrelin. Effects are presented in a forest plot as standardized regression coefficients (β*) with 95% CI of the corresponding linear mixed models (A-D) and linear models (E and F). All linear mixed models (A-D) were adjusted for sex, age, SES, waist-to-height ratio and the study wave. Models for active and total ghrelin were linear models considering only data from 2015, these models were adjusted for sex, age, SES and waist-to-height ratio. Associations that remained significant after correcting for multiple testing (FDR<0.05) are marked with an asterisk (*). **Abbreviations**: GLP-1, Glucagon-like-peptide-1; PYY, Peptide tyrosine tyrosine; PP, Pancreatic polypeptide; CI, Confidence interval; FDR, False discovery rate; SES, socioeconomic status.

436

437 ### 3.2.1 Sensitivity analyses

438 The results of the separate analysis per study wave are presented in **Table S2**. These results
439 confirm the findings of the analyses conducted on the entire study population. Similar trends
440 as reported in the main analyses were found per study wave, although not always significant.
441 The sensitivity analysis that removed waist-to-height ratio as potential collider from the models,
442 did not alter the results (**Table S3**). The associations for low-growing, high-growing and total
443 green space in multiple radii around the residence are summarized in **Table S4.** Similar as in
444 the main analyses, only for GLP-1 and leptin significant associations were observed. Leptin
445 was negatively associated with low, high and total green space close to the residence (50 to
446 500 m). GLP-1 was associated with low and high green space in the wider environment around
447 the residence, although the direction of the association was different for low (negatively) *vs*
448 high (positively) green space. Next, the role of traffic noise, air pollution, distance to major road
449 and neighborhood income in the association between residential green space and appetite
450 hormones was evaluated for the prementioned significant findings (**Table S5**). For GLP-1 only
451 the associations with high green space remained significant after adding $PM_{2.5}$ to the models.
452 Associations of residential green space and leptin were only diminished by addition of
453 neighborhood median income. Lastly, associations between air pollution markers and GLP-1
454 levels were not altered by adding neighborhood income to the models (**Table S6**).

455

456 ## 3.3 Multi-exposure score/Lifestyle score and fasting appetite hormone levels

457 **Figure 6** depicts the associations of the multi-exposure score and all appetite hormones. Only
458 total ghrelin levels were associated with the multi-exposure score (β*=-0.22; p=0.04) (**Table**
459 **S7**). An IQR increase of the multi-exposure score (=2.46) was associated with 11.15%
460 [95%CI:-20.5;-0.74%] decrease in total ghrelin levels. Moderation analyses revealed no
461 significant moderation effect of lifestyle score on the association between air pollution markers
462 or residential green space and appetite hormone values (**Table S8**).

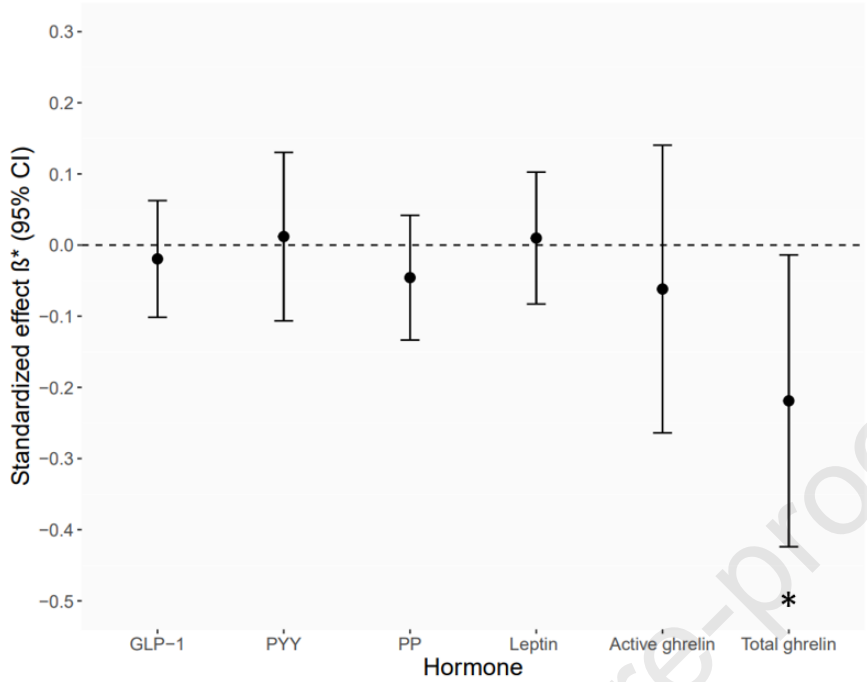

Figure 6: Associations between the multi-exposure score and appetite hormones. Effects are presented in a forest plot as standardized regression coefficients (β*) with 95% CI of linear mixed models (for GLP-1, PYY, PP and leptin) and the linear models (for active and total ghrelin). All linear models were adjusted for sex, age, SES, waist-to-height ratio. Linear mixed models were additionally adjusted for study wave. Note: models for active and total ghrelin were linear models considering only data from 2015. Associations that remained significant after correcting for multiple testing (p-value<0.05) are marked with an asterisk (*) **Abbreviations**: GLP-1, Glucagon-like-peptide-1; PYY, Peptide tyrosine tyrosine; PP, Pancreatic polypeptide; CI, Confidence interval; SES, socioeconomic status

4. Discussion

This study explores a number of associations between lifestyle and environmental exposures with appetite hormone levels in children and adolescents. Residential air pollution, residential green space and psychosocial stress (happiness and negative emotions) appeared to be associated with the appetite hormones GLP-1, leptin, active and total ghrelin. When combining all lifestyle and environmental factors in one multi-exposure score, a total of unhealthier exposures was associated with lower total ghrelin levels.

4.1 Environmental exposures

In this study, three air pollution markers ($PM_{2.5}$, BC, NO_2) were investigated. All of them showed robust inverse associations with GLP-1 levels. Our findings might have a significant public health impact as an IQR increase in BC, $PM_{2.5}$ and NO_2 was associated with a 13.3% [95%CI: 19.9 to 6.1%], 30.5% [95%CI: 43.1 to 15.1%] and 6.2% [95%CI: 10.3 to 2.0%] decrease in GLP-1 levels, respectively. Our observation in children and adolescents is supported by an animal study showing 64% lower GLP-1 levels in rats exposed to unfiltered air pollution compared to controls (Wei et al., 2016b). Furthermore, our results are also in line with a study including 80 Mexican children which reported lower GLP-1 levels in children living in high $PM_{2.5}$ polluted areas (mean annual $PM_{2.5}$ exposure 16.8 (± 8.3) µg/m³) in comparison to controls ($PM_{2.5}$ exposure <12 µg/m³) (Calderón-Garcidueñas et al., 2015).

With regard to potential mechanisms, we speculate that inflammation – either systemic and/or in the gut - and changes in the intestinal microbiome might potentially serve as mediators in the observed association between air pollution and GLP-1. As of today, there is no direct evidence for this, and it remains speculative. However, there is some literature that supports the direction of our thinking. Firstly, air pollution has been associated with increased production of inflammatory markers, which contribute to systemic inflammation. Secondly, via mucociliary clearance, inhaled air pollution particles such as black carbon can end up in the gastrointestinal tract where they can be involved in gut inflammation (Kish et al., 2013; Möller et al., 2004). This systemic and/or gut inflammation could potentially play a role in cellular dysfunction of the gastro-intestinal L-cells, responsible for GLP-1 secretion. Moreover, higher exposure to air pollution has been associated with lower intestinal microbial diversity, even in young children (Van Pee et al., 2023). Gut bacterial diversity is vital for GLP-1 production through various

mechanisms, including the production of metabolites such as short-chain fatty acids (Everard & Cani, 2014).

The findings of this study can be interpreted in the context of obesity as an appetite-related outcome. Numerous studies have yet reported associations between air pollution and obesity (Shi et al., 2022). Since air pollution is associated with lower GLP-1 levels in our healthy population and GLP-1 has appetite-suppressing effects, it may be plausible that air pollution is hence increasing appetite and may in this way contribute to the development of obesity. Remarkably and seemingly contrary to this hypothesis, obesity was associated with higher fasting GLP-1 levels in a large cohort study (n=4000) (Stinson et al., 2021). Two potential mechanisms could explain this observation. On the one hand, we could hypothesize that GLP-1's role as an appetite suppressor is secondary to its role as an incretin, whereby GLP-1 primarily stimulates insulin production and thus possibly contributes to insulin resistance. On the other hand, elevated levels of GLP-1 could be seen as a compensatory but futile attempt by the body to strive for homeostasis and thus weight reduction. Due to the lack of longitudinal studies, it is not clear yet whether this association between GLP-1 and obesity does indeed involve a causal relationship. Therefore, the possibility still exists that GLP-1 levels increase as a result of obesity, so the above reasoning of GLP-1 as underlying mechanism in the association between air pollution and obesity indeed applies, especially since the current study was conducted in a primarily healthy population. However, while delving into the impact of air pollution on GLP-1 and its potential connections to the development of obesity, it’s essential not to overlook the non-homeostatic or hedonic regulation of appetite, as both homeostatic and non-homeostatic appetite control can interact. In situations of relative energy abundance, the hedonic system can even override the homeostatic regulation of appetite (Lutter & Nestler, 2009).

In addition to its potential role in the development of obesity, the association between air pollution and GLP-1 could also have implications for insulin resistance. As mentioned earlier, GLP-1, as an incretin, stimulates insulin production. Thus, when GLP-1 levels are chronically lowered it could lead to insulin deficiency, resulting in elevated blood sugar levels. This hyperglycemia can damage blood vessels, potentially leading to cardiovascular disease over time.

Whereas air pollution was associated with lower GLP-1 levels, residential green space in the wider environment of the residence was associated with higher GLP-1 levels. Even after correcting the model for air pollution and neighborhood median income, high residential green space (vegetation height above 3 m) within a 1000 m and 2000 m radius remained associated with higher GLP-1 levels, suggesting an independent relationship between high residential green space and GLP-1 levels. Next to GLP-1, also leptin was associated with residential green space, although here in closer proximity around the residence. After correction of these models for air pollution and neighborhood median income, only the associations with the 100 m buffer of low and 300 m and 500 m of total residential green space remained, suggesting that air pollution and the socio-economic status play a role in the associations. Besides air pollution, also increased physical activity (James et al., 2015; Jennings & Bamkole, 2019) and stress reduction (Van Aart et al., 2018) could be proposed as potential mediators in the relationship between residential green space and impaired circulating appetite hormone levels. However, neither GLP-1 nor leptin were associated with physical activity in our study, possibly due to the rather subjective measurement of physical activity via questionnaires. A potential mediation by stress reduction seems out of question in our population since GLP-1 levels were positively associated with negative emotions and no associations were found for leptin.

4.2 Psychosocial stress

Significant hormone associations were observed with both positive (happiness) and negative (sadness, angriness and anxiety) emotions. Total and active ghrelin were positively associated with happiness and negatively with negative emotions. These findings are inconsistent with previous literature showing positive associations between both acute and chronic stress exposure and both active and total ghrelin (Bouillon-Minois et al., 2021; Ozsoy et al., 2014; Yousufzai et al., 2018). However, the aforementioned studies investigated severe forms of chronic stress like physician diagnosed depression or post-traumatic stress disorder (PTSD), while in our population none of the participants reported severe mental health problems. Seen the well-known association between chronic stress and obesity, one could expect that chronic psychosocial stress raises ghrelin levels, as ghrelin is proven to have anxiolytic effects, potentially leading to an increased appetite (Han et al., 2019). Paradoxically, the findings from our study challenge this expectation, revealing an inverse relationship between ghrelin levels and psychosocial stress, which contradicts the conventional line of reasoning.

4.3 Multi-exposure score

To our knowledge no previous studies investigated the combined effect of several lifestyle factors and/or environmental exposures towards appetite regulating hormones, although associations between individual lifestyle and environmental factors have been observed previously (Adams et al., 2011; Calderón-Garcidueñas et al., 2015). In this study, we found that children exposed to unhealthy lifestyle and environmental exposures had lower total ghrelin levels. This finding might seem contradictory seen the appetite-stimulating function of ghrelin and unhealthy lifestyle being a main predictor of obesity. However, studies have reported negative correlations between fasting ghrelin levels and BMI (Korek et al., 2013; Papandreou et al., 2017; Tschöp et al., 2001; Wang et al., 2022). Our observed association seems also more logic when considering that total ghrelin mainly reflects the inactive or des-acyl form (Wang et al., 2022), which could potentially counter the appetite stimulating effects of the acyl or active form of ghrelin (Iwakura et al., 2023). Besides its suggested role in appetite regulation, other health promoting functions of des-acyl ghrelin have been described: e.g., improvement of glucose tolerance and insulin sensitivity, reducing oxidative stress in vascular endothelial cells and the prevention of apoptosis (Iwakura et al., 2023) . In perspective of these functions, our observed association between a reduced ghrelin levels and more unhealthy exposures seems more obvious, as some of these functions may play an indirect role in the development of obesity. Altogether, the underlying mechanisms of the associations that were found in this study with the hormone ghrelin remain unclear. Further studies addressing the distinct physiological actions of acyl and des-acyl ghrelin are crucial to fully understand the relevance of the current observations to human health and disease.

4.4 Strengths and limitations

To our knowledge, this is the first study exploring the associations between a combination of exposures and appetite hormone levels in children and adolescents. However, we need to acknowledge some study limitations. First, with 534 children distributed over two study waves, our sample size was rather small, especially in the case of ghrelin as this hormone was only measured in the last study wave (n=157). Despite this rather small sample size, we were still able to observe statistically significant associations with some of the exposures. However, this study would have benefited from having larger power, especially regarding the moderation analysis and the large set of confounders in our analyses. Besides the modest sample size, our study population might not be entirely representative of the general population since

participants were all recruited in the same municipality, the parental education level was quite high and prevalence of obesity was rather low compared to the average Belgian population. In addition, some of the population characteristics differed significantly between both study waves which could have influenced the results. The measurements of certain exposures could be improved methodologically. For instance, physical activity was measured using questionnaires, while the use of more objective physical activity measurements by accelerometers could have improved the accuracy of our data. Moreover, the use of different immunoassays for leptin measurements in each study wave may have led to batch differences in the leptin data between the waves. In this study, appetite hormone levels were measured after overnight fasting to improve comparability and minimize the influence of circadian rhythms. However, studies addressing the association between obesity and appetite hormone levels do not only show alterations in fasting levels, but also alterations in circadian rhythms, or attenuations of post-prandial responses in circulating appetite hormone levels. These findings highlight the importance of more in-depth research targeting associations between lifestyle, environmental exposures and appetite hormone levels covering a 24-hour cycle, even though the more difficult practicality of this study design, especially when considering children. Lastly, longitudinal studies addressing the current research question might provide more insight into causation, whereas now only associations could be observed.

5. Conclusions

This study found associations of several appetite hormones with environmental quality (air pollution and residential green space) and psychosocial stress (happiness and negative emotions). For GLP-1, we observed negative associations with air pollution markers and positive associations with high residential green space, suggesting an appetite stimulating effect of air pollution and a contrasting appetite reducing effect of residential green space. However, negative associations of leptin with residential high green space (e.g. trees) suggest appetite stimulating effects for residential green space. Concerning psychosocial stress, the associations of ghrelin with happiness and negative emotions suggest appetite stimulatory effects of happiness while appetite-inhibitory effects of negative emotions. Finally, more unhealthy exposures associating with lower ghrelin levels also indicate appetite lowering effects. However, appetite regulation is a complex process involving multiple interacting

hormones, whereby also hedonic systems cannot be overlooked. Therefore, we should be careful with straightforward interpretations of the found associations towards appetite. Nevertheless, these results provide valuable new insights into the associations of external factors with appetite hormones, which is of high interest for preventive obesity research. More in-depth research in large longitudinal cohorts is recommended to reveal the underlying mechanisms (e.g., gut microbiome and inflammation) and causality of the observed associations.

Author contributions

Thaïs De Ruyter: Writing- Original Draft, Formal Analysis, Data Curation, Methodology, Project administration, Visualisation. **Dries S. Martens:** Writing - Review & Editing, Supervision. **Esmée M. Bijnens:** Software, Writing - Review & Editing. **Tim S. Nawrot:** Funding acquisition, Conceptualization, Writing - Review & Editing, Supervision. **Stefaan De Henauw:** Writing - Review & Editing, Supervision**. Nathalie Michels:** Funding acquisition, Conceptualization, Investigation, Methodology, Data Curation, Writing - Review & Editing, Project administration, Supervision.

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EXPLORING THE IMPACT OF LIFESTYLE AND ENVIRONMENTAL EXPOSURES ON APPETITE HORMONE LEVELS IN CHILDREN AND ADOLESCENTS: AN OBSERVATIONAL STUDY

Highlights:

- Associations of 14 exposures with 5 appetite hormones were studied in children.
- Air pollution is negatively associated with fasting Glucagon-like-peptide 1 levels.
- Residential green space is negatively associated with fasting leptin levels.
- Happiness is positively associated with active and total ghrelin levels.
- Negative emotions show negative associations with total ghrelin levels.

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: