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Long-term residential exposure to air pollution is associated with hair cortisol concentration and differential leucocyte count in Flemish adolescent boys

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ABSTRACT

Background: Exposure to air pollution and traffic noise are associated with adverse health outcomes in adolescents. Chronic endocrine stress and systemic inflammation have been hypothesized to underlie the adverse health effects. Simultaneous assessment of inflammation and chronic endocrine stress in epidemiological studies is lacking. The aim of the study was to investigate biomarkers of chronic endocrine stress and inflammation in relation to long-term residential exposure to air pollution and traffic noise in adolescents.

Methods: In Flemish adolescents (14–15 years), we determined hair cortisol concentration (HCC) as a chronic stress biomarker in 3-cm scalp-near hair sections (n = 395), and leucocyte and leucocyte subtype counts (neutrophils, monocytes, lymphocytes) as inflammatory biomarkers in peripheral blood (n = 385). Daily particulate matter ($PM_{2.5}$, PM_{10}), nitrogen dioxide (NO_2) and black carbon (BC) concentrations were modelled at the residential address and averaged over 3-month and 1-year periods prior to sampling. Residential traffic noise level was estimated and classified in 5 dB intervals. Sex-specific associations between residential exposures and effect biomarkers were studied using linear regression models, adjusted for *a priori* selected covariates.

Results: In boys, HCC increased with a factor 1.30 (95% CI: 1.10, 1.54) for an increase in 1-year mean NO_2 from the 25th to 75th percentile (p75/p25), after adjustment for age, BMI, personal and neighborhood socioeconomic status. The corresponding estimate for PM_{10} was 1.24 (95% CI: 1.02, 1.51). Total leucocyte count in boys, adjusted for the aforementioned covariates and recent health complaints, was positively associated with $PM_{2.5}$, PM_{10}, NO_2 and BC. In particular, the neutrophil count increased with a factor 1.11 (95% CI: 1.03, 1.19) for a (p75/p25)-factor increase in 1-year mean BC, corresponding estimates for $PM_{2.5}$, PM_{10} and NO_2 were 1.10 (95% CI: 1.01, 1.19), 1.10 (95% CI: 1.01, 1.20) and 1.08 (95% CI: 1.00, 1.16). Lymphocyte count increased with a factor 1.05 (95% CI: 1.01, 1.10) for a (p75/p25)-factor increase in 1-year mean NO_2. Similar results were observed for 3-month mean exposures. Results were robust to adjustment for recent air pollution exposure. In

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girls, air pollutants were not associated with HCC or differential leucocyte count. Residential traffic noise level was not associated with HCC or leucocyte counts in boys nor girls.
Conclusions: Long-term residential exposure to air pollutants was positively associated with chronic endocrine stress and inflammation in adolescent boys, not in girls. This study may contribute to a better understanding of the early pathophysiological changes that may underlie adverse health effects of air pollution exposure in adolescents.

1. Introduction

Despite considerable improvements in ambient air quality in the past decades, air pollution remains a major global environmental health hazard (Sun and Zhu, 2019). The impact of air pollution may be even more severe during developmental periods such as adolescence (Braga et al., 2001). Long-term air pollution exposure during adolescence has been related to increases in incidence of bronchitis, asthma, metabolic syndrome and hypertension, and to a negative impact on neurobehavioral function (Braga et al., 2001; Fuertes et al., 2016; Gauderman et al., 2004; Götschi et al., 2008; Islam et al., 2007; Kicinski et al., 2015; Zhang et al, 2019, 2021). Although these diseases are unique and diverse, early pathogenesis may involve common pathways, and is likely to be multifactorial (Brook et al., 2018).

The premise that stress pathways play an important role in mediating health effects of air pollutants has only emerged in the last decade (Snow et al., 2018; Thomson, 2013). Animal studies first pointed to activation of the endocrine hypothalamic–pituitary–adrenal axis (HPA axis) as one of the early biological mechanisms, triggered by air pollution exposure (Snow et al., 2018; Thomson et al., 2013). Activation of the HPA axis is a crucial stress response system in animals and humans (Iob and Steptoe, 2019). The main downstream effector of the HPA axis in humans, the glucocorticoid (GC) cortisol, has wide-reaching actions in multiple tissues and organs, aimed at immediate defense and survival (McEwen, 2007). Conversely, chronic activation of the HPA axis, with long-term increased secretion of cortisol, has been associated with increased risks of cardiovascular and respiratory diseases, depression and reduced cognitive function (Chrousos, 2009). These chronic adverse health conditions have also been linked to long-term exposure to air pollution (Landrigan et al., 2018). It has been hypothesized that the continuous exposure to ambient air pollution in real-life settings may induce a steady, chronic stress load (Hajat et al., 2019). During adolescence, chronic stress may have implications for physical and mental health that reach into adulthood (Sheth et al., 2017). The cortisol concentration in hair (HCC) has recently been validated as a biomarker for long-term cortisol secretion, however, research of HCC in relation to air pollution exposure is still scarce (Binz et al., 2018; Gray et al., 2018; Verheyen et al., 2021).

In addition to an endocrine stress response, air pollution exposure has been hypothesized to elicit an inflammatory immune response (Glencross et al., 2020). Moreover, the endocrine and the immune system are tightly interwoven (Da Pozzo et al., 2018). An overactive immune response may induce systemic inflammation and damage healthy tissues, thus, subtle changes in immune regulation may have far-reaching health consequences (Spannhake et al., 2002). As the duration of an inflammatory response increases, susceptibility to respiratory viruses has been shown to increase, as well as the risk for exacerbations of asthma and the progression of cardiovascular diseases (Cohen et al., 2012). Total count of leucocytes and of leucocyte subtypes (neutrophils, lymphocytes, monocytes) in peripheral blood are established biomarkers of an inflammatory immune response, with absolute numbers of leucocyte subtypes providing biologically more reliable information than percentages (Oulhote et al., 2017; Vozarova et al., 2002). Exploring the associations of environmental exposures with differential leucocyte counts is critical to understand their impacts on the human immune system (Gao et al., 2019). In vitro and in vivo studies have shown that the release of inflammatory mediators into the circulation, following inhalation exposure to air pollutants, may stimulate the bone marrow to release leukocytes and prolongs their survival in the circulation (Van Eeden and Hogg, 2002). Significant positive associations between short-term exposure to air pollutants and leucocyte counts have been reported in adults (Brook et al., 2010; Newby et al., 2015; Steenhof et al., 2014). Epidemiological studies have also reported associations of long-term air pollution exposure with leucocyte and neutrophil counts in adults (Chen and Schwartz, 2008; Chuang et al., 2011). The body of literature on associations of air pollution exposure with leucocyte counts in adolescents is, however, small. Short-term air pollution exposure in adolescence has previously been linked to higher leucocyte, neutrophil and monocyte count (Gao et al., 2019; Poursafa et al., 2011; Prunicki et al., 2020; Zhang et al., 2013). Significantly higher monocyte counts were also observed in Chinese adolescents that lived in a highly polluted area compared to adolescents from a control area (Li et al., 2019).

More than 80% of the urban population is exposed to air quality levels that exceed the health-based guidance values of the World Health Organization (WHO), all regions of the world are affected (Attademo and Bernardini, 2017). Within Western-Europe, Flanders is a well-known air pollution hotspot, characterized by a high population density and a dense road network (Dons et al., 2014). According to the 2018 report on air quality in Flanders (Flanders Environment Agency, 2018) traffic was the main source of NO_2 emissions in Flanders (61%), industry was the second main contributor (17%). Households were the primary source of $PM_{2.5}$ and PM_{10} emissions in Flanders in 2018 (51% and 37% respectively), followed by traffic (23% and 21% respectively) and industry (16% and 22% respectively). Of note, next to generating air pollution, traffic is also known to generate noise (Moudon, 2009). Exposure to traffic noise has been related to an increase in cortisol levels and immune response alterations (Daiber et al., 2020; Kim et al., 2017). As a consequence, adolescents' exposure to air pollution and traffic noise may each independently or jointly have an impact on health (Bloemsma et al., 2019).

The objective of this study was to simultaneously investigate associations of long-term residential exposure to air pollution and traffic noise with the endocrine stress response and the immune response in a general population of Flemish adolescents (14–15 years), using hair cortisol concentration (HCC) and differential leucocyte count (total leucocytes, neutrophils, monocytes, lymphocytes) as biomarkers. We hypothesized that increased long-term residential exposure to air pollution and traffic noise would be associated with increased hair cortisol concentration and leucocyte counts. To the best of our knowledge, this study is the first to simultaneously assess HCC and differential leucocyte count in relation to residential exposure to air pollution and traffic noise.

Previous research in the Flemish Environment and Health (FLEHS) study has revealed sex-specific associations of environmental exposures i.e., chemical exposures and air pollution, at both gene expression and pathway levels (De Coster et al., 2013; Vrijens et al., 2017). These studies recommended that males and females be considered separately when analyzing associations of environmental exposures with biological responses. Furthermore, the responsivity of this HPA axis and the immune system may significantly differ by sex during adolescence (Da Pozzo et al., 2018; Ordaz and Luna, 2012; Oyola and Handa, 2017). Several observational studies found greater cortisol reactivity in adolescent boys compared to girls (Bouma et al., 2009; Klimes-Dougan et al., 2001). Boys have also been reported to produce higher

inflammatory responses than girls during puberty (Klein and Flanagan, 2016). The factors that contribute to differential stress and immune responses among boys and girls remain incompletely understood. Possibly, HPA-axis reactivity is influenced by the rise in sex hormones during pubertal maturation (Naninck et al., 2011). Estrogen attenuates cortisol secretion in response to acute stress in girls by enhancing oxytocin release from the hypothalamus, which inhibits the effect of the corticotropin-releasing hormone (CRH) on the pituitary (Ordaz and Luna, 2012). In contrast, the male stress response is potentiated by testosterone during puberty (Harden et al., 2016). The effects of sex steroids on immune cells may also play a prominent role in sex differences in inflammatory status during puberty (Klein and Flanagan, 2016). We stratified our analyses by sex to pick up potential differences in responses to air pollution exposure between boys and girls.

2. Materials and methods

2.1. Study population

The Flemish Environment and Health Study (FLEHS), an environmental health surveillance program, was established by the Flemish government in 2002 (Schoeters et al., 2012). This study was embedded in the fourth cycle of the Flemish human biomonitoring study, FLEHS-4, which included a representative sample of 428 adolescents (14–15 years) from the general population of Flanders. The FLEHS-4 study protocol was approved in June 2017 by the Antwerp University Hospital Ethics committee (Belgian registration number B300201732753). A stratified clustered multi-stage sampling strategy was applied to enroll equal numbers of participants across both sexes and to represent all educational levels. Participants were recruited in 20 schools, geographically representing all Flemish provinces. The inclusion criteria of the FLEHS-4 study were: informed consent signed by the adolescent and a parent, having lived in Flanders for at least 5 years, adolescent and parents mastered enough Dutch to fill out extensive questionnaires. Exclusion criteria of FLEHS-4 were: data of more than 1 questionnaire missing, blood and urine sample missing, being held back in school for more than 1 year, attending a boarding school, pregnancy. Participants were examined in schools between September 2017 and June 2018. Of the 428 participants, 8 could not participate in this part of the study because their scalp hair was too short. We excluded 17 participants because of residential mobility in the year before sampling and additionally excluded 7 participants because of missing information on socioeconomic status. One participant was excluded for following a growth hormone therapy. Thus, the final sample of this study included 395 adolescents.

During fieldwork at schools, blood and hair samples were taken by trained nurses, following strict protocols. Body length and weight were measured with standardized equipment; from this information, the body mass index (BMI, body weight in kg/(body length in m)^2) was calculated. Boys and girls were classified as underweight, normal weight, overweight or obese according to the sex- and age-specific 2004 Belgian growth curves (Roelants et al., 2009). The extensive questionnaires, filled out by adolescents and parents, covered lifestyle, health and sociodemographic characteristics. Participants provided information on smoking and secondhand smoking at home (yes/no), recent health status as the occurrence of any health complaint in the fortnight prior to sampling (yes/no), recent medication use, i.e., the use of systemic corticoids. Girls provided information on menarcheal status (pre-/-postmenarche). We assessed household SES based on parents' perceived income adequacy, ranging from difficult to very easy to make ends meet. Research has shown subjective income measures to be positively associated with health and well-being, above and beyond the health benefits associated with objective income measures (Hofmann et al., 2018). Country of birth was assessed as Belgium when the adolescent and his/her parents were born in Belgium, European Union (EU) when the adolescent or one of the parents was born in another EU country and outside-EU when the adolescent or one of the parents was born outside the EU.

2.2. Residential characteristics

Assessment of exposure variables and neighborhood SES was based on participants' geocoded home addresses. All analyses were carried out using Geographic Information System (GIS) functions with ArcGIS 10 software (http://www.esri.com/arcgis). We assessed the Area Deprivation Index (ADI) as an indicator of neighborhood SES. The ADI is calculated at a sub-municipality level in Flanders on a yearly basis (Guio and Vandenbroucke, 2019). The ADI considers all children, born in deprived households in a given neighborhood in Flanders in the past three years, divided by the total number of children born in the neighborhood during the same period. Selection criteria for deprivation are household income, educational attainment, employment situation, development of the children (fine and gross motor skills, communication skills, social behavior), housing and health. A lower limit is set for each criterion, if a household scores below this limit for at least three criteria, it is considered to be deprived (Verheyen et al., 2021).

Residential exposure to particulate matter ($PM_{2.5}$, PM_{10}), nitrogen dioxide (NO_2) and black carbon (BC) was modelled using a high resolution spatial temporal interpolation method. The ambient exposure to the aforementioned ambient pollutants of the participants was determined, based on their residential address, using a validated spatial and temporal high resolution interpolation method in combination with a dispersion model (Janssen et al., 2008; Lefebvre et al., 2013). This interpolation method uses hourly measured $PM_{2.5}$, PM_{10}, NO_2 and BC pollution data collected at the official fixed-site 34 monitoring stations for $PM_{2.5}$, PM_{10}, NO_2 and 16 for BC. Land-cover data obtained from satellite images were used. The model chain provides hourly air pollution values on a dense, irregular receptor grid by using data both from the Belgian telemetric air-quality network and emissions from point sources and line sources. In the Flemish region of Belgium, more than 80% ($R^2 = 0.8$) of the temporal and spatial variability was explained by this interpolation tool for PM_{10}, $PM_{2.5}$ and NO_2 and 74% for BC. The validity of the model with regard to personal exposure was further proven by the internal urinary carbon load which correlated with the modelled residential exposure (Saenen et al., 2017). We subsequently averaged the modelled daily pollutant concentrations over periods of 2 days, 3 months and 1 year prior to sampling.

Assessment of residential exposure to traffic noise was based on the noise exposure map developed for MIRA, the Flanders Environment Report (Flanders Environment Agency, 2019). MIRA assesses noise exposure in Flanders, expressed in L_{den}, in a high spatial resolution (20×20 m^2), using the Common Noise Assessment Methods (CNOSSOS-EU), noise propagation is calculated according to the IS09613-2 standards (Kephalopoulos et al., 2012). Noise exposure levels were classified in 5 dB-intervals, according to the European Noise Directive (2002/49/EC).

Data on local ambient temperature was provided by the Belgian Royal Meteorological Institute (KMI). In Flanders, Belgium, ambient temperature is continuously measured in 15 weather stations of the KMI. We used daily averaged data from the weather station closest to the adolescents' home to calculate the 3-month mean local temperature.

2.3. Sample collection and biomarker analysis

A strand of hair of at least 3 cm was cut close to the scalp from the posterior vertex of the adolescents' head. As human scalp hair grows at a rate of 1 cm per month, the first 3 cm most proximal to the scalp retrospectively reflect circulating cortisol levels over a period of 3 months prior to sampling (Gray et al., 2018). Hair samples were stored in paper envelopes at room temperature until analysis, performed within 18 months after collection of the first samples. Samples were analyzed at the Institute of Public Health, Department of Environmental Medicine of

the University of Southern Denmark (SDU), using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) as previously described (Verheyen et al., 2021). The limit of quantification (LOQ) for cortisol and cortisone was 0.3 pg/mg hair. The intra-day repeatability coefficient of variation (8.7%) and the inter-day reproducibility coefficient of variation (9.5%) complied well with recommendations for reliable bioanalytical analysis (European Medicines Agency, 2012; Thyagarajan et al., 2016). The blood sample of 6 mL was collected in an EDTA-tube and gently mixed, 1 ml was then aliquoted in a Sarstedt tube. Samples were stored at 4 °C until analysis by the accredited medical laboratory A.M.L. (Algemeen Medisch Laboratorium). Analysis was performed within 48 h after collection. Total leucocyte count and leucocyte subtype distribution (percentage of neutrophils, monocytes, lymphocytes) were assessed using a (Sysmex XE-2100) instrument for hematology analysis, which combines flow cytometry with fluorescence detection (https://www.sysmex-mea.com/products/xe-2100-892.html). Counts of leucocyte subtypes were subsequently calculated by multiplying the subtype percentage with the total leucocyte count. Leucocyte data was available for 386 participants, data of one participant with an elevated leucocyte count of >20 000 leucocytes/μl, indicative of an inflammatory condition or infection, was excluded from the analysis.

2.4. Statistical analysis

Statistical analysis was performed using SPSS Statistics (version 26; IBM, Armonk, NY, USA) and R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria). For three HCC values below the LOQ, values were imputed by using a truncated lognormal distribution. First, a truncated lognormal distribution was fitted for the observed values (the values above the LOQ). This resulted in an estimate of the mean and standard deviation (SD) of the lognormal distribution of all values (below and above the limit). For values below the limit, random values were imputed, taken between 0 and the limit from the lognormal distribution with the estimated mean and SD. Descriptive statistics provide an overview of study population characteristics and exposure variables of all participants and of boys and girls separately.

Before statistical analysis, the distribution of all continuous independent and dependent variables was visually inspected. Air pollutant concentrations, HCC, leucocyte and leucocyte subtype counts (neutrophils, lymphocytes, monocytes) were logarithmically transformed (ln-scale) because of skewed distributions. Residential outdoor air pollutant concentrations, HCC and leucocyte counts of boys and girls were compared using an independent samples *t*-test. Spearman rank correlations between residential outdoor exposure variables, household and neighborhood SES variables were assessed, as well as Pearson's correlations between measured biomarkers.

We investigated sex-specific associations of each exposure variable (air pollutants, traffic noise) with HCC and with differential leucocyte count in sex-stratified single-exposure linear regression models. We specified two models with increasing level of adjustment for covariates. The selection of covariates was based on literature (Gray et al., 2018; Penz et al., 2018; Verheyen et al., 2021). Model I was adjusted for age. In model II, associations of exposure variables with HCC were adjusted for age, BMI, household and neighborhood SES. Associations of exposure variables with leucocyte counts were additionally adjusted for recent health complaints, to account for acute fluctuations in leucocyte counts in response to recent health complaints (Chabot-Richards and George, 2014). For girls, model II was additionally adjusted for menarcheal status (pre-or postmenarcheal status). We considered model II as our main model.

For all models, assumptions of linear regression were checked. We checked multicollinearity between variables in each linear regression model by evaluating the Variance Inflation Factor (VIF < 3 was set as cut-off). Reported *p*-values are two-sided with level of significance for associations set at $p \leq 0.05$. To quantify associations, the estimated factor change in the outcome variable (β) is presented with 95% confidence interval (95% CI) for an increase in exposure from the 25th to the 75th percentile (p75/p25) for continuous variables (air pollutants) and as the estimated factor change in outcome (β) compared to the reference category for categorical data (traffic noise).

To check the robustness of our findings in adolescent boys, we performed several sensitivity analyses. We first adjusted all air pollutant models for traffic noise levels to evaluate whether the associations of air pollutants with our outcomes were influenced by traffic noise levels. Exposure to traffic noise has previously been associated with altered stress and immune responses (Daiber et al., 2020; Kim et al., 2017).

Second, we additionally adjusted our models for active smoking and passive smoking at home to verify whether the associations of air pollutants with our outcomes were independent of smoking status and passive smoking. Active and passive smoking may have an impact on the endocrine system and on immune responses (Sunyer et al., 1996; Tweed et al., 2012).

Third, we additionally adjusted all models for 3-month mean local temperature to evaluate whether the associations of air pollutants with our outcomes were influenced by variations in ambient temperature. Fluctuations in ambient temperature may have an impact on leucocyte counts and HCC (Boesch et al., 2015; Gao et al., 2019).

Leucocyte counts may be influenced by recent events, including recent exposure to air pollution. Therefore, we evaluated boys' leucocyte count in relation to recent exposure to air pollutants (2-day mean concentration prior to sampling) in single-exposure models and additionally adjusted our main air pollutant models for 2-day mean exposure to the pollutant of interest. We also performed a subgroup analysis of boys' leucocyte count in relation to long-term air pollution exposure, in which we excluded participants with recent health complaints from the analysis. In the subgroup analysis, the estimated factor change in the outcome variable (β) is presented with 95% confidence interval (95% CI) for the same factor increase in exposure as in our main analysis to enhance comparability of results.

We evaluated the role of HCC in associations between air pollutants and leucocyte counts in boys. First, associations of HCC with leucocyte counts were evaluated, adjusted for the aforementioned covariates. Next, associations between air pollutants and leucocyte counts were additionally adjusted for HCC, to evaluate the influence of chronic endocrine stress on the associations.

We evaluated all associations in girls without adjusting for menarcheal status to allow comparison of effect estimates in identical models for boys and girls.

Last, our cortisol dataset contained 3 outliers above the mean ± 3 standard deviations (SD). Such outliers in hair cortisol levels are commonly observed in healthy subjects (Binz et al., 2018; Bossé et al., 2018; Penz et al., 2018). To evaluate their influence on statistical analysis, the data points are generally winsorized to the mean ± 3 SD (Wilcox, 2005). Accordingly, we evaluated associations of air pollution and traffic noise with HCC after winsorizing the outlying data points.

3. Results

3.1. Study population and residential characteristics

Study population and residential characteristics of 395 adolescents are summarized in Table 1. Our study population included slightly more girls (53.7%) compared to boys (46.3%), but equal distribution of the sexes is approached. The mean (SD) age of the study population was 14.8 (±0.5) year for both sexes. BMI was normal for 77.6% of boys and 68.4% of girls, in line with the percentage of Flemish boys and girls (10–17 years) with a normal BMI (77.6% and 69% respectively) (Steunpunt Milieu en Gezondheid, 2020). None of the participants reported the use of systemic glucocorticoid medication. Income adequacy was perceived as difficult by almost one third of the parents (27.8%). Belgium was the country of birth for 80.8% of adolescents and parents,

Table 1
Study population characteristics (n = 395).

Characteristics	All (n = 395)		Boys (n = 183)		Girls (n = 212)	
	n	%	*n*	%	*n*	%
Adolescent						
Age						
[13.5–14.5]	105	26.6	47	25.7	58	27.4
[14.5–15.5]	259	65.6	121	66.1	138	65.1
>15.5	31	7.8	15	8.2	16	7.5
Body Mass Index[a]						
Underweight	32	8.1	15	8.2	17	8.0
Normal weight	287	72.7	142	77.6	145	68.4
Overweight, obese	76	19.2	26	14.2	50	23.6
Recent health complaints						
No	278	70.4	134	73.2	144	67.9
Yes	116	29.4	49	26.8	67	31.6
Missing	1	0.3	0	0.0	1	0.5
Active smoking						
No	377	95.4	173	94.5	208	96.2
Yes	18	4.6	10	5.5	8	3.8
Passive smoking at home						
No	353	89.4	164	89.6	189	89.2
Yes	41	10.4	19	10.4	22	10.4
Missing	1	0.3	0	0	1	0.5
Perceived income adequacy[b]						
Difficult to make ends meet	110	27.8	52	28.4	58	27.4
Rather easy to make ends meet	131	33.2	62	33.9	69	32.5
Easy to make ends meet	154	39	69	37.7	85	40.1
Country of birth						
Belgium	319	80.8	151	82.5	168	79.2
EU	32	8.1	14	7.7	18	8.5
Outside EU	44	11.1	18	9.8	26	12.3
Residence						
Area deprivation index						
0–5.3%	97	24.6	41	22.4	56	26.4
5.4–9.3%	98	24.8	38	20.8	60	28.3
9.4–15.5%	95	24.1	47	25.7	48	22.6
>15.5%	105	26.6	57	31.1	48	22.6
Traffic noise level (Lden)						
<50 dB	60	15.2	26	14.2	34	16.0
[50–55 dB]	84	21.3	44	24.0	40	18.9
[55–60 dB]	117	32.2	57	31.1	70	33.0
[60–65 dB]	77	19.5	38	20.8	39	18.4
>65 dB	74	11.9	18	9.8	29	13.7
3-month mean temperature						
<6 °C	141	35.7	60	32.8	81	38.2
6–12 °C	170	43.0	101	55.2	69	32.5
>12 °C	84	21.3	22	12.0	62	29.2

[a] BMI classes based on age- and sex-specific Belgian growth curves (www.vub.be/groeicurven/groeicurven.html) .
[b] Reported by parents. EU European Union.

this proportion reflects the general Flemish population (79.5% born in Belgium) (Steunpunt Milieu en Gezondheid, 2020). The average ADI of 12% (±8.7%) in our study population was slightly lower than the 2018 mean ADI of Flanders (14.05%) (kindengezin, 2018). The average traffic noise exposure in Lden was 57 (±6) dB, 3-month mean temperature was 8.3 (±3.6) °C. Table 2 describes the distribution of residential air pollutant concentrations. The one year mean residential concentrations of NO_2 and BC in our study population (16.5 and 0.89 μg/m^3, respectively) were slightly higher than the 2018 annual mean concentrations in Flanders (15.5 and 0.87 μg/m^3, respectively), whereas one year mean $PM_{2.5}$ and PM_{10} concentrations (11.9 and 18.2 μg/m^3) were slightly lower than the 2018 Flemish annual means (13.2 μg/m^3 for $PM_{2.5}$ and 21.2 μg/m^3 for PM_{10}) (IRCEL) (www.irceline.be). The European Union applies legally binding annual limit values for $PM_{2.5}$ (25 μg/m^3), PM_{10} (40 μg/m^3) and NO_2 (40 μg/m^3) (Héroux et al., 2015). The World Health Organization (WHO) provides health-based air quality guideline values for annual mean exposure for $PM_{2.5}$ (10 μg/m^3), PM_{10} (20 μg/m^3) and NO_2 (40 μg/m^3) (Krzyzanowski, 2008). There are no annual limit values or guideline values available for BC exposure levels. None of the participants exceeded the European annual mean limit values for NO_2, PM_{10} or $PM_{2.5}$. More than 4 out of 5 participants (83.1%) were exposed to annual mean $PM_{2.5}$ concentrations that exceeded the WHO health-based guideline; the WHO annual mean guideline for PM_{10} was exceeded by 21.1% of the participants. We observed no exceeding of the WHO guideline of 40 μg/m^3 for annual mean NO_2 concentrations. However, 21.4% of the participants exceeded an annual mean NO_2 concentration of 20 μg/m^3, a policy objective of the Flemish government by 2030 (Department of Environment & Spatial Development, n. d.). We observed significantly higher concentrations of 1-year mean BC, $PM_{2.5}$ and PM_{10} at residence for girls and significantly higher means of 3-month mean $PM_{2.5}$ and PM_{10} for boys. There were no significant differences in mean NO_2 concentrations.

Spearman rank correlations between residential outdoor air pollution, traffic noise and SES variables are presented in supplementary material, Table S1. We observed weak but significant positive correlations of all air pollutants and traffic noise with neighborhood deprivation (Spearman ρ between 0.11 and 0.33). Household SES was not significantly associated with neighborhood SES, nor with air pollution or traffic noise.

Biomarker measurements are described in Table 3. Geometric mean HCC was higher for girls than for boys (3.27 (95% CI: 2.95, 3.63) versus 2.96 (95% CI: 2.61, 3.35) pg/mg), the difference was not statistically significant ($p = 0.142$). We measured a significantly lower geometric mean leucocyte count in boys compared to girls ($p = 0.006$, 6498 (95% CI: 6262, 6742) vs. 6963 (95% CI: 6743, 7191) cells/μl), together with a significantly lower neutrophil count ($p < 0.001$, 3194 (95% CI: 3021, 3377) vs 3846 (95%CI: 3655, 4047) cells/μl). The geometric mean lymphocyte and monocyte counts did not differ significantly between boys and girls ($p = 0.073$ and $p = 0.287$ respectively). Pearson's correlations between measured biomarkers are presented in Table S2, we observed no significant correlations between HCC and leucocyte counts. The significance of associations between each potential covariate and the effect biomarkers is described in Table S3.

3.2. Associations of residential exposure to air pollutants and traffic noise with hair cortisol concentration

Sex-specific associations of residential air pollution and traffic noise with HCC are presented in Table 4. We observed significant positive associations of 3-month mean and 1-year mean NO_2 concentrations with boys' HCC after adjustment for age, BMI, household and neighborhood SES (model II, $p = 0.003$). For an increase in 3-month mean NO_2 concentration from the 25th to the 75th percentile with a factor 1.35, an increase in boys' HCC with a factor 1.21 (95% CI: 1.02, 1.43) was estimated. For a corresponding increase in 1-year mean NO_2, an increase in boys' HCC with a factor 1.30 (95% CI: 1.10, 1.54) was estimated. We observed no significant associations of BC, $PM_{2.5}$ or 3-month mean PM_{10} concentrations with HCC. 1-year mean PM_{10} concentration was significantly associated with boys' HCC ($p = 0.034$), an increase in boys' HCC with a factor 1.24 (95% CI: 1.02, 1.51) was estimated for a (p75/p25)-factor increase in 1-year mean PM_{10}. We did not observe significant associations of air pollutants with girls' HCC. Associations of air pollution with HCC are illustrated in Fig. 1. Residential traffic noise levels were not associated with HCC in boys nor girls (model II $p = 0.834$, $p = 0.404$, respectively).

3.3. Associations of residential exposure to air pollutants and traffic noise with leucocyte counts

3.3.1. Total leucocyte count

Associations of residential outdoor air pollution and traffic noise with total leucocyte counts are described in Table 5 (boys) and Table 6 (girls). In boys, 3-month mean concentrations of BC, NO_2, $PM_{2.5}$ and PM_{10} were all significantly associated with total leucocyte count after adjustment for age, BMI, household and neighborhood SES and recent

Table 2
Air pollutant exposure characteristics (μg/m^3, n = 395).

Air pollutant	ALL (n = 395)				BOYS (n = 183)				GIRLS (n = 212)				Difference by sex
	GM (95%CI)	p25	p75	p75/p25	GM (95%CI)	p25	p75	p75/p25	GM (95%CI)	p25	p75	p75/p25	*p*-value
3-Month NO_2	18.3 (17.8, 18.8)	15.8	21.6	1.37	18.7 (18.1, 19.3)	15.9	21.5	1.35	18.0 (17.2, 18.8)	15.8	21.9	1.39	0.182
1-Year NO_2	16.5 (16.1, 16.9)	13.6	19.3	1.42	16.1 (15.6, 16.7)	13.5	18.3	1.36	16.8 (16.2, 17.4)	13.7	20.3	1.48	0.134
3-Month BC	0.95 (0.93, 0.97)	0.83	1.1	1.33	0.96 (0.93, 0.99)	0.85	1.1	1.29	0.95 (0.92, 0.98)	0.8	1.11	1.39	0.574
1-Year BC	0.89 (0.87, 0.91)	0.75	1.03	1.37	0.86 (0.83, 0.89)	0.74	0.98	1.32	0.92 (0.89, 0.95)	0.76	1.06	1.39	**0.010**
3-Month $PM_{2.5}$	13.5 (13.2, 13.6)	11.1	17.4	1.57	14.1 (13.7, 14.6)	11.8	17.7	1.50	13.0 (12.6, 13.5)	10.8	16.1	1.49	**0.001**
1- Year $PM_{2.5}$	11.9 (11.7, 12.1)	10.6	13.1	1.24	11.5 (11.3, 11.8)	10.3	12.9	1.25	12.2 (11.9, 12.4)	10.9	13.3	1.22	**0.001**
3-Month PM_{10}	19.8 (19.3, 20.2)	16.6	25.5	1.54	20.6 (19.9, 21.4)	17.5	25.9	1.48	19.1 (18.5, 19.6)	16	23.5	1.47	**0.001**
1-Year PM_{10}	18.2 (18.0, 18.4)	16.2	19.8	1.22	17.9 (17.5, 18.3)	15.8	19.7	1.25	18.4 (18.1, 18.8)	16.5	20	1.21	**0.036**

Abbreviations: BC black carbon, NO_2 nitrogen dioxide, $PM_{2.5}$ and PM_{10} particulate matter with an aerodynamic diameter smaller than 2.5 and 10 μm respectively, GM geometric mean, p25 –p75 25th and 75th percentile. Statistically significant differences ($p \leq 0.05$) in mean air pollutant concentrations by sex in independent samples *t*-tests are highlighted in bold.

Table 3
Biomarker characteristics (n = 395).

Biomarker	All (n = 395)			Boys (n = 183)			Girls (n = 212)			Difference by sex
	GM (95% CI)	p25	p75	GM (95% CI)	p25	p75	GM (95% CI)	p25	p75	*p*-value
Hair cortisol (pg/mg hair)	3.12 (2.88, 3.38)	2.10	4.32	2.96 (2.61, 3.35)	1.90	3.88	3.27 (2.95, 3.63)	2.34	4.62	0.124
Leucocytes (cells/μl)[a]	6740 (6577, 6903)	5653	7860	6498 (6262, 6742)	5390	7675	6963 (6743, 7191)	5943	8105	**0.006**
Neutrophils (cells/μl)[a]	3523 (3389, 3661)	2757	4617	3194 (3021, 3377)	2474	4132	3846 (3655, 4047)	3071	4911	**<0.001**
Lymphocytes (cells/μl)[a]	2248 (2193, 2305)	2280	2618	2303 (2223, 2385)	1970	2630	2200 (2124, 2280)	1918	2606	0.287
Monocytes (cells/μl)[a]	560 (544, 576)	452	685	569 (546, 593)	462	694	551 (529, 575)	442	678	0.073

Leucocyte data available for 385 adolescents, of which 182 boys and 203 girls. GM geometric mean, p25 – p75 25th and 75th percentile. Statistically significant differences ($p \leq 0.05$) in biomarker concentrations by sex in independent samples *t*-tests are highlighted in bold.

health complaints (model II, $p = 0.004$, p = 0.047, $p = 0.023$ and $p = 0.026$ respectively). For an increase in 3-month mean BC from the 25th to the 75th percentile with a factor 1.29, an increase in boys' leucocyte count with a factor 1.08 (95% CI: 1.02, 1.13) was estimated. For an increase in 3-month mean NO_2 from the 25th to the 75th percentile, an increase with a factor 1.05 (95% CI: 1.00, 1.10) was estimated. For 3-month mean $PM_{2.5}$ and PM_{10}, respective increases in boys' leucocyte count with a factor 1.08 (95% CI: 1.01, 1.15) and 1.07 (95% CI: 1.01, 1.14) were estimated. Furthermore, 1-year mean concentrations of BC, NO_2 and PM_{10} were significantly associated with boys' total leucocyte count (model II, $p = 0.006$, p = 0.036, $p = 0.028$ respectively), the association of 1-year mean $PM_{2.5}$ with boys' leucocyte count was borderline significant ($p = 0.076$). The estimated increase in boys' leucocyte count for an increase in 1-year mean air pollutant concentrations from the 25th to 75th percentile ranged from 1.05 (95% CI: 0.99, 1.12) for $PM_{2.5}$ to 1.07 (95% CI: 1.02, 1.12) for BC. In girls, we observed no significant associations of air pollutants with leucocyte count. Associations of air pollution with leucocyte count are illustrated in Fig. 2. Traffic noise levels were not significantly associated with total leucocyte count in boys nor girls.

3.3.2. Neutrophil, monocyte and lymphocyte count

Associations of residential outdoor air pollution and traffic noise with leucocyte subtype counts are described in Table 5 (boys) and Table 6 (girls) and illustrated in Fig. 3. In boys, 3-month mean concentrations of BC, $PM_{2.5}$ and PM_{10} were significantly associated with neutrophil count (model II $p = 0.002$, $p = 0.014$, $p = 0.025$ respectively). The association of 3-month mean NO_2 with neutrophil count was borderline significant (Model II $p = 0.064$). An increase in boys' neutrophil count with a factor 1.13 (95% CI: 1.05, 1.22) was estimated for an increase in 3-month mean BC from the 25th to 75th percentile. For corresponding increases in 3-month mean NO_2, $PM_{2.5}$ and PM_{10}, increases in neutrophil count with a factor 1.07 (95% CI: 1.00, 1.15), 1.13 (95% CI: 1.03, 1.25) and 1.11 (95% CI: 1.01, 1.22) were estimated. Similar associations were observed for 1-year mean BC, NO_2, $PM_{2.5}$ and PM_{10} with neutrophil count in boys (model II, $p = 0.006$, $p = 0.054$, $p = 0.025$, $p = 0.023$ respectively). The estimated increase in boys' neutrophil count for a 25th to p75th percentile factor increase in 1-year means was 1.11 (95% CI: 1.03, 1.19) for BC, 1.08 (95% CI: 1.00, 1.16) for NO_2, 1.10 (95% CI: 1.01, 1.19) for $PM_{2.5}$ and 1.10 (95% CI: 1.01, 1.20) for PM_{10}. In girls, we observed no significant associations of air pollution with neutrophil count. Traffic noise levels were not significantly associated with neutrophil count in boys nor girls.

We found no significant associations of residential exposure to air pollutants or residential traffic noise levels with monocyte count.

In boys, we observed significant associations of 3-month mean BC and NO_2 with lymphocyte count (model II, $p = 0.037$, $p = 0.042$). The estimated increase in boys' lymphocyte count for a 25th to 75th percentile increase in 3-month mean air pollutant concentrations was 1.06 (95% CI: 1.00, 1.11) for BC and 1.05 (95% CI: 1.00, 1.10) for NO_2. We observed similar associations of 1-year mean BC and NO_2 concentrations with lymphocyte count (Model II, $p = 0.052$, $\beta = 1.05$ (95% CI: 1.00, 1.10) and $p = 0.042$, $\beta = 1.05$ (95%CI: 1.01, 1.10)). Associations of 3-month mean $PM_{2.5}$ and PM_{10} with lymphocyte count were borderline significant (model II, $p = 0.092$, $p = 0.064$ respectively), associations of 1-year mean $PM_{2.5}$ and PM_{10} with lymphocyte count were not significant (model II, $p = 0.614$, $p = 0.105$ respectively).

Table 4
Associations of residential air pollutant concentrations and traffic noise levels with HCC in adolescent boys (n = 183) and girls (n = 212).

Exposure variable	BOYS (n = 183)		GIRLS (n = 212)	
	Model I	Model II	Model I	Model II
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Air pollutant				
3-Month BC	1.19 (1.00, 1.42)	1.14 (0.95, 1.38)	0.98 (0.85, 1.13)	1.00 (0.86, 1.16)
1-Year BC	1.18 (1.00, 1.38)	1.14 (0.96, 1.35)	1.06 (0.92, 1.22)	1.09 (0.92, 1.28)
3-Month NO_2	**1.23 (1.05, 1.46)**	**1.21 (1.02, 1.43)**	0.98 (0.88, 1.09)	0.99 (0.89, 1.11)
1-Year NO_2	**1.34 (1.13, 1.59)**	**1.30 (1.10, 1.54)**	1.08 (0.93, 1.26)	1.11 (0.94, 1.31)
3-Month $PM_{2.5}$	1.10 (0.87, 1.39)	1.13 (0.89, 1.43)	0.92 (0.78, 1.10)	0.93 (0.77, 1.12)
1-Year $PM_{2.5}$	1.10 (0.91, 1.33)	1.13 (0.93, 1.39)	1.00 (0.86, 1.15)	0.96 (0.70, 1.33)
3-Month PM_{10}	1.10 (0.89, 1.36)	1.14 (0.91, 1.42)	0.95 (0.78, 1.17)	0.98 (0.81, 1.19)
1-Year PM_{10}	1.19 (0.98, 1.45)	**1.24 (1.02, 1.51)**	0.99 (0.85, 1.15)	0.98 (0.82, 1.16)
Traffic noise level				
<50 dB	reference	reference	reference	reference
50–55 dB	1.25 (0.82, 1.91)	1.18 (0.77, 1.81)	0.96 (0.68, 1.37)	0.95 (0.67, 1.36)
55–60 dB	1.24 (0.83, 1.80)	1.21 (0.79, 1.83)	1.10 (0.80, 1.51)	1.14 (0.82, 1.58)
60–65 dB	1.15 (0.74, 1.78)	1.07 (0.69, 1.68)	1.27 (0.89, 1.80)	1.31 (0.91, 1.88)
>65 dB	1.42 (0.84, 2.41)	1.31 (0.76, 2.24)	1.18 (0.80, 1.73)	1.20 (0.81, 1.79)

Significant associations (p-value ≤ 0.05) are highlighted in bold. Model I adjusted for age. Model II adjusted for age, BMI, household SES and neighborhood SES, model including girls additionally adjusted for menarcheal status. Effect estimates (β) are presented with their 95% confidence intervals (95% CI) as a factor increase in HCC compared to the reference category and as a factor increase in HCC for a (p75/p25)-factor increase in air pollutant concentration. Abbreviations: BC black carbon, NO_2 nitrogen dioxide, $PM_{2.5}$ and PM_{10} particulate matter with an aerodynamic diameter smaller than 2.5 and 10 μm respectively, Db decibels.

Lymphocyte count in girls was not significantly related to air pollutants. We found no significant associations of traffic noise with lymphocyte count in boys nor girls.

3.4. Sensitivity analysis

As presented in Table S4, associations of air pollutants with HCC and leucocyte counts in boys remained robust after additional adjustment for traffic noise, for active and passive smoking at home, and for 3-month mean local temperature. Results of additional adjustments were generally similar to the main analyses in terms of strength of the association and direction.

We evaluated the impact of recent events on associations between long-term exposure to air pollutants and leucocyte counts. We observed no significant associations of recent exposure to air pollutants (2-day mean concentration prior to sampling) with leucocyte counts (results not shown). In addition, we evaluated the influence of recent exposure to air pollutants on associations of long-term air pollutant concentrations with boys' leucocyte counts in combined exposure models (Table S5). The additional adjustment was of little influence on significance and strength of the association. Second, in a subgroup analysis (n = 133), boys that reported a recent health complaint were excluded. As presented in Table S6, we observed slightly stronger positive associations of all air pollutants with leucocyte and neutrophil counts in the health complaint-free subgroup. The subgroup analysis was of little influence on significance and strength of associations between air pollutants and monocyte or lymphocyte counts. In line with our main findings, we did not observe significant associations of air pollutants with leucocyte counts in girls (n = 137, data not shown).

We assessed the influence of 3-month mean HCC on associations between long-term exposure to air pollutants and leucocyte counts (Table S7). We observed no significant associations of boys' HCC with total and differential leucocyte counts in models adjusted for age, BMI, household and neighborhood SES. Additional adjustment of associations in boys between long-term exposure to air pollutants and leucocyte counts for HCC was of minimal influence on the estimated strength of the associations.

Winsorizing outlying cortisol data points was of little influence on the significance and on the observed strength of associations (Table S8). Not adjusting our statistical models in girls for menarcheal status (Table S9) was of little influence on the strength and direction of associations.

4. Discussion

This study in a population-based sample of Flemish adolescents provides new insights into associations of residential exposure to air pollution and traffic noise with chronic endocrine stress and inflammation. The premise that endocrine stress-related pathways may be among the early biological changes, triggered by air pollution exposure, has only emerged in the past decade. Existing research has mostly focused on associations of short-term air pollution exposure with acute salivary or blood cortisol levels. Importantly, chronic endocrine stress during adolescence can lead to chronic physical and mental conditions (Chrousos, 2009). Given the continuous, mostly involuntary, exposure of adolescents to air pollution and traffic noise and the associated adverse health effects, identifying early pathophysiological changes in this vulnerable population subgroup is of public health importance. In accordance with our hypothesis, we found positive associations of long-term residential exposure to NO_2 and PM_{10} with hair cortisol concentration in boys. Simultaneously, long-term residential concentrations of all modelled air pollutants (BC, NO_2, $PM_{2.5}$ and PM_{10}) were positively associated with total leucocyte count in boys. In particular, we observed positive associations of BC, $PM_{2.5}$ and PM_{10} with neutrophil count, associations of NO_2 with neutrophil count were borderline significant, and positive associations of BC and NO_2 with lymphocyte count. All the observed associations were independent of age, BMI, household and neighborhood SES and remained robust after additional adjustment for traffic noise, smoking (active and passive) and 3-month mean ambient temperature. Recent exposure to air pollutants was of little influence on the associations between long-term exposure and boys' leucocyte counts. Our results did not support an influence of chronic endocrine stress on associations of air pollutants with leucocyte counts in adolescents. In girls, air pollutants were not associated with measured biomarkers. Residential traffic noise levels were not significantly associated with HCC or leucocyte counts in boys nor girls.

4.1. Heterogeneity of results by sex

In our study population of 14-15-year-old adolescents, we observed significant heterogeneity of our results by sex. The reasons for these differences in associations of air pollution with stress and immune responses among adolescent boys and girls are probably multifactorial. Previous studies have reported sex-specific associations of chemical exposures and air pollution exposure with gene expression and biological pathways (De Coster et al., 2013; Vrijens et al., 2017). Sex-specific associations of air pollutants with inflammatory biomarkers have also been reported. Among 4814 German adults, small increases in annual mean $PM_{2.5}$ (3.9 $\mu g/m^3$) were associated with increases in high-sensitivity C-reactive protein and in fibrinogen among men, but not among women (Hoffmann et al., 2009). Moreover, sex-dependent responsivity of the HPA axis during adolescence has previously been demonstrated (Ordaz and Luna, 2012). The rise in sex hormones during

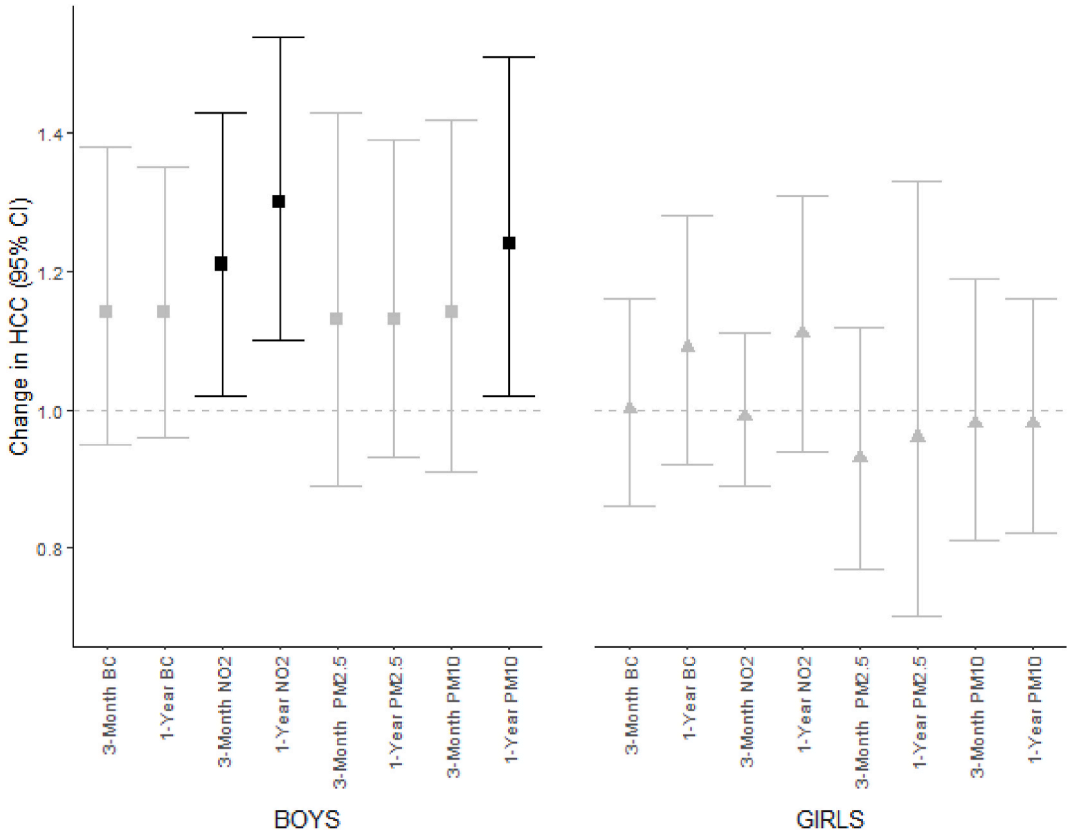

Fig. 1. Associations of air pollutants with HCC in adolescent boys (n = 183, squares) and girls (n = 212, triangles). Associations in boys are adjusted for age, BMI, household and neighborhood socioeconomic status and additionally adjusted for menarcheal status in girls. Estimates and their 95% confidence intervals are presented as the factor change in HCC for a (p75/p25)-factor increase in air pollutant concentration. Significant associations are marked in bold.

pubertal maturation of boys and girls may contribute to differential immune and stress responses (Klein and Flanagan, 2016; Naninck et al., 2011). Possibly, differences in exposure to air pollutants between boys and girls may have contributed to our findings. We observed significant differences in residential exposure to air pollution by sex. However, air pollutants that were associated with boys' HCC, i.e., 3-month and 1-year mean NO_2, 1-year mean PM_{10} were not significantly higher for boys compared to girls. The statistically higher exposure levels for boys with regard to 3-month mean $PM_{2.5}$ and PM_{10} may have contributed to stronger associations between these air pollutants, leucocyte and neutrophil counts in boys. However, significant associations of air pollutants with leucocyte and neutrophil counts were also observed exclusively in boys for air pollutants of which the means were significantly higher in girls, i.e., 1-year mean BC, $PM_{2.5}$ and PM_{10}. Taken together, these findings suggest that differences in mean residential exposure levels are of minor influence on the observed associations.

4.2. Residential exposures and hair cortisol concentration

A growing body of literature has linked exposure to air pollutants with endocrine stress pathways (Hajat et al., 2019; Snow et al., 2018; Thomson, 2019). Snow et al. (2018) only recently postulated that upon encountering pollutants, vagal sensory neurons in the respiratory tract may induce a generalized neural stress response, including HPA axis activation. Moreover, air pollutants may migrate via olfactory transport and directly impact the brain, including the hypothalamus (Calderón-Garcidueñas et al., 2015). A direct link between short-term exposure to concentrated ambient particles and acute HPA activation was first demonstrated in rodent studies (Sirivelu et al., 2006; Thomson et al., 2013) and later confirmed in observational studies in humans (Jia et al., 2018; Li et al., 2017; Niu et al., 2018). However, few studies have investigated chronic endocrine stress in relation to air pollution exposure. Given the important role of chronic endocrine stress in several chronic health conditions, understanding the link between air pollution and long-term cortisol secretion is even more relevant to public health. The findings presented in this study contribute to the existing literature by filling this knowledge gap in a general population of adolescents. Our results indicate positive associations of long-term exposure to NO_2 and PM_{10} with chronic stress levels in adolescent boys, not in girls. Our findings in adolescent boys are in line with emerging literature. In a cross-sectional analysis in 1793 adults from the MESA study, 1-year mean residential NO_2 exposure was associated with higher wake-up salivary cortisol (Hajat et al., 2019). In Flemish schoolchildren (7–12 years), residential 1-year mean BC and $PM_{2.5}$ were not significantly associated with HCC (Van Aart et al., 2018). However, adolescents may be more sensitive to stressors than children (Sheth et al., 2017). In IPANEMA, a Flemish urban pregnancy cohort study, we observed a positive association between long-term residential NO_2 and BC concentrations (3-months, 1-year) and HCC in the third pregnancy trimester (Verheyen et al., 2021). Similar to adolescence, pregnancy is known as a period of enhanced physiological responses to different types of stressors, including psychosocial stressors, physical stressors (nutritional deficiencies, fatigue, illness) and chemical stressors (environmental pollution) (González-Ochoa et al., 2018).

In our study population, residential exposure to traffic noise was not associated with HCC. Traffic noise has been associated with increased cortisol levels in adults (Daiber et al., 2020); studies on the association of traffic noise with cortisol levels in adolescents are sparse. Our results are in line with results of the IPANEMA study in which no significant associations of residential exposure to traffic noise with HCC were observed in pregnant women (Verheyen et al., 2021). Furthermore, a study in Swedish adolescents found no associations between residential

Table 5
Associations of residential exposure to air pollution and traffic noise with white blood cell counts in boys (n = 182).

Exposure	Leucocytes		Neutrophils		Monocytes		Lymphocytes	
	Model I	Model II	Model I	Model II	Model I	Model II	Model I	Model II
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Air pollutant								
3-Month BC	**1.10 (1.05, 1.16)**	**1.08 (1.02, 1.13)**	**1.16 (1.08, 1.25)**	**1.13 (1.05, 1.22)**	1.02 (0.96, 1.08)	1.00 (0.94, 1.06)	**1.06 (1.01, 1.12)**	**1.06 (1.00, 1.11)**
1-Year BC	**1.09 (1.04, 1.14)**	**1.07 (1.02, 1.12)**	**1.14 (1.06, 1.22)**	**1.11 (1.03,1.19)**	1.02 (0.97, 1.08)	1.01 (0.96, 1.07)	**1.05 (1.00, 1.10)**	1.05 (1.00, 1.10)
3-Month NO_2	**1.07 (1.02, 1.12)**	**1.05 (1.00, 1.10)**	**1.09 (1.02, 1.17)**	1.07 (1.00, 1.15)	1.02 (0.97, 1.08)	1.01 (0.95, 1.07)	**1.07 (1.02, 1.12)**	**1.05 (1.01, 1.10)**
1-Year NO_2	**1.08 (1.03, 1.14)**	**1.06 (1.01, 1.11)**	**1.11 (1.03, 1.20)**	1.08 (1.00, 1.16)	1.03 (0.97, 1.09)	1.02 (0.96, 1.08)	**1.06 (1.01, 1.12)**	**1.05 (1.01, 1.10)**
3-Month $PM_{2.5}$	**1.07 (1.00, 1.15)**	**1.08 (1.01, 1.15)**	**1.11 (1.00, 1.23)**	**1.13 (1.03, 1.25)**	1.04 (0.96, 1.12)	1.04 (0.96, 1.12)	1.06 (1.00, 1.13)	1.06 (0.99, 1.13)
1-Year $PM_{2.5}$	1.05 (0.99, 1.11)	1.05 (0.99, 1.11)	**1.10 (1.01, 1.20)**	**1.10 (1.01, 1.19)**	0.98 (0.93, 1.05)	0.99 (0.93, 1.06)	1.01 (0.96, 1.07)	1.01 (0.96, 1.07)
3-Month PM_{10}	1.06 (0.99, 1.13)	**1.07 (1.01, 1.14)**	1.09 (0.98, 1.21)	**1.11 (1.01, 1.22)**	1.03 (0.96, 1.11)	1.04 (0.97, 1.12)	1.06 (1.00, 1.13)	1.06 (1.00, 1.13)
1-Year PM_{10}	**1.06 (1.00, 1.12)**	**1.06 (1.01, 1.13)**	**1.08 (1.00, 1.17)**	**1.10 (1.01, 1.20)**	1.01 (0.95, 1.08)	1.02 (0.95, 1.09)	1.05 (0.99, 1.11)	1.05 (0.99, 1.11)
Traffic noise								
<50 dB	reference	reference	reference	reference	reference	reference	reference	reference
50–55 dB	1.07 (0.94, 1.20)	1.05 (0.93, 1.18)	1.01 (0.84, 1.21)	0.98 (0.82, 1.17)	1.11 (0.97, 1.28)	1.09 (0.95, 1.16)	1.15 (1.03, 1.29)	1.15 (1.02, 1.29)
55–60 dB	1.09 (0.97, 1.23)	1.07 (0.95, 1.20)	1.12 (0.93, 1.33)	1.08 (0.90, 1.29)	1.14 (1.01, 1.30)	1.12 (0.97, 1.28)	1.05 (0.94, 1.17)	1.04 (0.93, 1.17)
60–65 dB	1.16 (1.02, 1.31)	1.10 (0.97, 1.25)	1.21 (1.00, 1.46)	1.13 (0.93, 1.37)	1.11 (0.96, 1.28)	1.07 (0.92, 1.24)	1.15 (1.02, 1.29)	1.12 (0.98, 1.27)
>65 dB	1.16 (0.99, 1.35)	1.09 (0.94, 1.27)	1.14 (0.91, 1.44)	1.06 (0.84, 1.33)	1.09 (0.92, 1.30)	1.05 (0.88, 1.25)	1.18 (1.02, 1.36)	1.15 (0.98, 1.33)

Significant associations (*p*-value ≤ 0.05) are highlighted in bold. Model I adjusted for age. Model II including boys adjusted for age, BMI, household SES and neighborhood SES, recent health complaints Effect estimates (β) are presented with their 95% confidence intervals (95% CI) as a factor increase in leucocyte counts compared to the reference category and as a factor increase in leucocyte counts for a (p75/p25)-factor increase in air pollutant concentration. Abbreviations: BC black carbon, NO_2 nitrogen dioxide, $PM_{2.5}$ and PM_{10} particulate matter with an aerodynamic diameter smaller than 2.5 and 10 μm respectively, dB decibel.

Table 6
Associations of residential exposure to air pollution and traffic noise with white blood cell counts in adolescent girls (n = 204).

Exposure	Leucocytes		Neutrophils		Monocytes		Lymphocytes	
	Model I	Model II	Model I	Model II	Model I	Model II	Model I	Model II
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Air pollutant								
3-Month BC	0.99 (0.95, 1.03)	0.99 (0.95, 1.04)	0.98 (0.92, 1.05)	0.98 (0.90, 1.06)	0.97 (0.92, 1.03)	0.97 (0.91, 1.03)	0.99 (0.94, 1.04)	1.00 (0.94, 1.05)
1-Year BC	1.00 (0.96, 1.04)	1.01 (0.96, 1.06)	1.00 (0.94, 1.07)	1.02 (0.94, 1.11)	1.00 (0.95, 1.06)	1.01 (0.95, 1.08)	0.96 (0.91, 1.00)	0.96 (0.92, 1.02)
3-Month NO_2	0.99 (0.96, 1.02)	0.98 (0.95, 1.02)	0.99 (0.94, 1.04)	0.97 (0.92, 1.03)	0.97 (0.93, 1.01)	0.96 (0.91, 1.00)	1.00 (0.96, 1.04)	1.00 (0.96, 1.04)
1-Year NO_2	0.99 (0.95, 1.04)	1.00 (0.95, 1.05)	1.00 (0.93, 1.08)	1.01 (0.93, 1.10)	0.97 (0.92, 1.03)	0.97 (0.90, 1.03)	0.96 (0.91, 1.01)	0.96 (0.91, 1.03)
3-Month $PM_{2.5}$	0.99 (0.94, 1.05)	0.99 (0.93, 1.05)	0.96 (0.88, 1.06)	0.96 (0.87, 1.05)	0.98 (0.91, 1.05)	0.98 (0.91, 1.06)	1.05 (0.98, 1.12)	1.06 (0.99, 1.13)
1-Year $PM_{2.5}$	0.99 (0.95, 1.04)	1.00 (0.95, 1.05)	0.98 (0.91, 1.05)	1.00 (0.92, 1.08)	1.00 (0.95, 1.06)	1.02 (0.95, 1.11)	0.96 (0.91, 1.01)	0.97 (0.92, 1.03)
3-Month PM_{10}	0.99 (0.94, 1.04)	1.00 (0.94, 1.07)	0.97 (0.88, 1.06)	0.97 (0.88, 1.08)	0.99 (0.92, 1.07)	1.01 (0.93, 1.09)	1.04 (0.97, 1.11)	1.05 (0.98, 1.13)
1-Year PM_{10}	0.98 (0.94, 1.04)	1.01 (0.95, 1.07)	0.98 (0.91, 1.06)	1.00 (0.92, 1.10)	1.01 (0.95, 1.08)	1.04 (0.97, 1.12)	0.96 (0.91, 1.02)	0.98 (0.92, 1.04)
Traffic noise								
<50 dB	Reference	reference	reference	reference	reference	reference	reference	reference
50–55 dB	0.98 (0.88, 1.09)	0.98 (0.87, 1.10)	0.97 (0.81, 1.16)	0.97 (0.81, 1.17)	1.03 (0.90, 1.20)	1.04 (0.90, 1.20)	0.95 (0.84, 1.07)	0.94 (0.83, 1.07)
55–60 dB	0.92 (0.84, 1.02)	0.95 (0.85, 1.06)	0.97 (0.82, 1.14)	0.98 (0.83, 1.16)	0.96 (0.84, 1.09)	0.96 (0.83, 1.09)	0.89 (0.80, 0.99)	0.88 (0.79,1.02)
60–65 dB	0.99 (0.89, 1.10)	0.99 (0.88, 1.11)	1.01 (0.85, 1.21)	1.01 (0.84, 1.22)	1.02 (0.89, 1.18)	1.01 (0.87, 1.17)	0.94 (0.83, 1.06)	0.95 (0.84, 1.07)
>65 dB	0.99 (0.88, 1.11)	1.00 (0.88, 1.14)	0.99 (0.81, 1.19)	1.00 (0.81, 1.22)	0.98 (0.84, 1.15)	0.98 (0.83, 1.15)	0.94 (0.82, 1.07)	0.93 (0.81, 1.06)

Significant associations (*p*-value ≤ 0.05) are highlighted in bold. Model I adjusted for age. Model including boys adjusted for age, BMI, household SES and neighborhood SES, recent health complaints; model including girls additionally adjusted for menarcheal status. Effect estimates (β) are presented with their 95% confidence intervals (95% CI) as a factor increase in leucocyte counts compared to the reference category and as a factor increase in leucocyte counts for a (p75/p25)-factor increase in air pollutant concentration. Abbreviations: BC black carbon, NO_2 nitrogen dioxide, $PM_{2.5}$ and PM_{10} particulate matter with an aerodynamic diameter smaller than 2.5 and 10 μm respectively, dB decibel.

exposure to traffic noise and saliva cortisol levels (Wallas et al., 2018). The researchers in this study found indications that associations between noise and stress in adolescents may be associated with noise annoyance rather than noise levels.

4.3. Residential exposures and differential leucocyte count

A vast body of literature has pointed towards systemic inflammation as a key biological mechanism, underlying adverse health effect of air pollutant exposure (Brook et al., 2018; Newby et al., 2015). However, there is still a lack of epidemiological evidence in adolescents (Gao et al.,

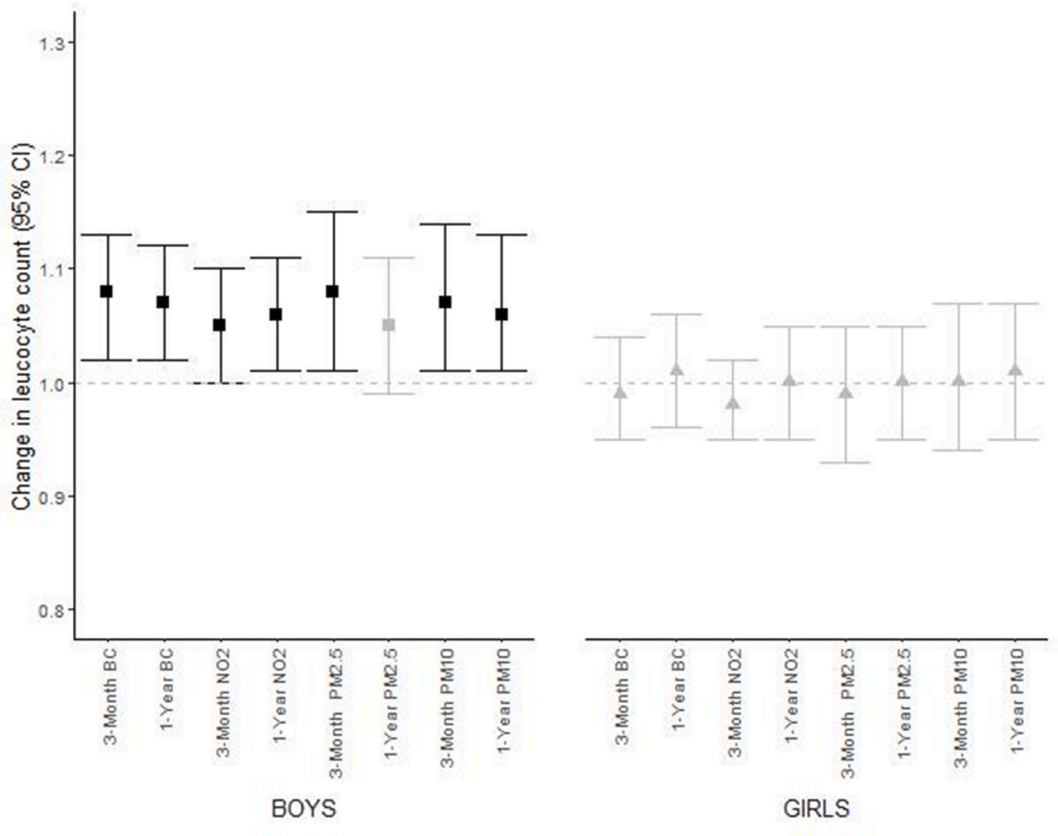

Fig. 2. Associations of air pollutants with leucocyte count in adolescent boys (n = 182, squares) and girls (n = 203, triangles). Associations in boys are adjusted for age, BMI, household and neighborhood socioeconomic status, recent health complaints and additionally adjusted for menarcheal status in girls. Estimates are presented with their 95% confidence intervals as the factor change in leucocyte count for a (p75/p25)-factor increase in air pollutant concentration. Significant associations are marked in bold.

2019), especially regarding long-term exposure. Our results contribute to existing literature by demonstrating that long-term residential exposure to all air pollutants (BC, NO_2, $PM_{2.5}$ and PM_{10}) was positively associated with total leucocyte count in boys, not in girls. In particular, we observed positive associations of long-term residential exposure to BC, $PM_{2.5}$ and PM_{10} with boys' neutrophil count, associations of NO_2 with neutrophil count were borderline significant in our main analysis and significant in a subgroup analysis of boys without recent health complaints. We also observed positive associations between long-term residential exposure to BC and NO_2 and lymphocyte count. Neutrophils, the most abundant leucocytic subtype, are of particular importance when considering air pollution pathogenesis, given their ability to release oxidant producing enzymes (Anderson et al., 2012). The presence of these oxidants in neutrophils means that transition metals, such as those present within PM, can further catalyze oxidative damage to biological systems; this functionality positions neutrophils as a significant threat to healthy tissue (Delfino et al., 2011). Lymphocytes, with B cells and T cells as the two main subtypes, are the second most abundant leucocyte subtype, and play an important role in immune responses and inflammation by producing cytokines, that help to model the adaptive immune response (Gasteiger and Rudensky, 2014). Lymphocytes can generate highly-effective immune responses or can, on occasion, cause inappropriate immune activation leading to pathology such as autoimmune conditions (Glencross et al., 2020).

Our findings in adolescent boys are in line with experimental and epidemiological literature. After exposing rodents to repeated inhalation of BC over a period of 90 days, leucocyte and neutrophil counts were significantly increased (Chu et al., 2019). Controlled exposure studies in healthy adults have demonstrated that short-term exposure to $PM_{2.5}$ and ambient urban air can induce significant alterations in total leucocyte count, neutrophils and lymphocytes in healthy subjects (Frampton et al., 2004; Salvi et al., 1999; Steenhof et al., 2014). Epidemiological studies with leucocyte counts as a metric are more scarce, due to the need for analysis in fresh blood samples (Gao et al., 2019). In the Third National Health and Nutrition Examination Survey (NHANES), a positive association between estimated long-term 1-year exposure to PM_{10} and leucocyte count in adults was observed (Chen and Schwartz, 2008). A German study in 4814 participants, however, found no significant associations of leucocyte count with long-term exposure to PM (Viehmann et al., 2015). In the Social Environment and Biomarkers of Aging Study (Taiwan) among the elderly, 1-year mean $PM_{2.5}$, PM_{10} and NO_2 were positively associated with neutrophil count (Chuang et al., 2011). In adolescents, short-term air pollution exposure has previously been linked to higher leucocyte, neutrophil and monocyte count (Gao et al., 2019; Poursafa et al., 2011; Prunicki et al., 2020; Zhang et al., 2013). Previous research in China also observed significantly higher monocyte counts in 13–14 year old adolescents that lived in a highly polluted area compared to adolescents from a control area (Li et al., 2019). We did not observe significant associations of air pollutants with monocytes. However, air pollution exposure levels were not known in the Chinese study, levels in the highly polluted area may have exceeded the Flemish levels. Monocytes are white blood cells of the innate immune system and play a role in inflammation (Prunicki et al., 2020). To the best of our knowledge, results of epidemiological studies on associations of long-term exposure to air pollution and differential leucocyte counts in adolescents have not yet been reported.

In this study, residential exposure to traffic noise was not associated with leucocyte counts. To our knowledge, the association between traffic noise exposure and leucocyte counts in adolescents has not been evaluated previously. It has been hypothesized that continuous exposure

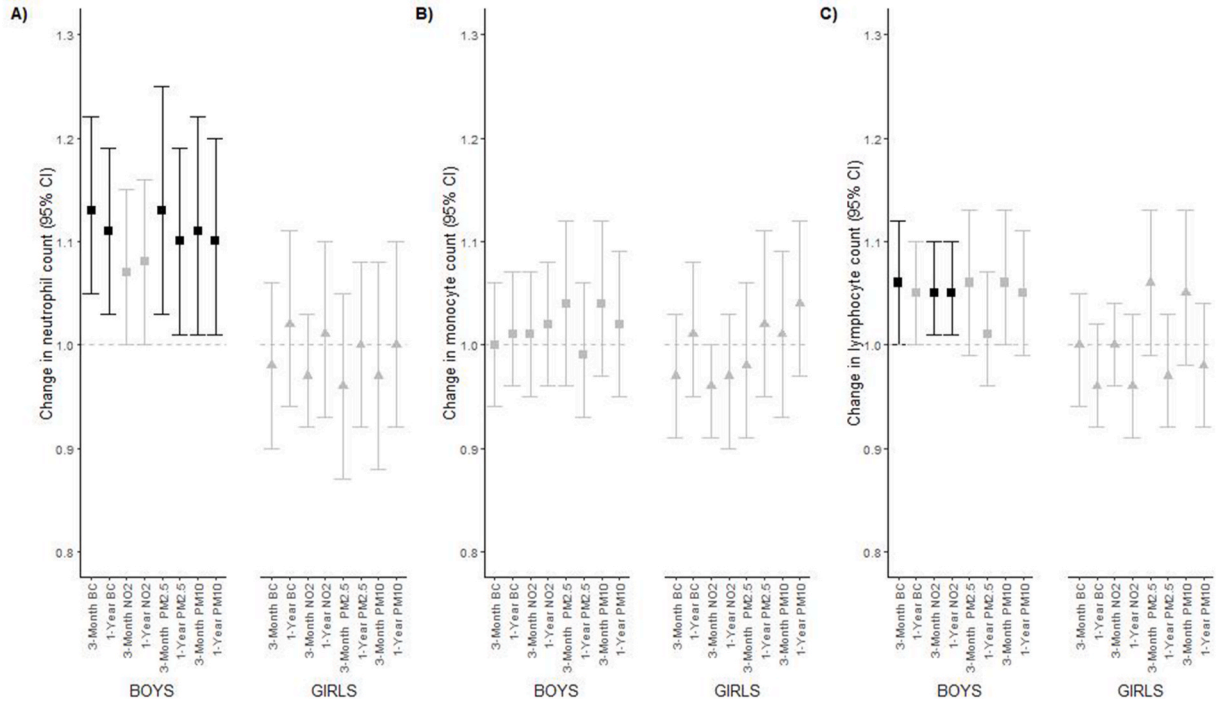

Fig. 3. Associations of air pollutants with neutrophil, monocyte and lymphocyte count in adolescent boys (n = 182, squares) and girls (n = 203, triangles). Associations in boys are adjusted for age, BMI, household and neighborhood socioeconomic status, recent health complaints and additionally adjusted for menarcheal status in girls. Estimates are presented with their 95% confidence intervals as the factor change in neutrophil (A), monocyte (B) and lymphocyte (C) count for a (p75/p25)-factor increase in air pollutant concentration. Significant associations are marked in bold.

to noise may have an effect on immune function through increased cortisol levels and through an increase in the levels of interleukin-12 (IL-12), a pro-inflammatory cytokine (Kim et al., 2017). However, IL-12 acts on neutrophils through stimulation of phagocytic and microbicidal activities, rather than neutrophil recruitment per se (Moreno et al., 2006). Furthermore, research indicates that cortisol is more strongly affected by sensitivity to noise than by road traffic noise levels (Kim et al., 2017; Wallas et al., 2018).

In literature, a direct contribution of air-pollution induced cortisol secretion to inflammation has been postulated (Hajat et al., 2019). When stress hormones were depleted by adrenalectomy in rats, air pollution-induced inflammation and lung injury was diminished (Snow et al., 2018). Our findings did not show significant associations of leucocyte counts with cortisol secretion in the 3-month period prior to sampling. Given the complex relation between the endocrine and immune system, interactions between the two systems through different immune cells (e.g., cytokines), cannot be ruled out (Clougherty et al., 2010).

4.4. Strengths and limitations

Our study has several strengths. The added value of a human biomonitoring study, a form of molecular epidemiology, is the possibility to examine early pathophysiological mechanisms, triggered by environmental exposure, in real-world settings, before the onset of disease. The number of people affected by air pollution-induced diseases is only the tip of the iceberg, compared to those affected by sub-clinical physiological changes that could also be of major public health importance (Van Brusselen et al., 2016). Molecular epidemiology has a greater sensitivity than classical epidemiology in terms of detecting the link between exposures and chronic diseases (Vineis and Perera, 2007). Whereas most air pollution health studies have focused on adults, FLEHS-4 included adolescents, a population subgroup that is known to be more vulnerable to both air pollution exposure and stress (Braga et al., 2001; Sheth et al., 2017). FLEHS-4 recruited a representative sample of Flemish adolescents (14–15 year), which suggests that our results are representative for Flemish adolescents within that age range. We collected detailed information through extensive questionnaires, allowing us to consider potential confounding factors. We had information on residential mobility and were able to limit our study population to participants that did not move in the year prior to sampling. In our study, a small team of trained nurses collected all samples, following a strict protocol to ensure low variability in sampling method across our study population. In the past decade, the cortisol concentration in scalp hair has been validated as a biomarker of long-term HPA-axis activity (Iob and Steptoe, 2019). Sampling is easily conducted and non-invasive, a single hair sample retrospectively captures several months of cortisol secretion (Binz et al., 2016). The use of HCC in epidemiological research is still novel. Traditional assessment of HPA axis function through measurement of cortisol levels in saliva, blood or urine, reflects cortisol concentrations over minutes to 24-h prior to sampling. Obtaining valid information on long-term cortisol secretion is difficult using these matrices (Iob and Steptoe, 2019). We used a sensitive LC-MS/MS method to measure hair cortisol, as recommended in literature (Binz et al., 2018).

Some limitations of the study need to be addressed. Our adolescent study population comprised a narrow age range. Results may therefore only be representative for this age group and further studies looking at different age groups are warranted. Furthermore, pubertal developmental may differ greatly between boys and girls at 14–15 years, the onset of puberty generally occurs at a younger age in girls (8–13 years) than in boys (9–14 years) (Naninck et al., 2011). We had information on menarcheal status in girls, the most frequently used indicator of pubertal development in girls (Hvidt et al., 2019), but no recent information on pubertal stage in boys. In future studies, physical measurement of pubertal development, i.e., evaluating the Tanner stage at the moment of sampling, would be of value to investigate differences in observed associations across pubertal development stages in both boys and girls. We assessed air pollutant concentrations and traffic noise levels at residence by estimation, not by measurement. Air pollution interpolation models

may not fully capture fine-scale variability in air pollutants that may be caused by local traffic conditions or street canyon effects (Kumar et al., 2019). Furthermore, exposure assessment was limited to the residential address. However, previous research in Flemish children has demonstrated that the internal urinary carbon load, a biomarker of personal exposure to air pollution, correlated well with RIO-IFDM modelled residential exposure (Saenen et al., 2017). Estimated residential traffic noise levels may not reflect the total soundscape, including other common sources of noise such as noise from neighbors, construction sites and wind turbines, and do not account for noise annoyance (Kim et al., 2017). The lack of information on noise annoyance may have contributed to our null findings for traffic noise exposure. Future studies may consider a more personal air pollution and noise exposure estimation, possibly through a participatory approach that takes personal mobility into account (Boniardi et al., 2021). Notwithstanding this limitation, including traffic noise levels in our models may have minimalized exposure misclassification for air pollution. Evaluation of associations of air pollution with health outcomes, ignoring potential confounding by traffic noise, could lead to an overestimation of the true effect of air pollution exposure (Klompmaker et al., 2019). Although we accounted for residential traffic noise levels, household and neighborhood SES and performed several sensitivity analyses, we cannot exclude residual confounding by other environmental and/or social stressors. Future studies, including a larger study population, could include a wider range of potential stressors in their analysis. Growing attention is currently being paid to the exposome concept, the totality of environmental exposures and their endogenous response in shaping disease risk and disease development (Tamayo-Uria et al., 2019; Vrijheid et al., 2020). We measured leucocyte counts at one time point and related the counts to long-term exposure to air pollution and traffic noise, whereas leucocytes in peripheral blood have a lifespan ranging from hours to days (Tak et al., 2013). However, associations of leucocyte counts with persistent or long-term environmental exposures have been evaluated in a multitude of epidemiological studies (Chen and Schwartz, 2008; Kumar et al., 2014, 2014; Oulhote et al., 2017). A low level of intra-individual variation in leucocyte count over time was first demonstrated in a 6.5-year longitudinal study in healthy men (Sunyer et al., 1996). A high level of intra-individual stability in leucocyte count was observed, based on 12 semiannual repeated measures. Results in this study underpinned the utility of a single measurement of leucocyte count as a biomarker in epidemiologic studies. We observed moderate increases in leucocyte and neutrophil counts in relation to long-term air pollution exposure. However, the immunological and cardiovascular relevance of moderately elevated leucocyte counts is well-established in all age groups, including adolescents (Agostinis-Sobrinho et al., 2018; Prunicki et al., 2020; Vozarova et al., 2002). The use of HCC as a biomarker of chronic stress in epidemiological studies is fairly recent. Information on clinical implications of elevated HCC during adolescence is still limited. Since adolescence is a vulnerable period for stress, chronically elevated cortisol levels may contribute to adolescents' allostatic load, which may lead to adverse health effects that persist into adulthood (Sheth et al., 2017; Thomson, 2019). In general populations of adults, research has linked increased HCC with cardiovascular risk factors (Iob and Steptoe, 2019; Lehrer et al., 2016; Stalder et al., 2013) and with burnout symptomatology (Penz et al., 2018).

5. Conclusions

For the first time, we found that long-term ambient air pollution levels were associated with increased chronic stress levels and increased leucocyte, in particular neutrophil count in adolescent boys. By investigating two different biological pathways simultaneously, this study may contribute to a better understanding of the early pathophysiological changes that may underlie adverse health effects of air pollution exposure in adolescents. From a public health perspective, it is important to draw attention to the fact that these associations were observed in a study population that was exposed to air pollution levels well below the current European limit values for long-term exposure to PM and NO_2. As is the case for 80% of the population worldwide, our study population was exposed to annual mean air pollutant concentrations above the WHO health-based guidelines. Regulation on air pollutants relies to a large extent on the available data of their adverse health effects. Results in this study suggest that the general population of Flemish adolescents, especially boys, may benefit from current and future measures to reduce ambient air pollutant levels.

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Ethics

The study protocol has been revised and approved by the Ethics committee of the Antwerp University Hospital (Belgian Registry Number: B300201732753).

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111595.

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