International Journal of Hygiene and Environmental Health xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Associations between ambient air pollution and bone turnover markers in 10-year old children: Results from the GINIplus and LISAplus studies

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ARTICLE INFO

Article history:
Received 24 February 2014
Received in revised form 21 July 2014
Accepted 25 July 2014

Keywords:
Ambient air pollution
Bone health
Bone turnover markers
Children
Epidemiology

ABSTRACT

Negative associations between bone turnover markers and bone mineral density have been reported. In order to study the association between ambient air pollution and bone turnover markers, as indicators of bone loss, we investigated associations between land-use regression modeled air pollution (NO_2, $PM_{2.5}$ mass, $PM_{2.5-10}$ [coarse particles], PM_{10} mass and $PM_{2.5}$ absorbance) and bone turnover markers in 2264 children aged 10 years. Serum osteocalcin and C-terminal telopeptide of type I collagen (CTx), measured by Modular-System (Roche), were the two bone turnover markers considered in this analysis. In total population, NO_2, $PM_{2.5-10}$ and PM_{10} mass exposure were positively and significantly associated with both osteocalcin and CTx. A 2.5 (95% CI: 0.6, 4.4) ng/ml increase in osteocalcin and a 24.0 (95% CI: 6.7, 41.3) ng/L increase in CTx were observed per IQR (6.7 $\mu g/m^3$) increase in NO_2, independent of socioeconomic status, sex, age, pubertal status, fasting status and total physical activity. The estimated coefficients were 3.0 (95% CI: 0.1, 5.8) for osteocalcin and 32.3 (95% CI: 6.1, 58.5) for CTx with $PM_{2.5-10}$; 3.2 (95% CI: 0.0, 6.4) for osteocalcin and 30.7 (95% CI: 1.7, 59.7) for CTx with PM_{10}. Children living close to a major road ($\leq$ 350 m) had higher levels of both osteocalcin (1.4 [−1.2, 4.0] ng/ml) and CTx (16.2 [−7.4, 39.8] ng/L). The adverse impact of ambient air pollution on bone turnover rates observed in one of the study areas showed stimulation of more such studies.

Introduction

Historic studies, which have used X-rays (Schlipkoter et al., 1986) to measure bone maturation status, have reported significant bone age retardation in children living in areas with heavy air pollution. However, these studies cannot be replicated nowadays due to ethical constraints. Instead, biomarkers of bone metabolism (bone turnover) are increasingly used as indicators of bone health, as summarized in a previous review (Szulc et al., 2000). Serum osteocalcin, or bone Gla protein, reflects osteoblastic activity (Liu and Peacock, 1998) and is a sensitive and specific marker of bone formation (Gundberg et al., 2012; Szulc et al., 2000). The concentration of osteocalcin increases during high bone turnover and decreases during low bone turnover (Szulc et al., 2000). C-terminal telopeptide of type I collagen (CTx) is a marker of bone resorption, and reflects the rate of bone loss (Delmas et al., 2000). CTx increases when bone mass is rapidly lost (Delmas et al., 2000).

Both osteocalcin (Cheng et al., 2002; Krall et al., 1997; Liu and Peacock, 1998; Minisola et al., 1997) and CTx (Okuno et al., 2005; Rosen et al., 2000) have been negatively associated with bone

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[1] The GINIplus Study Group see Acknowledgement.
[2] The LISAplus Study Group see Acknowledgement.

http://dx.doi.org/10.1016/j.ijheh.2014.07.006

mineral density (BMD) in both men (Cheng et al., 2002; Krall et al., 1997; Okuno et al., 2005; Rosen et al., 2000) and women (Cheng et al., 2002; Krall et al., 1997; Liu and Peacock, 1998; Minisola et al., 1997; Okuno et al., 2005; Rosen et al., 2000). In addition, weak but significantly inverse associations have been found between total BMD and air pollution (NO_2, $PM_{2.5}$ and PM_{10} [particles with aerodynamic diameters below 2.5 and 10 μm, respectively]) among 590 men 75 – 76 years old (Alvaer et al., 2007; Alver et al., 2010). Based on these previously published studies, it is possible to hypothesize that exposure to ambient air pollution may cause an increase in bone turnover markers (osteocalcin and CTx).

Childhood is a crucial period for bone development (Calderon-Garciduenas et al., 2013). The majority of bone mass accumulates during this period (Bailey et al., 1999). However, the role of exposure to ambient air pollution on bone markers in children has never been previously explored.

The primary purpose of this study was to examine associations between NO_2, $PM_{2.5}$, $PM_{2.5-10}$, PM_{10} and $PM_{2.5}$ absorbance and the distance to a major road from a child's home address, as approximations of ambient air pollution exposure, and two bone turnover markers (osteocalcin and CTx, as surrogates of bone formation and resorption, respectively) in 10-year old children.

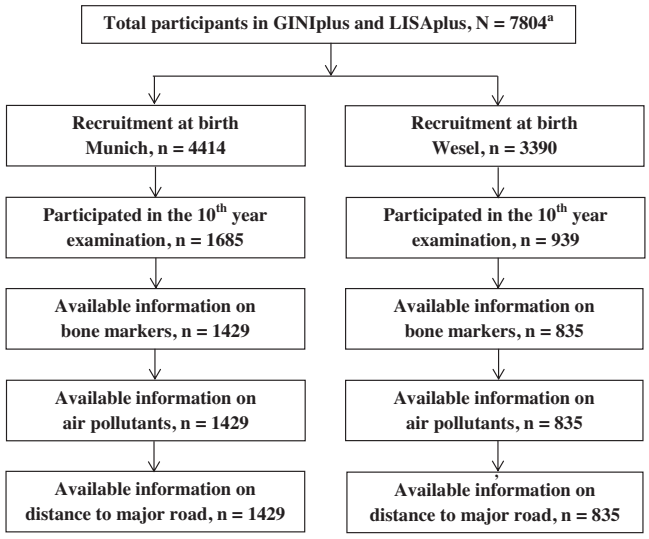

Fig. 1. Selection of study population. *N*: total participants in original cohorts; n: the number of study participants in each subgroup. [a]1282 children (976 from Leipzig and 306 from Bad Honnef) of the original cohorts are not shown in the flow chart due to lack of information on air pollution and bone turnover markers.

Methods

Study population

The study population consists of children from two German birth cohorts of healthy full-term neonates. The German Infant Nutritional Intervention plus environmental and genetic influences on allergy development study (GINIplus) was designed to prospectively investigate the effects of a nutrition intervention during infancy, as well as air pollution and genetics, on allergy development. Details on the design, recruitment and follow-up of this intervention study have been previously published (Filipiak et al., 2007; von Berg et al., 2010). Briefly, a total of 5991 newborns were recruited in obstetric clinics in Munich and Wesel, Germany, between September 1995 and July 1998. Follow-up occurred at the age of one, two, three, four, six and ten years of age. The Lifestyle-Related factors on the Immune System and the Development of Allergies in Childhood plus the influence of traffic emissions and genetics (LISAplus) population-based study aimed to assess the influence of lifestyle-related factors on the immune system, as well as the effects of air pollution and genetics on the development of allergies in childhood. In total, 3097 healthy full-term neonates were recruited from 14 obstetrical clinics in Munich, Leipzig, Wesel and Bad Honnef between November 1997 and January 1999. Follow-up for this study occurred at the age of six, twelve and eighteen months, and two, four, six and ten years. After the six-year follow-up, two children removed their consent to participate in the LISAplus study, and are thus not included in this analysis. A detailed description of the screening and recruitment of these studies has been described elsewhere (Heinrich et al., 2002; Zutavern et al., 2007).

We included children who lived in Munich or Wesel at the age of 10 years, for whom data on air pollution estimates at the home address and bone turnover markers were available (total number is 2264, of which 1429 and 835 were from Munich and Wesel, respectively). In addition, the distance between the home address and the nearest major road was also included as an indicator of traffic-related air pollution exposure. A detailed description on the selection of participants can be found in Fig. 1.

For both cohort studies, ethical approval was obtained by the medical ethical committees. Written informed consent was obtained from the parents of all participants.

Exposure assessment

Long-term (one-year) air pollution concentrations

Estimates of modeled annual average concentrations of NO_2 and particulate matter (PM; including $PM_{2.5}$, $PM_{2.5-10}$, PM_{10} and $PM_{2.5}$ absorbance) were derived from city-specified land-use regression models developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE) collaboration (http://www.escapeproject.eu/manuals).

In brief, NO_2 and PM were monitored at 40 and 20 sites, respectively, between October 2008 and November 2009. Each site was monitored for two consecutive weeks, three times (during warm, cold and intermediate temperature seasons). These discontinuous measurements were then adjusted for the actual long-term average of the observation period, according to data from one additional background site that measured air pollution concentrations using the same instruments continuously for a complete year. Using these data, land use regression models were developed and used to assign individual air pollution exposure estimates to each participant's home address at the age of 10 years. Details of this procedure have been previously published (Beelen et al., 2013; Cyrys et al., 2012; Eeftens et al., 2012a; Eeftens et al., 2012b).

Distance to major road

The closest distance between each child's home address and a major road, which was defined as a road with more than 5000 vehicles per day, was also included as an indicator of traffic-related air pollution exposure. Furthermore, as we found a sharp decrease of concentrations of air pollutants when the distance to a major road is bigger than 350 m, we also dichotomized this variable (= < 350 m versus >350 m to major road).

Bone marker concentration assessment

At the 10-year physical examination, serum bone turnover markers were measured in 2264 children. Of these children, 17.8% (402/2264) were also invited for over-night fasting blood sampling (the fasting children means that the children should not take food,

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juice, mild, etc. before the blood collection. children who are fasting have a significantly higher body mass index [BMI] compared to non-fasting children (Thiering et al., 2013)). The collected blood samples were centrifuged after collection and stored frozen at −80 °C until assayed for bone turnover marker concentrations.

Bone turnover marker concentrations for both Munich and Wesel were performed by the Modular-System (Roche) in the same lab. Intra- and inter-assay coefficients of variation for the precision of quality controls varied between 3.2% and 2.9% for the concentrations of 23.7 and 123.1 ng/mL for osteocalcin, and between 4.7% and 4.8% for the concentrations of 343 and 792 ng/mL for CTx, respectively.

Covariates and effect modifiers

Demographic, health, and lifestyle information on the study participants was collected using self-administered questionnaires completed by the parents (sex, duration of total physical activity [hours per week], highest education level of either parent [low: both parents reported less than 10 years of school; high: at least one of the parents reported more than 10 years of school; medium: the rest of the study participants with available information], and pubertal status of child, which was assessed based on parental reported questionnaire (signs of pubertal, e.g. pubic hair or menarche) [pubertal versus non-pubertal]). Height and weight were measured at the 10-year physical examination and were used to calculate the BMI of children.

Statistical analysis

The analyses were carried out using the statistical software R (version 2.14.1) (R Core Team, 2013). Pearson's chi-square, the Student's *t*-test and the Wilcoxon rank sum test were used to assess differences between children included in this analysis and those from the original birth cohorts for categorical, normally distributed continuous variables, and continuous variables with a skewed distribution, respectively. Unadjusted correlations between ambient air pollutants and bone turnover markers were assessed with the Spearman correlation coefficient.

Associations between ambient air pollutants and bone turnover markers in children were explored with generalized additive models (GAM, MGCV package in R (Wood, 2004)), as non-linear associations were observed between some continuous confounders (BMI, age and physical activity) and the bone turnover marker concentrations. Air pollutant concentrations and the distance to a major road were included as linear terms, after testing their linearity with the bone turnover markers using GAM plots. The distance to a major road was also categorized in to ≤350 m versus >350 m.

Table 1
Distribution of bone turnover markers by participant characteristics ($N = 2264$).

	n	Osteocalcin (ng/ml)			CTx (ng/L)		
		Mean	SD	*P*-value	Mean	SD	*P*-value
Cohort							
GINIplus	1667	91.1	30.5	<0.001	580.3	290.9	<0.001
LISAplus	597	99.1	33.5		878.2	346.5	
City							
Munich	1429	95.8	32.0	<0.001	726.1	330.2	<0.001
Wesel	835	88.8	30.2		543.8	306.8	
Sex							
Male	1156	87.9	27.1	<0.001	643.2	315.0	0.022
Female	1108	98.8	34.7		675.3	351.1	
Puberty[a]							
No	1570	89.3	27.9	<0.001	638.6	313.1	<0.001
Yes	668	102.3	37.2		704.3	372.9	
BMI at 10-years[b]							
Normal	1787	93.1	31.1	0.049	658.4	331.6	0.916
Overweight	382	95.8	33.7		662.4	344.8	
Obesity	83	86.7	28.0		672.4	322.5	
Fasting							
Yes	402	106.3	34.3	<0.001	928.8	377.7	<0.001
No	1862	90.4	30.2		600.6	292.1	
Parental education[c]							
Low	146	90.5	32.5	0.219	625.9	348.5	<0.001
Medium	586	91.8	30.3		604.4	331.5	
High	1524	93.9	31.7		682.5	329.7	
Season							
Mar – May	548	95.2	32.8	0.007	676.3	350.9	<0.001
Jun – Aug	652	90.8	28.4		588.1	262.7	
Sep – Nov	577	91.9	33.2		647.8	352.2	
Dec – Feb	478	96.3	31.7		752.2	353.0	
Hours of physical activity per week[d]							
Low (≤10.5 h)	563	93.6	31.9	0.915	675.3	334.5	0.033
Medium (10.5 ~ 26 h)	1063	93.2	31.3		666.6	332.2	
High (≥26 h)	548	92.8	31.8		627.5	330.5	
Age[d] (in months)							
Low (109 ~ 120)	545	93.6	27.6	0.014	665.4	310.4	0.038
Medium (121 ~ 123)	1224	91.7	30.7		644.9	335.5	
High (124 ~ 143)	486	96.7	37.1		689.9	352.0	

CTx, C-terminal telopeptide of type I collagen; BMI, body mass index.
[a] Defined based on a parental report of signs of puberty (e.g. pubic hair, menarche).
[b] According to the World Health Organization guidelines (available from: http://www.who.int/growthref/who2007_bmi_for_age/en/index.html).
[c] Categorized according to the highest number of years either parent attended school: low < 10 years, medium = 10 years and high > 10 years.
[d] Defined according to quartiles: low < 25%, medium = 25-75% and high > 75%.
Student's t-test was used to compare bone markers levels in children with different characters in cohort, city, sex, pubertal or fasting status; the one-way analysis of variance was used for children with different characters in BMI, parental education level, season, hours of physical activity per week and age.

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Three models were applied to test the associations between each of the exposure indicators (NO_2, $PM_{2.5}$, $PM_{2.5-10}$, PM_{10}, $PM_{2.5}$ absorbance, distance to a major road, living within 350 m of a major road versus not) and bone turnover marker concentrations. Model 1 included adjustments for child sex and city. Model 2 included further adjustments for BMI, age, as well as fasting and pubertal status. Model 3 additionally included adjustments for cohort (GINIplus; LISAplus), duration of total physical activity, parental education level, and the season during which the blood was drawn. Identical models, but without adjustment of city, were applied for city-stratified analyses.

Beta estimates are presented per interquartile range increase for the continuous exposures (IQR, 6.7 $\mu g/m^3$ for NO_2, 4.0 $\mu g/m^3$ for $PM_{2.5}$, 2.5 $\mu g/m^3$ for $PM_{2.5-10}$, 4.9 $\mu g/m^3$ for PM_{10}, 0.4×10^{-5} m^{-1} for $PM_{2.5}$ absorbance, and 442.2 m for distance to a major road, in the total data). For the models which include distance to a major road as a dichotomized variable, the coefficients reported represent an increase in bone turnover marker concentrations for children living close to a major road, compared to those living >350 m away from a major road. All results are presented as regression coefficients with corresponding 95% confidence intervals (CI). *P*-values below 0.05 were used to indicate statistical significance.

Results

In total, 2264 children were included in the present study, 63.1% ($n = 1429$) and 36.9% ($n = 835$) of which were from the Munich and Wesel areas, respectively. The distributions of bone turnover markers by participant characteristics are given in Table 1. Levels of both osteocalcin and CTx were significantly higher among females, children who had reached puberty and those fasting. The season at which blood samples were collected and age of the child were also associated with serum levels of bone turnover markers. Comparisons of characteristics between the original cohort participants who were not included in this analysis and those who were included are given in the Supplemental Material, Table S1. Children who are included in this analysis tended to have parents with a higher level of education compared to those not included.

Table 2 shows the distribution of ambient air pollutants and the distance to a major road. In the total population, the mean and standard deviation of the air pollutants were 21.2 ± 4.9 $\mu g/m^3$ for NO_2, 14.8 ± 2.2 $\mu g/m^3$ for $PM_{2.5}$, 7.1 ± 1.5 $\mu g/m^3$ for $PM_{2.5-10}$, 22.0 ± 3.3 $\mu g/m^3$ for PM_{10} and $1.5 \pm 0.3 \times 10^{-5}$ m^{-1} for $PM_{2.5}$ absorbance. Fifty-seven percent (1280 out of 2264) of the study participants lived close to a major road (≤350 m), and were exposed to higher concentrations of NO_2 (22.3 ± 5.2 $\mu g/m^3$), $PM_{2.5-10}$ (7.3 ± 1.5 $\mu g/m^3$), PM_{10} (22.1 ± 3.2 $\mu g/m^3$) and $PM_{2.5}$ absorbance ($1.6 \pm 0.3 \times 10^{-5}$ m^{-1}) compared to those who did not live close to a major road (19.6 ± 4.1 $\mu g/m^3$ for NO_2, 6.9 ± 1.4 $\mu g/m^3$ for $PM_{2.5-10}$, 21.9 ± 3.4 $\mu g/m^3$ for PM_{10} and $1.3 \pm 0.2 \times 10^{-5}$ m^{-1} for $PM_{2.5}$ absorbance). No significant difference for $PM_{2.5}$ was observed. Children from Munich had significantly lower concentrations of NO_2, $PM_{2.5}$, $PM_{2.5-10}$ and PM_{10}, compared to those from Wesel. Small crude correlation coefficients were found between the investigated exposures and bone metabolism markers (Table 3). The correlation coefficients in Munich and Wesel were provided in Supplemental Material, Table S2. The crude exposure response of bone turnover markers to ambient air pollutants were given in Supplemental Material, Figure S1.

Adjusted associations between air pollutant estimates and the distance to a major road with bone turnover markers are provided in Table 4. Positive and significant associations were found between NO_2 with both osteocalcin and CTx across different models. A 2.5 (95% CI: 0.6, 4.4) ng/ml increase in osteocalcin and a 24.0 (95% CI: 6.7, 41.3) ng/L increase in CTx were associated with a IQR (6.7 $\mu g/m^3$) increase in NO_2, after adjusting for sex, age, BMI, pubertal status, fasting status, parental education level, physical activity and season at which blood samples were collected. Also in the fully adjusted models, both osteocalcin and CTx were positively associated with $PM_{2.5-10}$ (3.0 [95% CI: 0.1, 5.8] ng/ml increase in osteocalcin and 32.3 [95% CI: 6.1, 58.5] ng/L increase in CTx per IQR [4.9 $\mu g/m^3$] increase in PM_{10}) and PM_{10} (3.2 [95% CI: 0.0, 6.4] ng/ml increase in osteocalcin and 30.7 [95% CI: 1.7, 59.7] ng/L increase in CTx per IQR [4.9 $\mu g/m^3$] increase in PM_{10}). Both osteocalcin and CTx were negatively associated with an increasing distance to a major road, which is in line with our findings for the estimated air pollution concentrations. Children living within 350 m of a major road had higher levels of both osteocalcin (1.4 [−1.2, 4.0] ng/ml) and CTx (16.2 [−7.4, 39.8] ng/L), although these results were not statistically significant. In the city-stratified analyses, associations for children from Munich were similar to those for the pooled population. The results for children from Wesel were all insignificant, rather inconstant and not in line with those for the pooled population nor for children from Munich (see Supplemental Material, Table S3). No substantial changes were observed after additional adjusted for moving history (see Supplemental Material, Table S4).

With respect to the covariates, pubertal status, fasting status and season of blood assessment were significantly associated with both osteocalcin and CTx. Sex was only significantly associated with osteocalcin. Across different exposure models, children who had reached puberty had higher levels of both osteocalcin (mean coefficient estimate = 9.1) and CTx (mean coefficient estimate = 42.3), fasting children had higher levels of both osteocalcin (mean coefficient estimate = 13.2) and CTx (mean coefficient estimate = 280.1),

Table 2
Distribution of environmental exposures.

Exposure	Munich (*N* = 1429)	Wesel (*N* = 835)	Total (*N* = 2264)
NO_2, $\mu g/m^3$			
Mean	19.7	23.7	21.2
5th percentile	13.5	19.9	13.9
Median	18.7	23.2	21.3
95th percentile	28.8	28.5	28.7
Interquartile range	6.6	3.3	6.7
$PM_{2.5}$, $\mu g/m^3$			
Mean	13.3	17.4	14.8
5th percentile	11.9	16.4	12.1
Median	13.2	17.3	13.9
95th percentile	14.8	18.7	18.2
Interquartile range	1.0	0.9	4.0
$PM_{2.5-10}$, $\mu g/m^3$			
Mean	6.3	8.4	7.1
5th percentile	4.6	7.7	4.9
Median	6.1	8.4	7.3
95th percentile	8.6	9.5	9.2
Interquartile range	1.6	0.6	2.5
PM_{10}, $\mu g/m^3$			
Mean	20.0	25.4	22.0
5th percentile	16.2	24.0	16.6
Median	20.4	25.2	21.6
95th percentile	23.5	28.0	26.7
Interquartile range	2.9	1.5	4.9
$PM_{2.5}$ absorbance, 10^{-5} m^{-1}			
Mean	1.6	1.2	1.5
5th percentile	1.4	1.0	1.0
Median	1.6	1.2	1.5
95th percentile	2.0	1.5	1.9
Interquartile range	0.2	0.2	0.4
Distance to major road, m			
Mean	485	633.2	539.6
5th percentile	30.2	47.6	34.4
Median	257.7	375	294.9
95th percentile	1786.6	2361.0	2036.2
Interquartile range	395.3	540.0	442.2
≤350 m, %	61.5	48.0	56.5

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Table 3
Spearman correlations between ambient air pollution and bone turnover marker concentrations.

	Osteocalcin	CTx	NO_2	$PM_{2.5}$	$PM_{2.5-10}$	PM_{10}	$PM_{2.5}$ absorbance
Osteocalcin	1.00						
CTx	0.58**	1.00					
NO_2	−0.02	−0.09**	1.00				
$PM_{2.5}$	−0.12**	−0.26**	0.53**	1.00			
$PM_{2.5-10}$	−0.07**	−0.18**	0.83**	0.71**	1.00		
PM_{10}	−0.09**	−0.22**	0.68**	0.81**	0.87**	1.00	
$PM_{2.5}$ absorbance	0.12**	0.24**	−0.04	−0.48**	−0.25 **	−0.38**	1.00
Distance to a major road	−0.06**	−0.09**	−0.36**	−0.02	−0.19**	−0.06**	−0.42**

CTx: C-terminal telopeptide of type I collagen.
** *P*-value < 0.01.

and females had higher osteocalcin levels (mean coefficient estimate = 8.0).

Discussion

In the present study, a cross-sectional study using data from two German birth cohorts was conducted to investigate associations between long-term ambient air pollution exposure and bone metabolism markers (osteocalcin and CTx) in 2264 children aged 10 years. We also explored the impact of living close to a major road on these markers. Significantly positive associations were observed between NO_2, $PM_{2.5-10}$ and PM_{10} with both markers in the pooled study population, independent of socioeconomic status, sex, age, pubertal status, fasting status and total physical activity. Also, negative associations were found between the distance from the home address to the closest major road and bone metabolism markers. Finally, children living within 350 m of a major road had higher levels of both osteocalcin and CTx. The results were driven by the subgroup of children from Munich, while no or negative associations were observed in Wesel.

As this is the first study to examine associations between individual-level ambient air pollution exposure and bone turnover markers in children, we are unable to compare the present study results with previous studies. However, one study conducted in elderly men reported that air pollution was inversely associated with total body BMD (Alvaer et al., 2007). The authors recruited 590 men aged 75 – 76 years as study participants. Exposure to $PM_{2.5}$, PM_{10} and NO_2 was modeled at each participant's home address and BMD was measured with dual-energy X-ray absorptiometry (DXA). The results showed that, after adjustment for BMI, smoking, physical education and education levels, total body BMD changed by −47 (95% CI, −77, −17), −28 (−48, −8) and −7 (−13, 0) mg/cm^2 with every 10 μg/m^3 increase in $PM_{2.5}$, PM_{10} and NO_2, respectively. It is not clear whether the aforementioned negative associations can be extrapolated to children. Another recent study (Calderon-Garciduenas et al., 2013) explored the impact of urban air pollution on bone health in children aged six years. The authors selected 20 children from a highly polluted area and 15 children from a control area (with $PM_{2.5}$ and ozone concentrations above and below the US EPA annual standards, respectively). No significant differences in BMD *z*-scores were observed between those two groups of children. However, this null association may be attributable to the small sample size used in this study.

The non-significant findings in city-stratified analyses observed in the current study for Wesel might also be due to limited statistical power. Although not significant, the direction of risk estimates for Wesel was not in line with that from Munich and the pooled population. Given that most children from Wesel are part of GINIplus cohort (87.3%, 729/835), we further investigated associations in GINIplus-Wesel (n = 729) and GINIplus-Munich (n = 938) separately, but found no indication of a cohort effect [data not shown]. It is also interesting that significant associations between ambient air pollution and bone turnover markers were observed in children from Munich only, which had lower concentrations of NO_2, $PM_{2.5}$ and PM_{10} than Wesel. Furthermore, we found that bone turnover markers were positively associated with $PM_{2.5-10}$ and

Table 4
Estimated change in osteocalcin (ng/ml) and CTx (ng/L) concentrations per corresponding interquartile range increase in air pollution exposures and distance to a major road.

	Model 1 (N = 2264)	**Model 2 (N = 2264)**	**Model 3 (N = 2264)**
Associations with osteocalcin			
NO_2	2.4 (0.5, 4.3)*	2.3 (0.5, 4.2)*	2.5 (0.6, 4.4)*
$PM_{2.5}$	−3.0 (−9.4, 3.3)	−3.6 (−9.8, 2.6)	−3.9 (−10.2, 2.3)
$PM_{2.5-10}$	2.8 (−0.1, 5.7)*	2.8 (0.0, 5.7)*	3.0 (0.1, 5.8)*
PM_{10}	3.1 (0.0, 6.3)	3.2 (0.1, 6.3)*	3.2 (0.0, 6.4)*
$PM_{2.5}$ absorbance	1.4 (−1.5, 4.4)	1.2 (−1.7, 4.1)	1.3 (−1.8, 4.3)
Distance to major road	−0.4 (−1.2, 0.3)	−0.4 (−1.4, 0.3)	−0.4 (−1.1, 0.3)
>350 m	ref	ref	ref
≤350 m	1.7 (−0.9, 4.3)	1.3 (−1.2, 3.9)	1.4 (−1.2, 4.0)
Associations with CTx			
NO_2	29.5 (10.0, 49.1)*	25.2 (6.8, 43.6)*	24.0 (6.7, 41.3)*
$PM_{2.5}$	−41.8 (−107.6, 24.0)	−50.4 (−112.2, 11.5)	−53.7 (−109.9, 2.7)
$PM_{2.5-10}$	42.6 (12.5, 72.7)*	36.9 (8.6, 65.2)*	32.3 (6.1, 58.5)*
PM_{10}	34.9 (1.7, 68.0)*	35.0 (3.8, 66.1)*	30.7 (1.7, 59.7)*
$PM_{2.5}$ absorbance	26.5 (−4.2, 57.3)	20.8 (−8.1, 49.7)	15.3 (−12.5, 43.1)
Distance to major road	−6.2 (−13.6, 1.3)	−5.8 (−12.9, 1.2)	−3.6 (−10.2, 2.9)
>350 m	ref	ref	ref
≤350 m	23.9 (−3.1, 50.8)	18.2 (−7.2, 43.7)	16.2 (−7.4, 39.8)

CTx: C-terminal telopeptide of type I collagen.
Model 1, adjusted for sex and city. Model 2, additionally adjusted for body mass index, age, fasting and pubertal status. Model 3, additionally adjusted for season, parental education level, hours of physical activity per week and cohort.
* *P*-value < 0.05.

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PM_{10} but negatively with $PM_{2.5}$ in pooled population and children from Munich. This interesting finding indicates that maybe it is only coarse particles and PM_{10} that are biological responsible for the increase of bone turnover markers. This might be another explanation for the null finding reported by Calderon-Garciduenas et al. (2013), in which study, $PM_{2.5}$ concentrations were selected as the main exposure. Further research untangle these associations is warranted.

Negative associations between bone turnover markers and bone health have been well established in adults (Ross and Knowlton, 1998). Higher levels of bone turnover markers can cause rapid bone loss (Melton et al., 1997; Ross and Knowlton, 1998), reduce bone strength (Einhorn, 1992; Melton et al., 1997), cause loss of structural elements in bone (Melton et al., 1997; Parfitt, 1984), and contribute to bone fracture (Melton et al., 1997). Slemenda et al. (1997) conducted a study among 45 monozygotic twin pairs aged 6 – 14, and observed negative associations between both bone formation (osteocalcin) and resorption (tartrate-resistant acid phosphatase) markers with bone mass. Although CTx was not included as a bone resorption marker in this previous study, it is expected to have similar associations with bone mass as does tartrate-resistant acid phosphatase. The observed associations between bone markers and bone mass have been replicated by two other studies conducted in boys (Jurimae et al., 2009) and girls (Libanati et al., 1999).

In our study, we found that exposure to ambient air pollution and road traffic is associated with enhanced bone turnover markers. As bone turnover markers are negatively associated with bone mass in children (Slemenda et al., 1997), these results suggest that ambient air pollution may negatively impact bone development in children. As the serum levels of bone markers may also be affected by many aspects, such as nutrition, medication, chronic inflammation, bone fracture, etc., we cannot conclude which changes in the levels of these markers are associated with bone loss. In adults, high bone turnover, i.e. an increase of bone resorption and formation markers as in postmenopausal osteoporosis, is indicative of a high rate of bone loss, and most anti-resorptive therapies address this mechanism. Based on this, we may only learn that bone mineral density inversely associated with bone markers, the exact cut-off point for this cannot be concluded.

Although the exact mechanism for this potential impact remains unclear, there are two potential pathways. The first is via air pollution induced inflammation (Krishna et al., 1996; Peden, 2001). Systematic inflammation may cause the release of interleukin 6 (IL-6), which is involved in immune regulation. Higher levels of IL-6 could in-turn lead to increases in bone turnover and bone loss (Nishimoto and Kishimoto, 2006). The other potential explanation is related to the polycyclic aromatic hydrocarbons (PAHs) in automobile exhausts, although the role of PAHs on bone heath has not been studied previously. There is however one study on tobacco smoke, which also has high PAHs concentrations, and bone health in rats (Lee et al., 2002). In the study from Lee et al., two PAHs (benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene) found in the tar fraction of cigarette smoke were reported to increase bone turnover and ultimately lead to bone loss. As these PAHs are also components of automobile exhausts (Alvaer et al., 2007), exposure to these compounds via traffic-related air pollution may contribute to elevated levels of bone turnover markers, which is consistent with our findings. However, neither IL-6 nor current exposure to tobacco smoke were associated with the two bone turnover markers in our study [data not shown]. As these two potential mechanistic pathways are highly speculative, further research on potential pathways is needed.

In the present study, one interquartile range increase in ambient air pollution concentrations is associated with a 2.5 – 3.2 ng/ml increase in osteocalcin and a 24.0 – 32.3 ng/L increase in CTx. These effect sizes are significantly associated with bone density loss, according to published papers in adults. Quantitative assessments between bone markers and bone mineral density are still lacking for children. It has nevertheless been reported that the odds of rapid bone loss increase by 1.8 (95% CI: 1.3 – 2.5) and 1.7 (95% CI: 1.24 – 2.40) for every 2.6 ng/ml increase in osteocalcin (Ross and Knowlton, 1998) and when serum CTx concentrations increase more than 50 ng/l (Okuno et al., 2005) in adults. In this case, we believe that the elevation of bone marker levels in our study might indicate rapid bone loss.

This study is unique in the following aspects. First, this is the first study to examine associations between ambient air pollution and bone health in children using a rather large sample size. As the risk of fracture is determined by the peak skeletal mass that accumulates early in life (Slemenda et al., 1997), it is important to increase our knowledge on the factors of bone development, especially among children. Second, bone metabolism markers are used as outcomes. These markers are not only informative for monitoring bone development (Jurimae et al., 2009), but also provide realistic and dynamic insights into bone metabolism (Jurimae, 2010). Using bone metabolism markers requires less stringent ethical considerations than assessing bone health using X-rays. Thus, the use of these markers is a promising approach for further epidemiological studies. Third, the levels of bone metabolism markers were assessed in serum. The metabolism markers in serum are much more stable compared to those collected in urine (de Ridder and Delemarre-van de Waal, 1998), thereby providing a more precise outcome measurement.

There are also limitations which should be considered when interpreting the present results. First, we have data on only one formation and one resorption marker. Further studies should include more markers to better explore bone metabolism status (Jurimae, 2010), and could also consider including BMD measurements by DXA. Second, data on fracture history was not available. According to Michalus et al. (2008), children which have fractured bones in the past have lower levels of bone formation markers and higher levels of bone resorption markers. Although some important covariates, such as sex, age, fasting status, physical activity and pubertal status, have been included in the current analyses, the inclusion of fracture history as a covariate may help to better explore the observed associations between ambient air pollution and bone turnover markers. Third, given that the present analysis is cross-sectional, we cannot infer any causal associations between exposure to ambient air pollution and variation in serum bone marker levels. Fourth, as we could not observe internal consistency in the two study areas, the statistically significant associations in Munich might be due to random chance. More studies are needed to better understand the true associations between ambient air pollution and bone turnover markers in children.

Conclusions

Exposure to ambient air pollution, especially NO_2, $PM_{2.5-10}$ and PM_{10}, may be associated with increased bone turnover rate in children. However, the inconsistent findings in two study areas showed stimulation of more such studies.

Conflict of interest statement

There is no conflict of interest associated with this manuscript.

Acknowledgments

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Declaration of all sources of funding

The GINI study was funded by Helmholtz Zentrum München and grants of the Federal Ministry for Education, Science, Research and Technology 292 (Grant No. 01 EE 94014), and the 10-year follow-up of the GINIplus study was partly funded by the Federal Ministry of Environment (IUF, FKZ 20462296). This study was supported by Ludwig-Maximilians-University's innovative research priority project MC-Health (subproject I). The LISAplus study was funded by Helmholtz Zentrum München, Munich Center of Health (MC Health) and partly by grants of the Federal Ministry of Environment (BMU) (for IUF, 298 FKZ 20462296), and Federal Ministry for Education, Science, Research and Technology (Nos. 01 EG 9705/2 and 01EG9732). Both studies were co-funded by the German Network of Competency on Adiposity.

The research leading to the ESCAPE results has received funding from the European Community's Seventh Framework Program (FP7/2007-2011) under grant agreement number: 21125

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijheh.2014.07.006.

References

Alvaer, K., Meyer, H.E., Falch, J.A., Nafstad, P., Sogaard, A.J., 2007. Outdoor air pollution and bone mineral density in elderly men—the Oslo Health Study. Osteoporos. Int. 18, 1669–1674.

Alver, K., Meyer, H.E., Falch, J.A., Sogaard, A.J., 2010. Outdoor air pollution, bone density and self-reported forearm fracture: the Oslo Health Study. Osteoporos. Int. 21, 1751–1760.

Bailey, D.A., McKay, H.A., Mirwald, R.L., Crocker, P.R., Faulkner, R.A., 1999. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. J. Bone Miner. Res. 14, 1672–1679.

Beelen, R., Hoek, G., Vienneau, D., Eeftens, M., Dimakopoulou, K., Pedeli, X., Tsai, M.-Y., Künzli, N., Schikowski, T., Marcon, A., Eriksen, K.T., Raaschou-Nielsen, O., Stephanou, E., Patelarou, E., Lanki, T., Yli-Tuomi, T., Declercq, C., Falq, G., Stempfelet, M., Birk, M., Cyrys, J., von Klot, S., Nádor, G., Varró, M.J., Dėdelė, A., Gražulevičienė, R., Mölter, A., Lindley, S., Madsen, C., Cesaroni, G., Ranzi, A., Badaloni, C., Hoffmann, B., Nonnemacher, M., Krämer, U., Kuhlbusch, T., Cirach, M., de Nazelle, A., Nieuwenhuijsen, M., Bellander, T., Korek, M., Olsson, D., Strömgren, M., Dons, E., Jerrett, M., Fischer, P., Wang, M., Brunekreef, B., de Hoogh, K., 2013. Development of NO_2 and NO_x land use regression models for estimating air pollution exposure in 36 study areas in Europe—the ESCAPE project. Atmos. Environ. 72, 10–23.

Calderon-Garciduenas, L., Mora-Tiscareno, A., Francolira, M., Torres-Jardon, R., Pena-Cruz, B., Palacios-Lopez, C., Zhu, H., Kong, L., Mendoza-Mendoza, N., Montesinoscorrea, H., Romero, L., Valencia-Salazar, G., Kavanaugh, M., Frenk, S., 2013. Exposure to urban air pollution and bone health in clinically healthy six-year-old children. Arh. Hig. Rada. Toksikol. 64, 23–34.

Cheng, S., Suominen, H., Vaananen, K., Kakonen, S.M., Pettersson, K., Heikkinen, E., 2002. Serum osteocalcin in relation to calcaneal bone mineral density in elderly men and women: a 5-year follow-up. J. Bone Miner. Metab. 20, 49–56.

Cyrys, J., Eeftens, M., Heinrich, J., Ampe, C., Armengaud, A., Beelen, R., Bellander, T., Beregszaszi, T., Birk, M., Cesaroni, G., Cirach, M., de Hoogh, K., De Nazelle, A., de Vocht, F., Declercq, C., Dėdelė, A., Dimakopoulou, K., Eriksen, K., Galassi, C., Grąulevičienė, R., Grivas, G., Gruzieva, O., Gustafsson, A.H., Hoffmann, B., Iakovides, M., Ineichen, A., Krämer, U., Lanki, T., Lozano, P., Madsen, C., Meliefste, K., Modig, L., Mölter, A., Mosler, G., Nieuwenhuijsen, M., Nonnemacher, M., Oldenwening, M., Peters, A., Pontet, S., Probst-Hensch, N., Quass, U., Raaschou-Nielsen, O., Ranzi, A., Sugiri, D., Stephanou, E.G., Taimisto, P., Tsai, M.-Y., Vaskövi, É., Villani, S., Wang, M., Brunekreef, B., Hoek, G., 2012. Variation of NO_2 and NO_x concentrations between and within 36 European study areas: results from the ESCAPE study. Atmos. Environ. 62, 374–390.

de Ridder, C.M., Delemarre-van de Waal, H.A., 1998. Clinical utility of markers of bone turnover in children and adolescents. Curr. Opin. Pediatr. 10, 441–448.

Delmas, P.D., Eastell, R., Garnero, P., Seibel, M.J., Stepan, J., 2000. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. Osteoporos. Int. 11 (Suppl 6), S2–S17.

Eeftens, M., Beelen, R., de Hoogh, K., Bellander, T., Cesaroni, G., Cirach, M., Declercq, C., Dedele, A., Dons, E., de Nazelle, A., Dimakopoulou, K., Eriksen, K., Falq, G., Fischer, P., Galassi, C., Grazuleviciene, R., Heinrich, J., Hoffmann, B., Jerrett, M., Keidel, D., Korek, M., Lanki, T., Lindley, S., Madsen, C., Molter, A., Nador, G., Nieuwenhuijsen, M., Nonnemacher, M., Pedeli, X., Raaschou-Nielsen, O., Patelarou, E., Quass, U., Ranzi, A., Schindler, C., Stempfelet, M., Stephanou, E., Sugiri, D., Tsai, M.Y., Yli-Tuomi, T., Varro, M.J., Vienneau, D., Klot, S., Wolf, K., Brunekreef, B., Hoek, G., 2012a. Development of Land Use Regression models for PM(2.5), PM(2.5) absorbance, PM(10) and PM(coarse) in 20 European study areas; results of the ESCAPE project. Environ. Sci. Technol. 46, 11195–11205.

Eeftens, M., Tsai, M.-Y., Ampe, C., Anwander, B., Beelen, R., Bellander, T., Cesaroni, G., Cirach, M., Cyrys, J., de Hoogh, K., De Nazelle, A., de Vocht, F., Declercq, C., Dėdelė, A., Eriksen, K., Galassi, C., Gražulevičienė, R., Grivas, G., Heinrich, J., Hoffmann, B., Iakovides, M., Ineichen, A., Katsouyanni, K., Korek, M., Krämer, U., Kuhlbusch, T., Lanki, T., Madsen, C., Meliefste, K., Mölter, A., Mosler, G., Nieuwenhuijsen, M., Oldenwening, M., Pennanen, A., Probst-Hensch, N., Quass, U., Raaschou-Nielsen, O., Ranzi, A., Stephanou, E., Sugiri, D., Udvardy, O., Vaskövi, É., Weinmayr, G., Brunekreef, B., Hoek, G., 2012b. Spatial variation of PM2.5, PM10, PM2.5 absorbance and PMcoarse concentrations between and within 20 European study areas and the relationship with NO_2—results of the ESCAPE project. Atmos. Environ. 62, 303–317.

Einhorn, T.A., 1992. Bone strength: the bottom line. Calcif. Tissue Int. 51, 333–339.

Filipiak, B., Zutavern, A., Koletzko, S., von Berg, A., Brockow, I., Grubl, A., Berdel, D., Reinhardt, D., Bauer, C.P., Wichmann, H.E., Heinrich, J., 2007. Solid food introduction in relation to eczema: results from a four-year prospective birth cohort study. J. Pediatr. 151, 352–358.

Gundberg, C.M., Lian, J.B., Booth, S.L., 2012. Vitamin K-dependent carboxylation of osteocalcin: friend or foe? Adv. Nutr. 3, 149–157.

Heinrich, J., Bolte, G., Holscher, B., Douwes, J., Lehmann, I., Fahlbusch, B., Bischof, W., Weiss, M., Borte, M., Wichmann, H.E., 2002. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. Eur. Respir. J. 20, 617–623.

Jurimae, J., 2010. Interpretation and application of bone turnover markers in children and adolescents. Curr. Opin. Pediatr. 22, 494–500.

Jurimae, J., Pomerants, T., Tillmann, V., Jurimae, T., 2009. Bone metabolism markers and ghrelin in boys at different stages of sexual maturity. Acta Paediatr. 98, 892–896.

Krall, E.A., Dawson-Hughes, B., Hirst, K., Gallagher, J.C., Sherman, S.S., Dalsky, G., 1997. Bone mineral density and biochemical markers of bone turnover in healthy elderly men and women. J. Gerontol. A Biol. Sci. Med. Sci. 52, M61–M67.

Krishna, M.T., Springall, D.R., Frew, A.J., Polak, J.M., Holgate, S.T., 1996. Mediators of inflammation in response to air pollution: a focus on ozone and nitrogen dioxide. J. R. Coll. Physicians Lond. 30, 61–66.

Lee, L.L., Lee, J.S., Waldman, S.D., Casper, R.F., Grynpas, M.D., 2002. Polycyclic aromatic hydrocarbons present in cigarette smoke cause bone loss in an ovariectomized rat model. Bone 30, 917–923.

Libanati, C., Baylink, D.J., Lois-Wenzel, E., Srinvasan, N., Mohan, S., 1999. Studies on the potential mediators of skeletal changes occurring during puberty in girls. J. Clin. Endocrinol. Metab. 84, 2807–2814.

Liu, G., Peacock, M., 1998. Age-related changes in serum undercarboxylated osteocalcin and its relationships with bone density, bone quality, and hip fracture. Calcif. Tissue Int. 62, 286–289.

Melton 3rd, L.J., Khosla, S., Atkinson, E.J., O'Fallon, W.M., Riggs, B.L., 1997. Relationship of bone turnover to bone density and fractures. J. Bone Miner. Res. 12, 1083–1091.

Michalus, I., Chlebna-Sokol, D., Rusinska, A., Jakubowska-Pietkiewicz, E., Kulinska-Szukalska, K., 2008. Evaluation of bone mineral density and bone metabolism in children with multiple bone fractures. Ortop. Traumatol. Rehabil. 10, 602–612.

Minisola, S., Rosso, R., Romagnoli, E., D' Erasmo, E., Manfredi, G., Damiani, C., De Antoni, F., Mazzuoli, G., 1997. Serum osteocalcin and bone mineral density at various skeletal sites: a study performed with three different assays. J. Lab. Clin. Med. 129, 422–429.

Nishimoto, N., Kishimoto, T., 2006. Interleukin 6: from bench to bedside. Nat. Clin. Pract. Rheumatol. 2, 619–626.

Okuno, S., Inaba, M., Kitatani, K., Ishimura, E., Yamakawa, T., Nishizawa, Y., 2005. Serum levels of C-terminal telopeptide of type I collagen: a useful new marker of cortical bone loss in hemodialysis patients. Osteoporos. Int. 16, 501–509.

Parfitt, A.M., 1984. Age-related structural changes in trabecular and cortical bone: cellular mechanisms and biomechanical consequences. Calcif. Tissue Int. 36 (Suppl 1), S123–S128.

Peden, D.B., 2001. Air pollution in asthma: effect of pollutants on airway inflammation. Ann. Allergy Asthma Immunol. 87, 12–17.

Rosen, H.N., Moses, A.C., Garber, J., Iloputaife, I.D., Ross, D.S., Lee, S.L., Greenspan, S.L., 2000. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. Calcif. Tissue Int. 66, 100–103.

Ross, P.D., Knowlton, W., 1998. Rapid bone loss is associated with increased levels of biochemical markers. J. Bone Miner. Res. 13, 297–302.

Schlipkoter, H.W., Rosicky, B., Dolgner, R., Pelech, L., 1986. Growth and bone maturation in children from two regions of the F.R.G. differing in the degree of air pollution: results of the 1974 and 1984 surveys. J. Hyg. Epidemiol. Microbiol. Immunol. 30, 353–358.

Slemenda, C.W., Peacock, M., Hui, S., Zhou, L., Johnston, C.C., 1997. Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass. J. Bone Miner. Res. 12, 676–682.

Szulc, P., Seeman, E., Delmas, P.D., 2000. Biochemical measurements of bone turnover in children and adolescents. Osteoporos. Int. 11, 281–294.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Thiering, E., Cyrys, J., Kratzsch, J., Meisinger, C., Hoffmann, B., Berdel, D., von Berg, A., Koletzko, S., Bauer, C.P., Heinrich, J., 2013. Long-term exposure to traffic-related air pollution and insulin resistance in children: results from the GINIplus and LISAplus birth cohorts. Diabetologia 56, 1696–1704.

von Berg, A., Kramer, U., Link, E., Bollrath, C., Heinrich, J., Brockow, I., Koletzko, S., Grubl, A., Filipiak-Pittroff, B., Wichmann, H.E., Bauer, C.P., Reinhardt, D., Berdel, D., 2010. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course—the GINIplus study up to the age of 6 years. Clin. Exp. Allergy 40, 627–636.

Wood, S.N., 2004. Stable and efficient multiple smoothing parameter estimation for generalized additive models. J. Am. Stat. Assoc. 99, 673–686.

Zutavern, A., Rzehak, P., Brockow, I., Schaaf, B., Bollrath, C., von Berg, A., Link, E., Kraemer, U., Borte, M., Herbarth, O., Wichmann, H.E., Heinrich, J., 2007. Day care in relation to respiratory-tract and gastrointestinal infections in a German birth cohort study. Acta Paediatr. 96, 1494–1499.

Please cite this article in press as: Liu, C., et al., Associations between ambient air pollution and bone turnover markers in 10-year old children: Results from the GINIplus and LISAplus studies. Int. J. Hyg. Environ. Health (2014), http://dx.doi.org/10.1016/j.ijheh.2014.07.006