Synergistic Effects of Prenatal Exposure to Fine Particulate Matter ($PM_{2.5}$) and Ozone (O_3) on the Risk of Preterm Birth: A Population-based Cohort Study

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Abstract

BACKGROUND: There is some evidence that prenatal exposure to low-level air pollution increases the risk of preterm birth (PTB), but little is known about synergistic effects of different pollutants.

OBJECTIVES: We assessed the independent and joint effects of prenatal exposure to air pollution during the entire duration of pregnancy.

METHODS: The study population consisted of the 2,568 members of the Espoo Cohort Study, born between 1984 and 1990, and living in the City of Espoo, Finland. We assessed individual-level prenatal exposure to ambient air pollutants of interest at all the residential addresses from conception to birth. The pollutant concentrations were estimated both by using regional-to-city-scale dispersion modelling and land-use regression–based method. We applied Poisson regression analysis to estimate the adjusted risk ratios (RRs) with their 95% confidence intervals (CI) by comparing the risk of PTB among babies with the highest quartile (Q_4) of exposure during the entire duration of pregnancy with those with the lower exposure quartiles (Q_1-Q_3). We adjusted for season of birth, maternal age, sex of the baby, family's socioeconomic status, maternal smoking during pregnancy, maternal exposure to environmental tobacco smoke during pregnancy, single parenthood, and exposure to other air pollutants (only in multi-pollutant models) in the analysis.

RESULTS: In a multi-pollutant model estimating the effects of exposure during entire pregnancy, the adjusted RR was 1.37 (95% CI: 0.85, 2.23) for $PM_{2.5}$ and 1.64 (95% CI: 1.15, 2.35) for O_3. The joint effect of $PM_{2.5}$ and O_3 was substantially higher, an adjusted RR of 3.63 (95% CI: 2.16, 6.10), than what would have been expected from their independent effects (0.99 for $PM_{2.5}$ and 1.34 for O_3). The relative risk due to interaction (RERI) was 2.30 (95% CI: 0.95, 4.57).

DISCUSSION: Our results strengthen the evidence that exposure to fairly low-level air pollution during pregnancy increases the risk of PTB. We provide novel observations indicating that individual air pollutants such as $PM_{2.5}$ and O_3 may act synergistically potentiating each other's adverse effects.

Key words: air pollution, fine particulates, ozone, prenatal exposure, preterm birth, interaction

Abbreviations:

FMI, Finnish Meteorological Institute;

SILAM, The system for integrated modelling of atmospheric composition;

UDM-FMI, Urban Dispersion Model-Finnish Meteorological Institute;

CAR-FMI, Contaminants in the Air from a Road-Finnish Meteorological Institute.

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Ethics approval:

The study protocol was approved by the Ethics Committee of Oulu University Hospital (Oulu, Finland).

1. Introduction

Preterm birth (PTB), defined as birth before 37 weeks of gestation is the leading cause of perinatal mortality and morbidity worldwide (Blencowe et al. 2013; Liu et al. 2012). A total of 14.9 million babies are born preterm each year worldwide (Blencowe et al. 2012; Goldenberg et al. 2008). In Finland, the occurrence of premature infants has been reported to be low, 5.7% of all births in 2013 (Vuori & Gissler 2014). The causes of preterm birth are not yet well understood, but there is evidence that environmental exposures play a role in the etiology of PTB (Wigle et al. 2008).

Previous systematic reviews and meta-analyses have provided consistent evidence that prenatal exposure to fine particulate matter ($PM_{2.5}$) during entire pregnancy increases the risk of PTB, with odds ratios ranging from 1.10-1.24 (point estimates) per 10μg/m^3 increase in concentration (Klepac et al. 2018; Stieb et al. 2012; Sun et al. 2015; Zhu et al. 2015). But the summary-effect estimates of prenatal exposure to sulfur dioxide (SO_2), nitrogen dioxide (NO_2), Ozone (O_3) and carbon monoxide (CO) on the risk of PTB have been inconsistent. (Klepac et al. 2018; Lamichhane et al. 2015; Stieb et al. 2012). Ambient air pollution is a complex mixture of particles and gaseous pollutants that are inhaled simultaneously; their combined toxicity could differ from that found in investigations with individual pollutants. Hence, the potential for synergy among air pollutant mixture components is a particular concern. A review of original laboratory studies among humans and animals elaborated the synergism between O_3 and different combinations of pollutants on different health effects (Mauderly and Samet 2009). Only a few previous epidemiological studies have elaborated the potential interaction between fine particles ($PM_{2.5}$) and oxidant gases (O_3, NO_2), focusing on the risk of cardiovascular diseases and mortality (Lavigne et al. 2018; Lin et al.2018; Yu et al. 2013). However, based on our systematic literature search there are no previous studies that have evaluated the joint effects of individual air pollutants on the risk of adverse pregnancy outcomes.

A few studies have elaborated the critical time period of air pollution exposure during pregnancy with inconclusive results (Huynh et al. 2006; Maroziene et al. 2002; Leem et al. 2006, Sagiv et al. 2005).

We conducted individual-level prenatal exposure assessment for the members of the Espoo Cohort Study by using regional-to-city-scale dispersion modelling and land-use regression (LUR)-based method combined with a Geographical Information System (GIS) data, from the estimated conception to the delivery on all the pollutants of interest (Jaakkola et al. 1993, Kukkonen et al. 2018). The study population was exposed to relatively low levels of air pollution with substantial seasonal variability including concurrent exposure to cold weather.

The overall objective of the present study was to assess the relation between prenatal exposure to air pollution and the risk of preterm birth. The novel objective was to elaborate potential joint effects of individual air pollutants with independent effects using average pollutant concentrations during entire pregnancy.

2. Methods

2.1. Study design

We conducted a population-based cohort study. We compared the risk of PTB among babies with the highest quartile (Q_4) of mean exposure during the entire duration of pregnancy, with the reference exposures, i.e. those with exposures in the lower exposure quartiles (Q_1-Q_3).

2.2. Study population

The study population comprised the members of the Espoo Cohort Study (Jaakkola et al. 1993; Jaakkola et al. 2005; Rantala et al. 2015). The source population included all the children of the city of Espoo, Finland, born between January 1, 1984 and March 31, 1990. Espoo is an urban–suburban municipality with a population of 279,000, located across the western border of Helsinki, the capital of Finland. We took a random sample of children living in Espoo in 1991 from the roster of Statistics Finland. The baseline study population included a total of 2,568 children (response rate 80.3%) whose parents filled in the questionnaire. The study was conducted in accordance with The Code of Ethics

of the World Medical Association (Declaration of Helsinki) for studies involving humans. The study protocol was approved by the Ethics Committee of Oulu University Hospital (Oulu, Finland).

2.3. Health outcome

The primary outcome of interest was preterm birth (PTB), which was defined as a live birth before the 37th week of pregnancy. We retrieved information on gestational age (GA) from the baseline data collection and if the information was missing then we retrieved the GA information from the Finland's Medical Birth Registry for children born on or later than January 1, 1987.

2.4. Exposure assessment

We assessed individual-level prenatal exposure to ambient air pollutants of interest based on exposure conditions at all the residential addresses from conception to birth, which accounted for residential mobility during pregnancy. The pollutant data, obtained from the Finnish Meteorological Institute (FMI), were based on a cascade of nested simulations of the system for integrated modelling of atmospheric composition (SILAM) (http://silam.fmi.fi, Sofiev et al. 2016). The model started from the global scale to capture the background levels of long-living pollutants and then zoomed to Europe and Northern Europe, finally reaching the spatial resolution of 0.1°×0.1° (Sofiev et al. 2015). This dataset was also used as a background by Kukkonen et al. (2018) for the fine resolution modelling of the concentrations of fine particulate matter ($PM_{2.5}$) in the Helsinki Metropolitan Area from 1980 to 2014. Details of the SILAM setup and the model evaluation can be found there. In the study area, the spatial resolution applied in this study corresponds to around 5.5 km in the east-west direction, and 11.1 km in the north-south direction. The home coordinates of the mothers were retrieved from the Population Register Centre of Finland, and Geographical Information System (GIS) was used to extract daily levels of air pollutants at the home coordinates. The exposures of interest included the following air pollutants: fine particulate matter with a dry diameter up to 2.5 μm ($PM_{2.5}$), particulate matter with a dry diameter up to 10 μm (PM_{10}), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon

monoxide (CO), and ozone (O_3). The exposure parameter of interest was the mean concentration of pollutants during entire pregnancy.

2.5. Covariates

We identified a set of determinants of PTB based on previous literature which were fitted systematically as potential confounders in the multivariable statistical models. They included maternal age (Fuchs et al. 2018), sex of the baby (Peelen et al. 2016), family's socioeconomic status (high vs. low or medium parental education and occupation) (Joseph et al. 2014), maternal smoking during pregnancy (Jaakkola and Gissler 2004), maternal exposure to environmental tobacco smoke during pregnancy (Windham et al. 2000) and single parenthood (Zeitlin et al. 2002). We also considered other air pollutants as potential confounders when assessing the effect of one pollutant. We first fitted one-pollutant models and then considered two-pollutant models by fitting one traffic related ($PM_{2.5}$, NO_2, CO) and one stationary fossil fuel combustion related pollutant (PM_{10}, SO_2). We also fitted two-pollutant models with O_3 as the main secondary pollutant and another pollutant. Finally, we considered three-pollutant models with one traffic related, one stationary fossil fuel combustion related pollutant and O_3. Family socioeconomic status was categorized as low (including both parents having no degree and having a vocational degree but being unemployed), high (including both parents having an academic degree and being white-collar workers or entrepreneurs) and middle (including all the other combinations of education and occupation, e.g. other parent low and high category and both parents' students). We have presented the above mentioned potential confounders used in this study with a Directed Acyclic Graph (DAG) as figure 1.

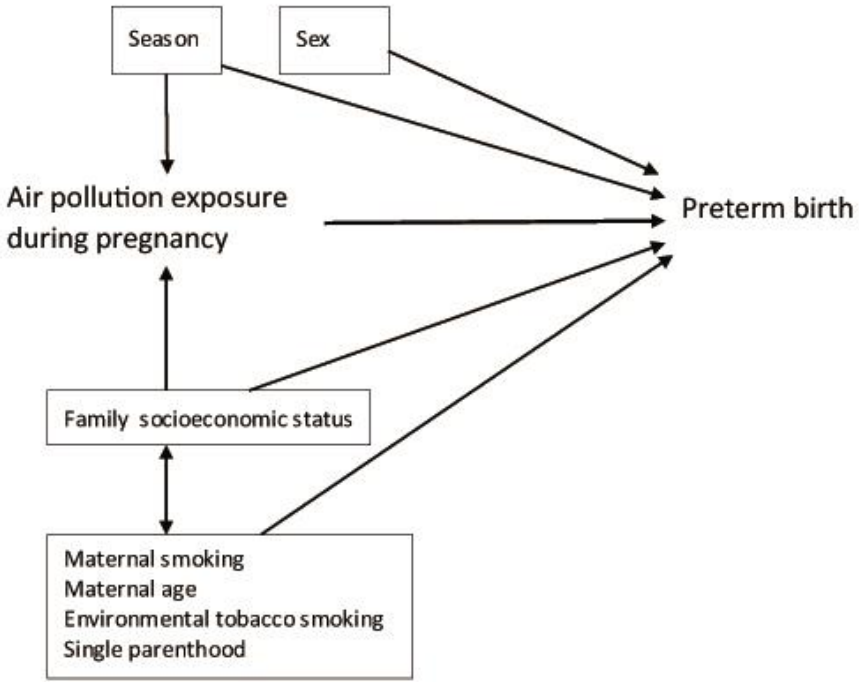

Figure 1. All the covariates in a Directed Acyclic Graph (DAG).

2.6. Statistical methods

We used risk ratio (RR) as the measure of the effect of prenatal exposure to air pollution on the risk of PTB. We compared the risk of PTB among babies with the highest quartile (Q_4) of exposure during the entire duration of pregnancy with the reference categories of exposure, i.e. those with exposures in the lower exposure quartiles (Q_1-Q_3). We performed Poisson regression analysis to estimate the adjusted RRs, with their 95% confidence intervals (95% CI). We fitted single, two, and three pollutant models.

We studied both the independent and joint effects of different air-pollutant exposures during the entire pregnancy on the risk of PTB. We estimated interactions on the additive scale, because their interpretation is most relevant from public health perspective (Rothman 1986, Rothman et al. 2008). This was performed by comparing the risk of PTB in three different exposure categories, defined as for example: 1) high $PM_{2.5}$ and low O_3 (i.e. A), 2) low $PM_{2.5}$ and high O_3 (i.e. B), and 3) high $PM_{2.5}$ and

high O_3 (i.e. AB), to the reference category of 'low $PM_{2.5}$ and low O_3' exposure. High and low refer to the exposure above and below to the Q_4 value, respectively. Estimates for the independent and joint effects were derived from the modified Poisson regression analysis by using both crude and adjusted models (Zou 2008). In the results section, we present excess relative risks (ERR) for the independent and joint effects of $PM_{2.5}$ and O_3 exposure categorized in the three different exposure categories described before. The relative risk due to interaction (RERI) was quantified on an additive scale by calculating the risk that is more than expected based on the independent effects of these factors. This can be expressed in terms of ERRs as follows:

$$\text{RERI} = \text{ERR (AB)} - \text{ERR (A)} - \text{ERR (B)}$$

We estimated the 95% CI for RERI using the method of variance estimates recovery (Zou 2008). For RERI, the null value corresponds to a statistical significance level p=0.05. Applying the same procedure, we also studied the independent and joint effects of exposure to other pollutants (i.e. between PM_{10} and O_3; SO_2 and O_3; NO_2 and O_3; and between CO and O_3) on the risk of PTB. For the analysis of joint effect, we chose the pollutants that were not strongly correlated to each other.

To examine potential modifying effect of season, we further performed analysis stratified by two birth seasons, i.e. warm season and cold season. The warm season included spring and summer (March to August) and the cold season included autumn and winter (September to February).

We conducted sensitivity analyses, by applying time to event approach with Cox regression model, and by applying air pollution data as continuous variables, giving the effect estimates for a 10-unit increase of each pollutant.

All the analyses were performed using PROC GENMOD procedure and for Cox regression model using PROC PHREG procedure in SAS 9.4 statistical software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of the study population

Table 1 shows the characteristics of the study population. In the analysis, the study population included only 2453 individuals, whose exposure data was available. There were altogether 195 cases of preterm birth, giving a prevalence of 7.8%. Almost half of the mothers in our total study population were older than 30 years and 23.4% belonged to the highest categories of socioeconomic status.

Table 1. Characteristics of the study population, The Espoo Cohort Study, 1984-1990.

Characteristic	*n* (%)
Total	2568 (100.00)
Preterm birth(PTB)	
<37 weeks of gestation	195 (7.82)
Mean gestational age for all PTB (Mean±SD)	34.48±2.34
Gender	
boy	1311 (51.05)
girl	1257 (48.95)
Maternal age at delivery (years)	
<25	370 (14.41)
25-30	960 (37.38)
>30	1216 (47.35)
missing	22 (0.86)
Family socioeconomic status	
High	597 (23.25)
Low or medium	1959 (76.29)
missing	12 (0.47)
Single parent or guardian	
yes	183 (7.13)
no	2385 (92.87)
Maternal smoking in pregnancy	
yes	364 (14.17)
no	2199 (85.63)
missing	5 (0.19)
Environmental tobacco smoke exposure of the pregnant mother	
yes	101 (3.93)
no	1874 (72.98)
missing	593 (23.09)

Note: SD, standard deviation

3.2. Exposure distributions

A summary of the air pollution distributions is presented in Table 2. The mean daily concentrations of $PM_{2.5}$, PM_{10}, CO, NO_2, O_3 and SO_2 during the entire pregnancy were 19.62 μg/m^3, 21.35 μg/m^3, 295.09 ppb, 4.31 ppb, 23.79 ppb and 3.94 ppb, respectively. The concentrations of $PM_{2.5}$ in this region

in the 1980's, including the study period, were approximately twice as high as the corresponding concentrations nowadays, as shown by Kukkonen et al. (2018). Also the concentrations of the other selected pollutants were substantially higher in this region during the 1980's.

Table 2. Distributions of air pollution parameters during entire pregnancy, Espoo 1 January 1983-31 March 1990.

Pollutants	Mean±SD	Minimum	25th percentile	Median	75th percentile	IQR	Maximum
$PM_{2.5}$($\mu g/m^3$)	19.62±4.50	3.01	16.93	19.53	22.10	5.17	38.48
PM_{10}($\mu g/m^3$)	21.35±5.12	3.17	18.47	21.15	24.06	5.59	43.78
CO(ppb)	295.09±51.50	119.89	264.02	295.97	329.74	65.72	482.45
NO_2(ppb)	4.31±1.23	0.21	3.63	4.41	5.07	1.44	8.73
SO_2(ppb)	3.94±1.95	0.19	2.76	3.75	4.71	1.95	15.88
O_3(ppb)	23.79±2.80	11.20	21.64	23.93	26.00	4.36	36.93

Note: All the distribution parameters are mean concentrations of the pollutants; CO, carbon monoxide; ; IQR, Interquartile range; NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter with a diameter of 2.5 micrometers; PM_{10}, particulate matter with a diameter of 10 micrometers; ppb, parts per billion; SD, standard deviation; SO_2, sulfur dioxide; $\mu g/m^3$, microgram per cubic meter.

The correlation matrix for all the studied pollutants during the entire pregnancy showed high correlation with each other, with the exception that O_3 showed a negative correlation with the other pollutants (Table S1). The negative correlation is resulted from the chemistry of the formation and depletion of ozone (Karppinen et al. 2000).

3.3. Air pollution exposure and preterm birth

Figure 2 presents the relations between the mean exposure to air pollutants during the entire pregnancy and the risk of PTB and the corresponding effect estimates are provided in Table S2. The risk of PTB was increased in relation to the highest quartile of mean $PM_{2.5}$ concentration, but the lower 95% confidence limit (95% CL) was < 1.00. The effect estimate was statistically significant among babies born during spring or summer (warm season), with an adjusted RR of 2.67 (95% CI: 1.43, 4.98) in the three-pollutant model that adjusted for SO_2 and O_3 exposures simultaneously (Figure 2 and Table S2).

The effect estimates for PM_{10} showed a similar pattern. The adjusted RR for babies born in spring or summer (warm season), was 3.05 (95% CI: 1.48, 6.28). Since $PM_{2.5}$ constitutes more than half of PM_{10} mass, the similarity is not surprising (Figure 2 and Table S2).

In contrast, the risk of PTB was not related to the NO_2 levels in any of the models. However, exposure to CO showed somewhat increased risk among babies born during spring or summer. The effect estimates for the mean SO_2 exposure during the entire pregnancy were elevated with an adjusted RR of 1.23 (95% CI: 0.75, 2.01) from the three-pollutant model adjusting for CO and O_3 and the effect estimates were greater for those babies born in the cold season, with an adjusted RR of 1.98 (1.05, 3.74) from the same model (Figure 2 and Table S2).

Furthermore, exposure to O_3 increased the risk of PTB, with an adjusted RR of 1.64 (95% CI: 1.14, 2.34) in the three-pollutant model. The effect estimate was substantially greater for babies born during the warm season, with the adjusted RR of 3.28 (95% CI: 1.85, 5.82). However, the effect estimate in the cold season was also slightly elevated (Figure 2 and Table S2).

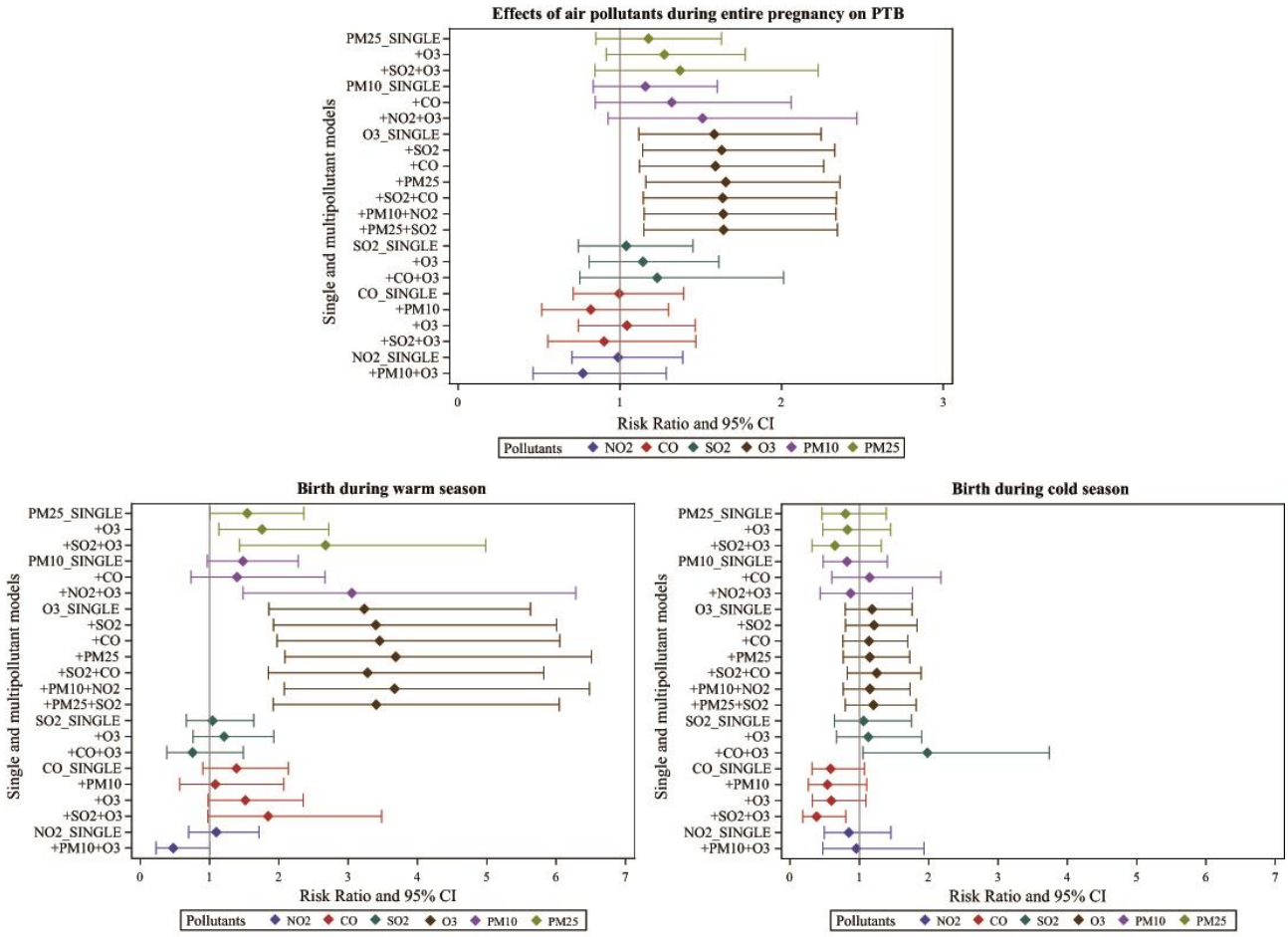

Figure 2. Adjusted risk ratios (RRs) with 95% confidence intervals (CI) of PTB by comparing highest quartile (Q_4) to lower three quartiles (Q_1-Q_3) of each pollutant exposure during entire pregnancy. CI, confidence interval; CO, carbon monoxide; NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter with a diameter up to 2.5 micrometers; PM_{10}, particulate matter with a diameter up to 10 micrometers; ppb, parts per billion; SO_2, sulfur dioxide; µg/m^3, microgram per cubic meter. Cut-offs for highest quartile of exposure: $PM_{2.5}$, ≥22.1µg/m^3; PM_{10}, ≥24.1µg/m^3; NO_2, ≥ 5.1 ppb; CO, ≥ 329.7 ppb; SO_2, ≥ 4.7 ppb; O_3, ≥ 26.0 ppb. Warm season included spring and summer (March to August) and the cold season included autumn and winter (September to February).

Cox regression analysis with time to event approach produced similar effect estimates for each pollutant. The main findings are given as supplementary Table S3. When we fitted air pollutant exposures as continuous variables in the models, only the effect estimates for $PM_{2.5}$ and NO_2 were somewhat elevated; the adjusted RR were 1.11 (95% CI: 0.70, 1.76) per 10 µg/m^3 and 1.12 (95% CI: 0.21, 5.81) per 10 ppb increase in concentration, respectively (Table S4).

3.4. Joint effects of air pollutants on the risk of preterm birth

Table 3 presents the joint effects of long-term exposure to $PM_{2.5}$ and O_3 on the risk of PTB. When assessing potential effects of simultaneous $PM_{2.5}$ and O_3 exposures, we detected that they had a synergistic effect on the risk of PTB: the joint effect of 'high $PM_{2.5}$ and high O_3' exposure on PTB showed an adjusted RR of 3.63 (95% CI: 2.16, 6.10). This was substantially higher than expected based on their independent effects on additive scale (RERI= 2.63 - 0.34 - (-0.01) =2.30; 95%CI: 0.95 to 4.57). In other words, there was a 230% (95% CI: 95% to 457%) excess risk associated with the joint effect of $PM_{2.5}$ and O_3 at their high exposure levels, when compared to the risk that would have been expected by summing their independent effects. The independent adjusted effect of high $PM_{2.5}$ was 0.99 (95% CI: 0.69, 1.42) and that of O_3 1.34 (95% CI: 0.90, 2.00).

Table 3. Joint effect of $PM_{2.5}$ and O_3 exposures during entire pregnancy on preterm birth, The Espoo Cohort Study, 1984-1990.

$PM_{2.5}$	O_3	n/N	% of PTB	Crude RR (95% CI)	Adjusted RR (95% CI) [a]	ERR (95% CI)[a]	RERI (95% CI)[a]
Low (< Q_4)	Low (< Q_4)	87/1287	6.8	1	1		
High (>Q_4)	Low (< Q_4)	37/551	6.7	0.99 (0.69,1.44)	0.99 (0.69,1.42)	-0.01 (-0.31,0.42)	
Low (< Q_4)	High (>Q_4)	50/553	9.0	1.34 (0.96,1.87)	1.34 (0.90,2.00)	0.34 (-0.10 ,1.00)	
High (>Q_4)	High (>Q_4)	15/62	24.2	3.58 (2.20,5.81)	3.63 (2.16,6.10)	2.63(1.16, 5.10)	2.30 (0.95, 4.57)

Note: All the estimates are from modified Poisson regression models. CI, confidence interval; ERR, Excess Relative Risk; O_3, ozone; $PM_{2.5}$, particulate matter with a diameter up to 2.5 micrometers; RERI, Relative Excess Risk Due to Interaction; RR, Risk Ratio.

[a] Adjusted for season of birth, gender, maternal age, family socioeconomic status, maternal smoking during pregnancy, environmental tobacco smoke exposure of pregnant mother and single parenthood

We also found a synergistic effect of high PM_{10} and high O_3 causing a 243% (95% CI: 110%, 467%) (Table S5), the joint effect of SO_2 and O_3 at their high exposure levels causing a 165% (95% CI: 26%, 416%) (Table S6), the joint effect of NO_2 and O_3 at their high exposure levels causing a 181% (62%, 378%) (Table S7) and the joint effect of CO and O_3 at their high exposure levels causing a 138% (31%, 309%) (Table S8) excess risk of PTB.

4. Discussion

4.1. Main findings

The results of our population-based cohort study showed that babies with the highest mean exposure to O_3 during the entire pregnancy had a 58 % (95% CI: 12%, 124%) increase in the risk of PTB from the single pollutant model compared to babies with lower exposure. This effect was even more pronounced for the babies born during the warm season of the year. Exposure to $PM_{2.5}$ during entire pregnancy and the birth during warm season increased the risk by 167% (95% CI: 43%, 398%), but there was no effect for the births taking place during the cold season. We were able to show, for the first time, that the highest exposures to $PM_{2.5}$ and O_3 during entire pregnancy had a synergistic effect on the risk of PTB causing a 230% (95% CI: 95% to 457%) excess risk, when compared to the risk that would have been expected based on adding their independent effects.

4.2. Validity of results

This population-based cohort study was based on a random sample of children from the Roster of Statistics Finland, and the response rate was high at 80.3%. Therefore, potential selection bias is minimized. The sample size is a limitation in our study in the sense that it would be better to have a larger study population with more cases of preterm birth. However, the individual-level exposure assessment compensates this limitation, which is indicated by statistically significant effect estimates with relatively narrow confidence intervals. Assessment of prenatal exposure to air pollution at individual level independently from the assessment of the health outcome (PTB) is a strength of our study, as it minimizes the likelihood of misclassification of exposure. Bell and Belanger (2012) paid attention to a potential misclassification of prenatal exposure to air pollution if there is a change of the place of residence during pregnancy that could not be identified. They estimated that approximately 9%–32% of women in the U.S. move during pregnancy (Bell and Belanger, 2012). To minimize such misclassification, we assessed exposure during the entire duration of pregnancy taking into account potential changes in residential address, in addition to exposure just prior to birth. Another strength of our study was the availability of high-quality exposure information: temporal resolution of one hour (averaged to daily for the analysis) and spatial resolution of 10 km of SILAM simulations

downscaled to hundreds of meters. 'These high resolution computations included both the emissions originated from vehicular traffic (separately exhaust and suspension emissions) and those from small-scale combustion, using the road network dispersion model CAR-FMI and the multiple source Gaussian dispersion model UDM-FMI, as described in Kukkonen et al. 2018. We were not able to take in to account exposure to indoor air pollution or maternal outdoor activity pattern, for example go far for work, shopping, travel etc.; which may have biased the exposure assessment to some extent. However, this type of bias in environmental epidemiological studies is rather common and needs to be accounted in future studies.

The assessment of the outcome of interest, i.e. preterm birth, was based on information available on the duration of pregnancy. Outcome information was collected already in the baseline data collection and if the gestational age information was missing in the baseline data collection then we retrieved that information from the Finnish Medical Birth Registry since January 1, 1987, which provides objective and physician-validated information from the official medical records of mothers in Finland. Questionnaire information on preterm birth could theoretically introduce a systematic error. However, systematic error is highly unlikely in our study, because at the time of the data collection, there was not yet any knowledge about potential effects of prenatal air pollution exposure on the risk of preterm birth. The possibility of outcome misclassification is reduced by the systematic use of ultrasound in Finland in the estimation of gestational age.

Extensive questionnaire information on maternal characteristics, environmental exposures at home and behavioral factors enabled adjustment for potential confounding. We were not able to take into account whether the PTB was natural or induced due to some medical conditions. Savitz and colleagues (2005) provided empirical evidence that spontaneous births and induced births have similar risk factors, such as smoking, and therefore information on the type of induction is not critical for the validity of the effect estimates. Also, Guan et al. 2019 provided similar evidence for the relations between exposure to $PM_{2.5}$ and risk of PTB, by showing that the effect estimates were similar for

natural preterm births and induced births. However, the context in Finland might be different from those studies, which leaves a potential limitation of our study.

Since our main objective was to assess the independent and joint effects of air pollutants during entire pregnancy, we did not look in to evaluating critical windows of exposure during pregnancy. Future studies should concentrate on the effects of air pollutants on PTB in different windows of gestation for example by using a distributed lag nonlinear model (DLNM).

4.3. Synthesis with previous knowledge

There is substantial previous evidence that prenatal exposure to particulate matter (PM) increases the risk of preterm birth (Klepac et al. 2018; Lamichhane et al. 2015; Sapkota et al. 2012; Stieb et al. 2012; Sun et al. 2015; Zhu et al. 2015). Klepac and colleagues (2018) analyzed six studies published up to June 2016, and reported a summary-effect estimate of 1.09 (95% CI: 1.03, 1.16) per a 10 μg/m^3 increase in PM_{10}. The corresponding effect estimate for $PM_{2.5}$ based on 13 studies was 1.24 (95% CI: 1.08, 1.41). In the present study, there was no clear linear increase in the risk of PTB in relation to exposure to PM. However, the risk of PTB was elevated in the highest quartile of exposure to both $PM_{2.5}$ and PM_{10} compared to the remaining quartiles, especially during the warm season. This is probably explained by the range of exposure (3.01-38.48 μg/m^3) which lies in the lowest part of the exposure-response function reaching harmful levels only in the highest quartiles. The contrast of Q_4 to Q_{1-3} represent approximately a 7.4 μg/m^3 difference in $PM_{2.5}$ levels and therefor the effect estimate of 1.37 is comparable to the estimate from the recent meta-analysis. This effect was stronger among babies born in warm season.

Our finding of the effect of prenatal exposure to O_3 is also consistent with the meta-analysis by Klepac and colleagues (2018) and other systematic reviews (Stieb et al. 2012).

Season was a significant effect modifier for the association between air pollution exposure during entire pregnancy and preterm birth. We found substantially greater effect estimates for $PM_{2.5}$ and

PM_{10} for babies born during summer season; their conception and the earlier period of pregnancy took place during the cold season, i.e. autumn and winter. Consistent with our findings, Jalaludin et al. 2007 found significant adverse effect of PM during the first trimester of pregnancy, if conception occurred in autumn or winter. Another study by Li et al.2018 also reported that the effect of $PM_{2.5}$ and PM_{10} were strongest among the women who conceived in autumn, so this is also consistent with our findings. For O_3, we found substantially greater effect estimates among babies born during summer season, i.e. conception and the earlier period of pregnancy took place during cold season, i.e. autumn and winter. However, for O_3 we also found increased risk among babies born during cold season, i.e. the conception and the earlier period of pregnancy took place in warm season (i.e. spring and summer). These findings for O_3 are consistent with the study conducted by Olsson et al 2012 in Sweden. That study found significantly increased risk of PTB for exposure to O_3 in the first trimester so their conception day was during spring, summer or winter. Season may affect both air pollution levels and other seasonal patterns of exposure, such as outdoor activities, Vitamin D levels that are related to sunlight exposure, and gestational age (Morley et al. 2006). Vitamin D status has been shown to have a clear seasonal variation in Scandinavian women (Moosgaard et al 2005). Deficiency of vitamin D is associated with increased amount of circulating inflammatory proteins, and it seems to modulate C-reactive protein levels (Ngo et al. 2010) which are associated with PTB.

We applied systematically multipollutant models to adjust for potential confounding, as well as to elaborate potential joint effects for pollutants with independent effects. To our knowledge, this is the first study that reports on the joint effect of prenatal exposure to PM and O_3 or any other air pollutant combinations on the risk of PTB. We found that high levels of $PM_{2.5}$ and O_3 exposure during entire pregnancy increase the risk of PTB synergistically with an excess risk of 230% above the additive effect.

4.4. Biological plausibility

Prenatal exposure to different air pollutants may increase the risk of PTB through several pathways, for example causing placental and fetal hypoxia, inflammation, or oxidative stress (Kannan et al. 2006; Roberts et al. 2003). Inhaled gaseous pollutants and particulate matter can potentially deposit deep in to the lungs and may enter the circulatory system and allow particles to reach the intrauterine compartment or induce systemic inflammation (Monn & Becker 1999). Existing evidence suggests that oxidant gas such as O_3 may modify PM health impacts. For example, O_3 deplete anti-oxidants in the lung lining fluid, which in turn may lower the natural defense mechanism against reactive oxygen species generated in response to $PM_{2.5}$ (Crobeddu et al. 2017; Lakey et al.2016). Moreover, the lung epithelium barrier is more permeable following ozone exposures, which may facilitate the absorption of particles directly into the circulatory system (Blomberg et al. 2003; Broeckaert et al. 2000; Ciencewicki et al. 2008), hence can reach the intrauterine compartment or induce systemic inflammation. Changes in the intrauterine inflammatory milieu may initiate cervical ripening, rupture of the amniotic sac, or increased myometrium contractility which in turn may lead to PTB (Challis et al 2009). The biological mechanisms of synergistic effect between SO_2 and O_3, between NO_2 and O_3 or between CO and O_3 on PTB are not clear. More research should be conducted in future to find out the possible biological mechanisms of different air pollutants on PTB.

5. Conclusions

In conclusion, our study conducted in Espoo, South Finland, provides a novel finding that exposure to highest quartiles of $PM_{2.5}$ and O_3 jointly increases the risk of PTB more than what would have been expected based on their independent effects. Our study also provides evidence that exposure to O_3 independently increases the risk of PTB. In summary, these results strengthen the evidence that prenatal exposure may induce premature delivery even among people living in an area with relatively low average concentrations of air pollution. Improvements in air quality by implementing laws and regulations, warning system or strict air pollution standards are needed even in the less polluted regions such as Finland. To prevent air pollution-induced PTB among future infants, more studies

should be conducted to identify potential interactions among different air pollutants, as well as to identify the potential threshold pollutant levels, so that sufficiently strict air pollution standards can be set.

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Table legends:

Table 1. Characteristics of the study population, The Espoo Cohort Study, 1984-1990.

Table 2. Distributions of air pollution parameters during entire pregnancy, Espoo 1 January 1983-31 March 1990.

Table 3. Joint effect of $PM_{2.5}$ and O_3 exposures during entire pregnancy on preterm birth, The Espoo Cohort Study, 1984-1990.

Figure legend:

Figure 1. All the covariates in a Directed Acyclic Graph.

Figure 2. Adjusted risk ratios (RRs) with 95% confidence intervals (CI) of PTB by comparing highest quartile (Q_4) to lower three quartiles (Q_1-Q_3) of each pollutant exposure during entire pregnancy. CI, confidence interval; CO, carbon monoxide; NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter with a diameter up to 2.5 micrometers; PM_{10}, particulate matter with a diameter up to 10 micrometers; ppb, parts per billion; SO_2, sulfur dioxide; $\mu g/m^3$, microgram per cubic meter. Cut-offs for highest quartile of exposure: $PM_{2.5}$, $\geq 22.1 \mu g/m^3$; PM_{10}, $\geq 24.1 \mu g/m^3$; NO_2, $\geq$ 5.1 ppb; CO, $\geq$ 329.7 ppb; SO_2, $\geq$ 4.7 ppb; O_3, $\geq$ 26.0 ppb. Warm season included spring and summer (March to August) and the cold season included autumn and winter (September to February)