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Gestational organophosphate pesticide exposure and childhood cardiovascular outcomes

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ABSTRACT

Introduction: The general population is chronically exposed to organophosphate pesticides through various routes including ingestion, hand-to-mouth contact, inhalation, and dermal contact. Exposure to organophosphate pesticides during pregnancy impairs fetal development, but the potential long-term effects of gestational organophosphate pesticide exposure are less well understood.

Methods: We investigated associations between gestational organophosphate pesticide exposure and cardiovascular outcomes in 643 children in the Generation R Study, a prospective pregnancy cohort based in Rotterdam, The Netherlands. Urinary organophosphate pesticide metabolites (dimethyl [∑DMAP], diethyl [∑DEAP], and total dialkyl phosphate [∑DAP] metabolites) were quantified in three urine samples collected from pregnant participants, and their children were followed until age 10 years at which time cardiac magnetic resonance imaging, ultrasonography, blood pressure, and serum biomarkers assessed cardiovascular health. Linear regression models estimated associations (β and 95 % confidence interval [CI]) between a one-interquartile range (IQR) increase in averaged gestational exposure biomarker concentrations and z-scored pediatric cardiovascular outcomes. We investigated effect modification of associations by PON1 genotype.

Results: Carotid intima-media thickness z-score was lower (β: -0.14 [95 % CI: −0.25, −0.02]) and HDL cholesterol z-score was higher (β: 0.14 [95 % CI: 0.02, 0.25]) for increases in ∑DEAP concentrations. Carotid intima-media distensibility z-score was lower (β: -0.08 [95 % CI: −0.19, 0.03]) for increases in ∑DMAP concentrations, and systolic blood pressure z-score was higher (β: 0.10 [95 % CI: −0.01, 0.21]) for increases in ∑DMAP and ∑DAP. Among those with PON1-161CC and PON1-L55MTT genotypes, higher organophosphate pesticide concentrations conferred an excess risk of adverse vascular and glycemic outcomes, respectively.

Conclusions: We observed heterogenous associations between gestational organophosphate pesticide exposure and pediatric cardiovascular health: an anti-atherogenic profile was observed for increases in ∑DEAP concentrations, and impairments in multiple aspects of cardiovascular health was observed for increases in ∑DMAP concentrations. PON1-161 and PON1-L55M single nucleotide polymorphisms modified associations for vascular and glycemic outcomes, respectively.

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1. Introduction

Organophosphate pesticides have historically accounted for a large share of insecticide use (Centers for Disease Control and Prevention, 2022). The general public may become exposed to organophosphate pesticides through ingestion of contaminated foods, hand-to-mouth contact with contaminated surfaces, and – to a lesser extent – inhalation and dermal contact (Centers for Disease Control and Prevention, 2022). Acute high-dose exposure can be highly risky for health, resulting in neurological dysfunction (Centers for Disease Control and Prevention, 2022). However, most exposed individuals receive low and chronic doses, the effects of which are less understood. Studies show that low-dose chronic organophosphate pesticide exposures inhibit acetylcholinesterase, downregulate muscarinic receptors, influence thyroid function, and exhibit cardiotoxic effects (Campos and Freire, Aug 2016; Eskenazi et al., 1999; Georgiadis et al., 2018/08/15/ 2018). These biological effects may translate to subclinical or clinical outcomes including dysregulated growth, cardiovascular, and respiratory diseases3, (Georgiadis et al., 2018). Early life represents a period of potentially increased susceptibility to organophosphate pesticide exposure, which can cross the placenta (Bradman et al., 2003) and has been linked to adverse fetal growth outcomes including low birthweight and reductions in fetal ultrasound indices (Ferguson et al., 2019; Bliznashka et al., 2022; Kamai et al., 2019).

Resulting from a series of studies in the 1980′s linking low birthweight to heart disease in adulthood, the developmental origins of health and disease hypothesis proposes that the *in utero* environment programs cardiovascular health across the lifecourse (Barker, 2007). The mechanisms underlying this association between the *in utero* environment and later cardiovascular health are unknown, but likely encompass anatomical, physiological, and epigenetic mechanisms. Fetal growth restriction is accompanied by cardiac remodeling and modifications in fetal hemodynamics, the effects of which may persist postnatally to influence cardiovascular outcomes10, (Toemen et al., 2019). Further, in a presentation dubbed the "Thrifty Phenotype" (Hales and Barker, 2001), smaller neonates tend to exhibit a pattern of rapid postnatal fat accretion followed by excess cardiovascular risk later in life (Singhal, 2017; Andersen et al., 2018; Sauder et al., 2021).

Prior studies have not directly examined associations between gestational organophosphate pesticide exposure and subsequent cardiovascular outcomes. However, a few studies have reported associations between questionnaire- or biomarker-based gestational organophosphate pesticide exposure and pediatric adiposity16, (Wohlfahrt-Veje et al., 2011), though not all studies have confirmed this association (Etzel et al., 2020/09/01/; Vrijheid et al., 2020; Blaauwendraad et al., Aug 2023). Furthermore, among highly-exposed pregnant greenhouse workers, pesticide exposure was associated with higher cardiometabolic risk profiles among offspring with the *PON1* 192QQ genotype (Andersen et al., 2012). Later studies confirmed differential methylation of the *PON1* 192R-allele in children with higher prenatal exposure levels (Declerck et al., 2017). However, though the majority of pesticides that workers were exposed to in these interaction studies were organophosphates, there were over 200 different pesticide formulations employed in this occupationally exposed cohort. Furthermore, the implication of these findings for the general population experiencing low-dose, chronic exposures is unclear. Finally, the role of PON1 in associations has been underexplored.

There are two major polymorphisms in the encoding region of the PON1 gene at Q192 and L55M, and additional polymorphisms of interest located in the promoter region of the PON1 gene (Shunmoogam et al., 2018/06/18). SNPs in the PON1 gene control the level and efficiency of paraoxonase, an HDL-associated enzyme that operates to detoxify organophosphate pesticides in the body and has atheroprotective properties8, (Andersen et al., 2012; Declerck et al., 2017). Low PON1 activity has been associated with increased cardiovascular disease risk (Shunmoogam et al., 2018/06/18). Further, certain PON1 genotypes (e.g., PON1-L55MTT, PON1-108TT, PON1-Q192TT) confer low detoxification of organophosphate pesticides and higher risk of cardiovascular disease, while other genotypes (e.g., PON1-L55MAA, PON1-108CC, and PON1-Q192CC) confer high detoxification of organophosphate pesticides and lower risk of cardiovascular disease8, (Verburg et al., 2008; Paul et al., 2017).

Operating through some of these mechanisms and aforementioned biological pathways, gestational organophosphate pesticide exposure may be associated with pediatric cardiovascular health. To test this hypothesis, we used data from the Generation R study to investigate associations between gestational organophosphate pesticide exposure and cardiovascular outcomes in children aged around 10 years, including effect modification by PON1 genotypes.

2. Methods

2.1. Study population & design

Analyses were conducted among children whose parents participated in the Generation R Study, a prospective cohort that aimed to recruit all pregnant persons with a delivery date between April 2002 and January 2006 in Rotterdam, the Netherlands (Kooijman et al., 2016). Among 8,879 mothers recruited during pregnancy, 778 from among 800 randomly selected participants who were pregnant between February 2004 and January 2006, provided spot urine samples in early (<18 weeks), mid (18–25 weeks; range 18 to 24 weeks), and late (>25 weeks; range 28 to 34 weeks) pregnancy, and had at least one follow-up visit were chosen for assessment of organophosphate metabolite concentrations (van den Dries et al., 2018). At 10 years of age, 661 of those mother–child pairs participated in the follow-up study. Of those, 643 had at least one measure of cardiovascular health and comprised our analytic sample.

Participants provided written informed consent prior to undergoing any study procedures and for each phase of the study. Approval for human subjects research was provided by the Medical Ethical Committee of the Erasmus Medical Centre, University Medical Centre, Rotterdam (MEC 198.782/2001/31).

2.2. Gestational organophosphate pesticide exposure assessment

Most (75 %) organophosphate pesticides are metabolized to dialkyl phosphate (DAP) metabolites, which are non-specific metabolites that can be measured in urine (Centers for Disease Control and Prevention. Organophosphorus Insecticides: Dialkyl Phosphate Metabolites. National Biomonitoring Program. Accessed September 6, 2022). Maternal urine samples from each of the three study visits during pregnancy were collected and stored in polypropylene containers at −20 °C until analysis (Kruithof et al., 2014). Most mothers in the analytic sample provided three urine samples (n = 637 with three samples, n = 4 with two samples, n = 2 with one sample). Concentrations of six urinary DAP metabolites were measured using gas chromatography coupled with tandem mass spectrometry at the Institute National de Santé Publique in Quebec, Canada as previously described28, (Jusko et al., 2019). Urinary DAP metabolites included: dimethylphosphate (DMP), dimethythiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). The Jaffe reaction was used to quantify creatinine concentrations for each sample.

Information on creatinine and DAP metabolite assessment and quality control and assurance has been published previously26, (Haines and Murray, Feb 2012; Jusko et al., 2019). In 45 participants with duplicate measurements per sample, creatinine concentrations had excellent reliability (intraclass correlation coefficient [ICC] 0.90–0.98) and total DAP metabolite concentrations varied from moderate to excellent reliability (0.81–0.95) (van den Dries et al., 2018).

2.3. Pediatric cardiovascular outcome assessment

Study outcomes included structural and functional cardiovascular measurements performed at a follow-up visit in children around 10 years of age.

Cardiac measures were assessed by cardiac magnetic resonance imaging using a wide-bore GE Discovery MR 750 3 T scanner (General Electric, Milwaukee, MI, USA) as previously described (Toemen et al., 2020) and included right ventricular end-diastolic volume (mL), right ventricular ejection fraction (%), left ventricular end-diastolic volume (mL), left ventricular ejection fraction (%), left ventricular mass (g), and left ventricular mass-to-volume ratio (g/L). End-diastolic volumes, ejection fractions, and left ventricular mass were converted to z-scores with adjustment for body surface area using generalized additive models for location, size, and shape (Toemen et al., 2020). Left ventricular mass-to-volume ratio was converted to z-scores without body surface area adjustment.

Vascular measures included carotid intima-media thickness (mm) and distensibility (kilopascal x 10^{-3}) as well as blood pressure (mmHg). Carotid intima-media thickness and distensibility were assessed using ultrasonography via the Logiq E9 (GE Medical Systems) as previously described (Blaauwendraad et al., 2022). We took the average of three readings and converted to z-scores derived from the study population for analyses. Systolic blood pressure and diastolic blood pressure were assessed four times at one minute intervals on the right brachial artery with the validated automatic sphygmomanometer Accutorr Plus (Datascope Corporation, Fairfield, NJ, USA). We took the mean values of the last three measurements and derived z-scores from the study population for analyses (Toemen et al., 2020).

Cardiovascular biomarkers were measured in venous blood samples and included non-fasting serum concentrations of glucose (mmol/L), insulin (pmol/L), total cholesterol (mmol/L), high-density lipoprotein (HDL) cholesterol (mmol/L), low-density lipoprotein (LDL) cholesterol (mmol/L), and triglycerides (mmol/L). The c702 module on the Cobas 8000 analyzer was used to determine glucose, cholesterol, and triglyceride concentrations. The electrochemiluminescence immunoassay (ECLIA) on the E411 module (Roche, Almere, The Netherlands) was used to determine insulin concentrations. Serum concentrations were converted to z-scores derived from the study population as previously described (Toemen et al., 2020) for analyses. Due to high correlations between total and LDL cholesterol (Figure S1, $r = 0.84$), we determined associations for LDL rather than total cholesterol.

2.4. Covariates

Mothers reported age, ethnicity (categorized as Dutch, European, non-European), parity (categorized as nulliparous, multiparous), pre-pregnancy weight, smoking (categorized as never during pregnancy, until pregnancy was known, during pregnancy) (Jaddoe et al., 2007), alcohol consumption (never during pregnancy, until pregnancy was known, during pregnancy), highest attained educational level (categorized as primary school, secondary school, higher), household income (<1200 euro per month which is below the Dutch social security level, 1200–2000 euro per month, >2000 euro per month), marital status (partner, no partner), and folic acid supplementation (supplementation, no supplementation) via prenatal questionnaire. Maternal height was measured without shoes at time of enrollment. Maternal body-mass indexes (BMI, kilograms [kg]/meter [m]^2) were calculated from self-reported pre-pregnancy weights and enrollment heights (Kooijman et al., 2016). Maternal daily fruit, vegetable, and total energy intake in kcal were collected in the first trimester via a modified validated semi-quantitative food frequency questionnaire covering the past three months (Klipstein-Grobusch et al., 1998/08/01 1998,; Slimani et al., 2002; Steenweg-de Graaff et al., 2014). Child's gestational age and sex assigned at birth were obtained from medical records.

We determined model adjustments using a directed acyclic graph (Figure S2) to identify potential confounders. Model covariates included maternal age, maternal ethnicity, parity, maternal pre-pregnancy BMI, maternal smoking, maternal alcohol consumption, maternal education, household income, maternal marital status, maternal folic acid supplementation, maternal fruit, vegetable, and total caloric intakes, and child's age and sex.

In a secondary analysis, we examined effect modification of associations by the child's *PON1* genotypes. Illumina 610 K and 660 W arrays were used to genotype cord blood. Single nucleotide polymorphisms (SNPs) for the PON1 gene included those in the coding (PON1-L55M [rs854560], PON1-Q192 [rs662]) and promoter (PON1-108 [rs705379], PON1-909 [rs854572], PON1-161 [rs705381]) regions, and were determined among a 430 children from our analytic sample, including those of both Dutch and non-Dutch national origin, as previously described6, (Jusko et al., 2019; Medina-Gomez et al., 2015).

2.5. Missing data imputation

Missing covariate data ranged from 0 (0 %) for maternal and child sociodemographics to 124 (19 %) for maternal dietary variables. Some organophosphate pesticide biomarker concentrations were missing due to insufficient sample or machine error (≤20 [3 %] of samples at each visit) and missingness due to concentrations below the LOD ranged from 0 (0 %) for DMP to 545 (85 %) for DEDTP. Creatinine was missing for a maximum of 5 (≤1%) of samples at each visit.

We used multiple imputation by chained equations to simultaneously impute missing exposures, covariates, and creatinine (qgcomp: Quantile G-Computation., 2022; R: a, 2022; van Buuren and Groothuis-Oudshoorn, 2011). In addition to these variables, imputation models included gestational age at each study visit, gestational age at birth, maternal marital status, and paternal education and BMI. Missing exposure concentrations were imputed from a left censored log-normal regression model with the maximum value set to the LOD for concentrations less than the LOD and set to the 95th percentile for concentrations missing due to insufficient sample or machine error (Welch et al., 2022). Otherwise, predictive mean matching was used to impute continuous variables and logistic or polytomous regression were used to impute categorical variables. Exposure biomarker concentrations at all visits were simultaneously included, but we restricted predictions involving gestational age and creatinine to exposure biomarker concentrations occurring at the same visit.

Missingness across outcomes ranged from 27 (4 %) for blood pressure to 230 (36 %) for structural cardiac measures. Missingness was similarly high in genotype data (n = 213 [33 %] for PON1-L55M, PON1-909, PON1-161, PON1-Q192; n = 217 [34 %] for PON1-108). We therefore did not impute study outcomes or genotype variables, nor were study outcomes and genotype variables included in imputation models.

2.6. Statistical analysis

Analyses were performed in R version 4.2.3 (Vienna, Austria). Prior to running imputation models, we examined the distributions and descriptive statistics (median [25th, 75th percentiles] and n [%]) for all covariates and outcomes. We also examined exposure descriptors including percent below LOD and percent missing due to machine error or inadequate sample. Subsequent analyses were run post-imputation.

Exposure biomarker concentrations were adjusted for urinary dilution using creatinine standardization based on the O'Brien method42, (O'Brien Katie et al., 2016; Butler AR. The Jaffé reaction. Part II. A kinetic study of the Janovsky complexes formed from creatinine (2-imino-1-methylimazolidin-4-one) and acetone. Journal of the Chemical Society, Perkin Transactions 2., 1975). Models were fit to predict urinary creatinine based on maternal characteristics including age, ethnicity, parity, BMI, smoking, alcohol consumption, education, household income, folic acid supplementation, and fruit, vegetable, and total caloric

intakes, as well as gestational age at assessment. We then standardized exposure biomarker concentrations for each participant using the following formula: $E_{cr} = E_o x \frac{Cr_p}{Cr_o}$, where E_{cr} is the creatinine-standardized exposure biomarker concentration, E_o is the observed exposure biomarker concentration, Cr_p is the predicted creatinine concentration from our model, and Cr_o is the observed creatinine concentration. The distribution (median [25th, 75th percentiles]) of creatinine-standardized exposure biomarker concentrations was examined at each visit.

As previously reported in this cohort, DAP metabolite concentrations demonstrated temporal variability across pregnancy with ICCs around 0.51 (95 % CI: 0.42–0.54) for total DAP metabolites in nmol/g creatinine26, (Spaan et al., 2015). We calculated the subject-specific geometric mean across pregnancy to determine gestational exposure biomarker concentrations for each participant (i.e., a pregnancy-average). Spearman's correlations were used to examine correlations among gestational exposure biomarker concentrations. Gestational exposure biomarker concentrations were log-transformed and standardized by the interquartile range (IQR) in subsequent analyses.

Parametric generalized linear regression models were used to estimate associations (β and 95 % confidence interval [CI]) between each gestational exposure biomarker concentration and cardiovascular outcomes in childhood. Betas represent the z-score difference in each cardiovascular outcome for a one-IQR increase in each gestational exposure biomarker concentration. Models were run separately for each exposure and outcome, and with and without adjustment for covariates. To facilitate comparisons to other studies and to investigate exposure to multiple organophosphate pesticides simultaneously, we calculated sums (ng/mL) of the three dimethyl metabolites (∑DMAP) from DMP, DMTP, and DMDTP, the three diethyl metabolites (∑DEAP) from DEP, DETP, and DEDTP, and the dimethyl and diethyl metabolites (∑DAP) from ∑DMAP and ∑DEAP. We then ran separate models to assess associations with ∑DAP, ∑DMAP, and ∑DEAP. We tested for non-linearity by introducing quadratic terms to models. Results from these analyses did not suggest deviation from linearity (p-value of quadratic terms ≥ 0.10) so we proceeded with analyses under the assumption of linearity.

We did two secondary analyses. Organophosphate pesticides and other chemicals that disrupt metabolism and endocrine function are frequently suspected of having sexually dimorphic effects8, (Le Magueresse-Battistoni and Tissue, 2020; Heindel et al., 2017). We therefore estimated sex-specific associations in models stratified by child's sex assigned at birth. To determine p-values for effect modification by child's sex, we added a single interaction term between exposure and child's sex to models from our primary analysis. A similar approach was used to examine genotype-specific associations; models were run separately for each SNP.

We had several sensitivity analyses to assess the robustness of our results. First, we re-ran primary analyses including inverse probability weights to adjust models for loss to follow-up and selection bias (van den Dries et al., 2020). We based these inverse probability weights on prior studies in the subset of participants with measured exposure, which found participants were more likely be Dutch, older, have a higher level of education, and a higher income compared with the full Generation R cohort (van den Dries et al., 2019). Next, to test the robustness of our imputation method, we re-ran primary analyses using a complete case dataset. For this complete case analysis, we excluded DEDTP due to high rates of concentrations below the limit of detection.

Due to multiple comparisons, we applied the Benjamini-Hochberg false discovery rate (Benjamini and Hochberg, 1995) using a q-value ≤ 0.10 to identify statistically significant associations (Stevens et al., 2023). In secondary analyses, an interaction p-value (p-int) ≤ 0.10 was used to determine potentially significant interactions; this p-int was not corrected for multiple comparisons. However, to determine which findings were notable, we considered the direction and magnitude of effect estimates, precision of confidence intervals, and consistency across biomarkers and models51, (Wasserstein and Lazar, 2016; STROBE, 2007).

3. Results

Our analytic sample consisted of gestational exposure to organophosphate pesticides measured up to three times in mothers and outcomes measured in their 643 children around 10 years of age (Table 1). During recruitment in 2004–2006, study participants were a median age of 31 years, had a median BMI of 22.3 kg/m^2, and were generally nulliparous (65 %) and socioeconomically advantaged (59 % highly educated and 73 % > 2000 euro per month). Genotyping data was available for 430 children (Table S1). Most organophosphate pesticide biomarkers had high (>80 %) detection during pregnancy with the exception of DEDTP, which was detectable in less than 20 % of urine samples at each visit (Table 2). Correlations between gestational exposure biomarker concentrations were low-to-moderate (Figure S3) with a maximum correlation of 0.59 (DMTP and DETP). Associations with individual metabolites were generally similar to those for summed biomarkers; we therefore present associations with individual metabolites supplementarily.

3.1. Associations of gestational organophosphate pesticide biomarker concentrations with child cardiac measures

There were few notable associations between gestational exposure biomarker concentrations and child cardiac outcomes, with findings similar in unadjusted (Table S2) and adjusted (Tables S3) models. In general, we observed negative estimates for left ventricular end diastolic volumes, and positive estimates for right ventricular ejection fractions, with increases in gestational exposure biomarker concentrations (Fig. 1**A**). There was some evidence of sex-specific associations for left ventricular ejection fraction (Table S4). For example, positive estimates for left ventricular ejection fraction were observed among female but not male children (p-int ≤ 0.10 for DMTP, ∑DMAP, and ∑DAP). There were no genotype-specific associations for summed gestational exposure biomarker concentrations and child cardiac outcomes (Fig. 2**A)** but one association of note for individual metabolites (Table S5-S9). For the PON1-909 SNP, smaller z-scores for right (β: −0.34 [95 % CI: −0.60, −0.09]) and left (β: −0.41 [95 % CI: −0.70, −0.11]) ventricular end diastolic volumes and left ventricular mass (β: −0.34 [95 % CI: −0.66, −0.02]) were observed for a one-IQR increase in gestational DMTP biomarker concentrations among those with the CC genotype.

3.2. Associations of gestational organophosphate pesticide biomarker concentrations with child vascular measures

Some gestational exposure biomarker concentrations were associated with lower carotid intima-media thickness and distensibility, as well as higher systolic blood pressure (Table S10-S11**,** Fig. 1**B**). After adjusting for covariates, a 0.14 (95 % CI: −0.25, −0.02) lower pediatric carotid intima-media thickness z-score was observed for a one-IQR increase in gestational ∑DEAP concentrations. Similarly, a 0.12 (95 % CI: −0.24, −0.01) and 0.11 (95 % CI: −0.23, 0.01) lower pediatric carotid intima-media distensibility z-score was observed for a one-IQR increase in gestational DMP and DMDTP concentrations, respectively; this was attenuated for ∑DMAP to a z-score difference of −0.08 (95 % CI: −0.19, 0.03). Finally, a 0.10 (95 % CI: −0.01, 0.21) and 0.10 (95 % CI: −0.01, 0.21) higher pediatric systolic blood pressure z-score was observed for a one-IQR increase in gestational ∑DMAP and ∑DAP concentrations, respectively.

There were no notable sex-specific associations for gestational exposure biomarker concentrations and child vascular outcomes (Table S12). However, we observed several genotype-specific associations for gestational exposure biomarker concentrations and child

Table 1
Characteristics of the study sample, consisting of 643 mother–child pairs in the Generation R Study. Mothers were recruited during pregnancy between 2004 and 2006. Children were followed up at age 10 years.

	Total Sample, n = 643
Parental characteristics	Median [IQR] or n (valid %)
Maternal age in years	31.3 [28.5, 34.0]
Maternal ethnicity	
Dutch	393 (61.1)
European	56 (8.7)
Non-European	194 (30.2)
Parity	
Nulliparous	415 (64.8)
Multiparous	225 (35.2)
Maternal pre-pregnancy body mass index in kg/m^2	22.3 [20.6, 25.0]
Maternal smoking	
Never during pregnancy	466 (78.1)
Until pregnancy was known	54 (9.0)
During pregnancy	77 (12.9)
Maternal alcohol consumption	
Never during pregnancy	210 (35.7)
Until pregnancy was known	107 (18.2)
During pregnancy	272 (46.2)
Maternal highest education attained	
Primary	28 (4.5)
Secondary	231 (36.7)
Higher	370 (58.8)
Household income	
<1200 euro per month	63 (11.0)
1200 – 2000 euro per month	92 (16.1)
>2000 euro per month	417 (72.9)
Maternal folic acid intake during pregnancy	
None	69 (13.2)
Started in first 10 weeks of pregnancy	175 (33.6)
Started preconception	277 (53.2)
Maternal fruit intake per day in kilocalories	191.9 [121.2, 230.8]
Maternal vegetable intake per day in kilocalories	139.8 [108.3, 180.1]
Maternal total energy intake per day in kilocalories	2072.1 [1739.6, 2417.0]
Marital status	
Partner	561 (89.6)
No partner	64 (10.4)
Paternal highest education attained	
Primary	23 (4.7)
Secondary	185 (37.4)
Higher	286 (57.9)
Paternal body mass index in kg/m^2	24.8 [22.7, 27.0]
Child characteristics	
Age child at visit in years	9.7 [9.6, 9.8]
Sex	
Male	325 (50.5)
Female	318 (49.5)
Gestational age at delivery	40.3 [39.4, 41.0]
Cardiac measures	
Right ventricular end-diastolic volume in mL	97.1 [85, 108.8]
Right ventricular ejection fraction in %	58 [54.3, 61]
Left ventricular end-diastolic volume in mL	96.9 [87.7, 106.8]
Left ventricular ejection fraction in %	58.3 [55.4, 61.5]
Left ventricular mass in g	45.3 [40.6, 51.5]
Left ventricular mass-to-volume in g/L	0.5 [0.4, 0.5]
Vascular measures	
Carotid intima-media thickness in mm	0.4 [0.4, 0.5]
Carotid intima-media distensibility per kilopascal x 10^{-3}	55.8 [48.8, 64.8]
Systolic blood pressure in mmHG	102.3 [97.7, 108.7]
Diastolic blood pressure in mmHG	58.5 [54, 63]
Cardiovascular biomarkers	
Glucose in mmol/L	5.3 [4.8, 6]
Insulin in pmol/L	194.6 [113.2, 308.6]
Total cholesterol in mmol/L	4.2 [3.8, 4.7]
High-density lipoprotein	1.4 [1.2, 1.7]
Low-density lipoprotein	2.3 [1.9, 2.7]
Triglycerides	1 [0.7, 1.3]

Missing per variable, n (%): parity, n = 3 (0.5); maternal pre-pregnancy body-mass index, n = 79 (12.3); smoking, n = 46 (7.2); alcohol consumption, n = 54 (8.4); education, n = 14 (2.2); income, n = 71 (11.0); dietary intake, n = 124 (19.3); marital status, n = 17 (2.6); folic acid intake, n = 122 (19.0); paternal education, n = 149 (23.2); paternal body mass index, n = 113 (17.6); structural cardiac measures, n = 230 (35.8); carotid intima-media thickness, n = 94 (14.6); carotid intima-media distensibility, n = 100 (15.6); blood pressure, n = 27 (4.2); glucose, n = 218 (33.9); insulin, n = 219 (34.1); total cholesterol, n = 217 (33.7); high-density lipoprotein cholesterol, n = 219 (34.1); low-density lipoprotein cholesterol, n = 220 (34.2); triglycerides, n = 218 (33.9).

vascular outcomes, primarily for systolic blood pressure (Fig. 2**B**, Table S13-S17). For example, those with the PON1-108 CC genotype generally had lower systolic blood pressure whereas those with the CT or TT genotype had higher blood pressure with an IQR increase in gestational exposure biomarker concentrations (7 of 9 biomarkers had a p-int $\leq$ 0.10). We also observed effect modification for all child vascular outcomes by the PON1-161 SNP. Among those with the CC genotype, carotid intima-media thickness and blood pressure were higher and carotid intima-media distensibility was lower for increases in gestational exposure biomarker concentrations; estimates were in the opposite direction among those with the GG genotype.

3.3. Associations of gestational organophosphate pesticide biomarkers concentrations with child cardiovascular biomarkers

Some gestational exposure biomarker concentrations were associated with higher HDL cholesterol, but few other associations were observed with child cardiovascular biomarkers (Table S18-S19**,** Fig. 1**C**). After adjusting for covariates, a 0.14 (95 % CI: 0.02, 0.25) z-score higher pediatric HDL cholesterol was observed for a one-IQR increase in gestational $\sum$DEAP concentrations.

There were no notable sex-specific associations for gestational exposure biomarker concentrations and child cardiovascular biomarkers (Table S20). However, notable effect modification of associations was observed, particularly for the PON1-L55M SNP genotypes and glucose and insulin (Fig. 2**C,** Tables S21-S25). Participants with the PON1-L55M TT genotype generally had higher glucose and insulin concentrations whereas those with the AA genotype had lower or no changes in glucose and insulin for an IQR increase in gestational exposure biomarker concentrations (at least 7 of 9 biomarkers had a p-int $\leq$ 0.10 for these outcomes). The association of $\sum$DEAP concentrations with triglycerides was modified (p-int $\leq$ 0.10) for 4 of 5 SNPs.

3.4. Sensitivity analyses

Associations were similar to our primary analysis in sensitivity analyses with inverse-probability weights for loss to follow-up and selection bias (Tables S26-S28). Compared to our primary analysis, we observed fewer statistically significant associations in complete case analysis though the direction and magnitude of estimates were similar to our primary analyses (Tables 29-S31).

4. Discussion

In this first human study to investigate gestational organophosphate pesticide exposure and cardiovascular outcomes, we observed that children possessing PON1 genotypes characterized by lower organophosphate pesticide detoxification were more likely to exhibit heightened risk of an adverse vascular profile and higher glucose and insulin levels associated with gestational organophosphate pesticide exposure. In our primary analysis without stratification by PON1 genotypes, findings for gestational DAPs and cardiovascular outcomes were heterogenous: we observed subclinical impairments in some measures of cardiovascular health for increases in gestational dimethyl biomarker concentrations and subclinical improvements in vascular health for increases in gestational diethyl biomarker concentrations. Associations were not modified by child's sex assigned at birth.

To our knowledge, prior studies have not evaluated child cardiovascular outcomes associated with gestational organophosphate

Table 2
Description of maternal gestational organophosphate pesticide biomarkers (ng/mL) in the Generation R study sample (n = 643).

Label	Biomarker	LOD (μg//L)	Early (<18 weeks)			Mid (18–25 weeks)			Late (>25 weeks)			Pregnancy-averaged
			% < LOD	% missing	Median (25th, 75th percentiles)	% < LOD	% missing	Median (25th, 75th percentiles)	% < LOD	% missing	Median (25th, 75th percentiles)	Median (25th, 75th percentiles)
Dimethyl metabolites	DMP	0.26	0.2	0.8	15 (9.3, 24.4)	0.0	0.3	14.4 (9.6, 22.5)	0.0	0.2	14 (9.1, 22.3)	14.8 (10.4, 20.5)
	DMTP	0.40	3.4	0.8	13.2 (6.4, 25.1)	3.7	0.2	13.3 (7.7, 23.6)	2.3	0.2	13 (7.2, 23.3)	13.1 (8.2, 20.1)
	DMDTP	0.09	19.0	0.8	0.5 (0.2, 1.1)	18.5	0.2	0.4 (0.2, 1)	17.0	0.2	0.4 (0.2, 1)	0.5 (0.3, 0.8)
	∑DMAP	–	–	–	28.9 (17.3, 49.9)	–	–	27.8 (17.8, 43.3)	–	–	27 (18.4, 44.5)	29 (20.5, 39.9)
Diethyl metabolites	DEP	0.50	3.1	0.9	4.7 (2.6, 8.3)	5.3	0.2	4 (2.3, 6.8)	3.9	0.2	4.2 (2.3, 7.8)	4.4 (2.9, 6.4)
	DETP	0.12	11.2	2.6	1.3 (0.5, 2.8)	11.8	3.1	1 (0.4, 2.3)	10.3	2.3	1.1 (0.5, 2.5)	1.2 (0.6, 2)
	DEDTP	0.06	80.7	0.8	0.1 (0, 0.1)	84.9	0.3	0.1 (0, 0.1)	85.2	0.3	0.1 (0, 0.1)	0.1 (0, 0.1)
	∑DEAP	–	–	–	6.5 (3.5, 11.7)	–	–	5.4 (3.2, 9.3)	–	–	5.7 (3.2, 10.6)	6 (4.1, 8.7)
Dialkyl phosphate metabolites	∑DAP	–	–	–	37.1 (23.5, 61)	–	–	34.9 (22.6, 54.4)	–	–	34.8 (23.1, 56.8)	36.7 (26.5, 49.9)

Abbreviations: Diethyldithiophosphate (DEDTP); Diethylphosphate (DEP); Diethylthiophosphate (DETP); Dimethyldithiophosphate (DMDTP); Dimethylphosphate (DMP), Dimethythiophosphate (DMTP), limit of detection (LOD); Sum of diethyl metabolites (∑DEAP); Sum of dimethyl metabolites (∑DMAP), Sum of dimethyl and diethyl metabolites (∑DAP).
Some exposure biomarker concentrations were missing due to insufficient sample or machine error. Missing exposure biomarker concentrations were imputed from a left censored log-normal regression model with the maximum value set to the LOD for concentrations less than the LOD and set to the 95th percentile for concentrations missing due to insufficient sample or machine error. After imputation, exposure biomarker concentrations were standardized for creatinine using the O'Brien method. For each exposure biomarker, the subject-specific geometric mean concentration of up to three urine samples (n = 637 with three samples, n = 4 with two samples, n = 2 with one sample prior to imputation) was calculated to obtain pregnancy-averaged exposure biomarker concentrations. The Median (25th, 75th percentiles) presented is the mean median and mean first and third quantiles from across the ten imputed datasets.

pesticide exposure specifically, though several studies have evaluated somewhat similar research questions (Tinggaard et al., 2016; Wohlfahrt-Veje et al., 2011; Etzel et al., 2020; Vrijheid et al., 2020; Blaauwendraad et al., Aug 2023; Andersen et al., 2012; Declerck et al., 2017). Two studies have reported positive associations between gestational organophosphate pesticide exposure and childhood adiposity16, (Wohlfahrt-Veje et al., 2011), while three studies – including the Generation R Study – did not find associations between prenatal exposure and obesity-related outcomes in childhood (Etzel et al., 2020/09/01/; Vrijheid et al., 2020; Blaauwendraad et al., Aug 2023). Andersen *et al* investigated occupational exposure to organophosphate and other pesticides during pregnancy (categorized as high, medium, or not exposed to pesticides based on toxicologist classification of greenhouse conditions) and excess cardiometabolic risk assessed using anthropometrics, blood pressure, heart rate, and metabolic biomarkers (leptin, insulin, insulin-like growth factor 1, and insulin-like growth factor binding protein 3) in 141 children 6–11 years of age21, (Declerck et al., 2017). This study reported that exposure was associated with higher abdominal circumference, body fat content, BMI Z-scores, blood pressure, and serum concentrations of leptin and insulin-like growth factor 1 only among those with the PON1 192R-allele21, (Declerck et al., 2017). The current study advances our knowledge from these prior studies by describing pediatric cardiovascular outcomes associated with gestational organophosphate pesticide exposure. We describe and interpret notable findings for each of our study outcomes in sections 4.1-4.3.

4.1. Interpreting findings for child cardiac measures

We observed weak evidence of associations between gestational organophosphate pesticide exposure and child cardiac measures, including specific measures of cardiac function and structure obtained from cardiac magnetic resonance imaging. End diastolic volumes (i.e., the amount of blood in the ventricles before contraction of the heart) were lower and right ventricle ejection fractions (i.e., the amount of blood the ventricle pumps out with each contraction of the heart) were higher for increases in gestational exposure biomarker concentrations. Secondary analyses suggested that, among those with the PON1-909 CC genotype, pediatric end diastolic volumes and left ventricular mass were lower for increased gestational DMTP concentrations.

While higher end diastolic volume and left ventricular mass and lower ejection fractions are considered markers of cardiac damage or disease in adults, the prognostic value of these measures in children are less clear (Toemen et al., 2020). Fetal growth restriction – an adverse health event linked to later cardiovascular morbidity and mortality (Barker, 2004) – has been associated with perinatal modifications in cardiomyocytes leading to lower right and left ventricular end diastolic volumes in children (Toemen et al., 2020). Furthermore, an unhealthy pattern of childhood growth characterized by higher childhood weight gain (Monteiro and Victora, 2005) has been associated with lower pediatric end diastolic volumes and left ventricular mass (Toemen et al., 2020). These lower cardiac indices may therefore be initial indicators of adverse cardiac remodeling in children, though further studies are needed to confirm these results and determine whether there are long-term implications of these findings.

4.2. Interpreting findings for child vascular measures

We observed evidence of pediatric vascular modifications following gestational organophosphate pesticide exposure. Findings were different by metabolites, with decreases in carotid intima-media thickness for increases in diethyl metabolite concentrations and impairments in carotid intima-media distensibility and systolic blood pressure for increases in dimethyl metabolite concentrations. Carotid intima-media thickness assesses the thickness of the inner layers of the carotid artery, reflecting morphological changes in the vascular tree; increased carotid intima-media thickness is an indicator of atherosclerosis (Epure et al., 2020). Thus, our findings of *decreased* carotid intima-media thickness for increases in diethyl metabolite concentrations is contrary to our hypothesis that exposure might induce adverse vascular modifications. The implications of these findings are unclear, especially given observed decreases in carotid intima-media distensibility and increases in systolic blood pressure for increases in gestational dimethyl metabolite concentrations. Carotid intima-media distensibility assesses elasticity of the inner layers of the carotid artery, reflecting functional

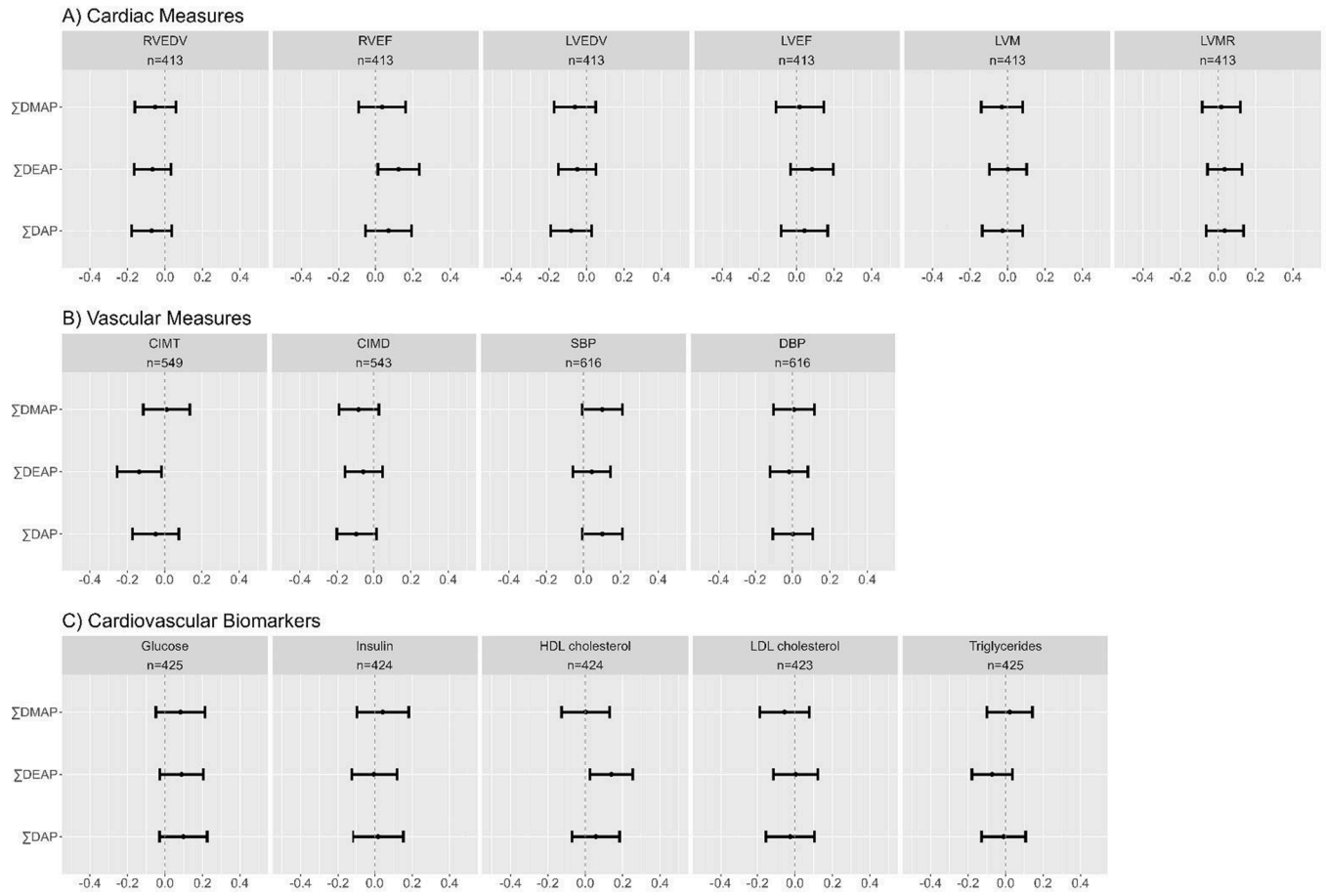

Fig. 1. Associations for gestational organophosphate pesticide biomarker concentrations and A) cardiac measures, B) vascular measures, and c) cardiovascular biomarkers in childhood in the Generation R Study sample (n = 643). Betas represent the z-score difference in pediatric outcomes at age 10 for a one-IQR increase in gestational exposure biomarker concentrations from a generalized linear regression model adjusted for maternal age, maternal ethnicity, parity, maternal BMI, maternal smoking, maternal alcohol consumption, maternal education, household income, maternal marital status, maternal folic acid supplementation, maternal fruit, vegetable, and total caloric intakes, and child's age and sex. Abbreviations: Carotid intima-media distensibility (CIMD); Carotid intima-media thickness (CIMT); Diastolic blood pressure (DBP); High-density lipoprotein (HDL); Left ventricular end-diastolic volume (LVEDV); Left ventricular ejection fraction (LVEF); Low-density lipoprotein (LDL); Left ventricular mass (LVM); Left ventricular mass-to-volume (LVMR); Right ventricular end-diastolic volume (RVEDV); Right ventricular ejection fraction (RVEF); Single nucleotide polymorphism (SNP); Sum of diethyl metabolites (∑DEAP); Sum of dimethyl metabolites (∑DMAP), Sum of dimethyl and diethyl metabolites (∑DAP); Systolic blood pressure (SBP).

changes in the vascular tree; lower carotid intima-media distensibility is an indicator of atherosclerosis. Blood pressure is independently inversely associated with arterial distensibility, and changes in both these measures associated with exposure indicate worse vascular health (Tran and Urbina, 2023).

When stratifying by genotype, we observed a worse vascular profile for increases in organophosphate pesticide exposure concentrations among those with the CC genotype of the PON1-161 SNP. Among these participants, we observed higher carotid intima-media thickness and blood pressures, but reduced carotid intima-media distensibility for increases in exposure concentrations. Vascular measures were the only outcomes with notable effect modification by this SNP, suggesting that this SNP directly moderates exposure-induced changes in vascular health rather than operating through or alongside pathways involving cardiac modifications. We additionally observed evidence that several PON1 genotypes modified associations between organophosphate pesticide biomarkers and systolic blood pressure, though effect sizes were modest.

4.3. Interpreting findings for child cardiovascular biomarkers

We observed higher HDL cholesterol in childhood, which may indicate an improved cardiovascular profile (Feingold, et al., 2000), for increases in gestational organophosphate pesticide exposure concentrations. Similar to findings for carotid intima-media thickness, these findings were for increases in diethyl metabolite concentrations but not dimethyl metabolite concentrations. HDL cholesterol is atheroprotective (Feingold, et al., 2000), suggesting HDL cholesterol may help explain why we observed an improvements in carotid intima-media thickness associated with diethyl exposure.

After stratifying by genotype, glucose and insulin levels were higher for increases in gestational organophosphate pesticide exposure concentrations among those with a TT genotype of the PON1-L55M SNP. These findings suggest exposure may be associated with gylcemic traits and a heightened risk of diabetes among those with this particular genotype (Huang et al., 2019) and are in line with the hypothesized association between the PON1-L55M SNP, organophosphate pesticides, and cardiovascular disease risk8, (Paul et al., 2017). To our knowledge, no prior studies have investigated effect modification of associations between organophosphate pesticide exposure and glycemic traits or diabetes by the PON1-L55M SNP.

4.4. Study Limitations

We evaluated associations within an urban cohort in which exposure to organophosphate pesticides likely occurred through ingestion of

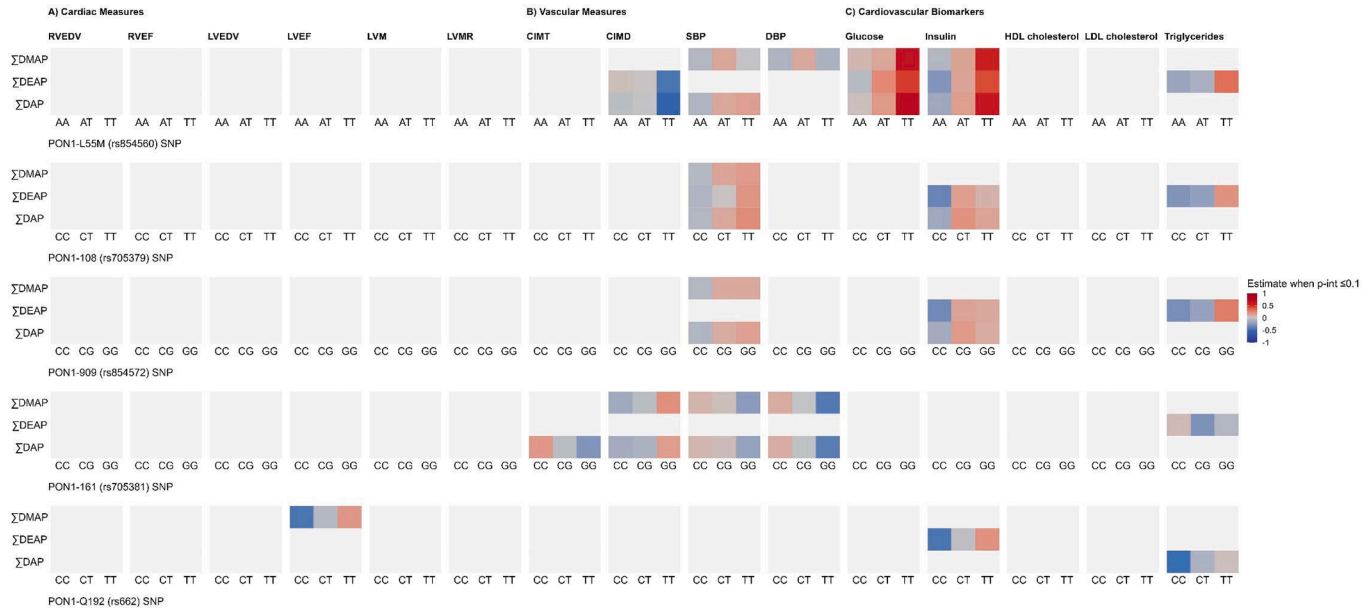

Fig. 2. Heatmap of genotype-specific associations for gestational organophosphate pesticide biomarker concentrations and A) cardiac measures, B) vascular measures, and c) cardiovascular biomarkers in childhood in the Generation R Study sample with genotyping data (n = 430). Boxes are colored according to the significance of the SNP interaction term p-value (p-int) and the magnitude and direction of effect estimates from generalized linear regression models examining the z-score difference in pediatric outcomes at age 10 for a one-IQR increase in gestational exposure biomarker concentrations. Models were adjusted for maternal age, maternal ethnicity, parity, maternal BMI, maternal smoking, maternal alcohol consumption, maternal education, household income, maternal marital status, maternal folic acid supplementation, maternal fruit, vegetable, and total caloric intakes, and child's age and sex. Abbreviations: Carotid intima-media distensibility (CIMD); Carotid intima-media thickness (CIMT); Diastolic blood pressure (DBP); High-density lipoprotein (HDL); Left ventricular end-diastolic volume (LVEDV); Left ventricular ejection fraction (LVEF); Low-density lipoprotein (LDL); Left ventricular mass (LVM); Left ventricular mass-to-volume (LVMR); Right ventricular end-diastolic volume (RVEDV); Right ventricular ejection fraction (RVEF); Single nucleotide polymorphism (SNP); Sum of diethyl metabolites (∑DEAP); Sum of dimethyl metabolites (∑DMAP), Sum of dimethyl and diethyl metabolites (∑DAP); Systolic blood pressure (SBP).

contaminated foods (van den Dries et al., 2018); despite adjusting for fruit and vegetable intakes, there may still be residual confounding by seasonality and behavioral or lifestyle factors. We were not able to assess exposure to organophosphate pesticides directly as DAP metabolites are non-specific and unable to be traced back to source pesticides (Margariti et al., 2007). However, organophosphate pesticide exposure assessment via urinary DAP metabolites concentrations is commonly used as it is easy to ascertain and non-invasive (Bravo et al., 2004). We interpret findings for DEDTP cautiously due to low detection of this metabolite, and present these results supplementarily for comparison to prior studies. Non-fasting blood samples were collected and used for assessment of cardiometabolic biomarkers. This may have increased outcome variability, leading to attenuation of associations (Toemen et al., 2020). Sample size was limited in this study relative to the full Generation R cohort, and missingness in PON1 genotypes led to even lower sample sizes in these secondary analyses. However, we found consistent results in sensitivity analyses limiting to complete case and correcting for potential loss to follow-up and selection bias.

4.5. Study strengths

Most prior studies of organophosphate pesticide exposure have been conducted in occupational settings. To understand associations with health outcomes in the general population, studies on non-occupational settings such as the Generation R sample are crucial. Organophosphate pesticides are non-persistent with short half-lives, and exposure assessment at multiple timepoints can help prevent exposure misclassification and attenuation bias (Vernet et al., 2019). Few prior studies have more than one timepoint with exposure assessment, yet the Generation R Study included three timepoints with urine sample collection for assessment of organophosphate pesticide exposure. In our sample, 99 % of participants had measured urinary DAP metabolites concentrations at all three timepoints.

4.6. Conclusions

In a cohort based in The Netherlands, we report findings from a study examining the average of three repeated measures of urinary concentrations of DAP metabolites obtained during pregnancy and cardiovascular outcomes assessed in 643 children at age 10 years. These findings suggest some subclinical impairments in cardiovascular health for increases in gestational exposure concentrations with effect modification by genotype. In particular, a more vulnerable PON1-161 and PON1-L55M genotype conferred excess risk of adverse vascular and glycemic outcomes, respectively, for increases in gestational organophosphate pesticide exposure concentrations. We also observed an atheroprotective profile in children for increases in gestational diethyl metabolite concentrations, which goes against our hypothesis that exposure may impair cardiovascular health. In the Netherlands, about a third of insecticides in 2004 were organophosphate insecticides generating dimethyl metabolites (e.g., Dimethoate, Malathion) whereas fewer than 1 % were organophosphate insecticides generating diethyl metabolites (e.g., chlorpyrifos, chlorfenvinphos) (van den Dries et al., 2018). These exposure discrepancies were reflected in the current study, which exhibited higher concentrations of dimethyl than diethyl metabolites. Thus, even if exposure to organophosphate pesticides generating diethyl metabolites is truly associated with an improved cardiovascular profile, exposure is infrequent and low relative to other pesticide exposures. However, overall high levels of organophosphate pesticide metabolites were observed among this urban cohort and our findings of adverse vascular and glycemic outcomes among gestationally exposed children with certain genotypes are concerning from a public health perspective, suggesting the adoption of exposure mitigation methods (e.g., consumption of organic foods Berman et al. (2016)). As this is the first study to evaluate cardiovascular outcomes following gestational organophosphate pesticide exposure specifically, further studies are needed with special attention to measurement of PON1 SNPs which appear to

play important roles in these relationships.

CRediT authorship contribution statement

Danielle R. Stevens: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Sophia M. Blaauwendraad:** Writing – review & editing, Software, Resources, Methodology, Investigation, Data curation. **Paige A. Bommarito:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Michiel van den Dries:** Writing – review & editing, Methodology, Investigation, Data curation. **Leonardo Trasande:** Writing – review & editing, Methodology, Investigation. **Suzanne Spaan:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Anjoeka Pronk:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Henning Tiemeier:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Romy Gaillard:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Vincent W.V. Jaddoe:** Writing – review & editing, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Kelly K. Ferguson:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.109082.

Data availability

Data will be made available on request.

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