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Exposure to endocrine disrupters and cardiometabolic health effects in preschool children: Urinary parabens are associated with wider retinal venular vessels

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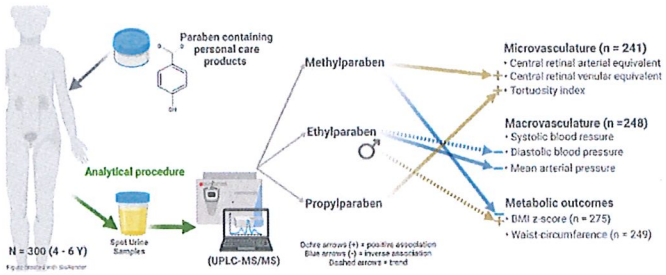
HIGHLIGHTS

- Early life exposure to urinary parabens was associated with cardiometabolic outcomes.
- Methyl paraben (MeP) was associated with a wider central retinal venular equivalent.
- Propyl paraben was associated with a higher retinal tortuosity index.
- MeP and the molar sum of parabens were inversely associated with BMI z-scores.
- In boys, ethyl paraben may be associated to higher BMI z-scores.

GRAPHICAL ABSTRACT



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ABSTRACT

Background and aim: Parabens are widely used as antimicrobial preservatives in personal care products. Studies investigating obesogenic or cardiovascular effects of parabens show discordant results, while data on preschool children are lacking. Paraben exposure during early childhood could have profound cardiometabolic effects later in life.

Methods: In this cross-sectional study paraben concentrations [methyl (MeP), ethyl (EtP), propyl (PrP), butyl (BuP)] were measured by ultra-performance liquid chromatography/tandem mass spectrometry in 300 urinary samples of 4-6-year-old children of the ENVIRONAGE birth cohort. Paraben values below the limit of quantitation (LOQ) were imputed by censored likelihood multiple imputation. The associations between log-transformed paraben values and cardiometabolic measurements (BMI z-scores, waist circumference, blood pressure and retinal microvasculature) were analyzed in multiple linear regression models with *a priori* selected covariates. Effect modification by sex was investigated by including interaction terms.

Results: Geometric means (geometric SD) of urinary MeP, EtP, and PrP levels above the LOQ were 32.60 (6.64), 1.26 (3.45), and 4.82 (4.11) μg/L, respectively. For BuP more than 96% of all measurements were below the LOQ. Regarding the microvasculature, we found direct associations between MeP and central retinal venular

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equivalent (β = 1.23, p = 0.039) and PrP with the retinal tortuosity index ($x10^3$)(β = 1.75, p = 0.0044). Furthermore, we identified inverse associations between MeP and ∑parabens with BMI z-scores (β = −0.067, p = 0.015 and β = −0.070, p = 0.014 respectively), and EtP with mean arterial pressure (β = −0.69, p = 0.048). The direction of association between EtP and BMI z-scores showed evidence for sex-specific differences with a direct trend in boys (β = 0.10, p = 0.060).

Conclusions: Already at young age paraben exposure is associated with potentially adverse changes in the retinal microvasculature.

1. Introduction

Parabens are low-cost, broad-spectrum antimicrobial agents in personal care products, food and pharmaceuticals (Elder, 1984; Ana and Paula, 2016). Methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP) and butyl paraben (BuP) are *p*-hydroxybenzoic acid esters with alkyl substituents of increasing length (Andersen, 2008). It has been suggested, that the widespread use of parabens (Elder, 1984; Kim and Chevrier, 2020) may contribute to the global metabolic and cardiovascular health epidemic due to potential endocrine disrupting properties (Heindel et al., 2015; Heindel and Blumberg, 2019). There are conflicting results in studies investigating links with metabolic outcomes (Kim and Chevrier, 2020; Reimann et al., 2021; Lee et al., 2019; Pazos et al., 2019; Quirós-Alcalá et al., 2018; Hu et al., 2013; Liu et al., 2019a), and parabens' general classification of potential cardiometabolic effects remains challenging.

The prevalence of cardiometabolic conditions is low at young age and subclinical changes in cardiometabolic risk factors may be missed or perceived as unharmful. However, this developmental period is sensitive. Exposure to endocrine-disrupting substances may have long-lasting effects (Birnbaum, 2013). Deviations from the normative developmental track in body weight and abdominal circumference in early childhood are linked with obesity in adulthood (Fang et al., 2019; Simmonds et al., 2016). Higher blood pressure (BP) levels in childhood and adolescence predict an increased risk of hypertension in later in life (Hardy and Urbina, 2021; Juhola et al., 2013). Mounting evidence suggests that cardiovascular risk markers in early life are also associated with structural changes in the retinal microvasculature (Newman et al., 2017) as endothelial dysfunction is detected in the microcirculation before macrovascular structures are affected (Rijks et al., 2018).

One study reported an increase in the odds of hypertension for the association with PrP exposure in middle-aged Mexican women (Zamora et al., 2021), while in children, prenatal and postnatal exposures to parabens were not linked to BP in 6–11-year old's (Warembourg et al., 2019a). However, the authors described that higher exposure to MeP and BuP were associated with smaller retinal vein widths (Montazeri et al., 2022).

Exposure to endocrine-disrupting chemicals during early childhood could indicate later health risks. However, the current body of literature does not inform us sufficiently about the harmful effects of paraben exposure during this sensitive developmental period. With this research, we aim to fill this gap, investigate multiple measures of cardiometabolic risk in preschool children exposed to parabens, and analyze the possibility of sex-specific differences in these associations.

2. Material and methods

2.1. Study population

Within the framework of the ongoing birth cohort Environmental Influence on Early Aging (ENVIRONAGE), mothers who did not have planned caesareans and could complete questionnaires in Dutch were enrolled on arrival at the delivery-ward at the East-Limburg Hospital in Genk, Belgium. Recruitment was conducted after providing written consent and according to procedures approved by the ethical committees of Hasselt University and the East-Limburg Hospital and conform to the principles outlined in the Helsinki Declaration.

When the child was between four and six years old, the parents were invited to fill in an online questionnaire. Mother-child pairs were invited for a follow-up (FU) examination. Before the start of the clinical examinations, mothers renewed their written informed consent, and the children gave their oral assent for the measurements. Examinations took place in a child-friendly room, and it was explained to both mothers and children that measurements were not obligatory and could be stopped at any moment.

The selection of mother-child pairs for the performed analyses is shown in Supplementary Fig. S1. Recruitment at birth started in February 2010 and this process is ongoing. FU examinations included in this study occurred between October 2014 and December 2020. During this period, 1367 children were aged between 4 and 6 years, of which 866 were eligible for the FU examination. In 494 of these cases, the consent was renewed, which brought the participation rate of the FU examination to 57.0%. Urinary paraben levels were quantified in 300 randomly selected urine samples of children that gave an adequate urine volume during the FU examination. Measurement of i) urinary osmolality was performed in 299 samples, ii) of BMI z-score in 298 samples, iii) of abdominal circumference in 264 samples, iv) of blood pressure in 263 samples and v) of retinal microvasculature in 291 samples. Information on household smoking was unavailable for 22 mother-newborn pairs, and for retinal microvasculature assessment, mean arterial pressure (MAP) values were missing in 37 cases. This resulted in a sample size of 275 for BMI z-score, 249 for abdominal circumference, 248 for blood pressure and 241 for retinal microvasculature in the statistical analysis (Supplementary Fig. S1).

2.2. Study procedure

2.2.1. Data collection

At birth medical information on newborns' sex, delivery date, and maternal parity from medical hospital records were retrieved. Parity was divided into three categories: mothers having their first, second, or third child or more. Additional information was obtained by a questionnaire during the mothers stay at the delivery ward. The child's ethnicity was considered to be European when two or more grandparents of the newborn were European; otherwise it was coded as non-European. Maternal smoking status was classified as never smoker, former smoker if she had stopped smoking before the index pregnancy, or smoking during pregnancy when the mother smoked at any point during the index pregnancy. From the online questionnaire at FU household smoking after birth was categorized with yes and no, depending on whether one or both of the parents smoked at any point after the birth. Maternal educational level was coded as "low" for mothers who did not obtain any diploma, "middle" when they obtained a high school diploma, and "high" when they obtained a college or university degree. In 23 cases where information about the maternal education level at the time of the FU was missing, the information provided in the questionnaire at birth was used. The child's exact age at FU was calculated as the difference between the numerical values of the date of FU participation and date of delivery and expressed with decimal precision as age in years plus the fractional age portion.

2.2.2. Assessment of retinal microvasculature characteristics

Fundus pictures of the left and right eye of the child were obtained with a Canon CR-2 plus 45° 6.3 megapixels digital nonmydriatic retinal camera (Hospithera, Brussels, Belgium) and further analyzed with the MONA-REVA vessel analysis software (version 2.1.1) developed by VITO (Mol, Belgium; http:\vito.be) as described previously (Luyten et al., 2020; Cox et al., 2020). Consistent retinal regions were obtained across all the fundus images in MONA REVA by defining an annular region centered on the optic disc, with the inner and outer radii of the annulus set at 1.0 and 3.0 times the radius of the optic disc, respectively. Next, the image analysis algorithm based on a multiscale line filtering algorithm automatically segmented the retinal vessels (Nguyen et al., 2013). Post-processing steps such as double thresholding, blob extraction, removal of small connected regions, and filling holes were performed. The diameters of the retinal arterioles and venules that passed entirely through the circumferential zone 0.5 to 1-disc diameter from the optic disc margin were calculated automatically. The trained grader verified and corrected vessel diameters and vessel labels (arteriole or venule) with the MONA REVA vessel editing toolbox. The diameters of the 6 largest arterioles and 6 largest venules were used in the revised Parr-Hubbard formula for calculating the Central Retinal Artery Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE) (Knudtson et al., 2003). The tortuosity index was computed as the mean tortuosity of the branch segments. Tortuosity of individual vessel segments was calculated as reported by Lisowska and coworkers (Lisowska et al., 2014). Both eyes' average CRAE, CRVE, and TI values were used in further analyses if both pictures were available. For 56 of 291 (19.2%) children with fundus pictures, only values of one eye were available due to missingness or insufficient quality of the second picture. In these cases, values from a single eye were used in the analyses.

2.2.3. Blood pressure measurements

Blood pressure was measured with an automated upper-arm blood-pressure monitor (Omron 705IT; Omron Corporation, Gent, Belgium), with a cuff accustomed to the arm size of children. Measurements were performed in a standardized way, as described previously (Flynn et al., 2017). In brief, after 5 minutes of rest in a supine position, a trained observer obtained five consecutive readings of the SBP and DBP at 1-min intervals. The mean values of SBP and DBP were based on the third, fourth and fifth measurements. Mean arterial pressure (MAP) was calculated by adding (2/3 × DBP) and (1/3 × SBP).

2.2.4. Determination of body mass index

A trained examiner measured children's height with a fixed stadiometer with an accuracy of 0.5 cm, and weight was measured to the nearest 0.1 kg with a digital scale. From the height and weight measurements, BMI z-scores were calculated according to the World Health Organization's (WHO) Child Growth Standards based on height, weight, age and sex of the child (World Health Organization (WHO) 2006). Abdominal circumference was measured at the level of the child's belly button. The examiner paid attention that the child was neither holding its breath nor blowing up its belly.

2.3. Urinary paraben concentrations

During the FU examination, spot-urine samples were collected in metal-free polypropylene containers (Yvsolab, Turnhout, Belgium) and temporarily stored on ice until the end of the examination. Then the samples were aliquoted into metal-free 50 ml Falcon tubes (VWR, Haasrode, Belgium) and stored at −20 °C until further processing.

For the mass spectrometric analysis, the samples were thawed, aliquoted into 15 ml tubes, and shipped on dry ice to the Laboratory of Clinical, Forensic and Environmental Toxicology, University of Liege, Belgium, where they were processed according to a previously described protocol (Dewalque et al., 2014a) (Supplementary Text 1), using a Quattro Premier XE mass spectrometer coupled to an Acquity UPLC system (Waters, Milford, MA, USA).

2.4. Statistics

In the descriptive statistics, the values of urinary parabens also include the trimmed mean as recommended for measurements with values below the LOQ (United States Environmental Protection Agency (EPA), 2000). For the regression analyses MeP, EtP and PrP exposure values below the LOQ were imputed via censored likelihood multiple imputation (CLMI) using the CRAN package lodi (Boss et al., 2019) as described in Supplementary Text 2. Additionally, a sensitivity analysis was performed for selected models including a complete case analysis and imputation with LOQ/2 to examine the robustness of the analysis with CLMI. In order to avoid simulation error, 20 rounds of imputation were performed, as suggested previously (van Buuren, 2018).

The molar sum of paraben concentrations (∑parabens) was calculated from the LOQ/2 imputed paraben concentrations in micrograms per liter divided by the molecular weight of the parabens and log-transformed for use in the analyses.

Various *a priori* selected variables described in the literature to be associated with the dependent variables, were added as covariates to the models: child's sex, age at FU examination, ethnicity and gestational age in weeks, parity, maternal early-pregnancy BMI, maternal education and smoking before and during pregnancy, current household smoking and osmolality of the child's urine. Furthermore, we adjusted in the analyses of different outcomes for additional variables described in the caption of Fig. 1 and in Supplementary Table S1.

Additionally, the interaction terms for the sex of the child and parabens were tested. If the interaction term was significant, the analysis was stratified for sex. Furthermore, we used quantile g-computation with the qgcomp package (Keil et al., 2020) as a sensitivity analysis to estimate the joint effects of paraben exposure in association with the cardiometabolic outcomes as described in Supplementary Text S3. Therefore we first imputed paraben values below LOQ with the mice. impute.leftcenslognorm function of the qgcomp package, conducted quantile g-computation on each of the 20 imputed datasets and then pooled the results from the models' fits using Rubin's method (Rubin, 2004). Collinearity of variables was examined by calculating the variance inflation factor with the R package *'faraway'* (Faraway, 2005). Statistical significance was defined as $p < 0.05$. All data analyses were performed in RStudio (RStudio Team, 2020) using R 4.1.2.

3. Results

3.1. Demographics

The children in this study were mostly of European origin (95.3%), had a mean (SD) gestational age of 39.1 (1.9) weeks, a mean (SD) birth weight of 3364 (491) g and were on average 4.5 (0.4) years at the time of examination (Table 1). There were fewer girls than boys in the subset of this study (41.8% vs. 58.2%). The mothers had an average pre-pregnancy BMI of 24.2 (4.3) kg/m^2; most had achieved a higher education level and never smoked. In 28.7% of the children's households, one of the parents had been smoking between birth and FU examination. For most mothers it was their first child (56.0%). The average values of the outcome variables are shown in Table 1.

3.2. Urinary paraben measurements

As more than 96% of the BuP measurements were below the LOQ, only the values of MeP (98.3% > LOQ), EtP (66% > LOQ) and PrP (85% > LOQ) were used for further statistical analysis. The trimmed means (SD) of urinary MeP (n = 290), EtP (n = 96), and PrP (n = 210) levels were 147.01 (316.89) μg/L, 0.55 (0.17), and 4.67 (4.08) μg/L respectively. The geometric means and additional descriptive statistics are shown in Supplementary Table S2 and Supplementary Fig. S2.

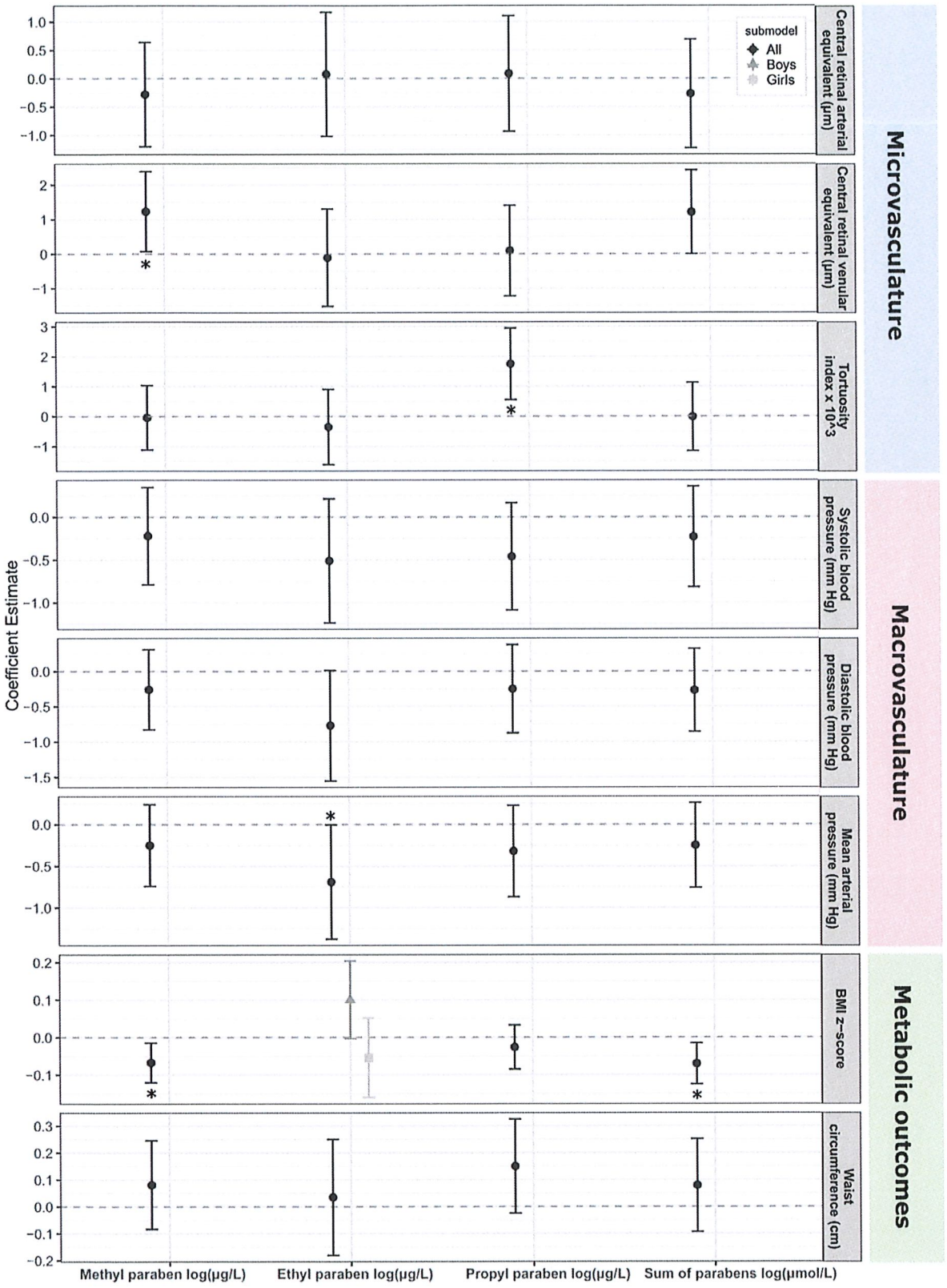

Fig. 1. Overview of the estimates and 95% confidence intervals in the analyses of the associations between MeP, EtP, PrP and ∑parabens with cardiometabolic outcomes. The multiple linear regression models were all adjusted for: child's sex, age, ethnicity and gestational age, parity, maternal early-pregnancy BMI, maternal education and smoking before and during pregnancy, current household smoking and osmolality of the child's urine. Additionally, adjustments were performed as follows: i) BMI z-scores for birthweight, ii) for abdominal circumference for birthweight and BMI z-score, iii) blood pressure measurements for BMI z-score and month and hour of the day of the examination, and iv) retinal microvasculature for BMI z-score, the month of examination, mean arterial pressure (MAP) and CRAE and CRVE respectively. If the interaction term for sex was significant, the analysis was performed in sub-models stratified for sex (indicated in light grey).* significance at $p < 0.05$.

Table 1
Population characteristics of n = 275 participants with available covariates.

Characteristics	Mean (±SD) or n (%)
Child Birth	
Girls, n	115 (41.8%)
European, n	262 (95.3%)
Birthweight, grams	3364 ± 491
Gestational age, weeks	39.1 ± 1.9
Child Follow-Up	
Age, years*	4.5 ± 0.4
BMI z-score	0.5 ± 0.9
Abdominal circumference[a], cm	53.3 ± 3.7
Urinary osmolality, mOsm/kg	704.7 ± 269.2
SBP[b], mmHg	98.2 ± 8.0
DBP[b], mm Hg	55.8 ± 8.2
MAP[b], mm Hg	69.9 ± 7.1
CRAE[c], μm	171.4 ± 16.2
CRVE[c], μm	241.2 ± 22.0
TI x 10^{3c}	890 ± 15
Mother	
Early pregnancy BMI, kg/m^2	24.2 ± 4.3
Education level at FU, n	
Low	15 (5.5%)
Middle	78 (28.4%)
High	182 (66.2%)
Smoking, n	
Never smoked	184 (66.9%)
Former smoker	61 (22.2%)
Smoked during pregnancy	30 (10.9%)
Smoking in household, n	79 (28.7%)
Parity, n	
1	154 (56.0%)
2	93 (33.8%)
≥3	28 (10.2%)

[a, b, c] Number of participants for the different outcome measures: a = 249, b = 248, c = 241.
CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; DPB = diastolic blood pressure; FU = follow-up; MAP = mean arterial pressure; SPB = systolic blood pressure; TI = tortuosity index.
*Age at FU examination was calculated as the difference between the numerical values of date of FU participation and date of delivery and expressed with decimal precision as age in years plus the fractional age portion.

3.3. Associations between urinary parabens and retinal vessel metrics

We found that MeP values in urine were associated with higher CRVE values (Fig. 1, Supplementary Table S3) in an adjusted model (β = 1.23, p = 0.039). This result translates into a 0.85 μm increase in CRVE for a doubling in urinary MeP values. For log-transformed PrP values the pooled analysis showed a significant association with TI x10^3 (β = 1.75, p = 0.0044), or an increase of 1.21 for a doubling in PrP exposure.

3.4. Association between urinary parabens and measures of blood pressure

Higher urinary EtP values were associated with lower mean arterial pressure (MAP), driven by a negative trend for DBP (Fig. 1, Supplementary Table S3). Specifically, an adjusted multiple linear regression model showed an inverse association between log-transformed EtP values and MAP (β = −0.69, p = 0.048), indicating a decrease in MAP of 0.49 mm Hg for a doubling in urinary EtP values. The same trend was visible for the association between EtP and DBP (β = −0.77, p = 0.058).

3.5. Associations between urinary parabens and BMI z-scores and abdominal circumference

Urinary MeP and ∑parabens levels were associated with BMI in preschool children (Fig. 1, Supplementary Table S3). Based on a pooled analysis of 20 adjusted linear regression models, an inverse association between log-transformed MeP values and BMI z-scores was found (β = −0.067, p = 0.015). This translates to a decrease of −0.046 points in the BMI z-score for a doubling of MeP values. Additionally, ∑parabens were significantly associated with BMI z-scores (β = −0.070, p = 0.014), representing a decrease of −0.049 points in BMI z-score.

In the case of EtP exposure the interaction term for sex was significant (p = 0.032), which resulted in a stratified analysis for the association between EtP and BMI z-score. In boys urinary EtP was borderline significantly associated with BMI z-scores (n = 160, β = 0.10, p = 0.060) but not in girls (n = 115, β = −0.054, p = 0.32) (Fig. 1, Supplementary Table S3).

Unlike the association with BMI z-scores, all parabens showed a positive trend for the association with waist circumference (Fig. 1, Supplementary Table S3).

3.6. Sensitivity analyses

In the sensitivity analysis the imputation by CLMI and LOQ/2 showed comparable estimates and p-values. In the complete case analyses the estimate for the association between EtP and MAP was more negative with a lower p-value (n = 167, β = −1.26, p = 0.0039) while the associations between MeP and CRVE and PrP and TIx10^3 showed lower, non-significant positive estimates (n = 238, β = 1.17, p = 0.058 and n = 209, β = 1.13, p = 0.13 respectively) (Supplementary Table S4).

Using quantile g-computation we did not observe any associations between the joint exposure to MeP, EtP and PrP with one of the cardiometabolic outcomes (Supplementary Table S5).

4. Discussion

The main finding of our study is the direct association between MeP and PrP with retinal microvasculature in preschool children. Furthermore, we present evidence of changes in other measures of cardiometabolic health in association with paraben exposure already in early childhood.

4.1. Paraben exposure in preschool children

We found three of the four parabens commonly used in personal care products (Food and Drug Administration, 2022, van der Schyff et al., 2022) in a large percentage of urinary samples of Flemish preschool children. MeP, with the shortest chain length and lowest antimicrobial activity, was present in almost all samples and in the highest concentrations. This observation is in line with other reports of paraben exposure in Chinese (Guo et al., 2017), Swedish (Larsson et al., 2014), US-American (Quirós-Alcalá et al., 2018), and Indian (Xue et al., 2015) children, though the concentration (GM of the uncorrected values) found in our study population was higher than those in the studies mentioned above (literature overview in Supplementary Table S6). One reason for this could be a difference in the concentrations of different parabens used in personal care products in different countries and age groups; for the age group of 1-6-year-olds a previous study of the Belgium population showed an even higher median concentration than observed in this study (Dewalque et al., 2014b).

Our study's high detection rate of PrP is in line with reports of widespread exposure to PrP in children elsewhere (Quirós-Alcalá et al., 2018; Guo et al., 2017; Xue et al., 2015). At the same time, EtP was found in only 66% of the urinary samples, less than in previous reports (Guo et al., 2017; Larsson et al., 2014; Xue et al., 2015) (Supplementary Table S6). The concentrations measured in our study were, on average higher than those found in other studies (Supplementary Table S6), which could be explained by the fact that in our study, we only reported values above the LOQ to ensure the reliability of the obtained values. To account for this, we additionally reported the trimmed means. Furthermore, differences in demographic and geographic characteristics of the study populations, including age and potential product use,

experimental parameters, and measuring sensitivity, could explain dissimilarities in the obtained concentrations.

4.2. Association between urinary parabens and retinal microvasculature

We found an association between urinary MeP and CRVE, indicating a link between higher MeP exposure and increased venular diameter. In adults, an increase in CRVE representative of venular widening indicates inflammation and atherosclerosis (Newman et al., 2017). Furthermore, wider venules were associated with higher BMI in the European-based prospective cohort study EPIC, comprising 7411 participants (Owen et al., 2019), and with higher levels of the inflammation parameters hsCRP and orosomucoid in the cross-sectional POLA cohort, including 1224 individuals aged 60 years (Daien et al., 2013). Also, in children, associations between wider CRVE and adverse cardiometabolic outcomes have been found. Evidence for an association between obesity and wider CRVE was shown in Australian pre-adolescent children (Gopinath et al., 2011), in 381 children aged 10–11 years (Siegrist et al., 2014), and in 578 school children aged 11.1 ± 0.6 years in Germany (Hanssen et al., 2012). In addition, we found urinary PrP concentrations to be associated with the TI of the retinal vasculature. Static retinal vessel metrics, including vascular tortuosity reflect the microvasculature's architecture and indicate potential deviation from an ideal configuration (Newman et al., 2017). An increase in TI has previously been associated with adverse cardiometabolic outcomes (Owen et al., 2011, 2019; Cheung et al., 2011; Sasongko et al., 2010). In children, increased arteriolar tortuosity was associated with cardiometabolic risk factors in a multiethnic study of UK primary school children (Owen et al., 2011) and patients with type 1 diabetes and higher hemoglobin type A1c (aged 12–20 years) (Sasongko et al., 2010).

For children, only one study reported a significant but inverse association between prenatal MeP exposure and CRVE in early adolescence (β = −0.71; 95% CI: −1.41, −0.01) (n = 416), (Montazeri et al., 2022). Because prenatal exposure may activate different biological mechanisms than postnatal exposure also potential associations may differ in their direction, reflecting distinct underlying pathways during different life stages. The increase in CRVE in our study, could indicate the first signs of an adverse effect of MeP exposure on cardiovascular health and may be associated with a changed disease risk contributable to early-life MeP exposure.

The sensitivity analysis with mixture models did not show significant associations for the joint exposure of MeP, EtP and PrP. This could be due to the fact that quantile g-computation allows for different directions of the individual exposure-outcome associations. Here the directions in the associations between MeP and PrP with CRVE and TI respectively differed from those of the other parabens (Supplementary Fig. S3 B and C) resulting in relatively small, non-significant estimates for the total mixture.

The underlying mode of action linking paraben exposure to potentially adverse effects in the parameters of the retinal microvasculature is not yet fully elucidated. A potential candidate mechanism may be oxidative stress and reactive oxygen species (ROS)-induced DNA damage (Fig. 2). *In vivo* (Nagar et al., 2020; Shah and Verma, 2011) and *in vitro* (Martín et al., 2010) studies have previously reported evidence of pro-oxidant effects of parabens. Oxidative stress is also a key indicator of cardiovascular disease (Dhalla et al., 2000; Elahi et al., 2009) through the increase of low-grade inflammation (Steven et al., 2019; Siti et al., 2015), and has been independently linked with several cardiovascular risk factors (Harrison and Gongora, 2009; Maritim et al., 2003; Harrison et al., 2003; Furukawa et al., 2017).

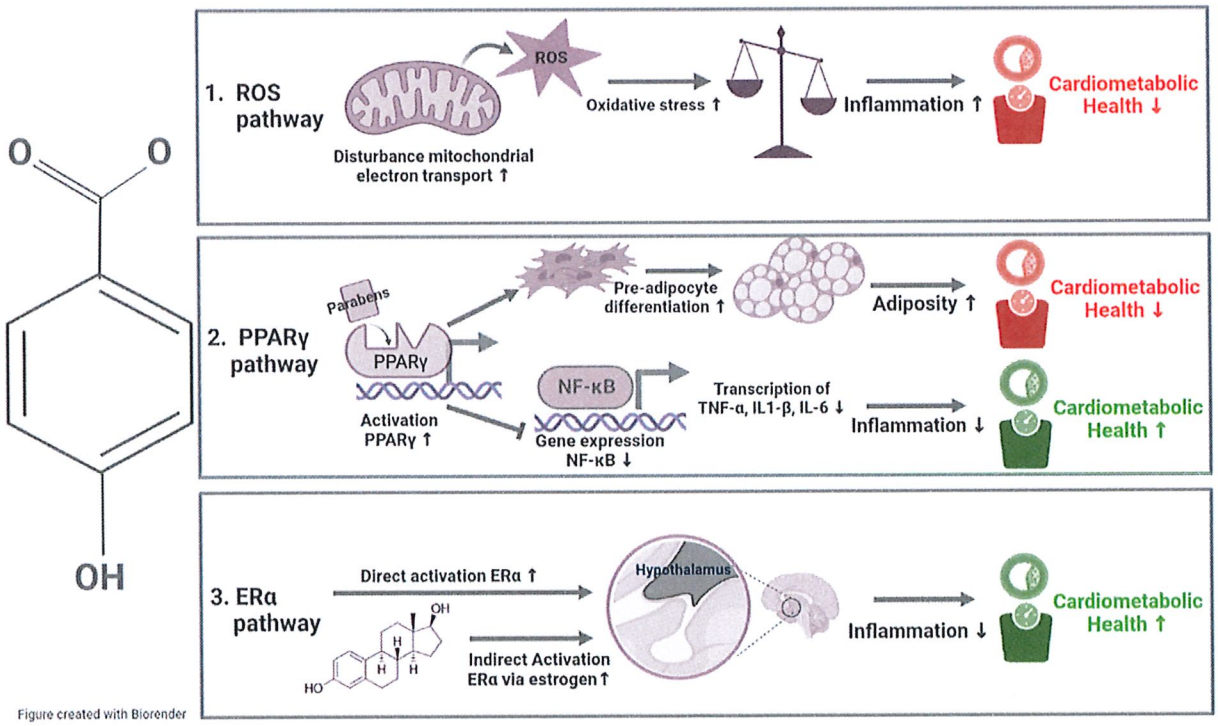

Fig. 2. Overview of the potential mechanisms linking paraben exposure to either negative or positive changes in cardiometabolic outcomes as proposed in the literature: (1.) the "ROS pathway" suggests a negative influence of parabens on cardiometabolic health through the disturbance of the mitochondrial electron transport chain increasing oxidative stress and subsequent inflammation, (2.) the "PPARγ pathway" links paraben exposure with a decrease in cardiometabolic health via the activation of peroxisome proliferator-activated receptor gamma (PPARγ), promoting pre-adipocyte differentiation and adipogenesis. Also increases in cardiometabolic health due to the blocking effects of PPARγ activation on Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) expression have been suggested, leading to the decreased expression of pro-inflammatory factors, (3.) in the "ERα pathway" direct or indirect activation of the hypothalamic estrogen receptor alpha (ERα) which plays an essential role in maintaining body weight and cardiovascular health could increase metabolic health.

4.3. Association between urinary parabens and blood pressure

Our study found EtP levels to be inversely associated with MAP, driven by the association with DBP. Four studies investigated SBP and DBP in relation to prenatal paraben exposure but did not report significant associations (Montazeri et al., 2022; Warembourg et al., 2019b; Liu et al., 2019b; Shiue, 2014). On the other hand, a study nested in the HELIX cohort, including 152 pregnant women, reported decreases in DBP for exposure to parabens in the second trimester but not for whole pregnancy exposure (Warembourg et al., 2019b). We are the first to observe potential associations between parabens exposure in childhood and MAP. MAP is maintained through regulation via the cardiovascular, renal, and autonomic nervous systems, including the renin-angiotensin-aldosterone system (DeMers and Wachs, 2022). PPARγ also modulates the renin-angiotensin-aldosterone system (Rőszer and Ricote, 2010; Sigmund, 2013). Since parabens have been shown to act as PPARγ agonists *in vitro* (Hu et al., 2013; Taxvig et al., 2012) (Fig. 2), they may act by modulating the renin-angiotensin system.

4.4. Association between urinary parabens and BMI z-scores

First, the discovery of an inverse direction of association between MeP and ∑parabens with BMI z-scores is consistent with the findings in several previous studies for the association with obesity in children and adults (Quirós-Alcalá et al., 2018), BMI in adults (Vindenes et al., 2021), and BMI only in women (Kim and Chevrier, 2020). Other studies reported no associations between MeP and weight measures in children (Guo et al., 2017; Xue et al., 2015; Lee et al., 2021). In contrast, in a study investigating the association between the self-reported dietary intake of parabens assessed by questionnaire and BMI in 585 Spanish adolescents, overweight/obese girls were more likely to belong to the highest tertile of measured MeP concentrations compared to those with a body mass index lower than 25 kg/m^2 (Monteagudo et al., 2021). The difference in the direction of the association reported in this latter study could be explained by a possible association between, on the one hand, potentially unhealthy processed foodstuff and parabens (Liao et al., 2013) and processed food and BMI (Rauber et al., 2021; Beslay et al., 2020), which could confound the association between parabens and BMI.

Second, our finding of an effect modification by sex already in early childhood and a suggestive trend for a positive association between EtP and BMI z-scores only in boys is corroborated by a previous report of significant associations between EtP and weight z-scores ($\beta = 0.16$, $p = 0.001$) in Chinese 3-year-old boys (Guo et al., 2017). Also, another study reported a trend for a positive association between EtP and BMI for boys ($\beta = 0.10$, $p = 0.5$), while the associations for all children and girls alone were reported to be inverse (Quirós-Alcalá et al., 2018).

Third, we found positive trends for the association between parabens and waist-circumference when correcting for BMI z-score, which could indicate a confounding effect of fat mass causing lower paraben levels more than being decreased by them. Even though parabens are not categorized as persistent pollutants, they are moderately lipophilic (El Hussein et al., 2007) and may accumulate to a certain degree in human fat stores (Kolatorova et al., 2018; Wang et al., 2015). Indeed, human adipose tissue can be an important repository of parabens (Wang et al., 2015). This could also explain inverse associations between parabens and weight measures, as one would expect in individuals with more extensive fat stores relatively more parabens to be sequestered in adipose tissue and less excreted via the urine.

Potential mechanisms linking paraben exposure to either negative or positive changes in cardiometabolic outcomes have been proposed from findings of *in vitro* and *in vivo* experiments (Hu et al., 2013; Collino et al., 2006; Ding et al., 2020; Kapadia et al., 2008; Villapol, 2018; Samarasinghe et al., 2018), as shown in Fig. 2. Peroxisome proliferator-activated receptor gamma (PPARγ) activation has been associated with obesogenic effects (Hu et al., 2013; Taxvig et al., 2012; Pereira-Fernandes et al., 2013), by promoting pre-adipocyte differentiation and adipogenesis (Hu et al., 2013, 2016; Lee et al., 2021) (Fig. 2). On the other hand, PPARγ activation also exhibits anti-inflammatory effects, and PPARγ agonists can show seemingly protective effects against oxidative stress (Collino et al., 2006; Ding et al., 2020; Kapadia et al., 2008; Villapol, 2018; Stein et al., 2013). As oxidative stress-related inflammation plays an important role in the development of obesity and its related adverse health outcomes (Furukawa et al., 2017; Marseglia et al., 2014; Fernández-Sánchez et al., 2011), this could explain our observed inverse associations with adverse metabolic outcomes (Fig. 2). Furthermore, estrogenic and anti-androgenic properties of parabens have been suggested explaining the inverse associations between parabens and metabolic outcomes in adults (Kim and Chevrier, 2020). Estrogens play an essential role in maintaining body weight and metabolic health and are linked to anti-obesogenic effects primarily through their actions on hypothalamic estrogen receptor alpha (ERα) (Musatov et al., 2007; Xu et al., 2011) (Fig. 2). In young children this effect may come into work even though the levels of circulating estrogens are much lower than in adolescents and adults (Frederiksen et al., 2020), explaining the sex-specific differences observed in our study. Significant differences in serum estrogen levels and estrogen/testosterone ratios between boys and girls before adrenarche have been described previously (Ikegami et al., 2001; Igarashi et al., 2021; Klein et al., 1994).

4.5. Strengths and limitations

We acknowledge the particular strengths and limitations of our present study. We are the first to investigate the association between parameters of retinal microvasculature and urinary paraben levels in preschool children. Because our study is nested in an established prospective birth cohort (ENVIRONAGE), our findings are generalizable. Additionally, we applied censored likelihood multiple imputation in the single pollutant models instead of imputing values below the LOQ with LOQ/2, which resulted in more robust and unbiased estimations for the linear regression models. Nevertheless, we also have to recognize potential limitations. The order of occurrence for paraben exposure and BMI z-scores cannot be determined with certainty because of the cross-sectional character of this study. On the one hand, paraben exposure could influence the BMI by obesogenic or leptogenic effects; on the other hand, it cannot be excluded that a higher BMI may cause lower urinary paraben concentrations through "fat trapping" (Wang et al., 2015; Artacho-Cordón et al., 2018). The sample size was relatively small, especially in the sex-stratified analyses, which might have resulted in limited power and prevented associations from reaching statistical significance. Furthermore, the reported p-values were not adjusted for multiple testing. We used spot-urine samples for assessing urinary paraben levels. Temporal variability in the urinary concentration of nonpersistent chemicals like parabens may cause exposure misclassification and attenuation bias in exposure-response functions (Casas et al., 2018). However, the occurrence of potential measurement errors may be limited as comparably high and reproducible intraclass correlation coefficients have been reported for urinary parabens (median = 0.52), presumably due to the relatively slow and constant dermal absorption of personal care products (Roggeman et al., 2022).

5. Conclusions

This study found associations between parabens and retinal microvasculature, which might indicate early signs of cardiovascular changes, linking early childhood paraben exposure with health complications later in life. Additionally, paraben exposure before puberty may affect human metabolism in a sex-specific way, with boys being more susceptible to adverse metabolic outcomes. Regarding the paraben usage in products for young children, our study shows that paraben exposure during early childhood may already be linked to subclinical changes in

cardiometabolic outcomes, which exemplifies the need for further research to reassess the safety of paraben use during this sensitive period.

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Credit author statement

Brigitte Reimann: Writing – original draft, Methodology, Formal analysis, Visualization. **Hanne Sleurs**: Investigation, Writing – review & editing. **Yinthe Dockx**: Investigation, Writing – review & editing.**Leen Rasking**: Investigation, Writing – review & editing. **Patrick De Boever**: Writing –review & editing. **Catherine Pirard**: Investigation, Resources, Writing–review & editing. **Corinne Charlier**: Investigation, Resources, Writing–review & editing. **Tim S. Nawrot**: Project administration, Supervision, Resources, Writing – review & editing. **Michelle Plusquin**: Conceptualization, Resources, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138570.

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