Phthalate and Bisphenol Urinary Concentrations, Body Fat Measures, and Cardiovascular Risk Factors in Dutch School-Age Children

Carolina C. V. Silva1,2, Vincent W. V. Jaddoe1,2, Chalana M. Sol1,2, Hanan El Marroun1,2,3,4, Maria-Pilar Martinez-Moral5,6, Kurunthachalam Kannan5,6,7,8, Leonardo Trasande8,9,10,11,12, and Susana Santos1,2

Objective: The purpose of this study was to investigate the associations of urinary phthalates and bisphenols at age 6 years old with body fat and cardiovascular risk factors at 6 and 10 years and with the change from 6 to 10 years.

Methods: Among 471 Dutch children, the phthalates and bisphenols urinary concentrations at 6 years and BMI, fat mass index, android fat mass, blood pressure, glucose, insulin, and lipids blood concentrations at 6 and 10 years were measured.

Results: An interquartile range increase in di-n-octyl phthalate (DNOP) metabolites concentrations at 6 years was associated with an increased risk of overweight at 6 and 10 years (odds ratio: 1.44; 95% CI: 1.11-1.87, and 1.43; 95% CI: 1.09-1.86, respectively). Also, higher DNOP metabolites concentrations were associated with higher fat mass index at 6 years, higher systolic blood pressure at 10 years, a decrease in high-density lipoprotein cholesterol, and an increase in triglycerides concentrations from 6 to 10 years (P<0.05). Higher total bisphenols and bisphenol A concentrations were associated with a decrease in BMI from 6 to 10 years (P<0.01).

Conclusions: DNOP metabolites are associated with overweight and an adverse cardiovascular profile in childhood. Total bisphenols and bisphenol A are associated with a decrease in BMI from 6 to 10 years.

Introduction
Endocrine disrupting chemicals (EDCs), such as phthalates and bisphenols, are adverse environmental factors that may affect childhood health (1,2). Phthalate metabolites are synthetic chemical esters of phthalic acid that are widely used in a variety of consumer products to impart flexibility and elasticity to plastics. Bisphenol A (BPA) is used to produce polycarbonate plastics and epoxy resins used in various products, including

Study Importance
What is already known?
► Phthalates and bisphenols interfere with metabolic processes and lead to cardiovascular disease in adults.
► Though many previous studies have examined fetal exposure to these chemicals, fewer have examined longitudinal effects of childhood exposure to phthalates and bisphenols on body fat and cardiovascular risk.

What does this study add?
► Adiposity in school-aged children may be influenced by phthalates and bisphenols exposure, specifically by di-n-octyl phthalate (DNOP) metabolites and bisphenol A.
► DNOP metabolites seem also to be associated with an adverse cardiovascular profile in childhood.

How might these results change the direction of research?
► Future research should further explore childhood as a vulnerable period of exposure to bisphenols and phthalates in relation to later adiposity and cardiovascular health and should explore the potential underlying mechanisms and causality.
High exposure to phthalate metabolites and bisphenols is increasingly reported to be associated with obesity, hypertension, insulin resistance, dyslipidemia, and cardiovascular disease among adults (6,7). In general, fetuses and children are likely to be more vulnerable to exposure to these chemicals than adults (8). Most previous studies have examined pregnancy as a vulnerable period rather than childhood. Results in children are mostly based on cross-sectional studies and they have not revealed expected effects consistently (9-22). Some cross-sectional studies have suggested associations of higher exposure to phthalate metabolites and BPA with higher BMI as well as hip and waist circumference (9-11,13). In contrast, another cross-sectional study among 845 Danish children aged 4 to 9 years reported that higher phthalate metabolites were negatively associated with height, weight, and BMI (19). Previous cross-sectional studies have found that higher phthalate metabolites and BPA concentrations were associated with adverse childhood cardiovascular profile, such as higher blood pressure, low-grade albuminuria, and insulin resistance (14-18). However, other studies have found negative or no associations between BPA exposure and childhood metabolic outcomes (20-22). This controversy of results from previous studies might be explained by differences in sample size, timing of collection of samples, and in the individual phthalates and bisphenols available. Also, a major literature gap is the lack of longitudinal data evaluating the association of childhood exposure to phthalate metabolites and bisphenols with body fat measures and cardiovascular risk factors (12,22). Assessing these associations using a longitudinal design in childhood and controlling for child’s diet and maternal exposure to phthalate metabolites and bisphenols during pregnancy will allow a better understanding of the influence of these chemicals on child’s health.

We hypothesized that increased childhood exposure to phthalate metabolites and bisphenols affects accretion of body fat and the development of elevated blood pressure and other adverse cardiovascular outcomes. We examined whether phthalate metabolites and bisphenols urinary concentrations at 6 years were associated with body fat measures and cardiovascular risk factors, including BMI, fat mass index, android fat mass, blood pressure, and glucose, insulin, cholesterol, and triglycerides concentrations at 6 and 10 years, as well as with the change in these outcomes from 6 to 10 years. Additionally, we also explored the associations of phthalate metabolites and bisphenols urinary concentrations with risks of overweight and clustering of cardiovascular risk factors in childhood.

**Methods**

**Study design**

This study was embedded in the Generation R Study, a prospective population-based cohort study from early fetal life onward in Rotterdam, the Netherlands (23). Phthalate metabolites and bisphenols urinary concentrations were measured among a subgroup of 775 singleton children aged 6 years. Children in this subgroup were similar to the broader Generation R cohort in terms of sociodemographic and lifestyle characteristics (Supporting Information Table S1). We excluded children with non-Dutch ethnicity because of potential ethnicity-specific differences in the associations (13). The population for analysis comprises 471 Dutch children with information on phthalate metabolites and bisphenols urinary concentrations and at least one measurement of body fat and cardiovascular risk factors at 6 or 10 years (Supporting Information Figure S1). The study was approved by the local Medical Ethics Committee of Erasmus MC (MEC 198.782/2001/31), and written informed consent was obtained from parents.

**Phthalate metabolites and bisphenols urinary concentrations**

Phthalate metabolites and bisphenols concentrations were measured in a spot urine sample obtained during the study visit at 6 years. As previously described, urine samples were collected between 8 am and 8 pm, stored at 4°C and transported within 24 hours of receipt to the Star Medisch Diagnostisch Centrum (STAR-MDC) laboratory to be frozen at −20°C. The urine specimens were shipped on dry ice in 4-mL polypropylene vials to the Wadsworth Center, New York State Department of Health, Albany, New York, for analysis (24). We grouped phthalate metabolites according to their molecular weight categories and parent compounds. BPA and BPS were grouped and used as proxy for total bisphenol exposure. Individual bisphenol and phthalate metabolites were only included in groups and assessed individually if less than 80% of the sample concentrations were below the limit of detection (LOD). We calculated the weighted molar sums for groups representing total bisphenols, low-molecular-weight (LMW) phthalates, high-molecular-weight (HMW) phthalates, and for two subgroups within HMW phthalates: di-2-ethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DNP) metabolites. Phthalic acid was analyzed separately as a proxy for total phthalate exposure. Phthalate metabolites and bisphenols concentrations below LOD were substituted by LOD/\(\sqrt{2}\) (25). Supporting Information Table S2 shows the metabolites included in all groups, their urinary concentrations, and detection rates at the age of 6 years. To account for urinary dilution, concentrations of phthalate metabolites and bisphenols were converted to microgram per gram of creatinine (for the separate metabolites) or micromole per gram of creatinine (for the metabolite groups).

**Body fat measures and cardiovascular risk factors**

Children were invited to visit our research center at 6 and 10 years. We calculated BMI (kilogram per meters squared) from height and weight, both measured without shoes and heavy clothing, and sex- and age-adjusted z scores of childhood BMI based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation) (26). BMI categories (normal weight and overweight/obesity) were obtained using the International Obesity Task Force cutoffs (27). We measured total body fat mass by dual-energy x-ray absorptiometry (Lunar iDXA GE 140; GE Healthcare, Chicago, Illinois; enCORE software v12.6) (28). We divided total fat mass by height\(^3\) at 6 years and by height\(^3\) at 10 years in order to obtain a fat mass index uncorrelated with height after estimating the optimal adjustment by log-log regression analyses (29,30). We also calculated android fat mass as a percentage of total fat mass.

Blood pressure was measured at the right brachial artery four times using the validated automatic sphygmomanometerDatascope Accutor Plus (Paramus, New Jersey). The mean value was calculated using
the last three measurements of each participant. Nonfasting blood samples were collected to determine serum concentrations of glucose, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Insulin concentrations were measured with electrochemiluminescence immunoassay on the E411 module (Roche, Almere, the Netherlands). Glucose (only available at 10 years), total cholesterol, HDL-cholesterol, and triglyceride concentrations were measured using the c702 module on the Cobas 8000 analyzer (Roche).

Based on previous literature, clustering of cardiovascular risk factors was defined as having three or more of the following components: systolic or diastolic blood pressure in the 75th percentile or above; android fat mass percentage in the 75th percentile or above; insulin concentration in the 75th percentile or above; and HDL-cholesterol in the 25th percentile or below or triglycerides in the 75th percentile or above (31).

Covariates
Maternal age and educational level were obtained by questionnaire at enrollment. Maternal phthalate metabolites and bisphenols urinary concentrations were measured at three time points during pregnancy (median 12.9 weeks of gestation [25th-75th percentiles 12.1-14.5]; median 20.4 weeks of gestation [25th-75th percentiles 19.9-20.9]; median 30.2 weeks of gestation [25th-75th percentiles 29.9-30.8]) and the pregnancy-averaged concentrations were calculated (32). Child sex and age were available from medical records. Child ethnicity was based on parental countries of birth obtained through questionnaire. Height was measured without shoes at both 6 and 10 years. The average television-watching time was obtained by questionnaires at both ages. Diet quality was determined by a parent-reported food frequency questionnaire assessed at the child’s mean age of 8.1 years. The algorithm to score adherence to Dutch dietary guidelines has been previously described and ranged from 0 to 10 on a continuous scale with higher scores reflecting better adherence to dietary guidelines (33).

Statistical analysis
Phthalate metabolites and bisphenols concentrations were natural log-transformed to reduce variability and account for right skewness of the distribution and standardized by the interquartile range to ease the interpretation of effect sizes. The distributions of fat mass index, insulin, and triglycerides concentrations were skewed and natural log-transformed. To enable comparison of effect sizes of different outcome measures, we constructed z scores [(observed value – mean)/SD]. We performed linear regression models to assess the associations of phthalate metabolites and bisphenols urinary concentrations at 6 years with body fat measures and cardiovascular risk factors at 6 and 10 years and with the change in these outcomes from 6 to 10 years. Nonlinearity was visually assessed using a scatterplot and ruled out. Additionally, we explored, using multinominal logistic regression models, the associations of phthalate metabolites and bisphenols with risks of overweight and clustering of cardiovascular risk factors at 6 and 10 years. Because of the small sample size, we were not able to assess these associations with the change from 6 to 10 years. Basic models included child’s sex, age, and height (only for blood pressure models) at outcome measurements. For the models with the change in the outcomes from 6 to 10 years, the corresponding change in age and height was included. Potential confounders were represented in a directed acyclic graph (Supporting Information Figure S2), and those that fulfilled the graphical criteria for confounding and changed the effect estimates >10% for at least one of the outcomes were included. Confounder models additionally included maternal educational level and child’s diet quality score and average television-watching time at 6 years. For the models with the change in the outcomes from 6 to 10 years, television-watching time at both ages was included because no multicollinearity issues were observed. Also, we performed a model in which we additionally adjusted for the corresponding phthalate metabolites or bisphenol pregnancy-averaged urinary concentrations. As sensitivity analyses, we additionally adjusted the models for the outcomes at 10 years and for the change in the outcomes from 6 to 10 years by the corresponding outcomes at 6 years. Based on previous literature, we tested for statistical interactions by child sex in these analyses, but none of these was consistently significant (10,11).

To maintain statistical power and reduce bias related to missing data on covariates, we performed multiple imputation according to the Markov Chain Monte Carlo method. The percentage of missing values for covariates ranged from 0% to 20%. Ten imputed data sets were created but no substantial differences were found between the original and imputed data sets. We presented results based on pooled imputed data sets. To correct for multiple hypothesis testing, each P value was compared with a threshold defined as 0.05 divided by the effective number of independent tests estimated based on the correlation between the exposures (P value threshold of 0.01) (34). All statistical analyses were performed using SPSS Statistics v.25.0 for Windows (IBM Corp., Armonk, New York).

Results
Subject characteristics
Table 1 shows the characteristics of the study population. The mean age of the children who attended the study visits at the research center was between 5.9 and 9.7 years, and more than half (52.4%) were boys. Overall, 11.4% and 15.0% of children had overweight, and 13.9% and 10.2% had clustering of cardiovascular risk factors at 6 and 10 years, respectively.

Phthalate metabolites and bisphenols urinary concentrations and body fat measures
In the confounder models, an interquartile range increase in HMW phthalates urinary concentrations was associated with higher childhood BMI at 10 years (P<0.05) (Table 2). Also, an interquartile range increase in DNOP metabolites urinary concentrations was associated with both higher childhood BMI at 6 and 10 years and an increase in childhood BMI from 6 to 10 years (P<0.05). The association of DNOP metabolites urinary concentrations with childhood BMI at 10 years remained significant after multiple testing corrections (z score: 0.16 [95% CI: 0.06 to 0.25]). In contrast, and after multiple testing correction, an interquartile range increase in total bisphenols and BPA urinary concentrations was associated with a decrease in childhood BMI from 6 to 10 years (z score: −0.13 [95% CI: −0.22 to −0.05], z score: −0.14 [95% CI: −0.23 to −0.05], respectively). An interquartile range increase in HMW phthalates and DNOP metabolites urinary concentrations was also associated with higher childhood fat mass index at 6 years (P<0.05). However, these results did not remain significant after multiple testing correction. No associations were observed for phthalate metabolites or bisphenols urinary
TABLE 1 Characteristics of mothers and their children

| Maternal characteristics | Total group (N = 471) |
|--------------------------|-----------------------|
| Age, mean (SD), y        | 31.4 (4.2)            |
| Education, n (%)         |                       |
| Lower                    | 6 (1.3)               |
| Middle                   | 161 (34.5)            |
| Higher                   | 300 (64.2)            |
| Sex, n (%)               |                       |
| Boys                     | 247 (52.4)            |
| Girls                    | 224 (47.6)            |
| Age at visit, mean (SD), y| 5.9 (0.2)            |
| Television-watching time, n (%)| 5.9 (0.2)            |
| <2 hours                 | 394 (89.5)            |
| ≥2 hours                 | 46 (10.5)             |
| Height, mean (SD), m     | 1.2 (0.0)             |
| BMI, mean (SD), kg/m²    | 15.9 (1.5)            |
| BMI categories, n (%)    |                       |
| Normal weight            | 397 (88.6)            |
| Overweight/obesity       | 51 (11.4)             |
| Fat mass index, median (25th,75th percentile), kg/m²| 3.1 (2.6,3.6) |
| Android fat mass, mean (SD), % | 3.8 (0.9)   |
| Systolic blood pressure, mean (SD), mm Hg | 101.7 (8.2) |
| Diastolic blood pressure, mean (SD), mm Hg | 60.0 (6.8) |
| Insulin, median (25th,75th percentile), pmol/L | 127.4 (66.4,191.6) |
| Total cholesterol, mean (SD), mmol/L | 4.2 (0.6) |
| HDL-cholesterol, mean (SD), mmol/L | 1.3 (0.3) |
| Triglycerides, median (25th,75th percentile), mmol/L | 1.0 (0.8,1.4) |
| Clustering of cardiovascular risk factors, n (%) | Yes 43 (13.9) |
| Diet quality, mean (SD), score | 4.5 (1.2) |
| Child characteristics at age 10 |                       |
| Age at visit, mean (SD), y | 9.7 (0.2) |
| Television-watching time, n (%) | 9.7 (0.2) |
| <2 hours                 | 299 (79.5)            |
| ≥2 hours                 | 77 (20.5)             |
| Height, mean (SD), m     | 1.4 (0.1)             |
| BMI, mean (SD), kg/m²    | 17.0 (2.3)            |
| BMI categories, n (%)    |                       |
| Normal weight            | 318 (85.0)            |
| Overweight/obesity       | 56 (15.0)             |
| Fat mass index, median (25th,75th percentile), kg/m²| 2.0 (1.6,2.6) |
| Android fat mass, mean (SD), % | 4.0 (1.2) |
| Systolic blood pressure, mean (SD), mm Hg | 102.9 (7.6) |
| Diastolic blood pressure, mean (SD), mm Hg | 58.9 (6.3) |
| Glucose, mean (SD), mmol/L | 5.5 (0.9) |
| Insulin, median (25th,75th percentile), pmol/L | 193.8 (114.2,293.3) |
| Total cholesterol, mean (SD), mmol/L | 4.3 (0.6) |
| HDL-cholesterol, mean (SD), mmol/L | 1.5 (0.3) |
| Triglycerides, median (25th,75th percentile), mmol/L | 1.0 (0.7,1.3) |
| Clustering of cardiovascular risk factors, n (%) | Yes 25 (10.2) |

Values are means (SD), medians (25th,75th percentile), or numbers of subjects (valid %).
Table 2: Associations of phthalate metabolites and bisphenols urinary concentrations with body fat measures in childhood

|                      | BMI | Fat mass index | Android fat mass |
|----------------------|-----|---------------|------------------|
|                      | 6-10 years | 6-10 years | 6-10 years |
| Phthalic acid        | 0.02 (−0.07, 0.10) | 0.03 (−0.07, 0.11) | −0.02 (−0.09, 0.05) |
| LMW phthalate        | 0.08 (−0.02, 0.17) | 0.14 (−0.02, 0.26) | 0.05 (−0.08, 0.17) |
| HMW phthalate        | 0.06 (−0.05, 0.12) | 0.10 (−0.05, 0.20) | 0.06 (−0.05, 0.13) |
| DEHP metabolites     | 0.08 (−0.04, 0.16) | 0.16 (−0.04, 0.23) | 0.07 (−0.04, 0.13) |
| Total bisphenols     | −0.01 (−0.11, 0.10) | −0.12 (−0.11, 0.02) | −0.02 (−0.13, 0.09) |
| BPA                  | −0.02 (−0.13, 0.09) | −0.12 (−0.25, 0.02) | −0.03 (−0.23, 0.01) |

Values are linear regression coefficients (95% CI). All models are adjusted for maternal educational level, child sex, age, diet quality score, and television-watching time. Change from 6 to 10 years corresponds to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age (except for sex-adjusted BMI), diet quality score, and television-watching time.

Discussion

In a population-based study, we observed that higher DNOP metabolites urinary concentrations were associated with an increased risk of overweight and obesity and with lower HDL-cholesterol and tended to be associated with higher systolic blood pressure and higher triglycerides in school-age children. Higher total bisphenols and BPA urinary concentrations were associated with lower BMI and tended to be associated with higher diastolic blood pressure and lower insulin in school-age children.

Interpretation of main findings

As a result of the widespread use of phthalate metabolites and bisphenols-related products, children can be exposed to these potential harmful chemicals through different pathways, such as ingestion, inhalation, and dermal contact. Phthalates and bisphenols may interfere with endocrine processes, resulting in a deviation from the normal homeostatic control that may lead to an adverse cardiovascular profile. We hypothesized that increased exposure to phthalate metabolites and bisphenols affect body fat and cardiovascular development already in childhood.

A previous narrative review reported positive associations of exposure to phthalate metabolites with childhood BMI, subscapular skinfold thickness, and hip and waist circumferences in five studies, the associations of which were mostly observed among boys. However, a recent meta-analysis of 29 studies in children and adults has reported inconsistencies in results from published literature on the association between the exposure to phthalates and adiposity. In the current study, we observed that higher HMW phthalate concentrations, specifically DNOP metabolites, at 6 years were associated with higher BMI and an increased risk of overweight and obesity in school-age children. Contrarily to previous studies and surprisingly due to estrogenicity of bisphenols and antiandrogenicity of some phthalates, we did not observe a statistical interaction by child’s sex. However, we cannot exclude the possibility that our results might have been underpowered to detect differences by sex because of the small sample size. Most studies of bisphenols were only focused on BPA and reported that, in childhood, higher BPA concentrations were associated with increased BMI, hip and waist circumferences, and body fat. However, we observed that higher total bisphenols and BPA urinary concentrations were associated with a decrease in BMI from 6 to 10 years but not with fat mass index or android fat mass. Based on previous studies, we did not hypothesize this association beforehand. Although we cannot disregard the possibility of a true association, it might be due to residual confounding.

An accumulating body of evidence suggests that phthalate metabolites and bisphenols exposure may contribute to acute and chronic cardiovascular risks, altered blood pressure, and atherosclerosis, as well as...
mechanisms have been found for bisphenols (36,37). However, this is basal metabolism, may also have obesogenic effects (37,38). Similar thyroid hormones system, which are critical for the maintenance of development of obesity (36). Likewise, the perturbation of the steroid and pocyte-related hormones, leading to higher susceptibility for the devel-

Activation of PPARs can increase lipid accumulation and release adi-

tors (PPARs) and imbalance of steroid and thyroid hormones (36-38).

metabolites with overweight and an adverse cardiovascular profile. Also, other studies did not report associations of BPA exposure with childhood metabolic outcomes, including glucose, insulin resistance, and blood lipids from midchildhood until adolescence (21,22). In the present study and similarly to previous studies, we observed that higher phthalic acid and HMW phthalates, specifically DNOP metabolites, tended to be associated with higher childhood systolic blood pressure. We also observed that higher LMW phthalates, DEHP metabolites, and DNOP metabolites tended to be associated with lower HDL-cholesterol and higher triglycerides concentrations in school-age children. On the other hand, we observed that higher total bisphenols and BPA urinary concentrations tended to be associated with an increase in diastolic blood pressure from 6 to 10 years and with lower insulin at 10 years. The positive association of total bisphenols and BPA with diastolic blood pressure from 6 to 10 years should be interpreted with caution because the effect estimates attenuated after additional adjustment for the outcome at 6 years and a negative association was observed at 6 years.

The potential mechanisms underlying the associations of phthalate metabolites with overweight and an adverse cardiovascular profile might include the activation of peroxisome proliferator-activated receptors (PPARs) and imbalance of steroid and thyroid hormones (36-38). Activation of PPARs can increase lipid accumulation and release adipocyte-related hormones, leading to higher susceptibility for the development of obesity (36). Likewise, the perturbation of the steroid and thyroid hormones system, which are critical for the maintenance of basal metabolism, may also have obesogenic effects (37,38). Similar mechanisms have been found for bisphenols (36,37). However, this is not in line with our results, which showed an association of bisphenols with lower BMI in children.

Altogether, our results suggest that DNOP metabolites and bisphenols exposure may affect childhood BMI. The associations of phthalate metabolites and bisphenols with cardiovascular risk factors, except for DNOP metabolites and HDL-cholesterol from 6 to 10 years, were no longer significant after multiple testing correction and thus we cannot exclude the possibility of results being chance findings. The observed effect estimates might be small on an individual level but can be important on a population-based level, as children are widely exposed to these EDCs and overweight and obesity and adverse cardiovascular risk factors tend to track into poorer cardiovascular health later in life. Because of the observational design of this study, we cannot draw conclusions about causality. Further studies are needed to replicate these findings and investigate potential mechanisms.

Strengths and limitations
The major strengths of this study were the availability of urinary measurements of diverse phthalate metabolites and BPS in addition to the detailed data available on childhood body fat measures and cardiovascular risk factors. Also, contrary to most previous studies that were embedded in a cross-sectional design and thus can be affected by reverse causality, we were able to address the associations of exposure to phthalate metabolites and bisphenols at 6 years with body fat and cardiovascular risk factors at 10 years. This study also has limitations. This study was conducted in a low-risk small sample, which might have resulted in insufficient power to detect associations, especially after conducting multiple testing correction. No substantial differences in terms of sociodemographic and lifestyle characteristics were observed between children in this subgroup and in the broader cohort, and thus, although it cannot be excluded, selection bias seems unlikely. In our study, we relied on a single-spot urinary measurement of phthalate metabolites and bisphenols as an estimate of exposure. Both phthalate metabolites and bisphenols have short biological half-lives (39,40),
### TABLE 3 Associations of phthalate metabolites and bisphenols urinary concentrations with blood pressure in childhood

| Endocrine disrupting chemicals urinary concentrations | Systolic blood pressure | Diastolic blood pressure | Diastolic blood pressure: Change from 6-10 years |
|--------------------------------------------------------|-------------------------|--------------------------|-----------------------------------------------|
|                                                        | 6 years                 | 10 years                 | Change from 6-10 years                        |
| Phthalic acid                                          | 0.06 (−0.14,0.07)       | 0.02 (−0.10,0.12)        | 0.08 (−0.05,0.23)                            |
| LMW phthalate                                          | −0.00 (−0.09,0.09)      | 0.04 (−0.05,0.13)        | 0.10 (−0.04,0.22)                            |
| HMW phthalate                                          | 0.08 (−0.03,0.20)       | 0.12 (0.01,0.23)*        | 0.04 (−0.11,0.18)                            |
| DEHP metabolites                                       | 0.05 (−0.07,0.18)       | 0.10 (−0.03,0.23)        | 0.03 (−0.14,0.19)                            |
| HMW phthalate                                          | 0.06 (−0.03,0.15)       | 0.06 (−0.03,0.16)        | 0.04 (−0.08,0.16)                            |
| Total bisphenols                                       | −0.01 (−0.13,0.12)      | −0.02 (−0.15,0.11)       | −0.13 (−0.25,−0.00)*                         |
| BPA                                                   | −0.02 (−0.16,0.11)      | −0.02 (−0.16,0.12)       | −0.13 (−0.26,−0.09)*                         |
| BPS                                                   | 0.10 (−0.03,0.23)       | 0.08 (−0.06,0.21)        | 0.10 (−0.02,0.31)                            |

Values are linear regression coefficients (95% CI) and reflect the differences in the z scores of childhood blood pressure at 6 and 10 years and the change in the outcomes in z scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in micromoles per gram of creatinine. Changes from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age, height, diet quality score, and television-watching time.

*P < 0.05.

### TABLE 4 Associations of phthalate metabolites and bisphenols urinary concentrations with insulin and lipids profile in childhood

| Endocrine disrupting chemicals urinary concentrations | Insulin | Total cholesterol | HDL-cholesterol | Triglycerides | Difference (95% CI) in z scores |
|--------------------------------------------------------|---------|-------------------|-----------------|--------------|--------------------------------|
|                                                        | 6 years | 10 years          | Change from 6-10 years | 6 years | 10 years | Change from 6-10 years | 6 years | 10 years | Change from 6-10 years | 6 years | 10 years | Change from 6-10 years |
| Phthalic acid                                          | −0.06 (−0.18,0.06) | −0.03 (−0.16,0.11) | 0.04 (0.17,0.24) | 0.04 (−0.08,0.16) | 0.09 (−0.14,0.08) | 0.06 (−0.50,0.18) | −0.05 (−0.18,0.08) | −0.07 (−0.20,0.05) | 0.01 (−0.05,0.01) | 0.06 (−0.07,0.19) | −0.02 (−0.21,0.17) |
| LMW phthalate                                          | −0.04 (−0.14,0.07) | 0.04 (−0.08,0.16) | 0.05 (−0.30,0.23) | 0.06 (−0.05,0.16) | 0.09 (−0.03,0.20) | 0.03 (−0.13,0.00) | 0.01 (−0.10,0.10) | −0.12 (−0.23,−0.00)* | −0.09 (−0.20,0.05) | 0.06 (−0.03,0.18) | 0.09 (−0.03,0.20) | −0.01 (−0.18,0.15) |
| HMW phthalate                                          | −0.07 (−0.20,0.06) | 0.01 (−0.14,0.16) | 0.11 (−0.11,0.33) | 0.02 (−0.11,0.15) | 0.03 (−0.11,0.17) | −0.00 (−0.12,0.12) | 0.07 (−0.50,0.20) | 0.03 (−0.11,0.17) | −0.09 (−0.22,0.06) | −0.02 (−0.15,0.11) | 0.14 (−0.01,0.28) | 0.16 (−0.04,0.33) |
| DEHP metabolites                                       | −0.06 (−0.22,0.06) | 0.04 (−0.12,0.20) | 0.15 (−0.10,0.40) | 0.03 (−0.12,0.17) | 0.07 (−0.09,0.22) | 0.02 (−0.11,0.15) | 0.10 (−0.40,0.23) | 0.07 (−0.08,0.23) | 0.03 (−0.18,0.11) | 0.03 (−0.12,0.17) | 0.19 (−0.03,0.34)* | 0.14 (−0.08,0.33) |
| HMW phthalate                                          | −0.06 (−0.14,0.07) | −0.00 (−0.13,0.12) | 0.07 (−0.12,0.28) | 0.01 (−0.09,0.12) | −0.03 (−0.15,0.10) | 0.00 (−0.10,0.10) | 0.07 (−0.50,0.20) | 0.03 (−0.11,0.17) | −0.09 (−0.22,0.06) | −0.02 (−0.15,0.11) | 0.14 (−0.01,0.28) | 0.16 (−0.04,0.33) |
| Total bisphenols                                       | −0.03 (−0.18,0.13) | −0.17 (−0.33,−0.01)* | −0.17 (−0.43,0.09) | 0.10 (−0.05,0.25) | −0.07 (−0.16,0.15) | −0.12 (−0.25,0.03) | 0.17 (−0.02,0.23)* | 0.01 (−0.15,0.16) | −0.07 (−0.22,0.08) | 0.01 (−0.14,0.17) | −0.06 (−0.25,0.10) | −0.13 (−0.37,0.10) |
| BPA                                                   | −0.03 (−0.19,0.13) | −0.19 (−0.36,−0.03)* | −0.19 (−0.46,0.07) | 0.07 (−0.09,0.23) | −0.04 (−0.20,0.12) | −0.12 (−0.26,0.02) | 0.20 (0.04,0.35)* | 0.01 (−0.15,0.17) | −0.06 (−0.24,0.07) | 0.02 (−0.14,0.18) | −0.02 (−0.18,0.14) | −0.09 (−0.30,0.16) |
| BPS                                                   | −0.02 (−0.17,0.13) | 0.06 (−0.10,0.22) | 0.07 (−0.17,0.31) | 0.14 (−0.01,0.29) | 0.04 (−0.12,0.19) | −0.05 (−0.17,0.08) | −0.02 (−0.16,0.13) | −0.04 (−0.19,0.11) | 0.04 (−0.10,0.18) | 0.10 (−0.06,0.25) | −0.10 (−0.25,0.06) | −0.15 (−0.37,0.08) |

Values are linear regression coefficients (95% CI) and reflect the differences in the z scores of childhood insulin and lipids profile at 6 and 10 years and the change in the outcomes in z scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in micromoles per gram of creatinine. Changes from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age, height, diet quality score, and television-watching time.

*Result remained significant after multiple testing correction.

*P < 0.05.
although it has been suggested that a single urine sample for phthalate concentrations reasonably reflects exposure for up to 3 months (41). Thus, measurement error may have led to underestimation of the effect estimates, especially for bisphenols. Moreover, the use of nonfasting blood samples of childhood glucose, insulin, and lipids profile may also have led to underestimation of the observed associations. However, previous studies have shown that semfasted insulin resistance is moderately correlated with fasting values (42) and that nonfasting blood lipids levels can accurately predict increased risks of cardiovascular events later in life (43). We collected information on many potential confounding variables, but residual confounding due to unmeasured lifestyle variables might still be an issue. Previous evidence supports a link between early puberty and adiposity (44). We do not have information on pubertal development. Future studies should assess these associations while considering pubertal status of the children. The current study was focused on phthalate and bisphenol urinary concentrations. Other EDCs, such as pesticides, might be related with adiposity outcomes in children (45). These associations should be explored in future studies.

Figure 2 Associations of phthalate metabolites and bisphenols urinary concentrations with risk of clustering of cardiovascular risk factors in childhood. Values are odds ratios (95% CI) on a logarithmic scale and represent the risk of clustering of cardiovascular risk factors at 6 and 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in micromoles per gram of creatinine. Models are adjusted for maternal educational level and child sex, age, diet quality score, and television-watching time. *P<0.05.

Funding agencies: The general design of the Generation R Study was made possible by financial support from the Erasmus MC, University Medical Center, Rotterdam, the Netherlands; the Organization for Health Research and Development (ZonMw); and the Ministry of Health, Welfare and Sport. This study was supported by the US National Institutes of Health (NIH) (grants RO1 ES022972 and RO1 ES029779). The content is solely the responsibility of the authors and does not represent the official views of NIH. VJ received an additional grant from the European Research Council (ERC Consolidator grant, ERC-2014-CoG-64916). All funding sources had no involvement in study design, the collection, analysis, and interpretation of data, the writing of the report, and in the decision to submit the article for publication.

Disclosure: The authors declared no conflict of interest.

Author contributions: CCVS, VJ, LT, and SS: designed and conducted the study. MM and KK: performed the analysis of samples at Wadsworth Center for phthalate metabolites and bisphenols. CCVS and SS: analyzed the data. CCVS, VJ, and SS: wrote the manuscript. VJ, LT, and SS: contributed to the interpretation of the data and gave input at all stages of the study. CCVS and SS: had primary responsibility for final content. CMS, HM, MM, KK, and LT: advised and reviewed the manuscript. All authors read and approved the final version of the manuscript.

Supporting information: Additional Supporting Information may be found in the online version of this article.

Conclusion

Our study suggests that adiposity in school-aged children may be influenced by phthalate metabolites and bisphenols exposure, specifically by DNOP metabolites and BPA. DNOP metabolites seem also to be associated with an adverse cardiovascular profile in childhood. Further studies are needed, both to replicate our findings and to explore the potential mechanisms involved.

Acknowledgments

We gratefully acknowledge the contribution of the participating children, their parents, general practitioners, hospitals, midwives, and pharmacies in Rotterdam, the Netherlands.

References

1. Mansouri V, Ebrahimpour K, Poursafa P, et al. Exposure to phthalates and bisphenol A is associated with higher risk of cardiometabolic impairment in normal weight children. Environ Sci Pollut Res Int 2019;26:18604-18614.
2. Soleciki R, Kortenkamp A, Bergman A, et al. Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement. Arch Toxicol 2017;91:1001-1006.
3. Philips EM, Jaddoe VWV, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol 2017;68:105-118.
4. Newbold RR, Padilla-Banks E, Snyder RJ, Phillips TM, Jefferson WN. Developmental exposure to endocrine disruptors and the obesity epidemic. Reprod Toxicol 2007;23:290-296.
5. Stojanoska MM, Milosevic N, Milic N, Abenavoli L. The influence of phthalates and bisphenol A on the obesity development and glucose metabolism disorders. Endocrine 2017;55:666-681.
6. Rancière F, Lyons JG, Loh VHY, et al. Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence. Environ Health 2015;14:46. doi:10.1186/s12940-015-0036-5
7. Posnack NG. The adverse cardiac effects of Di(2-ethylhexyl)phthalate and bisphenol A. Cardiovasc Toxicol 2014;14:339-357.
27. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, obesity prevalence in children and adolescents. JAMA 2012;308:1113-1121.

25. Horning RW, Murray HE, Scinicariello F. Age and sex differences in childhood and adult obesity association with phthalates: analyses of NHANES 2007-2010. Int J Hyg Environ Health 2014;217:687-694.

23. Kooijman MN, Kruithof CJ, van Duijn CM, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol 2016;31:1243-1264.

21. Lee HA, Kim YJ, Lee H, et al. Effect of urinary bisphenol A on androgenic hormones in Chinese school children. PLoS One 2013;8:e56800. doi:10.1371/journal.pone.0056800.

20. Teitelbaum SL, Mervish N, Moshier EL, et al. Associations between phthalate metabolites and age-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect 2013;121:501-506.

18. Trasande L, Sathyanarayana S, Spanier AJ, Blustein J. Racial/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect 2013;121:747-753 e1.

16. Trasande L, Sathyanarayana S, Trachtman H. Dietary phthalates and low-grade albuminuria in US children and adolescents. Clin J Am Soc Nephrol 2014;9:100-109.

14. Trasande L, Sathyanarayana S, Trachtman H. Bisphenol A exposure is associated with low-grade urinary albumin excretion in children of the United States. Kidney Int 2013;83:741-748.

12. Teitelbaum SL, Mervish N, Moshier EL, et al. Associations between phthalate metabolites and body size measures in New York City children. Environ Res 2012;112:186-193.

10. Buser MC, Murray HE, Scinicariello F. Age and sex differences in childhood and adult obesity association with phthalates: analyses of NHANES 2007-2010. Int J Hyg Environ Health 2014;217:687-694.

8. Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder SG. Nahrung. A. Environ Health Perspect 2014;99:2557-2566.

7. Andra SS, Makris KC. Thyroid disrupting chemicals in plastic additives and thyroid health. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2012;30:107-151.

6. Mattison DR, Karrysian A, Goodman M, LaKind JS. Pharmacokinetics and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol 2014;44:696-724.

5. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children’s health. Curr Opin Pediatr 2013;25:247-254.

4. Hauser R, Meeke JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Mol Cell Endocrinol 2012;36:106-115.

3. Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. J Endocrinol 1998;158:327-339.

2. Andra SS, Makris KC. Thyroid disrupting chemicals in plastic additives and thyroid health. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2012;30:107-151.

1. Mattison DR, Karrysian A, Goodman M, LaKind JS. Pharmacokinetics and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol 2014;44:696-724.