Obesity or diet? Levels and determinants of phthalate body burden A case stud

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A B S T R A C T

In this study we analyzed one of the most comprehensive sets of 21 urinary phthalate metabolites representing exposure to 11 parent phthalates (DEP, DMP, DiBP, DnBP, BBzP, DEHP, DiNP, DiDP, DCHP, DnPeP, DnOP) in first morning urine samples of 112 Portuguese children (4–18 years) sampled in 2014/15. The study population consisted of two groups: group 1 with normal weight/underweight children (N = 43) following their regular diet and group 2 with obese/overweight children (N = 69) following a healthy diet (with nutritional counselling). Most of the metabolites were above the limits quantification (81–100%) except for MCHP, MnPEP and MnOP. Metabolite levels were generally comparable to other recent child and general populations sampled worldwide, confirming the steady decline in exposures to most phthalates. Compared to Portuguese children sampled in 2011/2012, median urinary metabolite levels decreased by approximately 50% for DEHP, DnBP, DiBP and BBzP. Risk assessments for individual phthalates and the sum of the anti-androgenic phthalates did not indicate to attributable health risks, also at the upper percentiles of exposure. In the healthy diet group the median concentration of the DEHP metabolites was significant lower, while all phthalate metabolites except MEP tended to be lower compared to the regular diet group. Multiple log-linear regression analyses revealed significantly lower daily intakes (DIs) for all phthalates in the healthy diet group compared to the regular diet group (geometric mean ratios (gMR) between 0.510–0.618; $p \leq 0.05$), except for DEP (gMR: 0.811; $p = 0.273$). The same analyses with the continuous variable body mass index instead of the diet groups also showed effects on the DIs (gMRs between 0.926–0.951; $p \leq 0.05$), however much smaller than the effects of the diet. The results indicate that obese children following a healthy diet composed of fresh and less packaged/processed food can considerably reduce their intake for most phthalates and can have lower phthalate intakes than regular weight/regular diet children.

1. Introduction

Phthalates are dialkyl or alkyl esters of the ortho-benzene dicarboxylic acid (phthalic acid). Depending on the length of the alkyl chain, phthalates can be classified as low (LMW) or high (HMW) molecular weight phthalates (Koch and Calafat, 2009; Wittassek et al., 2011). Di (2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP), and di-iso-decyl phthalate (DiDP) are HMW phthalates and most frequently used as plasticizers in soft polyvinyl chloride (PVC) and plastisol applications. LMW phthalates such as dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl benzyl phthalate (BBzP), di-n-butyl phthalate (DnBP) and di-iso-butyl phthalate (DiBP) are frequently used in personal care products, paints, adhesives or enteric-coated tablets (Koch and Angerer, 2012; Koch and Calafat, 2009; Wittassek et al., 2011). Diet is regarded as the main source of exposure to HMW phthalates. For LMW phthalates dermal and inhalation exposures (e.g. by product use, indoor air or dust) have been shown to be important exposure routes next to diet/nutrition (Koch et al., 2011; Lorber et al., 2016; Trasande et al., 2013b; Weschler et al., 2015; Zota et al., 2016).

Several phthalates, i.e. those with alkyl chain backbone lengths

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between 3–7 carbon atoms (ranging from DiBP to DiNP) are known endocrine disruptors and developmental and reproductive toxicants in rodents (Foster, 2006; Furr et al., 2014; Gray et al., 2016; Gray et al., 2000; Hannas et al., 2011b; Lioy et al., 2015; van den Driesche et al., 2015). Some epidemiological studies have directly linked phthalate exposure to health effects in humans (Braun et al., 2013; Hauser et al., 2016; Jurewicz and Hanke, 2011; Koch and Angerer, 2012; Swan, 2008; Swan et al., 2005). The European Union has successively classified several phthalates based on developmental effects and/or for fertility effects and restricted the use of DEHP, DnBP, BBzP, DiBP, DiNP, DiDP and DnOP (di-n-octyl phthalate) in toys and childcare articles (Regulation (EC) No 1907/2006) (EU, 2006). Since February 2015, phthalates listed in Annex XIV of the REACH regulation (currently DiBP, DnBP, BBzP and DEHP) may only be placed on the European market or used in the European Union if an authorization (for defined and limited applications) has been granted (Regulations (EU) No 125/2012 and (EU) No 143/2011) (EU, 2011, 2012). Furthermore, in 2017 the Committee for Risk Assessment (RAC) and the Committee for Socio-Economic Analysis (SEAC) agreed on a restriction proposal on four phthalates (DEHP, DnBP, DiBP and BBzP) in articles and on TDFA in sprays used by the general public (ECHA, 2017).

Human biomonitoring studies have shown the worldwide exposure to phthalates (CDC, 2017; Černá et al., 2015; Den Hond et al., 2015; Frederiksen et al., 2013b; Health Canada, 2015; Kasper-Sonnenberg et al., 2014; Koch and Calafat, 2009; Koch et al., 2017). Exposures to most phthalates have been shown to considerably decrease over the recent years probably due to regulatory measures and market changes (Frederiksen et al., 2014; Gyllenhammar et al., 2017; Katsikantami et al., 2016; Koch et al., 2017; Schoeters et al., 2017; Zota et al., 2014). However, these studies also reveal differences in phthalate exposures between countries/regions, between different ethnic groups, between genders, and also in relation to age. Generally, children have been reported to be exposed to most of the phthalates at higher levels than adults (Den Hond et al., 2015; Frederiksen et al., 2014; Hartmann et al., 2015; Kasper-Sonnenberg et al., 2012). Phthalate exposures of child populations (including prenatal exposures) therefore remain in the focus of scientific interest.

Several recent studies have examined associations of childhood phthalate exposure and adiposity/obesity. Some studies report positive associations between adiposity/obesity-related markers and childhood phthalate exposures (Hatch et al., 2008) while others demonstrate inverse relationship between obesity-related markers in children and the prenatal phthalate exposure (Buckley et al., 2016a, 2016b; Maresca et al., 2016; Valvi et al., 2015) but also no or only small relationships were detected (Buckley et al., 2016a, 2016b). A recent review by Braun (2017) concluded that the results from studies linking phthalate exposures and childhood obesity are inconsistent.

With this study we report on the recent (years 2014–2015) internal exposure of Portuguese children (4–18 years old) to a set of the most relevant phthalates (11 phthalates, 21 metabolites). Moreover we investigated possible differences in phthalate exposures between two different groups of children in this study: one group (obese/overweight children) that had been set on a healthy, calorie controlled diet in a weight management program while the other group (normal weight/underweight children) continued with their regular diet.

2. Material and methods

2.1. Study population

The present study is part of an ongoing study to assess possible differences between obese/overweight and normal weight/underweight children in regard to exposure to environmental chemicals. While at the onset of the study exposure to persistent endocrine disruptors and/or obesogens were in the focus, we included non-persistent chemicals such as phthalates, phthalate substitutes and bisphenol A in a later stage of the study. Principles of the study design are already given in Lessmann et al. (2017) and et al. (2017a, 2017b); . Children were recruited from the pediatric appointment at Hospital de S. João (obese/overweight), and several local schools (normal weight/underweight), in the years of 2014 and 2015. São João is a university general Hospital focused on providing the best health care, with high levels of competence and excellence, encouraging training and research.

The children came from two Portuguese districts, Oporto and Aveiro, belonging to the North and Central region of the country, respectively. In all, one hundred and twelve children (55 boys, 57 girls) participated in this study with an age range of 4–18 years (arithmetic mean ± standard deviation: 10.4 ± 3.38 years old).

The children were divided in two groups: 1. Normal weight/underweight children (N = 43) following their usual regular diet (regular diet group). 2. Obese/overweight children (N = 69) that were counselled for healthy and balanced nutrition and were set on a prescribed diet based on fresh food and less packaged and processed food items (healthy diet group). The adherence to the dietary recommendations was confirmed by the nutritionist appointed to each child. The normal weight/underweight and obese/overweight children were grouped according to the WHO BMI charts (WHO, 2007). All children were not hospitalized. The children in the weight management program provided their first morning urine samples after having been in the program for approximately three months. The normal weight/underweight children provided a first morning urine sample during a regular check up at the hospital. Depending on the time of appointment some samples were collected at home before the appointments. All the specimens were kept cool during transportation and then stored at −20 °C until analyses.

The study was approved by the ethics committee of the Centro Hospitalar S. João/FMUP (Medicine Faculty of Oporto University ref. 163.13) and all the parents provided written consent.

2.2. Analysis of phthalate metabolites in urine

Urine samples were analyzed for 21 phthalate metabolites (representing the exposure to 11 parent phthalates) via on-line HPLC–MS/MS with isotope dilution quantification after enzymatic deconjugation. Details of the method and quality assurance were published elsewhere (Kasper-Sonnenberg et al., 2012; Koch et al., 2003; Koch et al., 2017). Briefly, 300 µL urine aliquots were added with 100 µL of 1 M ammonium acetate (at pH 6.0–6.4), 10 µL of internal standard and 6 µL of β-glucoronidase (from *E. coli* strain K-12, without arylsulfatase activity, diluted 1:1 in ammonium acetate buffer). The samples were gently mixed and placed in water bath at 37 °C for 2 h. Then 10 µL of acetic acid were added to adjust pH, and samples were frozen at −18 °C overnight. The samples were then thawed and equilibrated at room temperature and centrifuged at 1900 × *g* for 10 min, and 10 µL supernatant were injected into an Agilent Technology LC 1260 system coupled with an AB Sciex TripleQuad4500 tandem mass spectrometer. A Capcell PAK 5 u C18 MG-II column for clean-up and enrichment and an Atlantis d C18 (2.1 × 150 mm; 3 µm) for chromatographic separation were used, the two column assembly was operated in back-flush mode. Detection was performed in negative ionization mode and quantification was performed by isotope dilution with deuterium labeled internal standards. Quality control materials (prepared from pooled native urine) were included in each batch together with the study samples. The phthalate metabolites analyzed (including the names of the respective parent phthalates) are shown in Table S1 (Supplement material). The sums of the metabolites of DiBP, DnBP, DEHP, DiNP, and DiDP were calculated by summation (Σ) of the corresponding metabolite concentrations in µg/L.

2.3. Determination of urinary creatinine

Urinary creatinine was measured with a modified Jaffe method

(Jaffé, 1986) on an Olympus AU5400® (Beckman-Coulter®, Porto, Portugal) at São João Hospital, Department of Clinical Pathology. Four out of the 112 children had creatinine concentrations below 0.3 g/L. However, all urine samples were included in the statistical analysis, because creatinine concentrations below 0.3 g/L (WHO, 1996) (a cut-off for adult populations) in children do not necessarily indicate excessive dilution, but can be indicative of lower muscle mass in children compared to adults (Barr et al., 2005; Koch et al., 2011).

2.4. Daily intake estimation

Individual daily phthalate intakes (DI) were calculated from urinary metabolites levels according to a creatinine based estimated models published elsewhere (Koch et al., 2007; Wittassek et al., 2007) and were adjusted to the height/age dependent reference values for creatinine excretion (Remer et al., 2002). Supplemental Table S2 shows the factors of urinary excretion (Fue) used for DI calculation.

2.5. Statistical analysis

Basic statistical analysis was performed using SPSS 20.0 (IBM Corporation). Several statistical data is presented, such as median, geometric and arithmetic means, maximum values and percentiles. Concentrations below the LOQ were set to ½ LOQ (Lotz et al., 2013). For the present analysis, age was divided in two groups in agreement with the European regulations for clinical studies in pediatric patients (ICH, 2000): (i) children from 2 to 11 years old; (ii) adolescents from 12 to 18 years old or was used as continuous variable for multiple log-linear regression analyses (see below).

The non-parametric Mann-Whitney *U* test was performed to assess possible differences across the distribution between region, sex, age, and the two study groups (regular diet vs. healthy diet) for the urinary metabolite concentrations (µg/L and µg/g creatinine) and the daily intakes (µg/kg bw/day).

To analyze influences of important factors (e.g. diet groups, age, sex) on the DIs, we performed multiple log-linear regression analysis using the ln-transformed DIs of the phthalates (µg/kg bw/day) as dependent variable. The changes of the DIs by a unit change of the independent variables are presented as geometric mean ratios (gMR) together with their corresponding 95% confidence intervals (95% CI) which can be interpreted similar to Odds Ratios. For this purpose the regression coefficients beta were back-transformed on their natural scale (exp (beta)). This part of the analysis was performed with the statistical software SAS 9.4 © SAS Institute Inc., Cary, NC, USA.

2.6. Cumulative risk assessment

For cumulative risk assessment we applied the approach as described by the Chronic Hazard Advisory Panel (CHAP) on Phthalates and Phthalate Alternatives (CHAP, 2014; Lioy et al., 2015).

In short, the CHAP derived potency estimates for anti-androgenicity (PEAA, expressed as daily intakes in µg/kg/day) for the individual phthalates. Five phthalates, known to possess anti-androgenic potency, were included in the calculations (BBzP, DiBP, DnBP, DEHP and DiNP). Because the CHAP had to fall back on different studies, the CHAP decided to base their calculations on three different cases. Case 1 includes health benchmark values used in a cumulative risk assessment for mixtures of phthalates and other endocrine disruptors (Kortenkamp and Faust, 2010), case 2 includes values derived from a more recent mixture study of phthalates in rats enabling a direct comparison of potencies (Hannas et al., 2011b), and case 3 includes values from CHAP`s de novo literature review of reproductive and developmental endpoints focused on reliable no observed adverse effect levels (NOAELs) and Points of departure (PODs) (see Supplementary Table S3 for the used PEAAs).

For each case, and each phthalate, the Hazard Quotient (HQ) was calculated as the ratio of exposure (estimate of daily intake) to the respective PEAA (see Eq. (2.1)). The HI is the sum of the individual HQs (see Eq. (2.2)):

$$\text{Hazard Quotient}(HQ_j) = \frac{DI_j(\mu g/kg/day)}{PEAA_j(\mu g/kg/day)} \quad (2.1)$$

$$\text{Hazard index}(HI) = \sum_c^{j=1} HQ_j \quad (2.2)$$

A HQ or a HI of larger than 1 indicates that individual (HQ) or cumulative (HI) exposure levels are higher than levels deemed acceptable; adverse health effects cannot be excluded anymore with sufficient certainty.

3. Results

The total study population of 112 children was composed of 49 % boys and 51 % girls, with a median age of 10 years old. The majority of the children was overweight/obese and underwent a diet with nutritional guidance (healthy diet group) (62 %; n = 69). From the 43 children of the regular diet group 42 were normal weight and only one child was underweight. A summary of anthropometric data of the study population, including the two groups is given in Table 1. The discriminators body weight and BMI differed significantly between the two groups but age, sex, height and urinary creatinine were equally distributed (Table 1).

3.1. Urinary metabolite levels

The concentrations of the 21 phthalate metabolites (representing exposure to 11 parent phthalates) in urine samples of the 112 Portuguese children are depicted in Table 2 (in µg/L) and in

Table 1
Characteristics of the study population.

Population characteristics	Total (n = 112)			Regular diet (n = 43)[a] Normal weight/underweight No nutritional guidance			Healthy diet (n = 69)[a] Overweight/obese Nutritional guidance			*p value*[*]
	Median	P95	Max.	Median	P95	Max.	Median	P95	Max.	
Age (years)	10.0	16.4	18.0	11.0	17.0	18.0	9.00	16.0	17.0	0.426
Sex (%)	51% Female 49% Male			44% Female 56% Male			55% Female 45% Male			0.265
Weight (kg)	44.7	80.6	120	34.8	64.8	75.0	45.5	82.3	120	< **0.001**
Height (cm)	142	171	184	143	181	184	142	168	182	0.643
BMI (kg/m^2)	22.2	30.3	42.3	17.1	23.0	24.1	24.6	34.2	42.3	< **0.001**
Creatinine (g/L)	0.94	2.59	3.81	0.88	2.55	2.64	0.98	2.79	3.81	0.907

[a] The underweight/normal weight and obese/overweight groups were defined according to the WHO charters (WHO, 2007); P95–95th percentile; Max. – Maximum value.
[*] Mann-Whitney-*U* test, 2-tailed; Significant differences between the regular and healthy diet groups ($p \leq 0.05$) are marked in bold.

Table 2
Urinary phthalate metabolite concentrations (in µg/L) in the total study population (n = 112).

Parent Phthalate	Metabolite	LOQ	%	µg/L				
		[µg/L]	≥LOQ	Geom. Mean	95% Confidence interval	Median	P95	Max.
DMP	MMP	1.00	91.0	3.10	2.60–3.70	3.16	13.0	54.5
DEP	MEP	0.50	100	58.3	46.6–72.9	59.4	494	1310
BBzP	MBzP	0.20	96.0	2.25	1.78–2.81	2.27	30.3	68.5
DCHP	MCHP	0.20	5.00	< LOQ	< LOQ	< LOQ	0.24	0.56
DnPEP	MnPeP	0.20	0.00	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
DiBP	MiBP	1.00	99.0	16.8	14.4–19.6	16.9	68.4	195
	OH-MiBP	0.25	100	6.54	5.56–7.70	7.08	28.0	57.6
ΣDiBP				23.5	20.1–27.6	23.7	94.0	248
DnBP	MnBP	1.00	99.0	12.8	10.6–15.1	12.7	55.4	80.4
	OH-MnBP	0.25	97.0	1.67	1.40–1.97	1.92	6.95	14.0
ΣDnBP				14.6	12.1–17.2	14.2	63.0	94.4
DEHP	MEHP	0.50	87.0	1.90	1.52–2.37	2.02	12.2	70.8
	OH-MEHP	0.20	100	10.9	9.23–13.0	11.5	58.2	183
	oxo-MEHP	0.20	100	7.62	6.47–9.03	8.39	37.9	112
	cx-MEPP	0.20	100	16.1	13.8–19.0	17.4	68.8	281
ΣDEHP				37.3	31.8–44.0	40.4	170	639
DiNP	OH-MiNP	0.20	99.0	5.57	4.51–6.79	5.47	38.8	98.0
	oxo-MiNP	0.20	97.0	2.23	1.74–2.80	2.03	31.7	61.2
	cx-MiNP	0.20	100	7.42	6.12–9.03	6.99	44.5	137
ΣDiNP				15.8	13.0–19.2	13.9	118	250
DiDP	OH-MiDP	0.20	94.0	1.31	1.06–1.59	1.34	8.69	14.6
	oxo-MiDP	0.20	93.0	0.71	0.59–0.84	0.71	4.15	12.2
	cx-MiDP	0.20	100	1.19	1.04–1.38	1.17	4.88	10.8
ΣDiDP				3.38	2.87–3.97	3.59	19.9	27.3
DnOP	MnOP	0.20	0.00	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
various	MCPP[a]	0.50	81.0	1.03	0.87–1.22	1.03	4.81	15.1

[a]MCPP is a metabolite of several HMW and LMW phthalates (currently known: DnBP, DnPeP, DnOP, DiNP, DiDP) LOQ – Limit of quantification; Geom. Mean – geometric mean; Max. – Maximum value; P95–95th percentile.

Supplementary Table S4 (creatinine adjusted concentrations). Almost all metabolites were detected in the majority of the samples (detection rates from 81 to 100%). Only for MnPeP, MCHP and MnOP detection rates were below 10%. These metabolites were excluded from further in depth investigation.

For the LMW phthalates the DEP metabolite MEP was present at highest median concentrations (59.4 µg/L), followed by the metabolites of DiBP (Σ23.7 µg/L), DnBP (Σ14.2 µg/L), MMP (3.16 µg/L) and MBzP (2.27 µg/L). For the HMW phthalates, DEHP metabolites were detected at highest median concentrations (ΣDEHP 40.4 µg/L), followed by DiNP (ΣDiNP 13.9 µg/L) and DiDP (ΣDiDP 3.59 µg/L).

A comparison of urinary metabolite levels determined in this study with other studies on child populations is presented in Supplementary Table S5. Both urinary levels and metabolite distributions are roughly within the same order of magnitude. Compared to Portuguese children of the European-wide DEMOCOPHES study (Den Hond et al., 2015), with samples collected in 2011/12, median urinary metabolite levels in our study are approximately 50% lower for DEHP, DnBP, DiBP and BBzP.

In regard to sex, we found only MEP metabolite levels were significantly higher in girls with approximately 50% higher urinary MEP concentrations ($p \leq 0.05$; both in µg/L and after creatinine adjustment). In regard to age (4–11 years vs. 12–18 years) we found approximately two-fold higher MMP and MEP levels in the older children ($p \leq 0.05$ for values in µg/L), however with no statistical difference after creatinine adjustment (data not shown). These findings point to increased personal care product use in older children, especially girls.

The urinary phthalate metabolite concentrations for the two groups of children (regular diet group and healthy diet group) are presented in the Supplementary Tables S6–S9 in µg/L and µg/g creatinine. A comparison between the two groups, based on median urinary metabolite levels (multiple metabolites per phthalate summed up), is depicted in Table 3.

For nearly all metabolites (resp. summed metabolites) median concentrations (both in µg/L and µg/g creatinine) were higher in the regular diet group compared to the healthy diet group. However, these differences reached statistical significance ($p \leq 0.05$) only for the DEHP metabolites (ΣDEHP 47.0 µg/L vs. 29.2 µg/L and 48.9 µg/g vs. 35.0 µg/g). DEP was the only exception, with the healthy diet group (obese/overweight children) excreting slightly higher urinary MEP levels than the regular diet group (normal weight/underweight children).

Table 3
Differences of the phthalate metabolite concentrations between the regular and healthy diet groups.

Metabolites	Group	µg/L		µg/g creatinine	
		Median	*p value**	Median	*p value**
MMP	regular diet	3.59	0.193	3.83	0.136
	healthy diet	2.83		3.10	
MEP	regular diet	52.4	0.424	60.6	0.378
	healthy diet	60.8		64.3	
MBzP	regular diet	2.46	0.163	2.78	0.206
	healthy diet	2.01		2.22	
ΣDiBP	regular diet	29.3	0.168	31.8	0.179
	healthy diet	21.5		22.2	
ΣDnBP	regular diet	15.1	0.785	18.0	0.753
	healthy diet	14.1		15.3	
ΣDEHP	regular diet	**47.0**	**0.016**	**48.9**	**0.007**
	healthy diet	**29.2**		**35.0**	
ΣDiNP	regular diet	16.2	0.265	17.4	0.155
	healthy diet	12.8		11.7	
ΣDiDP	regular diet	3.83	0.130	3.82	0.102
	healthy diet	3.03		3.19	
MCPP	regular diet	1.35	0.184	1.22	0.251
	healthy diet	0.86		0.87	

Regular diet n = 43; healthy diet n = 69.

* Mann-Whitney-*U* test, 2 tailed; significant differences between the regular and healthy diet groups ($p \leq 0.05$) are marked in bold.

Table 4
Calculated daily phthalate intakes for the total study population and the regular and healthy diet group.

Phthalates	Group	Daily intake (µg/kg bw/day)					
		Geometric Mean	95% Confidence interval	Median	P95	Max	*p value*[*]
DMP	*total*	*0.08*	*0.07–0.09*	*0.07*	*0.36*	*1.44*	–
	regular diet	0.12	0.09–0.15	0.10	0.74	1.16	< **0.001**
	healthy diet	0.07	0.05–0.08	0.06	0.24	1.44	
DEP	*total*	*1.62*	*1.36–1.94*	*1.46*	*10.7*	*44.2*	–
	regular diet	1.79	1.31–2.58	1.55	20.2	44.2	0.394
	healthy diet	1.52	1.23–1.88	1.39	8.08	17.7	
BBzP	*total*	*0.06*	*0.05–0.08*	*0.07*	*0.40*	*1.15*	–
	regular diet	0.09	0.07–0.12	0.09	0.43	1.15	**0.005**
	healthy diet	0.05	0.04–0.07	0.05	0.38	0.98	
DiBP	*total*	*0.54*	*0.46–0.62*	*0.51*	*1.95*	*9.12*	–
	regular diet	0.75	0.59–0.97	0.78	2.91	9.12	**0.001**
	healthy diet	0.43	0.37–0.51	0.41	1.38	2.08	
DnBP	*total*	*0.28*	*0.23–0.32*	*0.27*	*1.03*	*2.60*	–
	regular diet	0.40	0.31–0.50	0.48	1.86	2.60	**0.001**
	healthy diet	0.22	0.18–0.27	0.25	0.78	1.35	
DEHP	*total*	*1.74*	*1.49–2.02*	*1.89*	*6.61*	*24.0*	–
	regular diet	2.60	2.14–3.14	2.79	7.19	9.38	< **0.001**
	healthy diet	1.35	1.12–1.65	1.41	5.51	24.0	
DiNP	*total*	*1.18*	*0.99–1.42*	*1.04*	*6.77*	*41.7*	–
	regular diet	1.55	1.22–1.96	1.32	6.74	14.4	**0.002**
	healthy diet	1.00	0.79–1.29	0.72	7.06	41.7	
DiDP	*total*	*0.27*	*0.23–0.31*	*0.26*	*1.11*	*1.65*	–
	regular diet	0.38	0.30–0.46	0.35	1.17	1.65	< **0.001**
	healthy diet	0.22	0.18–0.26	0.20	1.15	1.42	

Max. – Maximum value; P95–95th percentile; Mann-Whitney-*U* test, 2 tailed; significant differences between the regular and healthy diet groups ($p \leq 0.05$) are marked in bold.

3.2. Calculated daily intakes (DI)

Daily intakes (median, 95th percentile and maximum values in µg/kg bw/day) calculated separately for both groups are shown in Table 4. DEHP was the phthalate with the highest median DI (1.89 µg/ kg bw/day) followed by DEP, DiNP, DiBP, DnBP, DiDP, with lowest intakes for DMP and BBzP. For all phthalates, median DIs were around 1.5–2 times higher for children on the regular diet than for children on the healthy diet (e.g. for ΣDEHP 2.79 µg/kg bw/day vs. 1.41 µg/kg bw/day or ΣDiBP 0.78 µg/kg bw/day vs. 0.41 µg/kg bw/day, respectively). These differences were statistically significant, except for DEP ($p = 0.394$).

3.3. Predictors of the body burden

In a log-linear regression analysis we found that daily intakes were significantly associated with the healthy diet group for all of the phthalates, except for DEP (Table 5). Compared to the regular diet group the healthy diet group had between 38 % (DiNP) to 49 % (DEHP) lower DIs (Fig. 1). Furthermore, age was negatively associated with DiBP, DnBP, DEHP, and DiNP. This is in line with the already known higher phthalate DI in younger children. However, the changes are rather low and lie between 2% to 7%. Sex emerged as a significant factor on the DI only for DEP. The girls had a 55% higher DI than the boys which is most likely related to the higher use of cosmetics in the girls.

In a second log-linear regression analysis, we also tested whether the continuous variable BMI instead of the two diet groups has an effect on the DI of phthalates (Table 6). Indeed, the BMI was significantly and negatively associated with the DI for all phthalates, except for DEP. However, compared to the predicted changes on the DIs between the healthy diet group versus the regular diet group, these changes were considerably lower (about 7% to 5% lower DIs with increasing BMI). With this additional analysis we can demonstrate that the BMI is negatively associated with the DI within our study but this effect is much smaller than the effect of the healthy diet in the two study groups.

Table 5
Influences of covariates (diet groups, age, sex) on the daily intake of phthalates. Log-linear regression analyses using ln-transformed daily intakes of phthalates (µg/kg bw/d) as dependent variable.

		Healthy diet vs. regular diet	Age (years)	Sex (female vs. male)
DMP	gMR	**0.563****	1.005	0.999
	95% CI (lower; upper)	0.412; 0.770	0.961; 1.051	0.738; 1.352
DEP	gMR	0.811	0.978	**1.550***
	95% CI (lower; upper)	0.558; 1.179	0.927; 1.033	1.080; 2.223
BBzP	gMR	**0.558***	0.968	0.831
	95% CI (lower; upper)	0.369; 0.844	0.912; 1.028	0.557; 1.240
DiBP	gMR	**0.523*****	**0.926*****	1.277
	95% CI (lower; upper)	0.402; 0.680	0.892; 0.962	0.991; 1.647
DnBP	gMR	**0.517*****	**0.951***	0.995
	95% CI (lower; upper)	0.378; 0.707	0.909; 0.995	0.736; 1.345
DEHP	gMR	**0.510*****	**0.952***	0.835
	95% CI (lower; upper)	0.383; 0.678	0.913; 0.992	0.634; 1.101
DiNP	gMR	**0.618***	**0.948***	0.991
	95% CI (lower; upper)	0.430; 0.888	0.899; 0.999	0.698; 1.407
DiDP	gMR	**0.575****	**0.981**	0.953
	95% CI (lower; upper)	0.430; 0.770	0.940; 1.023	0.719; 1.264

gMR = geometric mean ratio = exp(β); 95% CI: 95% confidence interval = exp(lower limit of β) and exp (upper limit of β).
* $p \leq 0.05$.
** $p \leq 0.001$.
*** $p \leq 0.0001$.

3.4. Risk assessment

A risk assessment of exposure to phthalates can be performed for each phthalate individually or on a cumulative basis, taking account of

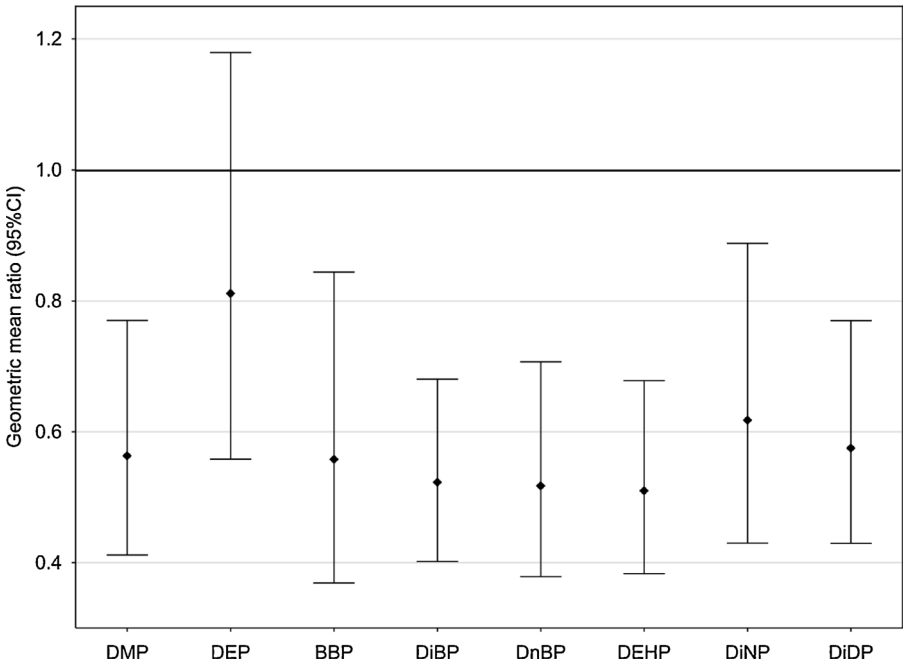

Fig. 1. Changes on the DIs of phthalates for children in the healthy diet group compared to children in the regular diet group (multiple log-linear regression analyses, adjusted for age and sex).

Table 6
Influences of covariates (body mass index, age, sex) on the daily intake of phthalates. Log-linear regression analyses using ln-transformed daily intakes of phthalates (µg/kg bw/d) as dependent variable.

		BMI (kg/m^2)	Age (years)	Sex (female vs. male)
DMP	gMR	**0.928*****	**1.053***	0.904
	95% CI (lower; upper)	0.902; 0.955	1.007; 1.102	0.678; 1.204
DEP	gMR	0.970	0.997	**1.491***
	95% CI (lower; upper)	0.936; 1.006	0.942; 1.054	1.043; 2.132
BBP	gMR	**0.930****	1.014	0.752
	95% CI (lower; upper)	0.894; 0.967	0.954; 1.078	0.510; 1.109
DiBP	gMR	**0.935*****	0.968	1.153
	95% CI (lower; upper)	0.912; 0.959	0.931; 1.007	0.898; 1.480
DnBP	gMR	**0.929*****	0.998	0.893
	95% CI (lower; upper)	0.902; 0.956	0.953; 1.045	0.667; 1.196
DEHP	gMR	**0.926*****	1.000	**0.747***
	95% CI (lower; upper)	0.902; 0.951	0.959; 1.043	0.574; 0.973
DiNP	gMR	**0.951***	0.979	0.918
	95% CI (lower; upper)	0.919; 0.985	0.927; 1.034	0.649; 1.300
DiDP	gMR	**0.942*****	1.020	0.872
	95% CI (lower; upper)	0.916; 0.968	0.976; 1.065	0.661; 1.149

gMR = geometric mean ratio = exp(β); 95% CI: 95% confidence interval = exp(lower limit of β) and exp (upper limit of β).

* $p \leq 0.05$.

** $p \leq 0.001$.

*** $p \leq 0.0001$.

concurrent phthalate exposures. For individual phthalates human biomonitoring assessment values have been derived based on the concentration of phthalate metabolites that are consistent with existing health-based exposure limit values such as the tolerable daily intake (TDI) or a reference dose (RfD). These values are either called Biomonitoring Equivalents (BE), derived by Summit Toxicology (Aylward et al., 2009a, 2009b; Hays and Aylward, 2009; Hays et al., 2011; Hays et al., 2007) or Human-Biomonitoring-Values (HBM-values), derived by the German Human Biomonitoring Commission (Angerer et al., 2011; Apel et al., 2016; Schulz et al., 2011, 2012). Urinary phthalate metabolites determined in the current study compared to these human biomonitoring assessment values are depicted in Table 7.

Median urinary metabolite concentrations were considerably below human biomonitoring assessment values for all phthalates, ranging between 0.07% of the BE of BBzP and 8.5% of the BE of DEHP. Also, at the 95th Percentile all urinary metabolites were consistently below the respective BE/HBM-I-values. The DEHP metabolites reflect 40.8% of the BE, followed by DnBP with 27.7% and DiNP with 6.6% of the BE. Only one child (from the healthy diet group) exceeded the BE for DEHP by a factor of 1.38.

We made similar observations comparing calculated DIs for the various phthalates in the Portuguese children with health benchmark doses for the respective phthalates (see Supplementary Table S10). The US EPA RfD for DEHP (20 µg/kg bw/day) was exceeded only by one child (same child as above) with a calculated daily intake of 24 µg/kg bw/day. At the 95th percentile, like for urinary metabolite levels, calculated DIs were consistently below respective health benchmark doses, with DEHP intakes reflecting 13.2% of the TDI and 33.1% of the RfD, while DnBP intakes reflecting 13.7% of the TDI and 1.4% of the RfD. DiNP and DiDP intakes reflect 4.5% and 0.7% of the TDI (nor RfD derived), respectively.

To evaluate the risk of the cumulative phthalate exposure we applied the Hazard Index (HI) approach as described by the Chronic Hazard Advisory Panel (CHAP) on Phthalates (CHAP, 2014; Lioy et al., 2015). This approach takes into account the common anti-androgenic mode of action in rodents as one of the critical effects of BBzP, DiBP, DnBP, DEHP and DiNP. The three cases used by CHAP for potency estimate derivation are depicted in Supplementary Table S3. Based on these PEAAs (three cases) we calculated the HIs for anti-androgenicity (Table 8). A HI > 1 indicates to cumulative exposure levels to the phthalates investigated that cannot be regarded as safe anymore.

In none of the three cases the HI exceeded the value of 1 (maximum HI: 0.81). At the 95th percentile the HIs were between 0.12 and 0.27. At the median, the values ranged between 0.03 and 0.10. Irrespective of

Table7
Humanbiomonitoringassessmentvalues(BEandHBM)incomparisontourinarymetaboliteconcentrationsmeasuredinthePortuguesechildren.

Phthalate	Human biomonitoring assessment values			Urinary metabolite concentrations in the Portuguese children								
				Total			Regular diet			Healthy diet		
	Type	Biomarkers/metabolites	Value (in µg/L)	Median	P95	Max	Median	P95	Max	Median	P95	Max
DEP	BE[a]	MEP	18000	59.4	494	1310	52.4	750	1310	60.8	397	965
BBzP	BE[a]	MBzP	3800	2.27	30.3	68.5	2.46	14.5	68.5	2.01	36.2	44.5
DnBP	BE[a,b]	MnBP	200	12.7	55.4	80.4	12.9	53.1	56.0	12.7	57.1	80.4
DEHP	HBM-I[c,d,e]	5OH-MEHP + 5oxo-MEHP	500	19.7	96.8	295	23.3	88.8	97.8	14.4	106	295
	BE[f,g]	MEHP + 5OH-MEHP + 5oxo-MEHP	260	22.2	106	**358**	26.6	95.2	114	17.4	118	**358**
DiNP	BE[h]	OH-MiNP + oxo-MiNP + cx-MiNP	1800	13.9	118	250	16.2	92.9	122	12.8	182	250

Exceedances highlighted in bold.
[a] Aylward et al. (2009a).
[b] Based on EFSA TDI (EFSA, 2005b).
[c] Schulz et al. (2012).
[d] Based on EFSA TDI of 50 µg/kg/d (EFSA, 2005a).
[e] Children 6–13 years.
[f] Aylward et al. (2009b).
[g] Based on US EPA RfD of 20 µg/kg/d (EPA).
[h] DiNP:(Hays et al., 2011).

Table 8
Hazard Indices (HI) for the Portuguese children, separately for the two groups of children.

	Group	Median	P95	Maximum
HI (case 1)	Regular diet	0.10	0.26	0.37
	Healthy diet	0.05	0.20	0.81
HI (case 2)	Regular diet	0.10	0.25	0.41
	Healthy diet	0.06	0.27	0.53
HI (case 3)	Regular diet	0.06	0.16	0.20
	Healthy diet	0.03	0.12	0.49

Regular diet- Normal weight/underweight on regular diet; Healthy diet – Obese/overweight with nutritional guidance.

the three calculation cases, median HIs of the healthy diet group were approximately half the median HIs of the regular diet group. Depending on the cases, either DEHP or DnBP was the major contributor to the HI (data not shown).

4. Discussion

4.1. Exposure and risk assessment

With this study we provide urinary excretion data for one of the most comprehensive sets of biomarkers of phthalate exposure (21 phthalate metabolites representing exposure to 11 parent phthalates). All children investigated were simultaneously exposed to a wide range of both LMW and HMW phthalates. Specific urinary metabolites of DEP, DiBP, DnBP, DEHP, DiNP and DiDP were quantifiable in ≥99% of all samples, followed by BBzP (96%) and DMP (91%). DCHP was detected only in 5% of the samples, generally at very low levels, and DnOP in none. DnPeP, one of the most critical phthalates in terms of anti-androgenicity (Hannas et al., 2011a) and only recently included in some biomonitoring studies (Silva et al., 2011), could not be detected in any sample analyzed.

In general, urinary metabolite levels quantified in this study are in the same order of magnitude as levels quantified in other studies on children in Europe, America and Asia (see Supplemental Table S5). For the LMW phthalate DMP and the HMW phthalates DiNP and DiDP, included only in few previous human biomonitoring studies on children, we provide first data for Portugal. DiNP and DiDP have been strictly regulated with regard to its use in children's toys and childcare articles in Europe and recently also in the US (CPSC, 2017). With our data we can confirm the ubiquitous exposure of children to these phthalates as reported previously for Germany, Sweden, Denmark and the US. However, some differences in urinary phthalate levels between countries and/or regions can still be observed. Den Hond et al. (2015) showed for Europe, that phthalate biomarker data clustered together by geographical grouping with the Southern European countries (Spain, Portugal) building their own cluster with generally higher urinary DEP and DEHP metabolite levels in these countries. These clusters and differences in exposure are probably due to different life-style habits, different product uses, or different market uses of the various phthalates in the different regions. We could confirm this by rather high DEP metabolite levels in our study population for Portugal. For DEP, Den Hond et al. (2015) identified the use of personal care products as the major determinant of exposure, in line with other studies (Larsson et al., 2014; Sathyanarayana et al., 2008b). Compared to the metabolite levels of Portuguese children in 2011/12, reported by Den Hond et al. (2015), most phthalate metabolites levels in this study, with samples collected in 2014/2015, are considerably lower (by a factor of 2–3). This is in accordance with the general decline in exposure observed for these phthalates over the recent years, due to regulatory measures, changes in market use, and the substitution of phthalates by non-phthalate alternatives (Correia-Sá et al., 2017b; Frederiksen et al., 2014; Gyllenhammar et al., 2017; Koch et al., 2017; Lessmann et al., 2017; Schoeters et al., 2017; Zota et al., 2014). For DEHP diet is generally considered the major route of exposure, for the LMW phthalates other sources such as consumer and personal care products use and indoor environment seem to be of additional relevance (Koch et al., 2013; Wormuth et al., 2006).

The risk assessment of exposure to the individual phthalates, either by comparing urinary metabolite levels with human biomonitoring assessment values (BE, HBM-I) or by comparing calculated daily intakes with health benchmark doses (TDI, RfD) resulted in similar findings, confirming that current exposure levels in this study population (with samples collected in 2014/2015) were consistently below these benchmark levels both at the median and in the upper percentiles. Only one child slightly exceeded the US EPA RfD derived benchmark level for DEHP. For DEHP and DnBP substantial and frequent exceedances of these health benchmarks have been reported in samples collected before the turn of the millennium (Koch et al., 2017) or in child populations sampled at the beginning of this millennium. In 239 children sampled in 2001/2002 as a pilot study of the German Environmental Survey on Children (GerES IV) health benchmark values were exceeded in up to 7.5 % of the children for DEHP (Wittassek et al., 2007) and in up to 37% of the children for DnBP (Koch et al., 2007).

The cumulative risk assessment based on the Hazard Index (HI) approach as described by the CHAP, taking into account the common anti-androgenic mode of action of phthalates (NRC, 2008) with three to nine carbon atoms in their alkyl side chain (DiBP, DnBP, BBzP, DEHP, DiNP) revealed no exceedance of the HI of 1. The highest HI observed was 0.81. Thus, we can assume that there is no attributable risk to anti-androgenic effects from the cumulative exposure to the phthalates investigated in this study. The CHAP, based on urinary metabolite data from NHANES (2005-06) and a mother-child cohort (SFF, sampling from 1999 to 2005) (Sathyanarayana et al., 2008a) found exceedances of the HI of 1.0 in about 10 % of pregnant women in the NHANES population and in about 4–5 % of mothers and infants in the SFF (Lioy et al., 2015). A recent application of the CHAP HI model to NHANES data from 2013/14 performed by the US CPSC (CHAP, 2017) found HI values > 1 only in less than 1–1.2 %, depending on the calculation case.

Frederiksen et al. (2013b) and Hartmann et al. (2015) calculated HI values for cumulative phthalate exposure in their population of children. These calculations are not directly comparable to the CHAP model because EFSA TDI's were used as potency estimates, which are not fully comparable to CHAPs anti-androgenicity based potency estimates. For Denmark, Frederiksen et al. (2013b) reported that more than 5 % of the children exceeded the HI of 1 with a maximum HI of 4.35. For Austria, Hartmann et al. (2015) reported that 4 % of the children aged 7–15 years had HIs larger than 1, based on EFSA TDIs. The HI calculations based on anti-androgenicity alone did not result in HI values > 1. Thus, considering the different years of sample collection and the different modes of HI calculation, results for our children are in good accordance to results for other child populations in Europe and populations in the US confirming the consistent decline in cumulative exposure to anti-androgenic phthalates to levels generally below a HI of 1.

4.2. Determinants of exposure

In a more in-depth investigation of the Portuguese data we laid special focus on the two different groups of children in this study, one group being overweight/obese children that had been set on a healthy diet in a weight management program, and the other being normal-weight children on their regular diet. As part of a larger study that was originally designed to assess possible differences between these two groups of children in regard to exposure to persistent environmental chemicals, phthalates were included at a later stage. Thus, unfortunately, no urine samples were collected at the onset of the study but only after the weight management program had already been initiated for approximately 12 weeks (3 months). Therefore, we could not evaluate the effect of the weight management program on urinary phthalate levels within the group of obese/overweight children. We can only compare the urinary phthalate levels of the obese/overweight children already under dietary guidance to regular weight children following their normal diet.

All urinary metabolite levels were higher in the regular diet group compared to the healthy diet group, except for the DEP metabolite MEP. However, these differences were only significant for the individual DEHP metabolites and the sum of DEHP metabolites, both in μg/L and after creatinine adjustment. In both cases DEHP metabolites were approximately 50% higher in the regular diet group compared to the healthy diet group. In line with the results of the urinary metabolite levels, calculated daily intakes were all higher in the regular diet group (normal weight children) compared to the healthy diet group (obese/overweight children). For daily intakes, all differences were statistically significant except for DEP. Multiple log-linear regression analyses further strengthened the above observed differences. Children in the healthy diet group showed 38 % (for DiNP) to 49 % (for DEHP) lower daily intakes of phthalates than children in the regular diet group.

Our findings are in contrast to recent studies reporting on associations of childhood exposure to some phthalates and adiposity/obesity. Hatch et al., 2008, reported an increase in body mass index (BMI) and waist circumference (WC) associated with urinary MEP concentrations among adolescent girls. Teitelbaum et al., 2012 found similar associations for MEP and the sum of LMW phthalates (MEP, MnBP and MiBP) for overweight children in the US. Three other studies found associations between the urinary levels of LMW phthalates and higher odds for obesity in male children and adolescents (Buser et al., 2014), in non-Hispanic blacks (Trasande et al., 2013a) or in girls aged 7–13 years (Deierlein et al., 2016). In Chinese children (8–15 years old) MEHP and MEP urinary levels, adjusted for age and sex, were positively associated with BMI or waist circumference (WC) (Wang et al., 2013). Several recent studies also evaluated prenatal phthalate exposure and childhood adiposity (Buckley et al., 2016a, 2016b; Harley et al., 2017; Maresca et al., 2016; Valvi et al., 2015). Some report positive associations and others inverse associations between obesity-related markers and prenatal phthalate exposures. Thus, more research is needed to unveil or negate links between prenatal and/or childhood phthalate exposures and childhood obesity.

One other study investigated urinary phthalate metabolite levels in adults who lost weight through either bariatric surgery or a conservative weight loss program with dietary and lifestyle counselling (Dirtu et al., 2013). They found no differences in metabolite levels and profiles between the obese individuals entering the program and regular weight controls. Furthermore, they reported a slight increase in urinary phthalate metabolite levels in parallel to the significant weight loss after 3–6 months. To some extent, the findings by Dirtu et al. (2013) contradict our findings of lower phthalate metabolite levels in obese children in the weight management program. However, the two studies are difficult to compare, because we only monitored the children at the onset of the weight management program (3 months) before a significant weight loss set in. Our weight management program was especially based on a healthy green diet consisting of fresh, unprocessed food, while the weight loss in the adult population of Dirtu et al. (2013) was achieved by different weight loss strategies.

Our findings are in very good accordance with a wealth of past studies that have identified contaminated food-stuff as a major contributor to exposure to HMW phthalates such as DEHP and DiNP. For DnBP, BBzP, DiBP and other LMW phthalates other sources (indoor environment, personals care product use, etc.) have been reported as additional contributors to exposure, often in a complex mixture of sources and routes. The LMW phthalate DEP is generally regarded as indicator to personal care product use (Ackerman et al., 2014; Colacino et al., 2010; Cutanda et al., 2015; Ferguson et al., 2016; Fromme et al., 2013; Hutter et al., 2016; Ji et al., 2010; Koch and Angerer, 2012; Koch and Calafat, 2009; Koch et al., 2013; Larsson et al., 2014; Rudel et al., 2011; Sathyanarayana et al., 2013; Serrano et al., 2014; Trasande et al., 2013b; Wormuth et al., 2006). Our study strongly suggests that healthy diet can have a significant effect on lowering exposures to both LMW (except DEP) and HMW phthalates. This finding has also to be put into context with the recent and rapid changes in market use of phthalates. We show that our child population has exceptionally low exposures to all phthalates compared to other populations investigated in earlier years. Most probably, exposures to LMW phthalates (except DEP) from personal care product use have dropped considerably due to a phase-out in these products. With exposures from these sources eliminated, other sources might surface as significant contributors such as contaminated foodstuff (or indoor environment), albeit at lower levels. Thus, the most probable explanation for the observed differences between the healthy and the regular diet group is the healthy diet of the obese/overweight children with focus on fresh unprocessed fruits and vegetables, whole grains, low-fat and nonfat dairy products, beans, fish, and lean meat. Additionally, the amount of calories was set according to their nutritional needs (energy intake is appropriate for the maintenance of a normal weight for height and for the adequate intake of micronutrients). Consequently, these children lowered their intake amount of foodstuff in general and of processed, packaged and fat rich foodstuff in special, probably with a combined effect on achieving

lower phthalate exposures. Nevertheless, we cannot separate the potential phthalate reducing effects in regard to reduction in quantity of the intake and the quality of the intake. Both are related to the term "healthy diet".

4.3. Limitations of the study

Certainly, this study has several important limitations. Due to study design, we could not observe the direct effect of nutritional guidance on urinary phthalate metabolites/daily intakes in the obese/overweight children. For future studies, especially for studies with non-persistent chemicals such as the phthalates, it would be advisable to collect biospecimens (urine samples) before the onset of the special diet and follow the trend of urinary metabolites during the course of study. Because we could compare our results only to regular weight children on their normal diet the actual differences in urinary metabolite levels/daily intakes might have been even bigger if urine samples of the obese children before starting the diet had been compared to their urine samples during the special diet.

The study has only been based on a single first morning urine sample from each child. Phthalate exposures are known to change over the course of the day, but also significant between-day differences have been shown (Frederiksen et al., 2013a; Koch et al., 2013; Preau et al., 2010; Watkins et al., 2014). To calculate daily intakes from spot urine samples we took account of the body height, the body weight and sex based reference values for urinary creatinine excretion. These reference values are derived from standard weight children (Remer et al., 2002) and their applicability to obese/overweight children has not been proven. Urinary flow rate and creatinine are known to be influence not only by age and sex, but also by BMI category (Barr et al., 2005; Hays et al., 2015). Urinary volume, urinary concentration and all calculations based on these measures (including daily intake calculations) might be biased by the inherent physiological differences between the obese/overweight and normal-weight children. To circumvent these issues, full 24h-urine samples need to be collected.

Although it is generally accepted that phthalate metabolites are rapidly metabolized and excreted after oral uptake with elimination half times shorter than a day, a minor share of the daily phthalate dose (or their metabolites) might accumulate in fatty tissues or other body compartments (such as the skin) and re-mobilized or released during a special diet or weight loss (Dirtu et al., 2013; Lorber and Koch, 2013). Such secondary effects might therefore add additional complexities and uncertainties to the extrapolation of urinary metabolite measures to daily intakes especially if urinary metabolite/exposure differences between populations or sub-populations are rather small.

5. Conclusions

With this study we could provide recent and novel phthalate exposure data for Portuguese children using the most extensive set of phthalate exposure biomarkers available. We could confirm the still ubiquitous exposure to a wide bandwidth of phthalates, but we could also show that exposure levels were rather low, confirming the general, worldwide trend to declining phthalate exposures, due to regulatory measures and the substitution of phthalates with non-phthalate alternatives. Neither individual risk assessments nor the anti-androgenicity based cumulative risk assessment indicated to health risks attributable to phthalate exposure in our population investigated.

With this study we could also provide valuable insights into phthalate exposures of children on a weight management program compared to children on their regular diet. We conclude that the green healthy diet, with a lot of fresh unprocessed food, resulted in the lower exposures (approx. 50%) to most phthalates in the healthy diet group. Only for DEP we suspect the use of personal care products as the dominant source of exposure. However, we also have to voice some words of caution. Obese and regular weight children differ strongly in many physical characteristics (e.g. urine flow rate, urinary volume, etc.) which can directly influence all measures ranging from urinary metabolite concentration to body weight related calculated (daily) intakes. This might explain inconsistent findings of several previous studies on urinary phthalate levels in relation to obesity.

Conflict of interest statement

The authors declare no conflicts of interest.

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