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Endocrine disrupting chemicals during diet-induced weight loss – A post-hoc analysis of the LOWER study

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ABSTRACT

The link between exposure to endocrine disrupting chemicals (EDCs) and the rapid increase in prevalence of obesity has recently been suggested. However, the magnitude and health impact of EDC exposure in at-risk populations remain largely unclear. In this study, we investigated the effect of a dietary intervention driven reduction in adipose tissue on the magnitude of urinary EDC exposure and mobilization, and whether higher EDC exposure leads to impaired weight loss in obese individuals. In this *post-hoc* analysis of the Lifestyle, OverWeight, Energy Restriction (LOWER) study from the Netherlands, 218 subjects were included. Five parabens, three bisphenols and thirteen metabolites of eight phthalates were measured in 24-h urine using LC-MS/MS, before and after three-months of a calory-restricted weight reduction intervention program. Associations between adiposity-related traits and EDCs were tested using multivariable linear regression and linear mixed effects models. A multiple testing correction based on the false discovery rate (FDR) was applied. After the 3-month intervention, urinary paraben and bisphenol excretions remained similar. Excretions of mono-butyl phthalates and most high-molecular-weight phthalates decreased, whereas mono-ethyl phthalate increased (all FDR<0.05). A reduction in adipose tissue was not associated with higher urinary EDC excretions. Higher baseline EDC excretions were associated with higher post-intervention body-mass index (methyl-, propylparaben), waist circumference (propylparaben, mono-n-butyl phthalate, mono-benzyl phthalate), and body fat percentage (mono-ethyl phthalate, mono-benzyl phthalate). Associations between parabens and body-mass index, and mono-benzyl phthalate and waist circumference and body fat percentage remained after multiple testing correction (all FDR<0.05). In a study of obese participants, we observed a reduction in most phthalates after a weight reduction intervention. A reduction in adipose tissue may not lead to mobilization and successively to higher urinary EDC excretions. Higher baseline paraben and phthalate exposures were associated with reduced weight loss, suggesting obesogenic properties.

1. Introduction

Obesity is a rapidly growing global problem. Comorbidities of this multifactorial chronic disease include (pre-)diabetes, cardiovascular diseases and musculoskeletal disorders (WHO, 2014). Underlying causes include a surplus of energy due to excessive caloric intake and reduced physical activity, and changes in the metabolism of this energy. Energy metabolism is closely controlled by the endocrine system, involving complex hormonal pathways. Endocrine disrupting chemicals (EDCs) are environmental factors that are proven to possess hormonal activity

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(Newbold et al., 2009). As such, they can potentially disrupt energy metabolism and may thereby exert obesogenic effects (Heindel et al., 2015; Darbre, 2017). Some EDCs are labelled as non-persistent due to half-life times of less than 24 h (24 h) (Anderson et al., 2001; Völkel et al., 2002; Janjua et al., 2008). These chemicals are metabolized quickly, partly via the first-pass effect of the liver and via the kidneys in the urine. EDCs are applied in a wide variety of consumer products that are used on a daily basis (e.g. plastics, personal care products), and EDCs have been detected ubiquitously in populations across the globe (Frederiksen et al., 2014; Kasper-Sonnenberg et al., 2014; Cutanda et al., 2015; Koch et al., 2017). Thus, despite their non-persistence, EDCs may exert obesogenic effects that contribute to the global burden of obesity due to their wide-spread and daily use.

One class of EDCs are phenols, which include parabens and bisphenols. Parabens possess estrogenic and anti-androgenic properties (Satoh et al., 2005; Watanabe et al., 2013), and have been shown to cause adverse effects on lipid metabolism through the activation of peroxisome-proliferator-activated receptors (PPARs) (Taxvig et al., 2012; Hu et al., 2016). Bisphenol A (BPA) possesses estrogenic properties, and induces adiposity in mice (Yang et al., 2016). Recently introduced analogues for BPA (i.e. bisphenol F, BPF, and bisphenol S, BPS) have endocrine disrupting potential similar to BPA (Rochester and Bolden, 2015). Another class of EDCs are phthalates, which are known for their anti-androgenic properties, and have also been shown to activate PPARs (Desvergne et al., 2009). In humans, exposure to high concentrations of phenols and phthalates has been associated with obesity and adiposity-related traits (Liu et al., 2017; James-Todd et al., 2016; Mouneimne et al., 2017; Hatch et al., 2008; Kolatorova et al., 2018; Stahlhut et al., 2007; Shankar et al., 2012; Song et al., 2014; Lind et al., 2012; Díaz Santana et al., 2019; Dirinck et al., 2015). To date, the obesogenic effects of EDCs have mainly been studied in healthy populations, whereas the degree and impact of EDC exposure in obese individuals remain largely unknown. Specifically, the examination of EDCs during a diet intervention in obese individuals may yield insights into exposure to EDCs, their metabolism, and their potential obesogenic effects.

Due to the different properties and application of the EDCs of interest, routes of exposure differ. Dietary intake is the main route of exposure for BPA and high molecular weight (HMW) phthalates (Koch et al., 2013; Christensen et al., 2012). Exposure to parabens and low molecular weight (LMW) phthalates is believed to occur largely through non-food products (e.g. personal care products) (Janjua et al., 2008; Koch et al., 2013). Therefore, we hypothesized that restricted dietary intake leads to lower excretions of urinary bisphenol and HMW-phthalates but does not affect excretions of urinary paraben and LMW-phthalates.

A calory restriction diet is often initiated to achieve weight loss through reduction of adipose tissue mass. More persistent EDCs have been shown to be stored in adipose tissue and are released in response to weight loss (Jandacek et al., 2005; Imbeault et al., 2002; Malarvannan et al., 2018). Parabens and bisphenols both show lipophilic properties due to their positive values (1.65–3.56) for the log of the octanol–water partition coefficient (K_{ow}), and have been widely detected in human adipose tissue (Soenen et al., 2012; Harris and Benedict, 1918; van der Meer et al., 2019). The lipophilic property of phthalates increases with the length of their alcohol chain and thus their molecular weight, making HMW-phthalates (molecular weight: $\geq$250 g/mol) more prone to be stored in adipose tissue ($\log(K_{ow})$: 4.73 to 8.83) than LMW-phthalates (molecular weight: $<$ 250 g/mol; $\log(K_{ow})$: 1.61 to 4.45). Yet both groups have been detected in adipose tissue (Bates et al., 2015; Team RDC, 2017). The molecular weight and $\log(K_{ow})$ for each EDC can be found in Supplementary table 1. Yet, for most of these EDCs, the exact metabolic rates in humans are unknown.

We hypothesized that a reduction in adipose tissue due to dietary intervention will lead to a release of EDCs from this tissue, resulting in higher urinary excretions of lipophilic EDCs.

Thus far, phenols and phthalates have been described to exert obesogenic effects over time in healthy populations (Song et al., 2014; Lind et al., 2012; Díaz Santana et al., 2019). We hypothesized that higher pre-intervention exposure to EDCs could impair weight loss response in the caloric restriction intervention.

In this study, we aimed to test the three hypotheses described above. To this end, we performed a *post-hoc* analysis of obese Dutch individuals that participated in a 3-month diet induced weight loss intervention program. We assessed exposure to EDCs (i.e. phenols and phthalates), as measured in 24 h urine voids, before (baseline) and after the 3-month intervention. Furthermore, we examined whether a reduction in adipose tissue resulted in higher urinary EDC excretion. Finally, we investigated associations between baseline EDC exposure and weight loss during the intervention.

2. Materials and methods

2.1. Study population

Subjects were enrolled in the Lifestyle, OverWeight, Energy Restriction (LOWER) study (clinicaltrials.gov, NCT00862953), an open label, randomized treatment intervention of which details of the study design and eligibility criteria have been previously reported (Soenen et al., 2012). In short, adults with a body mass index (BMI) above 27 kg/m^2 were randomized to one of four energy-restricted diet groups differing in protein and/or carbohydrate content: normal-protein normal-carbohydrate (NPNC), normal-protein low-carbohydrate (NPLC), high-protein normal-carbohydrate (HPNC), and high-protein low--carbohydrate (HPLC). Diet groups were formed based on the following criteria: age, BMI, bodyweight, Baecke's score for physical activity and Three Factor Eating Questionnaire. The intervention started with a two-week run-in period with 100% energy intake, followed by a three-month energy intake restriction to 33% of the age- and sex-based energy requirements. Required energy intake was based on the Harris-Benedict equation (Harris and Benedict, 1918). The primary outcome was bodyweight loss after three months. For this *post-hoc* analysis, subjects with available 24 h urine samples at both the end of the two-week run-in period (baseline) and at the end of the 3-month energy restriction period (follow-up) were included. Diabetes status was determined via questionnaire. Due to a lack of regulatory function in glucose homeostasis, subjects with Type 1 Diabetes were excluded. The Medical Ethics committee of the University Medical Centre Groningen approved the study, and all participants gave written informed consent.

2.2. EDC measurements

Urine samples were collected between 2008 and 2010. All urine voids were collected over 24 h, after which they were pooled, homogenized and an aliquot was taken and stored at −80 °C. A total of 21 EDCs was analyzed at baseline and 3 months follow-up using two offline isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) methods of which full analytical methods have been described previously (van der Meer et al., 2019). The first method included methyl, ethyl, propyl, n-butyl and benzyl paraben (MeP, EtP, PrP, n-BuP, BzP, respectively) and three bisphenols (BPA, BPF, BPS). The second method determined the mono-esters of four LMW-phthalates: methyl, ethyl, iso-butyl, and n-butyl phthalate (MMP, MEP, MiBP, MnBP), and the mono-esters of eight HMW-phthalates: 2-ethylhexyl, n-hexyl, 2-ethyl-5-hydroxyhexyl, 2-ethyl-5-oxohexyl, 2-ethyl-5-carboxypentyl, benzyl, iso-nonyl, hydroxy-iso-nonyl, iso-decyl phthalate (MnHP, MEHHP, MEOHP, MECPP, MBzP, MiNP, MHiNP, MiDP, respectively). A limit of detection (LOD) was determined at which the signal could be separated from noise and was calculated as "$3.3*S_0/b$", where S_0 is the standard deviation of the response and b the slope of the calibration curve. The limit of quantification (LOQ) was set where the imprecision

was ≤20% and the signal to noise ratio was >10 on six days. The LOD, LOQ and recovery have been described previously (van der Meer et al., 2019). For completeness, this information can be found in Supplementary Table 1a and b.

As the LOQ indicates a threshold at which an analyte can be precisely measured, only those EDCs of which the measured concentration was above the LOQ in at least 60% of the samples at the timepoint relevant for that analysis were eligible for analyses. For concentrations between LOD and LOQ, values generated by the LC-MS/MS system were used. The concentrations below LOD were imputed with their respective LOD divided by the square root of two ("$LOD/\sqrt{2}$"). The resulting imputed urinary EDC concentrations (ng/ml) were multiplied by the volume of the in total collected urine (ml) to calculate absolute EDC excretion over 24 h (ng/24 h). As the EDCs of interest are rapidly metabolized and excreted from the human body, absolute 24 h EDC excretion values were used as a proxy for daily EDC exposure. We transformed the 24 h excretion values of EDCs by their natural logarithm (lnEDC) to approximate a normal distribution.

2.3. Anthropometry

Extensive information on anthropometric measurements are reported elsewhere (Soenen et al., 2012). In short, waist circumference, weight and body composition were available for baseline and follow-up. BMI was calculated as "weight (kg)/(height (m) * height (m))". Body composition was assessed using the deuterium (^2H_2O) dilution technique, after which body fat was calculated as a percentage of total mass (i.e. body fat percentage, BF%).

2.4. Statistical analysis

2.4.1. Population characteristics

Participant characteristics were expressed as mean (standard deviation). Categorical variables were shown as number (percentage). Differences between baseline and follow-up were calculated using a paired *t*-test.

2.4.2. EDC exposure before and after a three-month diet-based weight loss intervention

The proportion of EDCs detected was expressed as percentage and excretions as median [25th quartile, 75th quartile]. Differences between lnEDC at baseline and follow-up were tested using linear mixed-effect models, implemented in the lme4 package (Bates et al., 2015). In these models, lnEDC was used as outcome, with fixed effects for sex and age and a random effect for intercept: "lmer(lnEDC ~ time + age + sex + (1 | ID), data)". Difference in lnEDC between diet groups was assessed using Analysis of covariance (ANCOVA), adjusting for age, sex, and diabetes status.

2.4.3. The effect of adipose tissue loss on urinary EDC excretion

Whether a loss of adipose tissue was associated with higher urinary EDC excretions was investigated using linear mixed-effect models, with fixed effects for age, sex, diabetes status and diet, and a random effect for intercept. As a proxy for adipose tissue loss, BMI, waist circumference and BF% were transformed into z-scores (i.e. adjusted to the mean, scaled by the standard deviation). Here, the association of change in adipose tissue with change in urinary EDC excretion over time was assessed by introducing an interaction term of EDC with time. With this interaction term, we examined whether change in body fat over time was associated with change in EDCs: "lmer(lnEDC ~ adiposity-related trait * time + (age + sex + diabetes + diet) * time + (1 | ID), data)".

2.4.4. Associations between adiposity-related changes and urinary EDC excretions at baseline

In order to investigate the obesogenic effects of EDCs during a dynamic setting of weight loss, effect sizes between EDC excretions at baseline and adiposity-related traits at follow-up were tested using multivariable linear regression models, additionally adjusting for the baseline level of the respective trait: "adiposity-related trait (follow-up) ~ lnEDC (baseline) + age + sex + diabetes + diet + adiposity-related trait (baseline)".

2.4.5. Multiple testing adjustment and sensitivity analysis

Due to the exploratory nature of this study, we report both raw p-values, as well as adjust for multiple testing using Benjamini and Hochberg False Discovery Rate (FDR) for all individual metabolites of respective groups (i.e. phenols, phthalates). Associations were considered significant at a two-sided FDR <0.05. Given that tests were not independent due to the correlation between EDC compounds, we consider this FDR cut-off conservative. In order to ascertain the robustness of our findings, we performed a sensitivity analysis by repeating the analyses after excluding subjects with T2D. All analyses were performed in R software version 3.5.3 (Team RDC, 2017).

3. Results

3.1. Population characteristics

In total, 219 out of 247 subjects had available 24 h urine at both time points. One subject was diagnosed with Type 1 Diabetes and was therefore excluded, resulting in a total of 218 subjects. Characteristics of the study population at baseline and follow-up are presented in Table 1. After three months, all adiposity-related traits had significantly decreased (all p-values < 0.0001).

3.2. EDC exposure before and after a three-month diet-based weight loss intervention

At baseline, all parabens were detected above LOD in ≥89% of the samples, except for BzP (2%). Bisphenols were detected in 97, 37 and 22% of the samples (BPA, BPF, BPS, respectively), whereas the presence of all phthalate metabolites, but MMP and MnHP (46, 84%, respectively) was ubiquitous (nearly 100%) (Table 2). MiNP, MHiNP and MiDP were not detected in any of the samples, and therefore not presented. Median excretions were similar within and between individuals at baseline and follow-up for all parabens and bisphenols. LMW-phthalate excretions were similar for MMP, whereas higher MEP and lower MiBP and MnBP excretions were detected at follow-up (FDR = 0.9; all other FDR < 0.0001). Regarding HMW-phthalates, all metabolite excretions but

Table 1
Anthropometric characteristics of the LOWER population (n = 218) at baseline and at 3-month follow-up.

	Baseline	Follow-up	*p*-value
Sex = Male, n (%)	32 (Hu et al., 2016)		
Age	52 (Koch et al., 2017)		
BMI	36.6 (5.6)	32.3 (5.1)	**<0.0001**
Waist circumference	109 (Watanabe et al., 2013)	98.0 (Satoh et al., 2005)	**<0.0001**
Body Fat Percentage	45.3 (6.3)	41.4 (6.6)	**<0.0001**
T2D, n (%)	19 (Kasper-Sonnenberg et al., 2014)		
Diet type, n (%)			
HPLC	54 (Song et al., 2014)		
HPNC	53 (Shankar et al., 2012)		
LPLC	54 (Song et al., 2014)		
LPNC	57 (Lind et al., 2012)		

Data expressed as mean (standard deviation) or number (proportion, %). Differences between baseline and follow-up were calculated using a paired *t*-test. Abbreviations: BMI, body-mass index; T2D, type 2 diabetes; HPLC, high-protein low-carbohydrate; HPNC, high-protein normal-carbohydrate; LPLC, low-protein low-carbohydrate; LPNC, low-protein normal carbohydrate; n, number.

Table 2
Comparisons between urinary excretions of measured EDCs at baseline and 3-month follow-up (μg/24 h).

		N > LOD (%)		N > LOQ (%)		median [25th quartile; 75th quartile]		FDR-value
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	
Parabens*								
Methyl paraben	MeP	218 (100)	217 (100)	215 (99)	212 (97)	51.3 [11.8; 148]	61.5 [16.3; 150]	0.5111
Ethyl paraben	EtP	212 (97)	203 (93)	143 (66)	149 (68)	2.41 [0.78; 13.3]	4.81 [1.05; 17.4]	0.1415
Propyl paraben	PrP	218 (100)	216 (99)	150 (69)	152 (70)	8.66 [1.54; 32.7]	8.81 [1.62; 38.1]	0.7205
Benzyl paraben	BzP	5 (Newbold et al., 2009)	4 (Newbold et al., 2009)	0 (0)	0 (0)	0.96 [0.32; 4.89]	1.07 [0.37; 5.19]	0.5367
n-Butyl paraben	n-BuP	195 (89)	196 (90)	72 (Imbeault et al., 2002)	74 (Malarvannan et al., 2018)	0.44 [0.19; 0.72]	0.35 [0.32; 0.46]	0.7979
Bisphenols**								
Bisphenol A	BPA	212 (97)	204 (94)	132 (61)	132 (61)	3.51 [2.05; 6.05]	4.06 [2.01; 8.11]	0.5367
Bisphenol F	BPF	80 (van der Meer et al., 2019)	66 (Koch et al., 2013)	25 (Koch et al., 2017)	19 (Kasper-Sonnenberg et al., 2014)	1.60 [0.76; 4.34]	1.51 [0.91; 4.16]	0.7205
Bisphenol S	BPS	49 (Kolatorova et al., 2018)	49 (Kolatorova et al., 2018)	10 (Anderson et al., 2001)	16 (Janjua et al., 2008)	0.36 [0.19; 0.85]	0.57 [0.22; 2.08]	0.5367
Low-Molecular-Weight phthalates*								
Mono-methyl phthalate	MMP	100 (Dirtu et al., 2013)	103 (Trasande et al., 2013)	15 (Janjua et al., 2008)	16 (Janjua et al., 2008)	1.90 [1.18; 3.22]	1.87 [1.32; 2.97]	0.8718
Mono-ethyl phthalate	MEP	218 (100)	217 (100)	218 (100)	217 (100)	217 [75.8; 588]	388 [107; 1146]	**<0.0001**
Mono-iso-butyl phthalate	MiBP	217 (100)	217 (100)	217 (100)	217 (100)	55.4 [36.9; 91.6]	48.0 [29.5; 78.7]	**<0.0001**
Mono-n-butyl phthalate	MnBP	217 (100)	217 (100)	217 (100)	217 (100)	45.6 [31.5; 66.5]	38.4 [25.3; 62.5]	**<0.0001**
High-Molecular-Weight phthalates**								
Mono-(2-ethylhexyl) phthalate	MEHP	218 (100)	217 (100)	136 (62)	91 (National Research Council, 2008)	5.44 [3.45; 8.98]	3.57 [1.98; 6.46]	**<0.0001**
Mono-n-hexyl phthalate	MnHP	184 (84)	155 (71)	9 (Darbre, 2017)	8 (Darbre, 2017)	0.36 [0.26; 0.52]	0.36 [0.27; 0.50]	0.4345
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	218 (100)	217 (100)	218 (100)	217 (100)	26.1 [18.4; 40.8]	20.1 [11.7; 27.6]	**<0.0001**
Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	217 (100)	216 (99)	217 (100)	216 (99)	17.7 [12.4; 27.0]	13.1 [7.94; 19.4]	**<0.0001**
Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	218 (100)	217 (100)	218 (100)	217 (100)	27.8 [18.9; 43.7]	21.1 [12.6; 32.9]	**<0.0001**
Mono-benzyl phthalate	MBzP	216 (99)	212 (97)	216 (99)	212 (97)	20.0 [10.4; 36.2]	13.8 [7.81; 30.0]	**<0.0001**

Differences between time-points were investigated for volume-adjusted, log-transformed 24 h EDC excretions, using linear mixed effect models adjusting for sex and age. FDR-values were calculated by correcting *p*-values for multiple testing (Benjamini-Hochberg False Discovery Rate < 0.05) and expressed bold when significant. Abbreviations: LOD, Limit of Detection; LOQ, Limit of Quantification; Mono-iso-nonyl phthalate, Mono-hydroxy-iso-nonyl phthalate, and Mono-iso-decyl phthalate did not exceed the LOD in any of the samples, and are therefore not displayed. Main route of exposure: *non-food products; **food-products.

MnHP (FDR = 0.4), were lower at follow-up (all FDR < 0.0001). Medians and percentiles of EDC excretions including samples < LOD are shown in Supplementary Table 2. No significant differences in EDC excretions either at baseline or at follow-up were observed between the different dietary groups (Supplementary Table 3a and b).

3.3. The effect of adipose tissue loss on urinary EDC excretion

Differences in adiposity-related traits within and between individuals during the intervention period did not result in a change in EDC excretions given that none of the tested associations were statistically significant (Fig. 1a–c and Supplementary table 4).

3.4. Associations between adiposity-related changes and urinary EDC excretions at baseline

To assess whether high pre-intervention EDC exposure impairs the response to a diet-induced weight loss intervention, associations between urinary EDC excretions at baseline and adiposity-related traits at follow-up were assessed (Fig. 2a–c). In the context of significant decreases in adiposity-related traits in the overall study population (Table 1), some EDCs were associated with an impaired reduction in these traits. Higher baseline excretions of the phenols MeP and PrP were associated with an impaired reduction in adiposity-related traits at follow-up (BMI: MeP, PrP; waist circumference: PrP, all $p \leq 0.028$). Regarding phthalates, higher excretions at baseline were associated with impaired reduction in waist circumference (MnBP, $p = 0.031$; MBzP, $p = 0.005$) and BF% (MEP, $p = 0.044$; MBzP, $p = 0.005$). After adjusting for multiple testing, associations between MeP and PrP, and BMI, and MBzP and waist circumference and BF% remained significant (FDR < 0.05). Effect sizes, confidence-intervals, p- and FDR-values are reported in Supplementary table 5. As sensitivity analysis, we repeated the analysis after excluding all subjects with T2D at baseline. Except from the association between BF% and MEP ($p = 0.3$), all associations retained their significance (Supplementary table 6).

4. Discussion

In an obese population, urinary excretion of phenols and phthalates was assessed before and after a 3-month dietary weight loss intervention program. We investigated the effects of dietary intervention on exposure to these EDCs, and whether a reduction of adipose tissue leads to

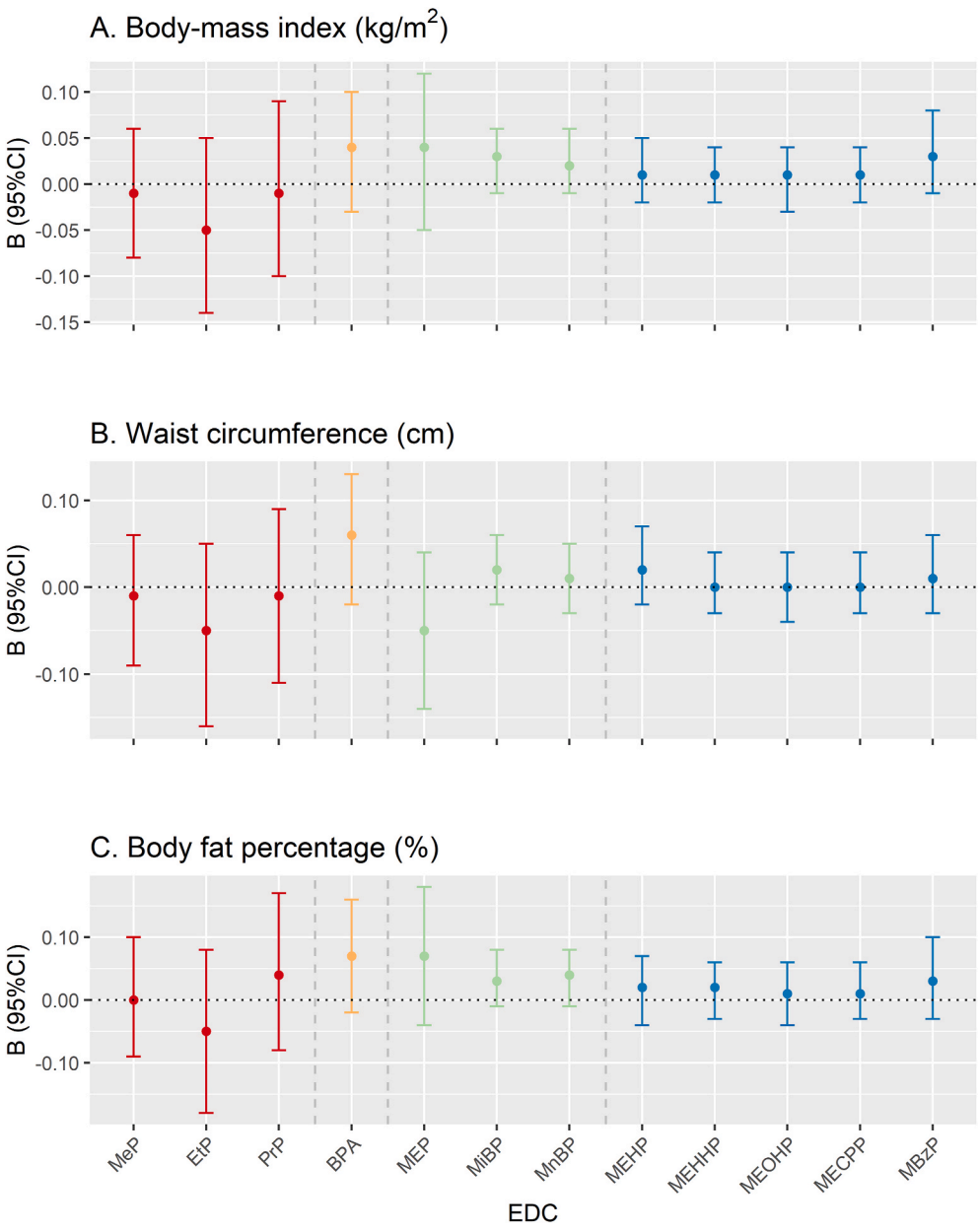

Fig. 1. Associations between change in adiposity-related traits within and between individuals and change in EDC excretions during a diet-induced weight loss program. Regression coefficients with 95% Confidence Intervals of associations for A. Body-mass index, B. Waist circumference and C. Body fat percentage and endocrine disrupting chemicals (EDCs). Formula: "*lmer(lnEDC ~ adiposity-related trait * time + (age + sex + diabetes + diet) * time + (1 | ID), data)*". EDC levels were imputed, volume-adjusted, and natural log-transformed. Linear mixed effect models with fixed effects for age, sex, diabetes status and diet, and random effects for intercept and time. An interaction term between adiposity-related trait and time was introduced in the models. Beta coefficients represent effects per *e*-fold increase per month in EDC parameter per month. For full names of compounds, see Table 2. For exact estimates, standard errors and raw *p*-values, see supplementary table 3. Red: parabens; orange: bisphenols; green: Low Molecular Weight-phthalates; blue: High Molecular Weight-phthalates. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mobilization and successively an increase in urinary excretions. Furthermore, we assessed the obesogenic effects of these chemicals in a setting of weight loss.

4.1. *Effects of a dietary weight loss intervention on urinary EDC excretion*

Main routes of exposure to phenols and phthalates include inhalation, ingestion and transdermal exposure, and largely depend on the foodstuff or consumer products in which these EDCs are contained. Uses of BPA and HMW-phthalates include food packaging and water bottles (Burridge, 2003; Serrano et al., 2014), and urinary excretions of these chemicals have been shown to rapidly decrease after a 48 h period of fasting (Koch et al., 2013; Christensen et al., 2012). On the other hand, parabens and LMW-phthalates are mainly used in personal care and cosmetic products (National Research Council, 2008), and their main route of exposure is believed to be mainly transdermal (Janjua et al., 2008). Consistent with this transdermal route of exposure, urinary LMW-phthalate excretions have been shown to remain stable during a period of fasting (Koch et al., 2013). In the present study, caloric intake was restricted to 33% of the daily required consumption. As exposure to bisphenols and HMW-phthalates is believed to be mainly through food products, we expected urinary excretions to decrease over time. Given the transdermal route of exposure of parabens and LMW-phthalates, and their lack of response to food deprivation, we hypothesized that urinary excretions of parabens and LMW-phthalates remain stable during the intervention.

After the intervention, all urinary HMW-phthalate excretions but MnHP decreased, corroborating the dietary source of these EDC exposures. Counter to expectation, urinary bisphenol excretions remained stable during the intervention. Two weeks prior to the start of the intervention, subjects were consulted by a dietician to meet an energy intake restriction to 100% of the calculated energy requirement. As the consumption of less calorie-dense food products has been associated with lower BPA concentrations (Artacho-Cordón et al., 2017a), this change in diet prior to the baseline measurement could have influenced BPA excretions. Median pre-intervention urinary BPA excretions were nearly half of those of a general Dutch population (van der Meer et al., 2020) (median [25th quartile; 75th quartile]: 3.51 [2.05; 6.05] μg/24 h

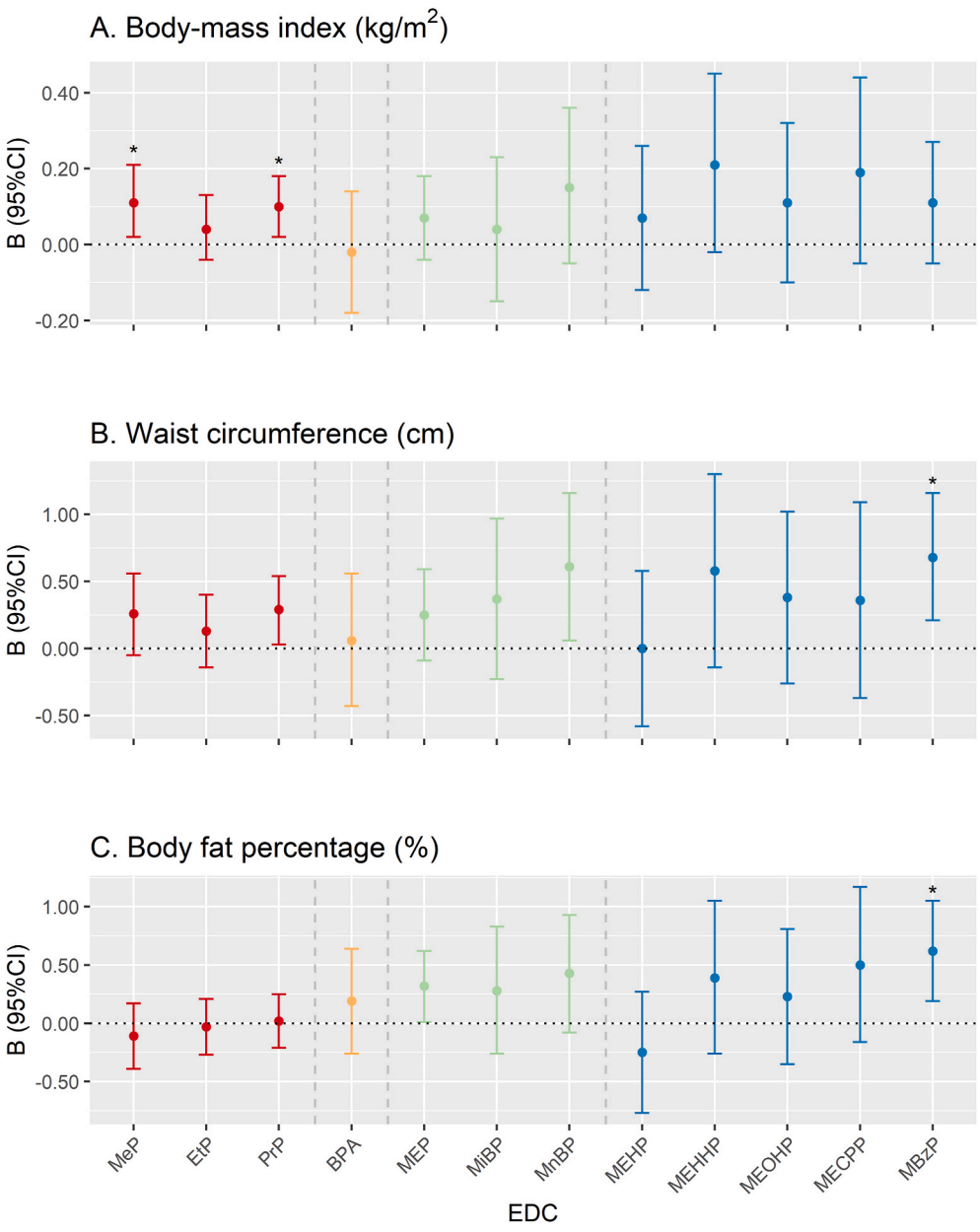

Fig. 2. Associations between baseline EDC excretions and adiposity-related traits after a diet-induced weight loss program. Regression coefficients with 95% Confidence Intervals derived from ordinary least squares linear regression for A. Body-mass index, B. Waist circumference and C. Body fat percentage and endocrine disrupting chemicals (EDCs). Formula: "*adiposity-related trait (follow-up) ~ lnEDC (baseline) + age + sex + diabetes + diet + adiposity-related trait (baseline)*". EDC levels were imputed, volume-adjusted, and natural log-transformed. Models were corrected for age, sex, diabetes status, diet, and respective trait at baseline. Regression coefficients represent effects per *e*-fold increase in EDC parameter over 3 months. Positive regression coefficients indicate impairment of reduction of the respective trait. For full names of compounds, see Table 2. For exact estimates, standard errors and raw *p*-values, see supplementary table 4. Red: parabens; orange: bisphenol A; green: Low Molecular Weight-phthalates; blue: High Molecular Weight-phthalates. *Benjamini-Hochberg False Discovery Rate <0.05. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

vs 1.65 [1.02; 2.87] μg/24 h). This implies that a change in type of food products rather than a quantitative restriction could lead to a decrease in exposure to BPA. This observation is consistent with the results of an intervention study by Rudel et al., which showed that BPA excretions were substantially reduced by avoiding cans, plastic food packaging and plastic cooking utensils (Rudel et al., 2011).

Consistent with our hypothesis, urinary paraben excretions did not differ pre- and post-intervention. Contrary to expectations however, the excretion of LMW-phthalates did change in response to the intervention. Urinary excretion of dibutyl phthalate metabolites were observed to decrease 1.2-fold from baseline, while MEP excretion showed a 1.8-fold increase. At baseline, we detected higher concentrations compared to a general Dutch population (median [25th quartile; 75th quartile]: 217 [75.8; 588] μg/24 h vs 83.5 [34; 247] μg/24 h), but similar a Belgian study investigating weight loss in an obese population (104 μg/ml vs 75 μg/ml) (Dirtu et al., 2013). Though Dirtu et al. observed a decrease in MEP concentrations over three months, the proportion of MEP relative to other phthalates increased. LMW-phthalates have proven to remain stable over a short period of fasting (Koch et al., 2013) and thus are considered to be unaffected by dietary sources. On the other hand, the sole structural change during the intervention being dietary in combination with the relatively short intervention period (i.e. 3 months) makes changes in other non-dietary sources of exposure such as personal care products unlikely. Moreover, several studies reported direct associations between higher MEP excretion and vegetable consumption (Trasande et al., 2013; Colacino et al., 2010). In the present study, subjects shifted to less calorie dense products such as vegetables to achieve a reduction in caloric intake. This shift to vegetables is a potential explanation of the increase in urinary MEP excretion during the intervention. Given its purported obesogenic properties (Díaz Santana et al., 2019), it is alarming that exposure to MEP almost doubled during the intervention, potentially counteracting weight loss in the longer term.

4.2. *Effects of adipose tissue loss on 24 h urinary EDC excretion*

A reduction in dietary intake had the expected results for urinary paraben and HMW-phthalate excretions, as excretions of these

compounds reduced during the study period. Unexpectedly, excretion of bisphenols remained stable over time, whereas MEP increased.

For persistent EDCs, weight loss has been shown to lead to a mobilization of chemicals, resulting in higher circulating concentrations (Jandacek et al., 2005; Imbeault et al., 2002; Malarvannan et al., 2018). Phenols and phthalates have been widely detected in human adipose tissue (Artacho-Cordón et al., 2017a, 2017b; Wang et al., 2015; Mes et al., 1974; Mathieu-Denoncourt et al., 2016). As these EDCs are suspected of obesogenic properties, an increase in exposure due to weight loss could in turn lead to weight gain creating a yo-yo effect. Quirós et al. found associations between higher urinary concentrations of parabens and lower body fat, which they hypothesized was due to an increase in storage capacity and therefore reduced excretion (Quirós-Alcalá et al., 2018). Based on those findings, we hypothesized that a reduction of adipose tissue would lead to the release of stored EDCs and therefore increase their urinary excretions. However, we found no significant associations between a decrease in adiposity-related traits and an increase in urinary EDC excretion. Therefore, we found no indication that a reduction of adipose tissue leads to the mobilization of phenols and phthalates and therewith increase in exposure and excretion.

4.3. Obesogenic effects of EDCs during a weight loss program

Consistent with our hypothesis, we observed that higher baseline EDC exposure was associated with an impaired reduction in BMI, waist circumference and BF% in response to a weight loss program. This suggests the existence of obesogenic effects of EDC that impair the response to weight loss through dietary intervention.

Parabens have been described to have obesogenic effects *in vitro* and *in vivo* (Taxvig et al., 2012; Hu et al., 2016), although cross-sectional studies in humans yielded inconclusive results (Kolatorova et al., 2018; van der Meer et al., 2020; Quirós-Alcalá et al., 2018). To the best of our knowledge, this is the first prospective study in humans that described obesogenic effects of parabens. Phthalates have been positively associated with adiposity-related traits in large cross-sectional studies (James-Todd et al., 2016; Hatch et al., 2008; Lind et al., 2012). Two studies prospectively investigated the effects of EDCs on adiposity-related traits. Song et al. found that higher urinary concentrations of MBzP and the sum of butyl phthalate metabolites were associated with weight gain after ten years in a healthy population (Song et al., 2014). A study conducted by Díaz Santana et al. found higher concentrations of the metabolites MEP and MnBP to be associated with weight gain after three years in post-menopausal women (Díaz Santana et al., 2019). These findings are consistent with the associations found between higher baseline excretions of MEP, MnBP, and MBzP and a reduced adipose tissue loss in current study. The association between MEOHP and weight gain reported by Díaz Santana et al. could not be reproduced. This could be due to a number of factors, including differences in population characteristics (postmenopausal women vs obese subjects), sample size ($n = 660$ vs $n = 218$), biological pathways involved (weight gain versus impaired weight loss) and study design (observational vs experimental).

Some modelling approaches better accommodate non-normally distributed data. Therefore, we repeated our analysis using untransformed EDC concentrations in generalized linear (mixed) models. Yet, generalized models failed to converge and therefore we do not report the results of this procedure.

4.4. Strengths and limitations

To our knowledge, this is the first study to investigate changes in body burden of this range of EDCs during a restrictive diet-based weight loss intervention. Due to the design we were able to obtain valuable insights in the effects of diet on exposure to EDCs, the effects of adipose tissue loss on EDC excretion and the obesogenic effects of EDCs in a setting of active weight loss intervention program in obese individuals. One of the strengths of the study is the availability of body composition information using the deuterium (^2H_2O) dilution technique. This way we were able to calculate the BF% and use this as an accurate marker for adipose tissue mass. A limitation of the study design is the lack of a control group. Although the intervention took a relative short period of time, potential bias in exposure to EDCs due to change in lifestyle factors other than dietary intake cannot be ruled out.

Phenols and phthalates are known to be rapidly metabolized and excreted from the body. Due to their short half-lives (Anderson et al., 2001; Völkel et al., 2002; Janjua et al., 2008), EDC excretions are prone to variate throughout the day resulting in intra-person variability (Preau et al., 2010). As 24 h urine is strenuous to collect, most studies use spot- or morning-urine samples yielding less precise estimations of daily exposure (Sun et al., 2017). By using 24 h urine in this study, we were able to provide a reliable estimation of the average daily excretion of these EDCs.

A major strength of the present study was that a wide variety of compounds, of two common classes of EDCs, were measured in this study. This facilitated a comprehensive assessment of EDCs and their relation to adiposity-related traits. Most previous studies only focused on a single compound, or class of compounds, and may therefore have missed relevant associations. However, this increased the multiple testing burden, necessitating correction based on the false discovery rate.

Several studies have reported associations between higher urinary BPA concentrations and higher adiposity-related traits (Liu et al., 2017; Song et al., 2014; Mouneimne et al., 2017). However, in the current study, BPA was not associated with BMI, waist circumference or BF%. This lack of findings might be explained by the fact that we observed much lower urinary BPA excretions at baseline compared to a general population, and therefore the BPA excretions detected in the current study might not be representative for long-term exposure. Although we measured EDC excretion at two different time points, the two-week run-in period prior to the first measurement could have affected exposure to BPA. Introducing urine measurements before and shortly (e.g. 48 h) after the start of the run-in period would give more insight in the short-term effects of product restriction on exposure to EDCs.

4.5. Implications

In this study, we gained insights in the restrictive dietary intervention program effects on the body burden of phenols and phthalates and their possible obesogenic effects in a dynamic setting of a 3-month weight loss program. Moreover, weight loss interventions might benefit from the reduction of EDCs. We did not find any indication of higher EDC excretion as a result of release from diminishing adipose tissue, and further research is warranted to replicate our findings in animal models.

5. Conclusion

This study assessed the exposure to common EDCs (i.e. phenols and phthalates), as measured by 24 h urine excretions of EDCs, in obese individuals before and after a restrictive diet-induced weight loss program. The body burden of butyl phthalate metabolites and HMW-phthalates decreased over the course of the intervention, while MEP concentrations increased. In contrast, the body burden of BPA and parabens remained unaffected by the dietary caloric restriction intervention program. The documented reduction in adipose tissue did not lead to higher urinary EDC excretion. Higher baseline urinary excretion of parabens and phthalates was associated with impaired loss of adipose tissue, suggesting obesogenic properties in a dynamic setting of weight loss.

Author contributions

TPvdM performed the analysis, interpreted data and wrote the manuscript; CHLT interpreted the data and analyses and wrote the manuscript; MvF coordinated and performed the measurements; APvB acquired data and/or provided study materials; HS contributed to interpretation of the data and analyses; FNRvB acquired data and/or provided study materials; IPK contributed to interpretation of the data and analyses; KCM contributed to interpretation of the data and analyses; BHRW acquired data and/or provided study materials; and JVvVO conceived, designed and implemented the study, was involved in data acquisition and contributed to writing the manuscript. All authors reviewed and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.110262.

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