An epigenome-wide association study identifies multiple DNA methylation markers of exposure to endocrine disruptors

BIOS Consortium; Lu, Xueling; Fraszczyk, Eliza; van der Meer, Thomas P; van Faassen, Martijn; Bloks, Vincent W; Kema, Ido P; van Beek, André P; Li, Shuang; Franke, Lude

Published in:
Environment international

DOI:
10.1016/j.envint.2020.106016

Publication date:
2020

Environment International 144 (2020) 106016

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

An epigenome-wide association study identifies multiple DNA methylation markers of exposure to endocrine disruptors

Xueling Lu[a,b,1], Eliza Fraszczyk[a,c,1], Thomas P. van der Meer[d], Martijn van Faassen[e], Vincent W. Bloks[f], Ido P. Kema[e], André P. van Beek[d], Shuang Li[g], Lude Franke[g], Harm-Jan Westra[g], BIOS Consortium, Xijin Xu[b], Xia Huo[h], Harold Snieder[a,*,1], Bruce H.R. Wolffenbuttel[d], Jana V. van Vliet-Ostaptchouk[a,g,i,*,1]

[a] Department of Epidemiology, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, the Netherlands
[b] Laboratory of Environmental Medicine and Developmental Toxicology, Shantou University Medical College, 515041 Guangdong, China
[c] Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), 3721 MA Bilthoven, the Netherlands
[d] Department of Endocrinology, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, the Netherlands
[e] Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, the Netherlands
[f] Department of Pediatrics, Section of Molecular Metabolism and Nutrition, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, the Netherlands
[g] Department of Genetics, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, the Netherlands
[h] Laboratory of Environmental Medicine and Developmental Toxicology, Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, 511443 Guangdong, China
[i] Genomics Coordination Centre, Department of Genetics, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, the Netherlands

ARTICLE INFO

Handling Editor: Shoji F. Nakayama

Keywords:
Endocrine disruptor
Metabolic trait
Human exposure
Epigenetics
DNA methylation
Epigenome-wide association study

ABSTRACT

Background: Exposure to environmental endocrine disrupting chemicals (EDCs) may play an important role in the epidemic of metabolic diseases. Epigenetic alterations may functionally link EDCs with gene expression and metabolic traits.

Objectives: We aimed to evaluate metabolic-related effects of the exposure to endocrine disruptors including five parabens, three bisphenols, and 13 metabolites of nine phthalates as measured in 24-hour urine on epigenome-wide DNA methylation.

Methods: A blood-based epigenome-wide association study was performed in 622 participants from the Lifelines DEEP cohort using Illumina Infinium HumanMethylation450 methylation data and EDC excretions in 24-hour urine. Out of the 21 EDCs, 13 compounds were detected in > 75% of the samples and, together with bisphenol F, were included in these analyses. Furthermore, we explored the putative function of identified methylation markers and their correlations with metabolic traits.

Results: We found 20 differentially methylated cytosine-phosphate-guanines (CpGs) associated with 10 EDCs at suggestive p-value $< 1 \times 10^{-6}$, of which four, associated with MEHP and MEHHP, were genome-wide significant (Bonferroni-corrected p-value $< 1.19 \times 10^{-7}$). Nine out of 20 CpGs were significantly associated with at least one of the tested metabolic traits, such as fasting glucose, glycated hemoglobin, blood lipids, and/or blood pressure. 18 out of 20 EDC-associated CpGs were annotated to genes functionally related to metabolic syndrome, hypertension, obesity, type 2 diabetes, insulin resistance and glycemic traits.

Conclusions: The identified DNA methylation markers for exposure to the most common EDCs provide suggestive mechanism underlying the contributions of EDCs to metabolic health. Follow-up studies are needed to unravel the causality of EDC-induced methylation changes in metabolic alterations.

* Corresponding authors at: Genomics Coordination Centre, Department of Genetics, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, the Netherlands (J.V. van Vliet-Ostaptchouk). Department of Epidemiology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, the Netherlands (H. Snieder).
E-mail addresses: h.snieder@umcg.nl (H. Snieder), j.v.van.vliet@umcg.nl (J.V. van Vliet-Ostaptchouk).
[1] Equal contribution.

https://doi.org/10.1016/j.envint.2020.106016
Received 14 May 2020; Received in revised form 2 July 2020; Accepted 26 July 2020

1. Introduction

Environmental endocrine disrupting chemicals (EDCs) are considered as obesogens and diabetogens that interfere with energy and macronutrient metabolism, consequently impairing metabolic health (Kassotis and Stapleton, 2019). Humans are ubiquitously exposed to non-persistent EDCs, including parabens, bisphenols, and phthalates, due to their widespread applications in miscellaneous consumer products (Li and Suh, 2019). Parabens are used as anti-microbial preservatives in a wide range of personal care products and food (Bledzka et al., 2014). Bisphenols are one of the highest volume chemicals produced worldwide and used for polycarbonate plastics and epoxy resins, e.g. plastic bottles, food containers, the interior lining of food cans, and thermal receipt papers (Andra et al., 2015). Phthalates can be extensively found in soft plastics, pharmaceutical and nutritional supplements, and cosmetics (Benjamin et al., 2017). Accumulating data demonstrated that EDC exposures can promote epigenetic changes by altering methyl donor availability, the activity of histone methyltransferases, and microRNA or noncoding RNA expression (Bowman et al., 2019; Martinez et al., 2019; Walker, 2016; Singh and Li, 2012). Thus, epigenetics may be a crucial mechanism linking environmental chemical exposures to underlying etiology of human metabolic diseases (Singh and Li, 2012).

Given the global epidemic of obesity and type 2 diabetes (T2D) and ubiquitous EDC exposure, evidence is emerging that apart from the unhealthy changes in diets and sedentary lifestyle (Unnikrishnan et al., 2017; Zobel et al., 2016; Cho et al., 2018), environmental EDC exposure might be an important contributor to explaining the magnitude and dramatic increase in the prevalence of metabolic diseases (Le Magueresse-Battistoni et al., 2017; Ranciere et al., 2019). Numerous population-based and animal studies have established that EDC exposure is associated with insulin resistance, alterations of glucose and lipid metabolism, the development of the metabolic syndrome and T2D (Regnault et al., 2018; Thayer et al., 2012; Sun et al., 2014). Furthermore, DNA methylation, to date the best-characterized epigenetic mechanism, plays an important role in the effects of environmental stimuli on the development of metabolic disorders (Rosen et al., 2018). Moreover, differential DNA methylation has been identified in genes for T2D and obesity pathogenesis (i.e. *GCK*, *PYY*) (Guida et al., 2019; Wang et al., 2019) and genes that impair insulin secretion (i.e. *CACNA2D2*) (Cromer et al., 2015). However, it remains to be established whether DNA methylation might link exposure to parabens, bisphenols and phthalates to adverse metabolic health.

Therefore, we carried out an epigenome-wide association study (EWAS) to investigate the effects of the most common EDCs, including five parabens, three bisphenols, and 13 metabolites of nine phthalates, on genome-wide DNA methylation patterns in 622 unselected samples from the Lifelines DEEP cohort. In this study, we address the knowledge gap in understanding the influence of these environmental exposures on DNA methylation in the general population.

2. Methods

2.1. Study population

A total of 622 adults (18–81 years) from the Lifelines DEEP cohort were included in this study based on available epigenome-wide methylation data and 24-hour (24 h) urine samples (general characteristics in Table 1). Lifelines DEEP is a randomly selected subpopulation of the Lifelines cohort from the north of The Netherlands (Tigchelaar et al., 2015; Scholtens et al., 2015). Blood samples in the fasting state were collected for analysis of laboratory markers and 24 h-urine was collected in containers that were accompanied by oral and written instructions (Scholtens et al., 2015). On the day of blood collection, whole blood levels of fasting blood glucose and glycated hemoglobin (HbA1c), and serum levels of blood lipids were measured. A standardized protocol was used to obtain metabolic traits [i.e. fasting glucose, HbA1c, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, diastolic blood pressure, systolic blood pressure] and anthropometric measurements (i.e. waist and hip circumferences, body height and weight), as described in detail elsewhere (Scholtens et al., 2015). All participants provided written informed consent. The study was approved by the Medical Ethical Committee of the University Medical Center Groningen (UMCG), Groningen, The Netherlands.

Table 1
General characteristics of the study population from the Lifelines DEEP cohort.

Characteristic	Value (N = 622)
Sex = Male [N (%)]	259 (42%)
Age (years)	46 [36, 55]
Weight (kg)	77.0 [67.0, 88.0]
Body mass index (kg/m^2)	24.7 [22.6, 27.5]
Waist circumference (cm)	88.0 [80.0, 97.9]
Waist-to-hip ratio *	0.92 (0.1)
Neutrophils (%) *	53.1 (8.2)
Lymphocytes (%)	34.3 [29.8, 38.9]
Monocytes (%)	8.2 [7.0, 9.7]
Eosinophils (%)	2.7 [1.8, 3.9]
Basophils (%)	0.5 [0.3, 0.7]
24-hour urine (mL)	1781.5 [1353.0, 2288.3]
Fasting glucose (mmol/L)	4.8 [4.6, 5.2]
HbA1c (%)	5.5 [5.3, 5.7]
Triglycerides (mmol/L)	0.90 [0.67, 1.32]
HDL cholesterol (mmol/L)	1.50 [1.20, 1.80]
LDL cholesterol (mmol/L)	3.10 [2.50, 3.80]
Total cholesterol (mmol/L)	4.95 [4.40, 5.70]
Diastolic blood pressure (mm Hg)	70 [64,76]
Systolic blood pressure (mm Hg)	117 [110,128]

Data are given as median [interquartile range] when not normally distributed. * Normally distributed data are expressed as mean (standard deviation). **Abbreviations:** HbA1c, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

2.2. Chemical analysis

Concentrations of five parabens, three bisphenols, and 13 metabolites of nine different phthalates were measured in 24 h-urine samples by offline isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) [compound details, abbreviations and limits of detection (LOD) in Table 2]. The technical specifications and validity of methods are described elsewhere (van der Meer et al., 2019). For each included compound, exposure levels below LOD were replaced by LOD/$\sqrt{2}$ (Frederiksen et al., 2013). In the EWAS analysis, we included 14 compounds, 13 of which had > 75% of samples measured above LOD and bisphenol F (BPF) with 52% of samples above LOD. We included BPF because it is a relatively new compound and widely used as a bisphenol A (BPA) substitute (Rochester and Bolden, 2015).

2.3. Genome-wide DNA methylation

500 ng of genomic DNA was bisulfite-converted using the EZ DNA Methylation kit (Zymo Research Corp., USA) and hybridized on Illumina Human Methylation 450 K BeadChip arrays (Illumina, Inc.) according to the manufacturer's protocols. The original IDAT files were generated by the Illumina iScan BeadChip scanner at the Human Genotyping facility (HugeF) of ErasmusMC, The Netherlands (http://www.glimDNA.org/). The R-package "minfi" was used to perform quality control checks on the probes and samples (Maksimovic et al., 2016). We removed samples with probes having a detection *p*-value > 0.01 in more than 1% of probes. Then we performed background correction and probe type normalization using "preprocessQuantile" implemented in the "minfi" package. Sites with single nucleotide polymorphisms (SNPs) were defined by the function

Table 2
Concentrations of endocrine disrupting chemicals in 24-hour urine collections (ng/mL).

Compounds	Phthalate metabolites		LOD (ng/mL)		N > LOD (%)	Mean	Min	Q25	Median	Q75	Max	Max /median
Parabens												
Methyl paraben	**MeP**			0.14	622(100)	63.42	0.62	5.73	25.31	76.02	4079.11	161
Ethyl paraben	**EtP**			0.09	609(98)	11.73	0.09	0.56	1.68	7.18	488.21	291
n-Propyl paraben	**PrP**			0.07	577(93)	23.11	0.07	0.7	3.41	21.63	1962.97	576
n-Butyl paraben	**n-BuP**			0.06	531(85)	1.65	0.06	0.1	0.21	0.9	64.48	307
Benzyl paraben	BzP			0.07	31(5)	0.23	0.07	0.12	0.18	0.28	0.71	4
Bisphenols												
Bisphenol A	**BPA**			0.22	588(95)	3.32	0.22	1.08	2.05	3.84	54.39	27
Bisphenol F	**BPF**			0.23	325(52)	1.66	0.23	0.39	0.65	1.44	56.44	87
Bisphenol S	BPS			0.06	57(9)	0.51	0.06	0.1	0.18	0.55	4.06	23
Phthalates												
Di-methyl phthalate	DMP	Mono-methyl phthalate	MMP	0.43	320(51)	1.9	0.43	0.69	1.09	1.89	37.58	34
Di-ethyl phthalate	DEP	Mono-ethyl phthalate	**MEP**	0.35	621(100)	145.28	2.7	20.3	47.58	131.17	6634.73	139
Di-*iso*-butyl phthalate	DiBP	Mono-*iso*-butyl phthalate	**MiBP**	0.33	622(100)	29.89	3.71	11.89	19.61	33.01	388.76	20
Di-n-butyl phthalate	DnBP	Mono-n-butyl phthalate	**MnBP**	0.22	622(100)	25.13	2.54	10.61	17.27	28.41	364.48	21
Di-(2-ethyl-hexyl) phthalate	DEHP											
		Mono-(2-ethylhexyl) phthalate	**MEHP**	0.12	516(83)	3.4	0.16	1.5	2.61	4.06	50.45	19
		Mono-(2-ethyl-5-hydroxyhexyl) phthalate	**MEHHP**	0.11	622(100)	11.64	1.79	6.11	9.1	13.74	182.69	20
		Mono-(2-ethyl-5-oxohexyl) phthalate	**MEOHP**	0.09	622(100)	7.99	0.82	4.08	6.1	9.45	146.13	24
		Mono-(2-ethyl-5-carboxypentyl) phthalate	**MECPP**	0.25	622(100)	12.98	1.74	6.63	10.12	15.44	306.88	30
Di-n-hexyl phthalate	DnHP	Mono-n-hexyl phthalate	MnHP	0.07	123(20)	0.52	0.07	0.1	0.16	0.39	18.22	114
Butylbenzyl phthalate	BBzP	Mono-benzyl phthalate	**MBzP**	0.22	620(100)	11.72	0.28	3.22	5.82	10.76	617	106
Di-*iso*-nonyl phthalate	DiNP	Mono-*iso*-nonyl phthalate	MiNP	0.10	5(1)	1.64	0.33	0.6	0.9	1.07	5.31	6
		Mono-hydroxy-*iso*-nonyl phthalate	MHiNP	0.29	0(0)	NA	NA	NA	NA	NA	NA	NA
Di-*iso*-decyl phthalate	DiDP	Mono-*iso*-decyl phthalate	MiDP	0.31	63(10)	0.41	0.31	0.32	0.34	0.36	2.9	9

Abbreviations: LOD, limit of detection; Q25, 25th quartile; Q75, 75th quartile; NA, not available. We performed EWAS for 13 compounds, measured above LOD in > 75% of the samples, and BPF, measured in > 52% of the samples. These 14 EDCs are indicated in **bold**.

"dropLociWithSnps" in the "minfi" package. Next, we dropped probes according to the following criteria: (a) a detection *p*-value > 0.01; (b) bead count < 3 in over 5% of samples; (c) location of a known SNP or SNP at the single base extension site or cytosine-phosphate-guanine (CpG) site; (d) all CpGs on the sex chromosomes; (e) cross-reactive probes (n = 29,233) (Chen et al., 2013) and multi-mapped probes (n = 33,457) (Benton et al., 2015). Ultimately, the probe exclusions resulted in 420,522 high quality CpGs. Prior to linear regression analysis, the methylation dataset was trimmed on: (25th percentile − 3 × IQR) and (75th percentile + 3 × IQR). Such outlying CpG values were set to "missing" and excluded from further analyses. Methylation level (β-value) at each CpG was expressed as the ratio of the methylated intensity over the total intensity, which was used for all subsequent statistical analyses and biological interpretation.

2.4. Statistical analyses

Urinary concentrations of the EDCs were measured (ng/mL) and the total excretion per day (ng/24 h) was calculated by multiplying the concentration with the 24 h-urine volume (mL/24 h). Due to non-normal distributions, the excretions of compounds per 24 h are presented as median [interquartile range], and Spearman correlation coefficients were used for the relationships among EDCs. To identify associations of differentially methylated probes with urinary concentrations of 14 EDCs, a robust linear regression analysis was performed using the R-package "MASS" (Ripley et al., 2013). In the regression model, DNA methylation levels (β-values) were used as dependent variables, and log10-transformed EDC excretions in 24 h (ng/24 h) as predictors. To minimize systematic bias of the heterogeneity in blood cell composition and technical array-related confounders, the regression models were adjusted for age, sex, body mass index (BMI), measured blood cell counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils), array number, and position on the array (Shiwa et al., 2016). Finally; we applied the R-package "QCEWAS" for quality control of EWAS results (Van der Most et al., 2017). In EWAS, we used the Bonferroni-corrected *p*-value < 1.19×10^{-7} as genome-wide significance threshold and *p*-value < 1×10^{-6} as a suggestive threshold.

2.5. Correlations between CpG methylation β-values and metabolic traits

To determine the associations of EDC-associated CpG methylation levels with metabolic traits, 200 Spearman correlations were calculated between 10 metabolic traits and the residuals from methylation proportions at 20 CpGs regressed on the covariates mentioned above (i.e. age, sex, BMI, blood cell counts, array number and array position). A false discovery rate (FDR) < 5% was used as the significant threshold. All EWAS and correlational analyses were conducted in R-studio (based on R v3.6.3).

2.6. Bioinformatics characterization of EDC-associated CpGs

We used Illumina's Infinium HumanMethylation450k v1.2 product files (Illumina,. https://support.illumina.com/) and the R-package "FDb.InfiniumMethylation.hg19" to annotate to the nearest gene for each CpG. The bioinformatics characterization of genes annotated to

Table 3
Top 20 CpG sites associated with log10-transformed EDC excretions in 24-hour urine at suggestive *p*-value $< 1 \times 10^{-6}$.

EDC	CpG	Nearest Gene	CHR	BP position	Location in gene	Relation to CpG-island	Effect size	Raw *p*-value
PrP	cg04229238	*RB1CC1*	8	53,627,628	TSS1500	Shore	−0.002	3.50×10^{-7}
BPA	cg08655701	*MRPL4*	19	10,363,255	Body	Island	−0.008	5.72×10^{-7}
BPF	cg09905416	*MS4A2*	11	59,861,219	Body		−0.005	9.45×10^{-7}
MEP	cg24882097	*BIRC3*	11	102,188,439	5′UTR	Island	−0.011	6.32×10^{-7}
MnBP	cg27454300	*TNKS*	8	9,414,031	1stExon	Island	−0.005	8.83×10^{-7}
MEHP	cg26094004	*PYY*	17	42,075,116	5′UTR	Shelf	−0.013	$\mathbf{1.41 \times 10^{-9}}$
	cg07484739	*MIR1246*	2	177,356,020		Shore	−0.017	$\mathbf{9.77 \times 10^{-8}}$
	cg20914725	*LOXL3*	2	74,776,831	Body	Island	0.008	$\mathbf{1.11 \times 10^{-7}}$
	cg05795313	*ZNF641*	12	48,745,136	TSS1500	Shore	0.011	2.71×10^{-7}
	cg04533116	*SLC6A19*	5	1,169,063		Shore	0.015	3.17×10^{-7}
	cg02566391	*IL12RB2*	1	67,805,528	Body		−0.014	3.40×10^{-7}
	cg21987356	*GCK*	7	44,199,597	Body		0.004	3.55×10^{-7}
	cg26325335	*CACNA2D2*	3	50,402,333	Body	Island	0.023	5.04×10^{-7}
	cg18291014	*FAM20C*	7	93,658			−0.008	6.56×10^{-7}
	cg08537847	*CARMN*	5	148,810,203	TSS200		0.012	7.21×10^{-7}
	cg21634100	*FECH*	18	55,254,527	TSS1500	Shore	0.005	8.94×10^{-7}
	cg01745867	*IER3*	6	30,710,816	TSS1500	Island	−0.004	9.90×10^{-7}
MEHHP	cg06890484	*PTPRJ*	11	48,001,940	TSS200	Island	−0.011	$\mathbf{3.74 \times 10^{-8}}$
MEOHP	cg06890484	*PTPRJ*	11	48,001,940	TSS200	Island	−0.01	1.44×10^{-7}
MECPP	cg05100540	*RPS12*	6	133,135,557	TSS200	Island	−0.005	6.47×10^{-7}
MBzP	cg26759551	*FBP1*	9	97,401,509	1stExon	Island	0.005	7.50×10^{-7}

> 75% of the samples detected above LOD (limits of detection) were used in the robust linear regression models, which were adjusted for covariates: age, sex, body mass index, array number and position on array, measured cell counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils). The numbers of CpGs associated with log10-transformed EDCs are four at Bonferroni-correction $< 1.19 \times 10^{-7}$ (in **bold**) and 20 at *p*-value $< 1 \times 10^{-6}$, respectively. **Abbreviations:** PrP, n-Propyl paraben; BPA, Bisphenol A; BPF, Bisphenol F; MEP, Mono-ethyl phthalate; MnBP, Mono-n-butyl phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, Mono-(2-ethyl-5-carboxypentyl) phthalate; MBzP, Mono-benzyl phthalate.

EDC-associated CpG sites (*p*-value $< 1 \times 10^{-6}$) from EWAS results were explored.

To clarify the putative function of the suggestive markers and association with any of the metabolic traits (i.e. blood pressure, waist circumference, obesity-related traits, glycemic traits, lipids, diabetes, hypertension), we queried gene names in the GeneCards (the Human Gene Database, https://www.genecards.org/) and the NHGRI-EBI GWAS Catalog (the database of SNP associations in published and peer-reviewed genome-wide association studies, https://www.ebi.ac.uk/gwas/). Moreover, to investigate the relationships of suggestive CpGs with metabolic traits, we searched annotated genes along with metabolic traits (i.e. insulin, glucose, lipids, adiposity, obesity, diabetes and blood pressure) in PubMed.

2.7. Expression quantitative trait methylation

To investigate the association between EDC-associated CpGs (at *p*-value $< 1 \times 10^{-5}$) and gene expression, we performed expression quantitative trait methylation (eQTM) analysis in 2905 whole blood samples from the Biobank-Based Integrative Omics Studies (BIOS) data (Bonder et al., 2017). Here, we performed constraint eQTMs analysis on the EDC-associated CpGs and the genes within 1Mb distance to the CpG sites, which in total result in 4127 tests. We corrected for multiple testing by calculating an empirical FDR estimate, where we created a null distribution by performing 10 permutations, each time swapping sample labels, and considered FDR < 5% as significant.

2.8. Comparative toxicogenomics database

To check the chemical-gene interactions, for each EDC compound, we queried the chemical and corresponding genes identified by eQTM analysis in the integrated Comparative Toxicogenomics Database (CTD, a public resource for toxicogenomic information from the peer-reviewed scientific literature, http://ctdbase.org/). CTD includes manually curated interaction types (i.e. either chemical effects on methylation and gene expression or gene effects on chemical degradation and abundance from population or experimental studies), which we extracted to validate our results.

3. Results

3.1. Exposure to common EDCs in the Dutch population

Table 2 shows concentrations of all measured compounds in 24 h urine voids (ng/mL). Five parabens (MeP, EtP, PrP, n-BuP, BzP), three bisphenols (BPA, BPF, BPS), and 13 metabolites of nine phthalates (MMP, MEP, MiBP, MnBP, MEHP, MnHP, MEHHP, MEOHP, MECPP, MBzP, MiNP, MHiNP, MiDP) were measured in all samples. Of these 21 EDC compounds, MeP, EtP, PrP, n-BuP, BPA, and eight metabolites of the phthalates (MEP, MiBP, MnBP, MEHP, MEHHP, MEOHP, MECPP, MBzP) were detected above the LOD in > 75% of samples; and BPF and MMP in > 50% of samples. Other chemicals (BzP, BPS, MnHP, MiNP, and MiDP) were detected in < 20% of samples, while MHiNP was not detectable at all. All 13 compounds detected in > 75% of samples (plus BPF) were further analyzed.

The correlation plot for the concentrations of these 14 EDC compounds showed high correlations within the same classes of EDCs, i.e., within the four parabens (r_s: 0.34–0.71) and within the eight phthalate metabolites (r_s: 0.16–0.97), while the correlations between BPA and BPF and across EDC classes were not significant (**Supplementary Figure 1**). The paraben MeP and the phthalate metabolite MEP were excreted in the highest median concentrations followed by MiBP, MnBP, and MECPP. The concentration ranges of the parabens and the phthalate metabolites were wide with maximum levels being 576- (PrP), 307- (n-BuP), 291- (EtP), 161- (MeP), and 139- (MEP), 114- (MnHP), 106- (MBzP) fold higher than the corresponding median concentrations. By comparison, the observed concentration ranges of other analytes were much more narrow [4- (BzP), 27- (BPA), and 87- (BPF), 23- (BPS), 34- (MMP), 20- (MiBP), 21- (MnBP), 19- (MEHP), 20- (MEHHP), 24- (MEOHP), 30- (MECPP), 19- (MEHP), 6- (MiNP), 9- (MiDP) fold higher, respectively].

3.2. 24 h EDC excretions and DNA methylation

The EWAS analysis revealed 21 associations for methylation levels of 20 separate CpG sites (cg06890484 was associated with both MEHHP and MEOHP) with 24 h EDC excretions at suggestive p-value $< 1 \times 10^{-6}$ (12 for MEHP, one each for PrP, BPA, BPF, MEP, MnBP, MEHHP, MEOHP, MECPP, and MBzP). Four CpGs remained significant at strict Bonferroni-correction (p-value $< 1.19 \times 10^{-7}$) and three of these were associated with MEHP (Table 3). Details on 204 identified CpGs at p-value $< 1 \times 10^{-5}$ from EWAS for a total of 14 compounds are provided in **Supplementary** Tables 1–14, where the effect changes of DNA methylation per unit increase in 24 h urinary excretions of log10-transformed EDCs are presented. EWAS-specific quantile-quantile plots with lambdas are shown in **Supplementary Figure 2**. Manhattan plots (**Supplementary Figure 3**) show the location of CpGs for 14 compounds throughout the genome.

3.3. CpG methylation levels and metabolic traits

We calculated correlations between the suggestive EDC-associated CpGs and fasting glucose, HbA1c, waist-to-hip-ratio, blood lipids and blood pressure. Table 4 shows significant correlations of the methylation levels with at least one of the metabolic traits. Nine out of the 20 CpGs were significantly correlated at FDR $< 5\%$, and 11 CpGs at FDR $< 10\%$.

Text box1 in Supplemental Materials describes putative functions of genes that were annotated to the suggestive CpGs in relation to metabolic traits based on the GWAS-catalog. A total of 18 out of 20 genes were assumed to play some part in metabolic health and nine out of 20 genes were reported to be associated with metabolic traits in the GWAS-catalog. Examples included metabolic diseases such as T2D, obesity, and hypertension, as well as continuous traits such as BMI, glycemic traits, and triglycerides.

3.4. Association with gene expression

We observed that EDC-associated CpGs (at p-value $< 1 \times 10^{-5}$) affect expressions at 46 genes (FDR $< 5\%$, **Supplementary Table 15**). Only one suggestive CpG (MEHP-associated cg08537847 at p-value $< 1 \times 10^{-6}$) was associated with higher gene expression of *PCYOX1L* and *CSF1R* in the eQTM analysis rather than with the annotated gene *CARMN*.

3.5. Differential methylation and known EDC-gene interactions

We identified 16 interactions between three chemicals (BPA, MnBP and MEHP) and genes identified in eQTM analysis (Table 5**)**. For other compounds, we found no overlap since these EDCs were not present in the CTD. For 11 CpG-gene combinations, the effect directions of interactions reported in the CTD were consistent with our results. For instance, MEPH-associated CpG cg21987356 was positively associated with expressions of *PCYOX1L* and *CSF1R*. Meanwhile, increased expression of *CSF1R* in response to MEHP was reported in the CTD. Moreover, for the rest of five CpG-gene combinations, the effect directions were not uniformly consistent within CTD reports. For example, we observed a positive association between MEHP-associated CpG cg04609694 and *VEGFA* expression, but there were one positive and two negative effects, and one reference does not describe a specific effect degree in the CTD.

4. Discussion

We assessed genome-wide DNA methylation patterns associated with exposure to 14 common non-persistent EDCs (four parabens, two bisphenols and eight phthalate metabolites) in the general Dutch population. EWAS analysis identified 20 CpG sites at suggestive p-value $< 1 \times 10^{-6}$ associated with 24 h-urine concentrations of 10 EDCs; four CpGs survived the Bonferroni-correction. Furthermore, 11 out of the 20 EDC-associated CpG sites were significantly correlated with multiple metabolic traits, which may indicate that the differential methylation markers functionally link EDC exposure to metabolic homeostasis.

We identified that 18 out of the 20 genes annotated to suggestive CpGs were reported to be involved in metabolic health. Three out of

Table 4
Spearman correlations between methylation levels of 20 CpGs and metabolic traits.

EDC	CpG	Nearest Gene	Metabolic trait (correlation coefficient)			
PrP	cg04229238	*RB1CC1*	\			
BPA	cg08655701	*MRPL4*	DBP (0.09)			
BPF	cg09905416	*MS4A2*	\			
MEP	cg24882097	*BIRC3*	\			
MnBP	cg27454300	*TNKS*	\			
MEHP	cg26094004	*PYY*	**Triglycerides (0.10)**	**HDL (−0.10)**		
	cg07484739	*MIR1246*	Triglycerides (0.09)	**DBP (0.15)**	**SBP (0.15)**	**WHR (0.11)**
	cg20914725	*LOXL3*	**HbA1c (0.11)**	**Cho (0.10)**		
	cg05795313	*ZNF641*	**HDL (0.13)**			
	cg04533116	*SLC6A19*	**HDL (0.12)**	Triglycerides (−0.09)	**DBP (−0.10)**	
	cg02566391	*IL12RB2*	**HbA1c (−0.10)**	HDL (−0.09)	SBP (0.09)	**DBP (0.11)**
	cg21987356	*GCK*	\			
	cg26325335	*CACNA2D2*	**Glucose (0.11)**			
	cg18291014	*FAM20C*	**HbA1c (−0.11)**	**Triglycerides (0.10)**	**HDL (−0.16)**	
	cg08537847	*CARMN*	HbA1c (0.09)	HDL (0.09)	DBP (−0.09)	
	cg21634100	*FECH*	\			
	cg01745867	*IER3*	\			
MEHHP	cg06890484	*PTPRJ*	\			
MEOHP	cg06890484	*PTPRJ*	\			
MECPP	cg05100540	*RPS12*	\			
MBzP	cg26759551	*FBP1*	**Glucose (−0.10)**	**Triglycerides (−0.10)**	HDL (0.09)	

Spearman correlations were adjusted for age, sex, body mass index, cell counts, array number and position on array. **Bold** traits: significant at FDR $< 5\%$. Other traits: significant at FDR $< 10\%$.

Abbreviations: FDR, false discovery rate; PrP, n-Propyl paraben; BPA, Bisphenol A; BPF, Bisphenol F; MEP, Mono-ethyl phthalate; MnBP, Mono-n-butyl phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, Mono-(2-ethyl-5-carboxypentyl) phthalate; MBzP, Mono-benzyl phthalate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; Cho, cholesterol; WHR, waist-to-hip ratio; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 5
The eQTM-identified genes known to interact with EDCs from the comparative toxicogenomics database (CTD).

EDC	CpG	eQTM gene	Effect size of CpG on gene expression	FDR	Gene-chemical interaction from the CTD
BPA	cg16711332	*PLEKHG5*	−11.16	< 0.001	**Expression (−); Methylation (−)**
MnBP	cg12427444	*HSPA1B*	−4.18	0.011	Expression (+4); Expression (affect 1)
MEHP	cg08537847*	*CSF1R*	3.64	0.036	**Expression (+)**
	cg08537847*	*PCYOX1L*	4.79	< 0.001	NA
	cg05006384	*DICER1*	−4.41	0.004	**Expression (−); Expression (affect 3)**
	cg23357708	*RPS28*	−6.16	< 0.001	**Expression (−3)**
	cg23357708	*SNAPC2*	−5.42	< 0.001	**Expression (−2)**
	cg02696067	*VARS2*	3.80	0.027	**Expression (+)**
	cg03065503	*NSG1*	4.04	0.010	Expression (−); Methylation (affect 1)
	cg22491680	*HAL*	−5.19	< 0.001	Expression (affect 2)
	cg02296171	*PTH2R*	−14.10	< 0.001	**Expression (−)**
	cg25143871	*FBXO21*	3.88	0.026	**Expression (+)**
	cg07043361	*TPCN1*	3.63	0.035	Expression (affect)
	cg10502324	*NAP1L1*	−3.83	0.026	**Expression (−2); Expression (affect 2)**
	cg03331229	*MMP1*	−4.82	< 0.001	**Expression (−2); Expression (affect)**
	cg04609694	*NFKBIE*	4.97	< 0.001	**Expression (+2)**
	cg04609694	*VEGFA*	4.69	< 0.001	Expression (−2); Expression (affect); Expression (+)

*CpG site survived at p-value $< 1 \times 10^{-6}$. The chemical-gene interaction queries were performed in the CTD database (http://ctdbase.org) for each compound together with the corresponding gene identified in Biobank-Based Integrative Omics Studies (BIOS) data using expression quantitative trait methylation (eQTMs) analysis. **Bold** interactions indicate that the effect of CpG on gene expression are directionally consistent with eQTM results. +/-, positive or negative effects; affect, the reference does not describe a more specific degree; e.g. expression (+2) means that 3 reports have increased gene expression in response to the corresponding chemical. **Abbreviations**: EDC, environmental disrupting chemical; FDR, false discovery rate; BPA, bisphenol A; MnBP, Mono-n-butyl phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; NA, not available.

four genome-wide significant methylation markers: cg26094004 in *PYY*, cg07484739 in *miR1246*, and cg20914725 in *LOXL3*, showed significant associations with MEHP and metabolic traits including HbA1c, triglycerides, HDL-cholesterol, total cholesterol, blood pressure, and waist-to-hip ratio. *PYY* encodes a member of the neuropeptide Y family of peptides. These peptides regulate pancreatic secretion, glucose metabolism and energy homeostasis, suggesting a close association with T2D and obesity (Guida et al., 2019; Wu et al., 2019). Mature miR1246 is incorporated into an RNA-induced silencing complex (RISC), which in human islets exerts an essential effect on islet function and T2D pathogenesis (Kameswaran et al., 2014). *LOXL3* interacts with STAT3 signaling pathway, which participates in the pathogenesis of inflammation and insulin resistance (Heo et al., 2019; Laurentino et al., 2019). The fourth methylation marker, cg06890484 in *PTPRJ*, was associated with both MEHHP and MEOHP, which may be explained by the high correlation between the two compounds. None of the metabolic traits were significantly associated with cg06890484. However, *PTPRJ* has been identified in a GWAS on hypertension (German et al., 2020). *PTPRJ* acts as a negative regulator of the insulin signaling pathway and suppresses insulin sensitivity in a mouse model (Kruger et al., 2015). In our analysis of 14 EDCs, MEHP showed to have the largest effect on methylation in terms of the identified number of CpGs mapped to the different genes, which was also reflected in its relatively large statistical inflation of the association p-value (lambda = 1.357; **Supplementary Figures 2 and 3**), indicating high potency of MEHP to promote epigenetic changes. These observations are consistent with previous findings showing that MEHP has larger metabolic impact than other phthalate metabolites (Piecha et al., 2016). Taken together, our EDC data and in particular the MEHP results suggest that disruption of DNA methylation might underlie the association between endocrine disruptors and metabolic alterations.

Additionally, EDC-associated CpGs likely contributed to altered gene expressions. Among the differential methylation CpGs were linked 16 genes known from experimental and toxicological studies to interact with EDCs, as reported in the CTD. In eQTM analysis, one CpG was significantly associated with expression of two genes (i.e. cg08537847 with higher *PCYOX1L* and *CSF1R* expression). *PCYOX1L*, known as prenylcysteine oxidase 1 like, is involved in prenylcysteine oxidase activity and oxidoreductase activity, which is important in protein metabolism and metabolic homeostasis. *CSF1R* encodes colony stimulating factor 1 receptor, and its increased expression was reportedly induced by exposure to di(2-ethylhexyl) phthalate (Fang et al., 2019). Moreover, *CSF1R* plays an important role in inflammation and mediates the pathological process of adverse metabolic effects (Theurich et al., 2017). Collectively, evidence above indicates that the EDC-associated CpGs could be suggestive markers for assessing the potential biological effects of EDCs on metabolic health.

5. Strengths and limitations

One of the strengths of our study is the use of EDC excretions in 24 h urine. As reported previously, 24 h-urine collections accurately reflect daily environmental exposures (Sun et al., 2017; Calafat et al., 2015). The analytical methodology has been validated in our technical report (van der Meer et al., 2019). Secondly, we adjusted EWAS analysis for measured blood cell counts and possible batch effects (array number and the position on array). The EDC effects on DNA methylation in our study are unlikely to represent the methylation shifts due to cell composition or technical bias. Finally, recent data indicate that methylation profiles in other tissues can be (partly) mirrored in blood (Wahl et al., 2017), supporting blood as a good proxy tissue to capture DNA methylation patterns.

Some limitations must be taken into account. Firstly, the cross-sectional design of our study is not optimal for estimating the causal effects of EDC exposures on epigenetic modifications. We acknowledge that mediation analysis, or even better Mendelian randomization analysis, would help explore possible casual relationships. However, we decided not to include such analyses in the current paper as the modest effect sizes of the Spearman correlations in combination with our relatively modest sample size of n = 622 indicate we would have limited power to successfully perform such analyses (Relton and Davey Smith, 2010). Further analyses in prospective populations are required to

establish the dynamic of the epigenetic changes in response to EDC exposures.

Secondly, some previous studies have reported EWAS results with some of the EDCs in this study, e.g. for BPA and phthalates (Miura et al., 2019; Grindler et al., 2018). However, we were unable to replicate our findings in independent samples due to the fact that the present study, to the best of our knowledge, is the first EWAS simultaneously investigating DNA methylation patterns associated with multiple common non-persistent EDCs. Moreover, variations between the populations, the collection of urine samples and the analytical methodology, may explain the differences. Nevertheless, we used different methods to support our findings (i.e. toxicogenomic-based approach to check known chemical-gene interactions and the GWAS-catalog) and also compared the results with the existing literature. Although the eQTM analysis did not reveal the effects of EDC-associated methylation at most of suggestive CpGs on the expression levels of the annotated genes, our findings were somewhat supported by the data and observations reported previously in both epidemiological and functional studies in the CTD database. We acknowledge though that, e.g., verification of our results in independent samples and functional studies would have been preferred to validate our results.

Thirdly, we recognize the potential of identification of false positive (while still unreplicated) results and the single measurement of EDC levels which can vary from day to day. We acknowledged that there might be some unexpected confounders because DNA methylation and EDC levels were measured in different tissues. However, blood is known to be a good proxy tissue reflecting the epigenetic profiles in other tissues (Wahl et al., 2017). Also, the analysis was adjusted for the relevant covariates. However, several of our findings are supported by data and observations reported previously in both epidemiological and experimental studies. We cannot exclude that DNA methylation is linked to the EDC excretions.

Lastly, we did not explore the combined biological effects of all measured EDCs in one model because of the potential collinearity from the close correlations between compounds. However, with one exception the top CpG sites were different for each compound, perhaps indicating that their specific methylation target sites may differ.

6. Conclusions

To conclude, our findings suggest that differential methylation markers associated with metabolic traits may partly be attributable to non-persistent EDC exposures (PrP, BPA, BPF, MEP, MnBP, MEHP, MEHHP, MEOHP, MECPP, MBzP). Replication samples and longitudinal studies are necessary to further examine the causal role of EDC-affected DNA methylation in the onset of metabolic diseases.

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.

Acknowledgments

The authors wish to acknowledge all participants of the Lifelines Cohort. We thank Hanne Frederiksen, Copenhagen University Hospital, for critical comments on the manuscript. We thank the Genome Analysis Facility of the University of Groningen, University Medical Center Groningen, for performing the Illumina 450 K methylation array experiments.

Funding

This work was supported by a Diabetes Funds Junior Fellowship from the Dutch Diabetes Research Foundation (to JVvVO, project no. 2013.81.1673), and by the National Consortium for Healthy Ageing (NCHA) (NCHA NGI Grant 050-060-810). XH was funded by the National Natural Science Foundation of China (21876065).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106016.

References

Andra, S.S., et al., 2015. Biomonitoring of human exposures to chlorinated derivatives and structural analogs of bisphenol A. Environ. Int. 85, 352–379.
Benjamin, S., et al., 2017. Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. J. Hazard. Mater. 340, 360–383.
Benton, M.C., et al., 2015. An analysis of DNA methylation in human adipose tissue reveals differential modification of obesity genes before and after gastric bypass and weight loss. Genome Biol. 16, 8.
Bledzka, D., Gromadzinska, J., Wasowicz, W., 2014. Parabens. From environmental studies to human health. Environ. Int. 67, 27–42.
Bonder, M.J., et al., 2017. Disease variants alter transcription factor levels and methylation of their binding sites. Nat. Genet. 49 (1), 131–138.
Bowman, A., et al., 2019. Phthalate exposures, DNA methylation and adiposity in Mexican children through adolescence. Front. Public Health 7, 162.
Calafat, A.M., et al., 2015. Optimal exposure biomarkers for nonpersistent chemicals in environmental epidemiology. Environ. Health Perspect. 123 (7), A166–A168.
Chen, Y.A., et al., 2013. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. Epigenetics 8 (2), 203–209.
Cho, N.H., et al., 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. 138, 271–281.
Cromer, M.K., et al., 2015. Neomorphic effects of recurrent somatic mutations in Yin Yang 1 in insulin-producing adenomas. Proc. Natl. Acad. Sci. USA 112 (13), 4062–4067.
Fang, H., et al., 2019. Di-(2-ethylhexyl)-phthalate induces apoptosis via the PPARgamma/PTEN/AKT pathway in differentiated human embryonic stem cells. Food Chem. Toxicol. 131, 110552.
Frederiksen, H., et al., 2013. Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother-child pairs. Int. J. Hyg. Environ. Health 216 (6), 772–783.
German, C.A., et al., 2020. Ordered multinomial regression for genetic association analysis of ordinal phenotypes at Biobank scale. Genet. Epidemiol. 44 (3), 248–260.
Grindler, N.M., et al., 2018. Exposure to phthalate, an endocrine disrupting chemical, alters the first trimester placental methylome and transcriptome in women. Sci. Rep. 8 (1), 6086.
Guida, C., et al., 2019. PYY plays a key role in the resolution of diabetes following bariatric surgery in humans. EBioMedicine 40, 67–76.
Heo, Y.J., et al., 2019. Visfatin induces inflammation and insulin resistance via the NF-kappaB and STAT3 signaling pathways in hepatocytes. J. Diabetes Res. 2019, 4021623.
Kameswaran, V., et al., 2014. Epigenetic regulation of the DLK1-MEG3 microRNA cluster in human type 2 diabetic islets. Cell Metab. 19 (1), 135–145.
Kassotis, C.D., Stapleton, H.M., 2019. Endocrine-mediated mechanisms of metabolic disruption and new approaches to examine the public health threat. Front. Endocrinol. (Lausanne) 10, 39.
Kruger, J., et al., 2015. Enhanced insulin signaling in density-enhanced phosphatase-1 (DEP-1) knockout mice. Mol. Metab. 4 (4), 325–336.
Laurentino, T.S., et al., 2019. LOXL3 function beyond amino oxidase and role in pathologies, including cancer. Int. J. Mol. Sci. 20 (14).
Le Magueresse-Battistoni, B., et al., 2017. Endocrine disrupting chemicals in mixture and obesity, diabetes and related metabolic disorders. World J. Biol. Chem. 8 (2), 108–119.
Li, D., Suh, S., 2019. Health risks of chemicals in consumer products: A review. Environ. Int. 123, 580–587.
Maksimovic, J., Phipson, B., Oshlack, A., 2016. A cross-package Bioconductor workflow for analysing methylation array data. F1000Res 5, 1281.
Martinez, R.M., et al., 2019. Urinary concentrations of phenols and phthalate metabolites reflect extracellular vesicle microRNA expression in follicular fluid. Environ. Int. 123, 20–28.
Miura, R., et al., 2019. An epigenome-wide analysis of cord blood DNA methylation reveals sex-specific effect of exposure to bisphenol A. Sci. Rep. 9 (1), 12369.
Piecha, R., et al., 2016. Urine levels of phthalate metabolites and Bisphenol A in relation to main metabolic syndrome components: dyslipidemia, hypertension and type 2 diabetes. A pilot study. Cent. Eur. J. Public Health 24 (4), 297–301.

Ranciere, F., et al., 2019. Exposure to Bisphenol A and Bisphenol S and incident type 2 diabetes: a case-Cohort Study in the French Cohort D.E.S.I.R. Environ. Health Perspect. 127 (10), 107013.
Regnault, C., et al., 2018. Unexpected metabolic disorders induced by endocrine disruptors in Xenopus tropicalis provide new lead for understanding amphibian decline. Proc. Natl. Acad. Sci. USA 115 (19), E4416–E4425.
Relton, C.L., Davey Smith, G., 2010. Epigenetic epidemiology of common complex disease: prospects for prediction, prevention, and treatment. PLoS Med. 7 (10), e1000356.
Ripley, B.V., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D., Ripley, M.B., 2013. Package 'MASS'. Available from: https://cran.r-project.org/web/packages/MASS/MASS.pdf.
Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A systematic review and comparison of the hormonal activity of Bisphenol A substitutes. Environ. Health Perspect. 123 (7), 643–650.
Rosen, E.D., et al., 2018. Epigenetics and epigenomics: implications for diabetes and obesity. Diabetes 67 (10), 1923–1931.
Scholtens, S., et al., 2015. Cohort Profile: LifeLines, a three-generation cohort study and biobank. Int. J. Epidemiol. 44 (4), 1172–1180.
Shiwa, Y., et al., 2016. Adjustment of cell-type composition minimizes systematic bias in blood DNA methylation profiles derived by DNA collection protocols. PLoS ONE 11 (1), e0147519.
Singh, S., Li, S.S., 2012. Epigenetic effects of environmental chemicals bisphenol A and phthalates. Int. J. Mol. Sci. 13 (8), 10143–10153.
Sun, Q., et al., 2014. Association of urinary concentrations of bisphenol A and phthalate metabolites with risk of type 2 diabetes: a prospective investigation in the Nurses' Health Study (NHS) and NHSII cohorts. Environ. Health Perspect. 122 (6), 616–623.
Sun, Q., et al., 2017. Reproducibility of urinary biomarkers in multiple 24-h urine samples. Am. J. Clin. Nutr. 105 (1), 159–168.
Thayer, K.A., et al., 2012. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. Environ. Health Perspect. 120 (6), 779–789.
Theurich, S., et al., 2017. IL-6/Stat3-dependent induction of a distinct, obesity-associated NK cell subpopulation deteriorates energy and glucose homeostasis. Cell Metab. 26 (1), 171–184.e6.
Tigchelaar, E.F., et al., 2015. Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics. BMJ Open 5 (8), e006772.
Unnikrishnan, R., et al., 2017. Type 2 diabetes: demystifying the global epidemic. Diabetes 66 (6), 1432–1442.
van der Meer, T.P., et al., 2019. Development and interlaboratory validation of two fast UPLC-MS-MS methods determining urinary bisphenols, parabens and phthalates. J. Anal. Toxicol. 43 (6), 452–464.
Van der Most, P.J., et al., 2017. QCEWAS: automated quality control of results of epigenome-wide association studies. Bioinformatics 33 (8), 1243–1245.
Wahl, S., et al., 2017. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature 541 (7635), 81–86.
Walker, C.L., 2016. Minireview: epigenomic plasticity and vulnerability to EDC exposures. Mol. Endocrinol. 30 (8), 848–855.
Wang, F., et al., 2019. Retinol binding protein 4 mediates MEHP-induced glucometabolic abnormalities in HepG2 cells. Toxicology 424, 152236.
Wu, Y., et al., 2019. The role of neuropeptide Y and peptide YY in the development of obesity via gut-brain axis. Curr. Protein Pept. Sci. 20 (7), 750–758.
Zobel, E.H., et al., 2016. Global changes in food supply and the obesity epidemic. Curr. Obes. Rep. 5 (4), 449–455.