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Relationship between endocrine disrupting chemicals (phthalate metabolites, triclosan and bisphenols) and vitamin D in female subjects: An exploratory pilot study

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Abbreviations: EDCs, endocrine disrupting chemicals; 25(OH)D_3, vitamin D_3: cholecalciferol; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; BMI, body mass index; CRP, C reactive protein; TSH, thyroid stimulating hormone; Free-T3, free triiodothyronine; Free-T4, free thyroxine; eGFR, estimated glomerular filtration rate; PDE, calcium/calmodulin-dependent phosphodiesterase; CaMK, calcium/calmodulin-dependent kinase; CaMKKα, calcium/calmodulin-dependent kinase kinase alpha; LMWP, low molecular weight phthalate; HMWP, high molecular weight phthalate; MMP, monomethyl-phthalate; MEP, monoethyl-phthalate; MiBP, mono-iso-butylphthalate; MBP, mono-butylphthalate; MCHP, mono-cyclohexyl phthalate; MCPP, mono-3-carboxypropyl-phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl)-phthalate; MECPP, mono(2-ethyl-5-carboxypentyl)-phthalate; MEOHP, mono(2-ethyl-5-oxohexyl)-phthalate; MEHP, mono(2-ethylhexyl)-phthalate; MBzP, mono-benzyl-phthalate; MOP, mono-*n*-octyl-phthalate; MNP, monononyl phthalate; MiDP, mono-iso-decyl phthalate; DMP, dimethyl phthalate; DEP, diethyl phthalate; DiBP, di-isobutyl phthalate; DBP, di-*n*-butyl phthalate; DCHP, dicyclohexyl phthalate; DnOP, di-*n*-octyl phthalate; DEHP, di-2-ethylhexyl phthalate; BBP, butyl benzyl phthalate; DOP, di-octyl phthalate; DNP, di-nonyl phthalate; DiDP, di-isodecyl phthalate; TCS, triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol); BPs, bisphenols; BPA, bisphenol A; BPS, bisphenol S; BPB, bisphenol B; BPF, bisphenol F; BP-AF, bisphenol-AF; CYPs, cytochrome P450 oxidases; IVF, in vitro fertilization; LC-MS/MS, liquid chromatography tandem mass spectrometry; ESI, electrospray ionisation; MRM, multiple reaction monitoring; LOQ, limit of quantification; SOMA, slow off-rate modified aptamer; SD, standard deviation; VDR, vitamin D receptor; SRC, steroid receptor coactivator.

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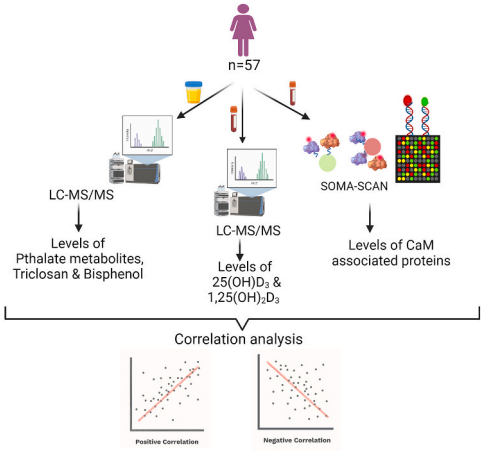
HIGHLIGHTS

- Phthalates are proposed to impact CYP enzymes involved in $1,25(OH)_2D_3$ metabolism.
- The relationship between phthalate metabolites and $25(OH)D_3$ in women was examined.
- MCPP negatively correlated with $25(OH)D_3$ in the study cohort.
- In deficient $25(OH)D_3$ women, MiBP and MBP negatively correlated with $25(OH)D_3$.
- Observed correlations are remarkably strong compared to other predictors of $25(OH)D_3$.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Evidence suggests that endocrine disrupting chemicals (EDCs), commonly used in plastics and personal care products, may be associated with reduced levels of vitamin D. Therefore, this study examined the relationship between phthalate metabolites, 5-chloro-2-(2,4-dichlorophenoxy)phenol (triclosan; TCS) and bisphenols (BPs) with vitamin D_3 ($25(OH)D_3$) and active 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), and their relationship to calcium homeostasis.

Methods: 57 female participants (age 31.8 ± 4.6 years; BMI 25.6 ± 3.7 kg/m^2) were analyzed for urinary levels of phthalate metabolites, TCS and BPs, and serum levels of $25(OH)D_3$ and $1,25(OH)_2D_3$, determined by isotope-dilution liquid chromatography tandem mass spectrometry. Serum calcium/calmodulin-dependent (CaM) associated proteins were determined by Slow Off-rate Modified Aptamer (SOMA)-scan.

Results: In the study cohort, $25(OH)D_3$ and $1,25(OH)_2D_3$ levels were 22.9 ± 11.2 ng/mL and 0.05 ± 0.02 ng/mL, respectively: mono-3-carboxypropyl-phthalate (MCPP) correlated negatively with $25(OH)D_3$ ($\rho = -0.53$, $p = 0.01$). 28 of the 57 women recruited were $25(OH)D_3$ deficient, <20 ng/mL (50 nmol/L): in this group, mono-isobutylphthalate (MiBP) and mono-butylphthalate (MBP) negatively correlated with $25(OH)D_3$; ($\rho = -0.47$, $p = 0.049$) and ($\rho = -0.64$, $p = 0.005$), respectively. EDCs did not correlate with $1,25(OH)_2D_3$, measures of renal function or CaM proteins.

Conclusion: These putative data indicate that MCPP is related to $25(OH)D_3$, while MiBP and MBP were related to vitamin D deficiency; however, no correlations were observed with TCS and BPs. No phthalate metabolites correlated with $1,25(OH)_2D_3$, CaM associated proteins or renal function, suggesting that effects occur earlier in the vitamin D pathway and not through modulation of cellular calcium flux. The observed correlations are surprisingly strong compared to other predictors of $25(OH)D_3$, and larger studies adjusting for potential confounders are warranted.

1. Introduction

Phthalates and bisphenols (BPs), endocrine disrupting chemicals (EDCs) with relatively short half-lives (Gore et al., 2015), are extensively used in industrial and consumer products as plasticizers. 5-Chloro-2-(2,4-dichlorophenoxy)phenol (triclosan; TCS), also with reported endocrine disrupting potential (Cai et al., 2023), is an antibacterial agent used in various personal hygiene products (Milanović et al., 2023). A recent study by Eales et al. (2022) supports the evidence of associated health risks of phthalates in adults such as fecundity (Radke et al., 2018), endometriosis (Cai et al., 2019b), type-2 diabetes and reproductive cancers (Fu et al., 2017); and in animal models, association with reproductive and cardiovascular effects (Mariana et al., 2016). Levels of di-2-ethylhexyl-phthalate (DEHP), di-*n*-butyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-isobutyl phthalate (DiBP) at ≥0.1% by weight are now restricted in certain plastic articles (Commission Regulation 2018/2005, 2018) Bisphenol A (BPA), with a tolerable daily intake of 0.2 ng/kg (EFSA Panel on Food Contact Materials et al., 2023), has been associated with reproductive hormone levels (Kandaraki et al., 2011; Ehrlich et al., 2012), type-2 diabetes (Kandaraki et al., 2011), breast cancer (Yang et al., 2009a), liver enzyme levels (Lang et al., 2008), hypertension and obesity (Shankar and Teppala, 2012) and oxidative stress markers (Yang et al., 2009b). Although BP analogues have been introduced as potentially safe alternatives, research indicates that bisphenol S (BPS) is as hormonally active as BPA (Rochester and Bolden, 2015), has equivalent obesogenic effects, correlates with metabolic disorders, is potentially more reproductively toxic and has an equivalent breast cancer promotion rate (Thoene et al., 2020). In women, TCS has been associated with thyroid hormone levels (Wang et al., 2017; Ha et al., 2019), gestational diabetes (Ouyang et al., 2018), infertility (Ye et al., 2018), osteoporosis (Cai et al., 2019a) and breast cancer (Cai et al., 2023). Such health effects has led to the ban of TCS as a human hygiene biocidal agent (Commission Implementing Decision 2016/110, 2016)

There is some evidence to suggest that exposure to phthalate metabolites and BPA may be associated with 1,25-dihydroxyvitamin D_3 (25

$(OH)D_3$) levels in the body. In the National Health and Nutrition Examination Survey (NHANES), urinary levels of mono(2-ethylhexyl)-phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl)-phthalate (MEHHP) and the sum of DEHP metabolites were associated with lower levels of serum $25(OH)D_3$ while monoethyl-phthalate (MEP) was positively associated, and BPA was inversely associated, but in women only (Johns et al., 2016). In a cohort of pregnant women, urinary mono-3-carboxypropyl-phthalate (MCPP) and the sum of DEHP metabolites associated with decreased levels of $25(OH)D_3$ and levels of both DEHP metabolites and BPA could account for a 20% increase in chance of vitamin D deficiency in the study cohort (Johns et al., 2017). Other studies have found an inverse association between $25(OH)D_3$ and BPA (Erden et al., 2014; Brandi et al., 2022).

Vitamin D_3 (cholecalciferol) is synthesized in the skin by the UV-B irradiation of 7-dehydrocholesterol in which the B ring is broken forming pre-vitamin D_3 that via a thermo-sensitive process subsequently undergoes isomerization to vitamin D_3 (Bikle, 2014). With prolonged exposure to UVB, pre-vitamin D_3 isomerizes to tachysterol $_3$ or lumisterol $_3$ that exhibit anti-proliferative, anti-inflammatory and anti-cancer properties (Slominski et al., 2020). Vitamin D_3 is then transported to the liver where it is hydroxylated to $25(OH)D_3$ primarily by cytochrome P450 oxidase (CYP) CYP2R1 which is subsequently transported to the kidneys or other extrarenal tissue where it is further hydroxylated to the active metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (Bikle, 2014). In the canonical pathway, the two hydroxylation steps in $1,25(OH)_2D_3$ metabolism, 25-hydroxylation and 1α-hydroxylation, are tightly controlled by, CYP2R1 or CYP27A1 and CYP27B1, respectively (Bikle, 2014; Slominski et al., 2023). Whereas in the non-canonical pathway, CYP11A1 catalyses vitamin D_3 resulting in several hydroxylated derivates that are biologically active (Slominski et al., 2015, 2023). Global vitamin D deficiency, <50 nmol/L $25(OH)D_3$, is reported as 47.9% [95% confidence interval (CI): 44.9–50.9] and is more prevalent in females (Cui et al., 2023). Vitamin D deficiency is associated with several health conditions including osteoporosis and increased risk of common cancers (Holick and Chen, 2008), cardiovascular disease (Butler et al., 2021), and increased mortality (Osorio Landa et al., 2020).

The mechanism by which phthalates and bisphenols may affect vitamin D levels is speculative. It has been suggested that DEHP (Liu et al., 2015) and BPA (Quesnot et al., 2014) may impact, directly and/or indirectly, CYP enzymes involved in $1,25(OH)_2D_3$ metabolism. Some evidence also exists that these EDCs may impact calcium homeostasis, indirectly impacting $1,25(OH)_2D_3$ levels, given that in mice BPA was found to alter the expression of calcium binding protein and serum calcium levels (Otsuka et al., 2012). Additionally, these EDCs may impact the production of hormones that play a role in vitamin D metabolism given that BPA was shown to positively correlate with parathormone (PTH) (Brandi et al., 2022) which is a major stimulator of $1,25(OH)_2D_3$ metabolism.

More research is needed to fully understand the relationship between phthalate metabolites, BPs and TCS to vitamin D deficiency; therefore, this study was undertaken to examine the relationships of phthalate metabolites, TCS and BPs (Table 1) with $25(OH)D_3$ and its active metabolite $1,25(OH)_2D_3$ in a group of non-obese healthy women. We also sought to determine if the impact of these EDCs may be through an indirect effect on calcium homeostasis, specifically via calcium/calmodulin-dependent (CaM) kinases (CaMK), CaMK kinase alpha (CaMKKα) and CaM phosphodiesterase (PDE) proteins.

2. Methods

2.1. Study design and patient recruitment

57 non-obese Caucasian women were sequentially recruited in 2015 from the Hull In Vitro Fertilization (IVF) Unit, UK, following ethical approval from The Yorkshire and The Humber NRES ethical committee, UK (approval number 02/03/043). All participants were from the same geographical area in northern England which was within a 20-mile radius from the IVF center under study. Exclusion criteria were known immunological disease, diabetes, renal or liver insufficiency, acute or chronic infections, inflammatory disease, age <20 or >45 years, body mass index (BMI) > 30 kg/m^2, taking prescription, over the counter medication or vitamin D supplements for nine months preceding the study. Overnight timed urine collection (Midnight to 08:00) into a glass container was performed. Samples were taken on day 21 of the menstrual cycle prior to IVF hormonal treatment. At the same time point, venous blood samples were drawn for biochemical measures and were centrifuged at 3500×*g* for 15 min at 4 °C and serum stored at −80 °C within 1 h of collection for further analysis. All participants gave written informed consent. No participant was on any prescribed or over-the-counter medication, including vitamin D supplementation.

Table 1
EDCs tested in the study.

Phthalate metabolites		Bisphenols	Other
Molecular weight	Parent phthalate		
Low		Bisphenol A (BPA)	Triclosan (TCS)
Monomethyl-phthalate (MMP)	Dimethyl phthalate (DMP)	Bisphenol B (BPB)	
Monoethyl-phthalate (MEP)	Diethyl phthalate (DEP)	Bisphenol S (BPS)	
Mono-iso-butylphthalate (MiBP)	Di-isobutyl phthalate (DiBP)	Bisphenol F (BPF)	
Mono-butylphthalate (MBP)	Di-*n*-butyl phthalate (DBP)	Bisphenol AF (BP-AF)	
Mono-cyclohexyl phthalate (MCHP)	Dicyclohexyl phthalate (DCHP)		
High			
Mono-3-carboxypropyl-phthalate (MCPP)	Di-*n*-octyl phthalate (DnOP)		
Mono(2-ethyl-5-hydroxyhexyl)-phthalate (MEHHP)	Di-2-ethylhexyl phthalate (DEHP)		
Mono(2-ethyl-5-carboxypentyl)-phthalate (MECPP)			
Mono(2-ethyl-5-oxohexyl)-phthalate (MEOHP)			
Mono(2-ethylhexyl)-phthalate (MEHP)			
Monobenzyl-phthalate (MBzP)	Butyl benzyl phthalate (BBP)		
Mono-*n*-octyl-phthalate (MOP)	Di-*n*-octyl phthalate (DOP)		
Monononyl phthalate (MNP)	Di-nonyl phthalate (DNP)		
Mono-iso-decyl phthalate (MiDP)	Di-isodecyl phthalate (DiDP)		

2.2. Phthalate metabolite, TCS and BP measurement

Phthalate metabolites, TCS and BPs concentrations were measured using a direct injection liquid chromatography tandem mass spectrometry (LC-MS/MS) method previously reported (Heffernan et al., 2020). Diluted urine samples were incubated with isotopically-labelled internal standards and β-glucuronidase at 37 °C for 90 min, before quenching the reaction with 0.5% formic acid solution. Centrifuged samples were analyzed by isotope-dilution LC-MS/MS in negative ion multiple reaction-monitoring mode and quantified using isotope dilution. A sum phthalate metabolite (ΣPhthalates) variable was calculated by adding the concentrations of each phthalate metabolite. In addition, a sum variable of low molecular weight phthalate metabolites (LMWPs), high

molecular weight phthalate metabolites (HMWPs), DEHP metabolites and BPs were also calculated. LMWPs were classified as those with ester side chain lengths of one to four carbons and HMWPs as five or more carbons (National Research Council Committee on the Health Risks of, 2008). Previous research in women reports that specific gravity is more effective than creatinine in adjusting for urine dilution (Adibi et al., 2008). As such, we used the formula EDCc = EDC × [(1.01205 − 1)/(SG − 1)], where EDCc is the specific gravity corrected concentration (μg/L), EDC is the experimental EDC concentration (μg/L), and SG is the specific gravity of the urine sample.

2.3. Vitamin D_3 and biochemical parameters

Biochemical and hormonal parameters were measured as previously detailed (Brennan et al., 2022). Briefly, serum 25(OH)D_3 and 1,25$(OH)_2D_3$ levels were quantified using isotope-dilution LC-MS/MS. Vitamin D metabolites and labelled internal standards were extracted from 250 μL serum using supportive liquid-liquid extraction and Diels-Alder derivatization prior to LC-MS/MS analysis. Chromatographic separations were achieved using a Hypersil Gold C18 column (150 × 2.1 mm; 1.9 μm) at flow rate 0.2 mL/min, operated in Electrospray Ionisation (ESI) positive mode and analyzed by the multiple reaction monitoring (MRM) method (Javed et al., 2019). The limit of quantification (LOQ) for 1,25$(OH)_2D_3$ was 10 pg/mL and for 25(OH)D_3 was 1.2 nmol/L (0.5 ng/mL). Vitamin D deficiency was defined as a cut off of 20 ng/mL (50 nmol/L) of 25(OH)D_3, as per the clinical practice guidelines of the Endocrine Society Task Force on Vitamin D (Holick et al., 2011).

2.4. CaM proteomic measurement

We examined seven circulating CaM associated proteins in study participants: PDE1A, CaMK2A, CaMK2B, CaMK1D, CaMK2D, CaMK1, and CaMKKα. CaM proteomic measurement was performed using Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement (Somalogic, Boulder, CO, USA) (Moin et al., 2021). Analyte–SOMAmer complexes were formed using a fully synthetic fluorophore-labelled SOMAmer, coupled to a biotin moiety through a photocleavable linker. The procedure involves a two-step capture of protein-SOMAmer complexes on two sets of streptavidin beads, the first through biotin-labelled SOMAmers and the second through biotin-labelled proteins (Kahal et al., 2020). The complete procedure, normalization of raw intensities, hybridization, median signal and calibration were performed as previously described (Moin et al., 2023).

2.5. Statistics

Descriptive data are presented as mean ± standard deviation (SD) for continuous data. As the study aimed to explore potential relationships, we carried out correlation analysis. Potential correlations between phthalate metabolites, BPs, TCS and 25(OH)D_3 and 1,25$(OH)_2D_3$ were examined using exploratory Spearman's rank order correlations due to non-normal distribution of the data. Correlations were carried out for the study cohort and separately for the sufficient 25(OH)D_3 and deficient 25(OH)D_3 subset. A p-value of <0.05 was considered to indicate statistical significance. Statistical analysis was conducted using Jamovi (version 2.0.0).

3. Results

3.1. Demographics and biochemical data

The mean age of the study cohort was 31.8 ± 4.6 years with a mean BMI of 25.6 ± 3.7 kg/m^2. Measures of inflammation, thyroid stimulating hormone (TSH), free triiodothyronine (Free-T3), free thyroxine (Free-T4) and C-reactive protein (CPR) were normal. Mean levels of measures of renal function, urea, creatinine and estimated glomerular filtration rate (eGFR), were 3.9 ± 0.9 nmol/L, 65.9 ± 8.9 nmol/L and 92.5 ± 14.9 mL/min/1.73 m^2, respectively. Mean levels of 25(OH)D_3 and 1,25$(OH)_2D_3$ were 22.6 ± 11.1 ng/mL and 0.05 ± 0.02 ng/mL, respectively (Table 2). 28 of the 57 women recruited were 25(OH)D_3 deficient; <20 ng/mL (50 nmol/L) 25(OH)D_3.

3.2. Phthalate metabolite, TCS and BP levels

Mean levels of individual phthalate metabolites, ΣPhthalates, ΣLMWPs, ΣHMWPs, and ΣDEHP metabolites, TCS, BPA, BPS, and ΣBPs are shown in Table 3. MEP, mono-iso-butylphthalate (MiBP), mono-butylphthalate (MBP) and MEHHP were the most frequently detected (93%), followed by mono(2-ethyl-5-oxohexyl)-phthalate (MEOHP) (91%), mono(2-ethyl-5-carboxypentyl)-phthalate (MECPP) (88%), monobenzyl-phthalate (MB_ZP) (86%), MEHP (75%), monomethyl-phthalate (MMP) (72%) and MCPP (51%) while mono-cyclohexyl phthalate (MCHP), mono-octyl phthalate (MOP), monononyl phthalate (MNP), and mono-isodecyl phthalate (MiDP) were not detected. TCS had a detection frequency of 93%. BPA and BPS had detection frequencies of 40% and 23%, respectively. Bisphenol F (BPF) and bisphenol AF (BP-AF) had detection frequencies less than 5% and were not examined, while bisphenol B (BPB) was not detected.

3.3. Whole group correlations

In the study cohort, MCPP correlated negatively with 25(OH)D_3 ($\rho = -0.53$, $p = 0.01$) (Fig. 1). There were no correlations found between 1,25$(OH)_2D_3$ and any of the individual phthalate metabolites, ΣPhthalates, ΣLMWPs, ΣHMWPs or ΣDEHP metabolites. There were no associations found between MCPP and measures of renal function: urea, creatinine and, eGFR. Levels of TCS, BPA, BPS and ΣBPs did not correlate with 25(OH)D_3 or 1,25$(OH)_2D_3$ in the study cohort.

In the study cohort, 25(OH)D_3 positively correlated with CaMK1D ($\rho = 0.29$, $p = 0.049$) (Fig. 2) but no associations were found with MCPP levels.

3.4. Subgroup correlations

Using a 25(OH)D_3 cut-off of 20 ng/mL (50 nmol/L), participants

Table 2
Demographics and biochemical measures in the study cohort.

	Female subjects (n = 57)	
	Mean	SD
Age (years)	31.8	4.6
BMI (kg/m^2)	25.6	3.7
CRP (mg/L)	2.6	2.5
TSH (mU/L)	2.1	0.9
Free-T3 (pmol/L)	4.8	0.7
Free-T4 (pmol/L)	11.3	1.8
Urea (nmol/L)	3.9	0.9
Creatinine (nmol/L)	65.9	8.9
eGFR (mL/min/1.73 m^2)	92.5	14.9
25(OH)D_3 (ng/mL)	22.6	11.1
1,25$(OH)_2D_3$ (ng/mL)	0.05	0.02
PDE1A (RFU)	789	1060
CAMK2A (RFU)	373	453
CaMK2B (RFU)	859	1423
CaMK1D (RFU)	1695	580
CaMK2D (RFU)	2379	3462
CaMK1 (RFU)	4681	1371
CaMKKα (RFU)	343	463

BMI, body mass index; CRP, C reactive protein; TSH, thyroid stimulating hormone; Free-T3, free triiodothyronine; Free-T4, free thyroxine; eGFR, estimated glomerular filtration rate; PDE: calcium/calmodulin-dependent phosphodiesterase; CaMK: calcium/calmodulin-dependent kinase; CaMKKα: calcium/calmodulin-dependent kinase kinase alpha.

Table 3
EDC levels in the study cohort.

	Female subjects (n = 57)			
	Mean	SD	Min	Max
LMWP metabolites				
MMP (μg/L)	3.2	4.3	0.8	27.1
MEP (μg/L)	94	156	4.9	940
MiBP (μg/L)	20.5	25.3	3.4	171
MBP (μg/L)	10.8	11.1	1.8	59.5
ΣLMWPs (μg/L)	128	167		
HMWP metabolites				
MCPP (μg/L)	1.8	2.3	0.3	10.7
MEHHP (μg/L)	5.5	4.2	0.6	19.8
MECPP (μg/L)	6.7	4.3	1.2	17.3
MEOHP (μg/L)	3.8	2.8	0.4	12
MB_ZP (μg/L)	3.3	10.7	0.2	66.2
MEHP (μg/L)	3.5	3.1	0.08	11.1
ΣHMWPs (μg/L)	22.5	17.7		
ΣDEHP (μg/L)	17.1	13		
ΣPhthalates (μg/L)	150	168		
TCS (μg/L)	90.1	373	0.2	2303
BPs				
BPA (μg/L)	6.5	15.1	0.7	73.6
BPS (μg/L)	0.23	0.3	0.1	1.4
ΣBPs (μg/L)	5.62	13.8		

LMWP, low molecular weight phthalates; MMP, monomethyl-phthalate; MEP, monoethyl-phthalate; MiBP, mono-iso-butylphthalate; MBP, mono-butylphthalate; HMWP, high molecular weight phthalate; MCPP, mono-3-carboxypropyl-phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl)-phthalate; MECPP, mono(2-ethyl-5-carboxypentyl)-phthalate; MEOHP, mono(2-ethyl-5-oxohexyl)-phthalate; MB_ZP, monobenzyl-phthalate; MEHP, mono(2-ethylhexyl)-phthalate; DEHP, di(2-ethylhexyl)-phthalate; TCS, Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol); BPs, bisphenols; BPA, bisphenol A; BPS, bisphenol S.

were categorized as 25(OH)D_3 sufficient (>20 ng/mL; >50 nmol/L) or deficient 25(OH)D_3 (<20 ng/mL; <50 nmol/L). In deficient 25(OH)D_3 women (n = 28), MiBP and MBP negatively correlated with 25(OH)D_3; ($\rho = -0.47$, $p = 0.049$) and ($\rho = -0.64$, $p = 0.005$), respectively (Fig. 1). MiBP and MBP in deficient women did not correlate with measures of renal function (urea, creatinine or eGFR). No other associations were observed between individual phthalate metabolites, ΣPhthalates, ΣLMWPs, ΣHMWPs and ΣDEHP metabolites, TCS, BPA, BPS or ΣBPs and 1,25$(OH)_2D_3$ in sufficient or deficient 25(OH)D_3 women who participated in the study.

In sufficient 25(OH)D_3 women (n = 29), 25(OH)D_3 positively correlated with PDE1A ($\rho = 0.37$, $p = 0.047$) (Fig. 2). In deficient 25(OH)D_3 women, no associations were observed between calcium/calmodulin-dependent proteins (PDE1A, CaMK2A, CaMK2B, CaMK1D, CaMK2D, CaMK1 and CaMKKα) and 25(OH)D_3, or the individual phthalate metabolites, MiBP and MBP.

4. Discussion

The data suggests that in the study cohort only the HMWP metabolite, MCPP, is related to 25(OH)D_3. MCPP is a metabolite of di-*n*-octyl phthalate (DnOP) which is commonly used in household items, such as wires and cables, and food packaging (Wang and Qian, 2021). This result is in accord with that of Johns et al. (2017) where MCPP was associated to a significant but more moderate 4.48% decrease [95% confidence interval (CI): 7.37, −1.58] in total 25(OH)D_3. In contrast to the studies on US populations (Johns et al., 2016, 2017), where 25(OH)D_3 levels were similar (26 ng/mL versus 23 ng/mL), we did not find any correlations between 25(OH)D_3 and individual DEHP metabolites or ΣDEHP metabolites. This is perhaps surprising given that DEHP is among the most common phthalate plasticizers used in polyvinyl chloride (PVC) products (Talsness et al., 2009) and its metabolites are among the top four detected in urine in biomonitoring studies (Wang et al., 2019). This could perhaps be attributed to the fact that median levels of DEHP metabolites in this study were much lower; lowest, MEHP 2.21 verses 10.1 μg/L and highest, MECPP 5.44 versus 40.6 μg/L. Interestingly, BPA did not correlate with vitamin D levels in the study cohort, in agreement with others (Johns et al., 2017). However, in the few population studies that have examined the relationship between BPA and 25(OH)D_3 (Johns et al., 2016; Brandi et al., 2022), results indicate a consistent negative association. In this study, although not significant, BPA negatively correlated with 25(OH)D_3 which is consistent with published studies and the lack of significance may be reflective of the smaller sample size. No metabolites correlated with 1,25$(OH)_2D_3$ suggesting that any effect of these metabolites is earlier in the vitamin D pathway and may not affect renal conversion to the active 1,25$(OH)_2D_3$ form. This may have been inferred by the lack of correlations of any of the metabolites measured with renal function parameters. However, it is important to note that 1,25$(OH)_2D_3$ is also activated in extrarenal tissues, activated locally by CYP27B1 (Bikle, 2014), and is not an established marker of vitamin D deficiency. Therefore, the lack of correlation is perhaps not surprising. The relationship between phthalates and kidney function is unclear. However, in a recent prospective population-based cohort examining maternal phthalate exposure and childhood kidney function, no consistent associations were observed (Sol et al., 2022).

When the study cohort was divided into two subgroups, sufficient or deficient based on their 25(OH)D_3 levels, in 25(OH)D_3 deficient women, it was surprising to see that a relationship was unmasked with MiBP and MBP. These are LMWP metabolite products of DiBP and DBP metabolism, respectively. DiBP and DBP are generally used in non PVC applications such as personal care products (Wittassek et al., 2011) and their metabolites are among the most commonly detected in urine (Wang et al., 2019). Why MiBP and MBP were not associated with vitamin D levels in sufficiency is unclear, but hypothetically vitamin D sufficiency may dampen any deleterious effects of MiBP and MBP that may then become overt in vitamin D deficiency (Fig. 3).

Of note, the strength of the correlations observed between phthalate metabolites and 25(OH)D_3 levels in this study are surprisingly strong compared to other published studies. This is further evidenced by the fact that a population-based study with a comprehensive set of genetic, anthropometric, dietary, and lifestyle predictors could not account for more than 32.8% of the variation in 25(OH)D_3 (Kühn et al., 2014).

As EDCs, phthalates and BPs impact steroid hormone action through nuclear receptor binding, acting as agonist or antagonists, thereby increasing, decreasing or blocking hormone action (Gore et al., 2015). It is therefore plausible that these EDCs may impact the vitamin D endocrine axis given that the active metabolite 1,25$(OH)_2D_3$ is a secosteroid hormone that exerts its biological effects through a genomic pathway (binding to nuclear vitamin D receptor (VDR) ultimately leading to altered expression of target genes) and a membrane initiated pathway (rapid activation of intracellular calcium signaling) (Ellison et al., 2005). Mechanistically, there is some evidence to suggest that these EDCs impact CYP enzymes which are responsible for hydroxylation of vitamin D_3 in the canonical (Quesnot et al., 2014; Liu et al., 2015) and non-canonical pathway (Källsten et al., 2022). Additionally, BPA has been shown to impact PTH involved in vitamin D metabolism (Brandi et al., 2022) and to alter expression of calcium transport genes, impact serum calcium levels and calcium absorption (Otsuka et al., 2012; Kim et al., 2013). CaM proteins have been shown to impact both the genomic and membrane initiated pathways of 1,25$(OH)_2D_3$ by increasing VDR phosphorylation, independent transcription activity of VDR coactivator steroid receptor coactivator (SRC) 1, and ligand-dependent interaction between VDR and SRC coactivator proteins (Ellison et al., 2005). As expected, although weak, we found correlations between 25(OH)D_3 and both PDE1A and CAMK1D. No relationships were found between the EDCs examined suggesting that their effects in humans on calcium and vitamin D is not through modulation of cellular calcium flux, potentially differing to that of animal models. It should be noted, however, that the BPA intake dosages used in the above animal models (20 mg/kg/day

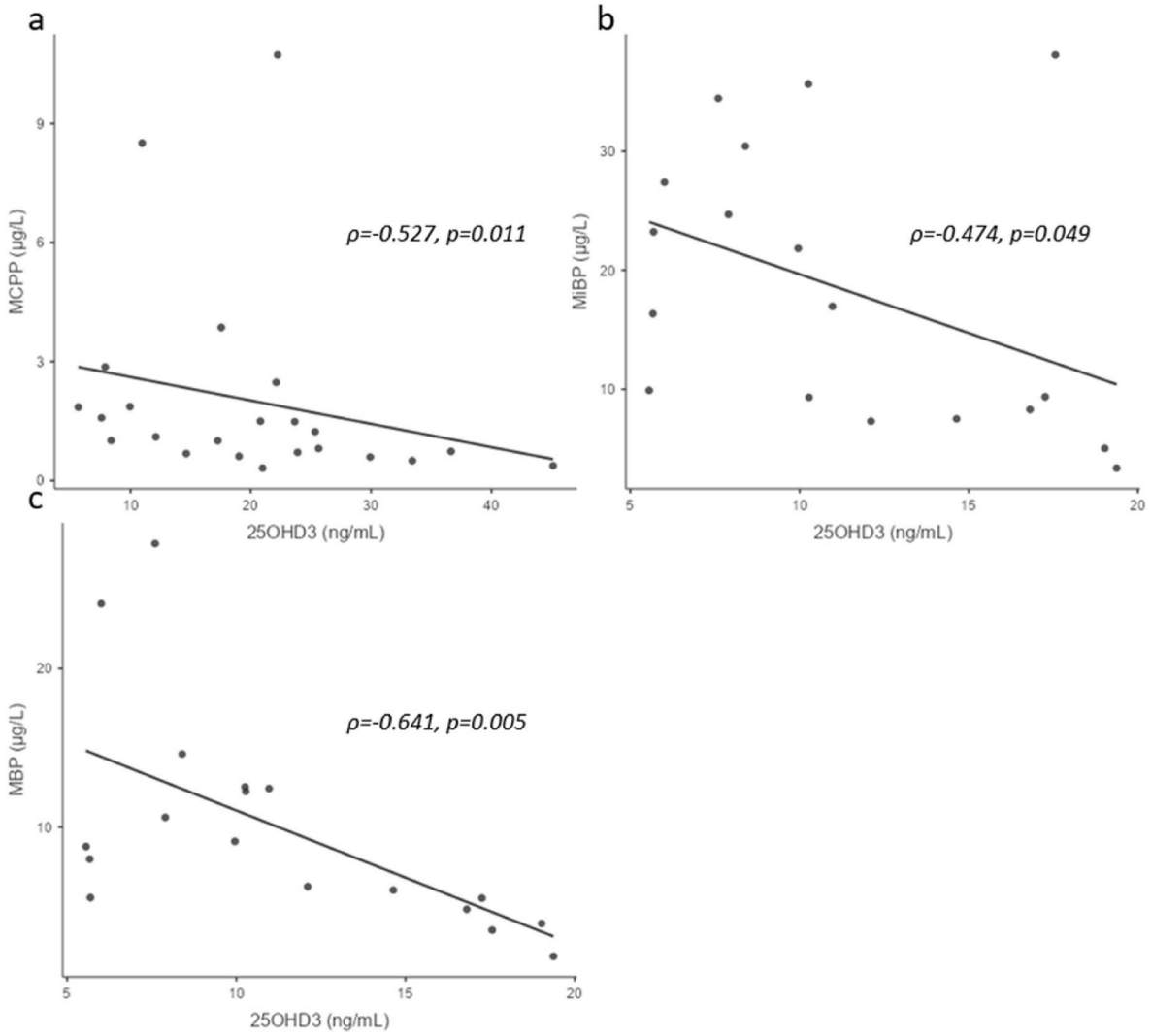

Fig. 1. Correlations of 25(OH)D_3 with phthalate metabolites in female subjects. In study cohort, (a) 25(OH)D_3 and MCPP; In vitamin D deficiency females (<20 ng/mL (50 nmol/L) 25(OH)D_3), (b) 25(OH)D_3 and MiBP and (c) 25(OH)D_3 and MBP.

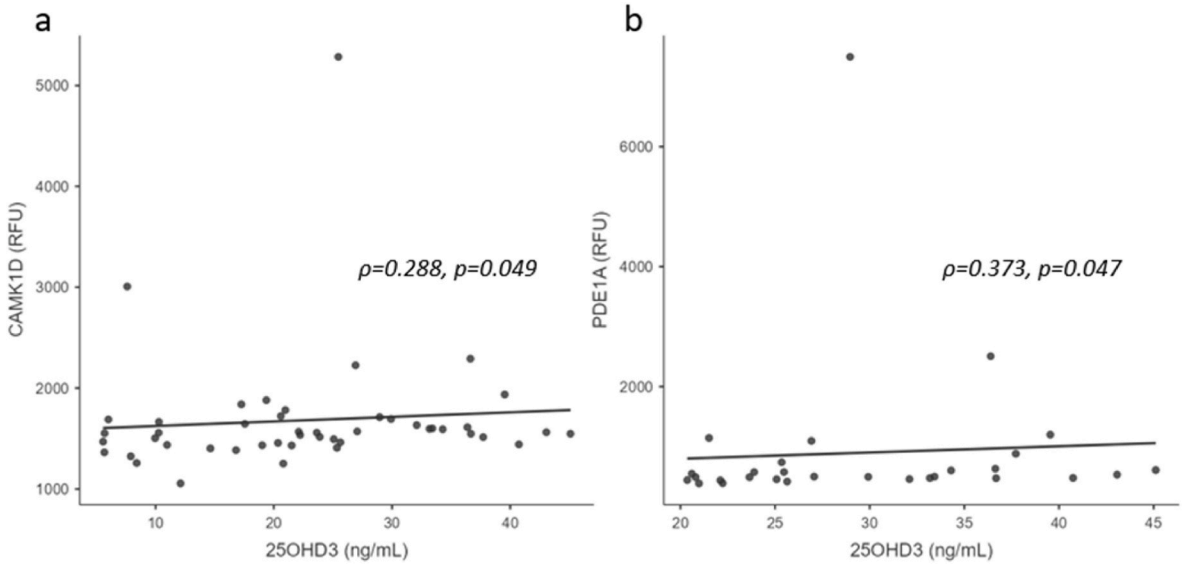

Fig. 2. Correlations of 25(OH)D_3 with calcium/calmodulin-dependent proteins female subjects. In study cohort, (a) 25(OH)D_3 and CaMKID; In vitamin D sufficient females (>20 ng/mL (50 nmol/L) 25(OH)D_3), (b) 25(OH)D_3 and PDE1A.

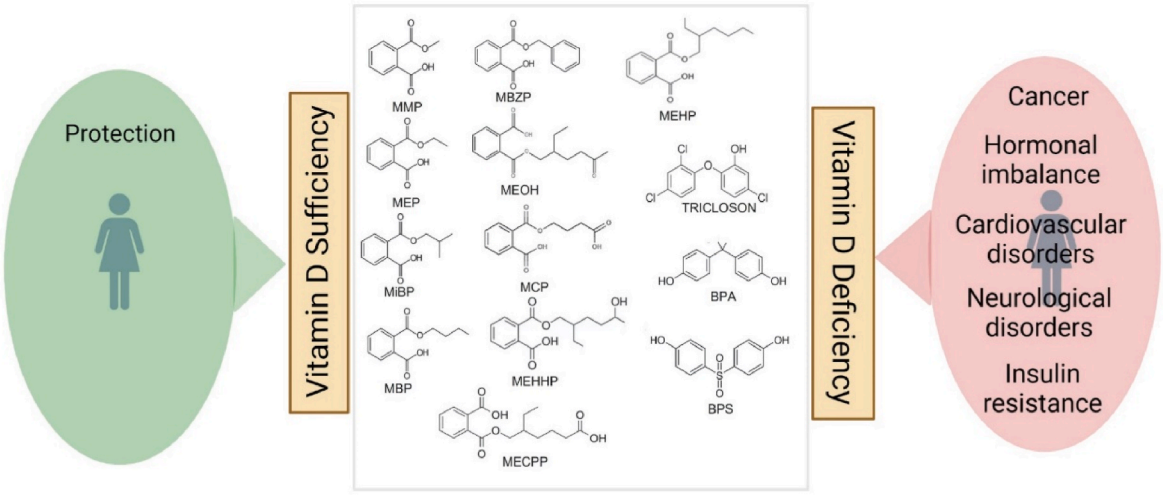

Fig. 3. A schematic to illustrate the relationship between phthalate metabolites, bisphenols and triclosan and 25(OH)D_3 and its active metabolite 1,25$(OH)_2D_3$. Vitamin D sufficiency mitigates the adverse effects and thereby provides protection from the associated conditions/disorders arising from exposure to phthalates metabolites, while vitamin D deficiency unmasks and aggravates the adverse effects of these endocrine disrupting chemicals. Low molecular weight phthalate (LMWPs) metabolites; MMP, monomethyl-phthalate; MEP, monoethyl-phthalate; MiBP, mono-iso-butylphthalate; MBP, mono-butylphthalate; high molecular weight phthalate (HMWP) metabolites; MCPP, mono-3-carboxypropyl-phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl)-phthalate; MECPP, mono(2-ethyl-5-carboxypentyl)-phthalate; MEOHP, mono(2-ethyl-5-oxohexyl)-phthalate; MB_ZP, monobenzyl-phthalate; MEHP, mono(2-ethylhexyl)-phthalate; triclosan and bisphenol A (BPA) and S (BPS) were analyzed.

(Kim et al., 2013), 5/50 mg/kg/day (Otsuka et al., 2012)), were higher than that reported in humans (34 ng/kg/day (Lakind and Naiman, 2011)). In accord with the pleiotropic effects of vitamin D_3, recent advancements highlight that vitamin D_3 derivates can also act as ligands for nuclear receptors other than VDR such as retinoid-related, orphan receptors α and γ, aryl hydrocarbon receptor, liver X receptor, and peroxisome proliferator-activated receptor γ (Slominski et al., 2020, 2022). Whether phthalates or BPs interact with VDR or alternative nuclear receptors requires investigation.

In a recent study, MEB, MiBP and MBP were the top phthalate metabolites detected in females (Urbancova et al., 2022) which is consistent with this study, although the levels were lower here; for example MBP median 7.7 μg/L versus 22.1 μg/L. Although the parent phthalates of the metabolites identified in this study have had their use restricted in recent years (Wang and Qian, 2021), the data here suggests that it is potentially important to further limit exposure to phthalates by avoiding plastic products that contain these chemicals and opting for safer alternatives. Additionally, maintaining adequate vitamin D levels through diet and/or supplements may help mitigate the potential effects of phthalate exposure, as when vitamin D deficiency evolves it may be exacerbated by MiBP and MBP. Thus, these findings suggest that exposure to phthalate metabolites may have negative implications for health outcomes associated with vitamin D deficiency. This is of particular concern if phthalate metabolites may potentiate vitamin D deficiency, a global health condition affecting over 50% of the world (Siddiqee et al., 2021; Cui et al., 2023). Even a modest effect on vitamin D deficiency may have a marked effect on those diseases associated with vitamin D deficiency, such as diabetes (Butler et al., 2020; Ahmed et al., 2021) and cardiovascular disease (Butler et al., 2021) (Fig. 3). In fact, higher urine levels of MiBP and MBP have been shown to be associated with increased risk of diabetes in women (Sun et al., 2014), and MCPP levels were higher in women with diabetes (Nam et al., 2020). In addition, there is evidence to suggest that phthalate exposure may contribute to adverse cardiovascular health, with changes in blood pressure and risk of atherosclerosis (Mariana and Cairrao, 2020). Therefore, phthalate exposure may potentiate the effects of vitamin D deficiency and vice versa. However, the clinical importance of the observations reported here requires validation with a larger cohort with a focus on the underlying mechanism of action to determine if there is causality.

The strengths of this study include the measurement of several EDCs of health concern including phthalate metabolites, TCS and BPs, and vitamin D (25(OH)D_3 and 1,25$(OH)_2D_3$). The limitations of this study include the small numbers of subjects and that they were all Caucasian females, so these findings may not be generalizable to male subjects or those of differing ethnicities. The low numbers compounded by the detection number may have resulted in a type 2 statistical error (false negative) and the analyses carried out were exploratory in nature. In addition, no confounding factors were accounted for in the present analysis. Lastly, there are limited epidemiological studies examining the association between vitamin D with EDCs available from which to draw comparisons. Therefore, this study would allow the determination of power for a larger study focusing on phthalate exposure and vitamin D deficiency.

5. Conclusion

In conclusion, these data indicate that phthalate metabolites through MCPP is related to 25(OH)D_3, and that MiBP and MBP are related to vitamin D deficiency; however, TCS and BPs did not correlate with vitamin D deficiency, and no metabolites correlated with 1,25$(OH)_2D_3$, CaM associated proteins or with renal function, suggesting that a phthalate metabolite effect is earlier in the vitamin D pathway and not through modulation of cellular calcium flux. The observed correlations are surprisingly strong compared to other predictors of 25(OH)D_3, and larger studies adjusting for potential confounders are warranted.

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Ethics approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of The Yorkshire and The Humber NRES ethical committee, UK (approval number 02/03/043).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Author contributions

Conceptualization, S.L.A and T.S.; methodology, S.L.A and T.S; validation, S.L.A; formal analysis, E.B, and AEB; investigation, K.T; resources, S.L.A.; data curation, S.L.A.; writing—original draft preparation, E.B, A.E.B, and S.L.A; writing—review and editing, E.B., A.E.B, M. N, S.L.A, K.T, and T.S.; visualization, E.B, A.E.B, M.N and S.L.A; All authors have read and agreed to the published version of the 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.

Data availability

Data will be made available on request.

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None.

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