Association of flame retardants, polybrominated diethyl ethers (PBDEs), with vitamin D in female subjects

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Abstract

Introduction. A class of flame retardants, polybrominated diethyl ethers (PBDEs), are known endocrine disrupters and may induce the hepatic enzymes CYP24 and CYP3A that promote 25-hydroxylation of vitamin D_3. Therefore, this study examined the association of PBDEs with vitamin D_3 ($25(OH)D_3$) and the active 1,25-dihydrovitamin D_3 ($1,25(OH)_2D_3$) in a cohort of non-obese women.

Methods. 58 female participants (age:31.9±4.6 years; body mass index (BMI):25.7±3.7 kg/m^2) had seven indicator PBDEs [PBDE28;PBDE47;PBDE99;PBDE100;PBDE153; PBDE154;PBDE183] measured using high resolution gas chromatography, with ΣPBDE level calculated. $25(OH)D_3$ and $1,25(OH)_2D_3$ levels were determined by isotope-dilution liquid chromatography tandem mass spectrometry. Plasma level of calcium/calmodulin-dependent protein kinase type 1 (CaMK1) was measured by Somascan proteomics.

Results. In this cohort, vitamin D_3 ($25(OH)D_3$) and $1,25(OH)_2D_3$ levels were 22.9±11.2 ng/mL and 0.05±0.02 ng/mL, respectively. Of those, 28 had vitamin D deficiency [$25(OH)D_3$ level <20 ng/mL (<50nmol/L)]. For the whole group, individual PBDEs (PBDE28; PBDE47;PBDE99;PBDE100;PBDE153;PBDE154;PBDE183) and ΣPBDEs did not correlate with $25(OH)D_3$ or its active metabolite $1,25(OH)_2D_3$ nor with BMI.

For the subset who were $25(OH)D_3$ sufficient, negative correlations were found for $1,25(OH)_2D_3$ with PBDE153 (ρ=-0.77;p=0.02) and PBDE100 (ρ=-0.72;p=0.005). In the subset of women who were $25(OH)D_3$ deficient, positive correlations were found for $1,25(OH)_2D_3$ with PBDE153 (ρ=0.68;p=0.02) and ΣPBDEs (ρ=0.57;p=0.03). Using sufficient and deficient subset categories, no correlations were seen with $25(OH)D_3$ nor any of the PBDEs, and PBDEs did not correlate to renal function (estimated glomerular filtration rate, eGFR). $1,25(OH)_2D_3$ was negatively associated with CaMK1 (r= -0.36;p=0.03) as was PBDE153 (r=-0.31;p=0.02).

Conclusion. PBDEs were not associated with $25(OH)D_3$, but PBDE100 and 153 correlated with its active $1,25(OH)_2D_3$ metabolite and PBDE153 correlated to the calcium modulator CaMKI, suggesting that PBDE effects could either be mediated through vitamin D status or that functional inactivation or inhibition of $1,25(OH)_2D_3$ may contribute to the impact of vitamin D deficiency.

Introduction

Polybrominated diethyl ethers (PBDEs) are a group of synthetic chemicals that are used as flame retardants and monitoring data has shown that their concentrations have increased rapidly in both animals and humans, particularly in infants and toddlers; they enter the body through inhalation and ingestion (Linares, Bellés et al. 2015). These chemicals exist as polybrominated formulations (tri, tetra, penta, hexa, hepta formulations for example) that may make up a large proportion of a product weight; for example, 15% of plastic may be PBDEs (Linares, Bellés et al. 2015). As they are resistant to degradation, they bioaccumulate (Law, Covaci et al. 2014), though highly brominated PBDEs such as dexa formulations may debrominate to lower brominated congeners that are more toxic (Law, Covaci et al. 2014); however, it is unclear if this happens in the body. Animal studies have suggested that PBDEs are associated with liver toxicity (Shockley, Cora et al. 2020), thyroid toxicity (Li, Gao et al. 2020), neurodevelopmental toxicity (Dorman, Chiu et al. 2018) and impaired sperm motility (Li, Gao et al. 2021), but no single congener appears to be more associated with health effects than another.

Vitamin D deficiency is associated with differing health conditions including osteoporosis, cancer, cardiovascular disease, autoimmune diseases and increased mortality (Bjelakovic, Gluud et al. 2014, Osorio Landa, Perez Diaz et al. 2020). Vitamin D_3 (cholecalciferol) is the result of UVB irradiation of 7-dehydrocholesterol; vitamin D_3 is the product of phototransformation of 7-dehydrocholesterol in which, after absorption of UVB, the B ring is broken and the D_3 configuration is thermally driven (Kim, Atigadda et al. 2020). Under high doses of UVB, pre-vitamin D_3 isomerizes to tachysterol or lumisterol that has anti-proliferative, anti-inflammatory and anti-cancer properties (Slominski, Chaiprasongsuk et al. 2020). Subsequently, there is hydroxylation of vitamin D to 25(OH)D_3 in the liver (Bikle 2014). Subsequently, 25(OH)D_3 needs to be converted to 1,25-dihydroxyvitamin D_3

95 ($1,25(OH)_2D_3$), its active metabolite that occurs primarily in the kidneys. In addition, there
96 are alternative pathways of vitamin D activation initiated by CYP11A1 that are biologically
97 active locally and may contribute systemically (Slominski, Kim et al. 2015, Slominski, Li et
98 al. 2015). PBDEs may induce the cytochrome P450 (CYP) enzymes through activation of
99 both the aryl hydrocarbon dependent and independent pathways (Sanders, Burka et al. 2005),
100 and the CYP enzymes mediate vitamin D metabolism (Kasarla, Garikapati et al. 2022).
101 Therefore, PBDEs may be associated with vitamin D levels, despite a report indicating that
102 PBDEs showed little association with vitamin D in pilot whales (Hoydal, Ciesielski et al.
103 2016). Therefore, this study was undertaken to look at the association of PBDEs (tri-
104 PBDE28, tetra-PBDE47, penta-PBDE99, penta-PBDE100, hexa-PBDE153, hexa-PBDE154
105 and hepta-PBDE183) with $25(OH)D_3$ and its active metabolite $1,25(OH)_2D_3$ in a group of
106 non-obese women prior to them undergoing *in vitro* fertilization (IVF).
107
108 **Methods**
109 Patient recruitment. Subjects were recruited from the Hull IVF Unit, UK, following ethical
110 approval from The Yorkshire and The Humber NRES ethical committee, UK (approval
111 number 02/03/043). Venesection was performed on 58 fasting, non-obese Caucasian women;
112 samples were taken at 0900 on day 21 of the menstrual cycle prior to IVF downregulation.
113 All participants gave written informed consent.
114
115 Polybrominated diethyl ethers measurement.
116 Samples were analyzed for 7 PBDEs [PBDE28, PBDE47, PBDE99, PBDE100, PBDE153,
117 PBDE154 and PBDE183], as previously described (Drage, Heffernan et al. 2019). Five mL of
118 serum was aliquoted into a 50 mL polypropylene centrifuge tube. Samples were spiked with 5
119 ng each of internal standards ($^{13}C_{12}$-labelled PBDEs -28, -47, -99, -100, -153, -154, -183).

120 Samples were vortexed for approximately 1 minute and left to stand for 30 minutes. 6 mL
121 acetonitrile, 3 mL milliQ, 5 g anhydrous $MgSO_4$ and 1 g NaCl were added along with a ceramic
122 homogenizer. Samples were manually shaken for 1 minute prior to centrifuging at 4500 RPM
123 for 8 minutes at 10 °C. The supernatant layer was collected and transferred to a glass tube. The
124 extract was evaporated to near-dryness on a hot plate using a gentle stream of nitrogen and
125 reconstituted in approximately 1 mL hexane. 1 mL >98% concentrated sulfuric acid was added
126 and the sample was vortexed for at least 30 seconds. The aqueous and organic layers were left
127 to separate overnight at <4 °C. The supernatant layer was transferred directly onto a silica solid
128 phase extraction cartridge (Supelco LC-Si 3mL/500 mg), preconditioned with 6 mL
129 dichloromethane, followed by 6 mL hexane. The sample was allowed to load onto the cartridge
130 gravimetrically. Target compounds were eluted into a glass tube using 6 mL hexane, followed
131 by 8 mL dichloromethane at approximately 2 mL/min. The sample was evaporated to near-
132 dryness and reconstituted in 100 µL iso-octane containing 2.5 ng $^{13}C_{12}$-PCB-141 as a recovery
133 standards. After analysis for PBDEs by high resolution gas chromatography coupled with high
134 resolution mass spectrometry (HRGC/HRMS).
135
136 Instrumental Analysis
137 For PBDE analysis by HRGC/HRMS,a Thermofisher TRACE 1300 gas chomatograph was
138 coupled to a Thermofisher DFS mass spectrometer. The injector was operated in splitless mode
139 with separation achieved on an Agilent DB-5ms column (30 m length x 0.25 mm in diameter
140 x 0.25 µm film thickness). Experiments were conducted in MID mode at 10,000 resolution
141 (10% valley definition). The inlet, transfer line and source were held at 250 ºC, 280 ºC and 280
142 ºC respectively. The flow rate was maintained at 1.0 mL/min. Details of acquisition ions for
143 PBDEs are outlined in (Drage, Heffernan et al. 2019) and quality assurance checks using

previously described methods (Wang, Banks et al. 2019). A sum PBDE (∑PBDE) variable was calculated by adding together the molar concentrations of the PBDE congeners analyzed.

Vitamin D_3 and biochemical parameters.

Biochemical and hormonal parameters were measured as previously detailed (Brennan, Kumar et al. 2022). Isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to determine vitamin D levels (Javed, Papageorgiou et al. 2019), with a 25(OH)D_3 cut off of 20ng/mL (50nmol/L) to define vitamin D deficiency.

Calcium/calmodulin-dependent protein kinase type 1 (CaMK1) proteomic measurement.

Circulating levels of complement pathway proteins were determined by Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement (Somalogic, Boulder , CO, USA), the details of which have been previously reported (Moin, Sathyapalan et al. 2021). Normalization of raw intensities, hybridization, median signal and calibration signal were performed based on the standard samples included on each plate, as previously described (Moin, Sathyapalan et al. 2023).

Statistics.

No previous studies were available to perform a power analysis, therefore this pilot study was designed according to Birkett and Day (Birkett and Day 1994). Data are presented as mean ± SD. PBDE levels, hormone concentrations, and metabolic markers were assessed for normality and Independent T or Mann-Whitney U tests were used to compare means/medians, as appropriate. Potential associations were examined using Spearman's rank order correlations or Pearson's product moment correlations. A p-value of <0.05 was

considered to indicate statistical significance. Statistical analysis was carried out using Jamovi (version 2.0.0).

Results

Whole cohort analysis. Demographic and biochemical data for this cohort are shown in Table 1. The participants had a mean age of 31.9±4.6 years and a mean body mass index (BMI) of 25.7±3.7 kg/m^2. Thyroid function and C-reactive protein, as a measure of underlying inflammation, were normal. Mean levels of vitamin D_3 ($25(OH)D_3$) and $1,25(OH)_2D_3$ were 22.9±11.2 ng/mL and 0.05±0.02 ng/mL, respectively.

Of the 58 women recruited, 28 had a $25(OH)D_3$ level less than 20 ng/mL (50 nmol/L). Levels of PBDEs, $25(OH)D_3$ and $1,25(OH)_2D_3$ are shown in Table 1. Those women that had vitamin D sufficiency were statistically older by 1.6 years ($p<0.01$) and had a higher T3 ($p<0.002$), but the TSH levels did not differ. None of the PBDEs differed significantly between sufficient and insufficient though PBDE100 and total PBDE were higher in sufficiency, but failed to reach significance ($p<0.06$ and 0.08, respectively). PBDE28, PBDE47, PBDE99, PBDE100 and PBDE153 had detection frequencies of 31%, 90%, 64%, 69% and 59%, respectively, whilst PBDE154 and PBDE183 were not detected.

Whole group correlations. Individual PBDEs (PBDE28, PBDE47, PBDE99, PBDE100, PBDE153, PBDE154 and PBDE183) and ΣPBDEs did not correlate with $25(OH)D_3$ or its active metabolite $1,25(OH)_2D_3$ nor with BMI (Table 2).

Subset correlations. When the subset of women who were $25(OH)D_3$ sufficient (>20 ng/mL; >50 nmol/L) were analyzed, strong negative correlations were found for $1,25(OH)_2D_3$ with PBDE153 ($\rho=-0.77$;$p=0.02$) and PBDE100 ($\rho=-0.72$;$p=0.005$) (Figure 1A, B). In the subset

of women who were 25(OH)D_3 deficient (<20 ng/mL; <50 nmol/L), strong positive correlations were found for 1,25$(OH)_2D_3$ with PBDE153 (ρ=0.68;p=0.02) and ΣPBDEs (ρ=0.57;p=0.03) (Figure 1C, D). Using sufficient and deficient subset categories, no correlations were seen with 25(OH)D_3 and any of the PBDEs.

Using sufficient and deficient subset categories, no correlations were found between eGFR and the individual PBDEs nor ΣPBDEs that associated with 1,25$(OH)_2D_3$ (data not shown).

Association with calcium/calmodulin-dependent protein kinase type 1 (CaMK1).

To investigate a possible mechanism by which PBDE may affect 1,25$(OH)_2D_3$ action, determination of plasma levels of calcium/calmodulin-dependent protein kinase type 1 (CaMK1), that may be indicative of 1,25$(OH)_2D_3$ action, was subsequently undertaken (Ellison, Dowd et al. 2005). Here, we found that 1,25$(OH)_2D_3$ was negatively associated with CaMK1 (r= -0.36, p=0.03), and that PBDE153 was also negatively associated with CaMK1 (r= -0.31, p=0.02) (Figure 2).

Discussion

These data show that 7 indicator PBDEs and ΣPBDEs do not appear to be associated with 25(OH)D_3 levels. Mechanistically, PBDEs activate CYP3A and CYP3B (Lundgren, Darnerud et al. 2007) that are involved in vitamin D metabolism by converting 25(OH)D_3 to inactive metabolites (Kasarla, Garikapati et al. 2022) and, therefore, it may have been anticipated that PBDEs would have been associated with vitamin D and perhaps more associated with vitamin D sufficiency, but this was not seen here. However, in the absence of an association of PBDEs with 25(OH)D_3, the lack of association with 1,25$(OH)_2D_3$ for the group as a whole was not unexpected. What was surprising was the negative correlation of PBDE153 and PBDE100 with 1,25$(OH)_2D_3$ in those subjects that were vitamin D sufficient.

This would suggest that the PBDEs are perhaps acting on CYP3, resulting in increased inactivation of $1,25(OH)_2D_3$, perhaps through CYP promoting 23R- and 24S-mediated conversion of $1,25(OH)_2D_3$ into inactive $1\alpha,23R,25(OH)_2D_3$ and $1\alpha,24S,25(OH)_2D_3$ (Kasarla, Garikapati et al. 2022). Conversely, in the subset of women who were $25(OH)D_3$ deficient, strong positive correlations were found for $1,25(OH)_2D_3$ with PBDE153 and ΣPBDE. Both PBDE100 and ΣPBDE were higher in deficiency though not statistically different, but could be considered to show a trend. Women with vitamin D sufficiency were older but likely this was not clinically significant, and had a higher T3, but the TSH as a measure of thyroid function did not differ between groups suggesting that whilst statistically different, it was not clinically important. What is well recognized is that production of $1,25(OH)_2D_3$ occurs in the renal cortex and is enhanced by vitamin D deficiency (Armbrecht, Zenser et al. 1981). Speculatively, PBDE may act through PPAR-γ (Tung, Boudreau et al. 2014) and it is known that PPAR may decrease CYP (Bouillon and Bikle 2019) that may reflect in the increase in $1,25(OH)_2D_3$. An alternate speculative scenario can be inferred from the fact that, in the presence of vitamin D deficiency in pigs, 1α-hydroxylase, the enzyme responsible for the production of $1,25(OH)_2D_3$, increases five to ten fold (Engstrom, Horst et al. 1984), so PBDE may be blunting the overall enzymatic response to $1,25(OH)_2D_3$ formation. It is likely that the diverse observations in the vitamin D sufficient and deficient groups cancelled each other out so that, overall, there appeared not to be an association of PBDE with $1,25(OH)_2D_3$. Specific studies are required to determine the underlying mechanisms for the observations reported for PBDE on vitamin D sufficient and deficient subjects, but currently the literature is scant to predict the underlying mechanism(s). Given that there was no correlation with eGFR as a marker of renal function, these effects on $1,25(OH)_2D_3$ are likely not mediated through a nephrotoxic mechanism. In addition, extrarenal tissues may also convert $25(OH)D_3$ to $1,25(OH)_2D_3$ although, notably, activation in renal and non-kidney tissues is regulated

243 differently with macrophage production of $1,25(OH)_2D_3$ through the type 2 interferon
244 response (Adams, Rafison et al. 2014); it has been shown that PBDE47 can modulate the
245 macrophage immune response (Longo, Longo et al. 2021), suggesting that this could be
246 another mechanism by which the PBDEs may modulate $1,25(OH)_2D_3$ effects *in vivo*.
247 Clinically, this observation of the association of PBDEs with $1,25(OH)_2D_3$ may be important
248 and requires validation with a larger series and a focus on the underlying mechanism of
249 action to determine if there is causality. Vitamin D deficiency affects 50% of the world,
250 reaching over 70% in countries such as Pakistan (Siddiqee, Bhattacharjee et al. 2021) and is
251 associated with differing health conditions including osteoporosis, cancer, cardiovascular
252 disease, autoimmune diseases, diabetes and increased mortality (Bjelakovic, Gluud et al.
253 2014, Osorio Landa, Perez Diaz et al. 2020). The PBDE global burden may not be distributed
254 equally with less industrialized regions showing accelerated growth of manufacturing
255 industries in their booming economies, leading to higher consumption with inappropriate
256 waste management practices causing increased PBDE concentrations in the environment and
257 in human tissues in some Asian and African countries (Abbasi, Li et al. 2019), but it is
258 unclear if the global PBDE burden maps to global vitamin D deficiency on a country by
259 country basis.
260 To investigate a possible mechanism by which PBDE may affect $1,25(OH)_2D_3$ action,
261 determination of plasma levels of CaMK1, that may be indicative of $1,25(OH)_2D_3$ action, was
262 undertaken. $1,25(OH)_2D_3$ exerts rapid actions at the cell membrane that include increasing
263 intracellular calcium levels through CaMKIV (Ellison, Dowd et al. 2005). CaMKIV signaling
264 stimulates vitamin D receptor (VDR)-mediated transcription by increasing phosphorylation
265 levels of VDR and enhancing autonomous steroid receptor coactivator (SRC) activity,
266 resulting in higher $1,25(OH)_2D_3$-dependent interaction between VDR and SRC coactivators
267 (Ellison, Dowd et al. 2005). In addition, there are alternative nuclear receptors for the

hydroxy-derivatives of vitamin D_3 including retinoic acid-related orphan receptors, the aryl hydrocarbon receptor and liver X receptors (Slominski, Kim et al. 2014, Slominski, Chaiprasongsuk et al. 2020, Slominski, Kim et al. 2021) that may be affected by PBDEs. The consensus sequences for phosphorylation by CaMKI and CaMKIV are similar, and hence these kinases phosphorylate the same substrates (Hook and Means 2001, Beghi, Furmanik et al. 2022). Here, we found that $1,25(OH)_2D_3$ was negatively associated with CaMK1, and that PBDE153 was also negatively associated with CaMK1, suggesting that increasing levels of PBDE153 may be acting at the level of calcium modulation through calmodulin-dependent kinases (Figure 3).

Strengths of this study include the state-of-the-art measurement of the PBDEs and vitamin D ($25(OH)D_3$ and $1,25(OH)_2D_3$). Limitations of the 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 selection of participants from women without obesity prior to undergoing in vitro fertilization may have introduced a selection bias to the present study as their lifestyle is focused upon optimizing their potential fertility. The low numbers compounded by the PBDE detection rate may have resulted in a type 2 statistical error (false negative); however, this study would allow the determination of power for a larger study focusing on vitamin D deficiency. One of the major limitations in the interpretation of the results is that there are very few studies in humans on this subject; therefore, the inference of the results is derived from animal studies in differing species that may not be directly comparable to man.

In conclusion, our findings show that PBDEs were not associated with $25(OH)D_3$, but PBDE100 and 153 correlated with its active $1,25(OH)_2D_3$ metabolite and PBDE153 correlated to CaMKI for calcium modulation, suggesting that PBDE effects could either be

mediated through vitamin D status or that functional inactivation or inhibition of $1,25(OH)_2D_3$ may contribute to the impact of vitamin D deficiency.

DECLARATIONS

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of The Yorkshire and The Humber NRES ethical committee, UK (approval number 02/03/043) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication: All authors gave their consent for publication.

Availability of data and materials: All the data for this study will be made available upon reasonable request to the corresponding author.

Competing interests: No authors have any conflict of interest or competing interests to declare.

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Authors' contributions:

AEB analyzed the data and wrote the manuscript. TS supervised clinical studies and edited the manuscript. DSD performed the PBDE analyses. SLA and EB contributed to study design, data interpretation and the writing of the manuscript. All authors reviewed and approved the final version of the manuscript. Alexandra E Butler is the guarantor of this work.

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442 **Table 1.** Demographics of the 58 female subjects in the population studied. Data are presented for the whole cohort as well as for the vitamin D
443 sufficient and deficient subgroups.
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	Female subjects (n=58)		Vitamin D sufficient female subjects (n=30)		Vitamin D deficient female subjects (n=28)		P-value Vitamin D sufficient vs deficient
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	31.9	4.6	33.4	4.6	30.3	4.2	0.01
BMI (kg/m^2)	25.7	3.7	26.3	3.5	25	3.9	0.17
CRP (mg/L)	2.6	2.5	2.4	2.5	2.8	2.5	0.63
TSH (mU/L)	2.4	2.2	2	0.9	2.8	3	0.22
Free-T3 (pmol/L)	4.8	0.7	5.1	0.7	4.5	0.6	0.002
Free-T4 (pmol/L)	11.3	1.8	11.5	1.9	11.2	1.7	0.53
$25(OH)D_3$ (ng/mL)	23	11.2	29.9	7.5	11.4	4.9	<0.0001
$1,25(OH)_2D_3$ (ng/mL)	0.05	0.02	0.05	0.02	0.04	0.02	0.02
PBDE28 (ng/g Lipid)	0.15	0.05	0.15	0.05	0.15	0.06	0.99
PBDE47 (ng/g Lipid)	0.7	0.69	0.59	0.49	0.85	0.87	0.18
PBDE99 (ng/g Lipid)	0.26	0.17	0.23	0.1	0.28	0.21	0.33
PBDE100 (ng/g Lipid)	0.36	0.37	0.26	0.24	0.48	0.47	0.06
PBDE153 (ng/g Lipid)	2.33	2.26	1.79	0.76	2.87	3.05	0.17

PBDE154 (ng/g Lipid)	<0.3		<0.3		<0.3		
PBDE183 (ng/g Lipid)	<0.4		<0.4		<0.4		
∑PBDEs (ng/g Lipid)	2.45	2.65	1.88	1.62	3.11	3.39	0.08
eGFR (mL/min/1.73m^2)	92.3	14.8	84.6	7.4	86.1	6.9	0.48

BMI: Body mass index; CRP: C reactive protein; TSH: thyroid stimulating hormone; Free-T3: Free Triiodothyronine; Free-T4: Free Thyroxine; PBDE: polybrominated diethyl ether; eGFR: estimated glomerular filtration rate.

Table 2. Spearman's rho (p) correlations between PBDEs, 25(OH)D_3, 1,25$(OH)_2D_3$ and BMI.

	PBDE28	PBDE47	PBDE99	PBDE100	PBDE153	ΣPBDEs
25(OH)D_3 (ng/mL)	-0.104 (0.70)	-0.122 (0.43)	-0.153 (0.41)	-0.216 (0.23)	-0.025 (0.89)	-0.275 (0.06)
1,25$(OH)_2D_3$ (ng/mL)	0.092 (0.75)	-0.298 (0.09)	-0.252 (0.26)	-0.35 (0.10)	0.079 (0.74)	-0.061 (0.72)
BMI	0.011 (0.96)	0.037 (0.79)	0.114 (0.50)	0.253 (0.12)	-0.032 (0.86)	-0.018 (0.89)

PBDEs: polybrominated diethyl ethers; BMI: Body mass index

Figure legends

Figure 1. Correlations of $1,25(OH)_2D_3$ with PBDEs in female subjects categorized according to vitamin D sufficiency or deficiency. A cut off value for $25(OH)D_3$ of 20ng/mL (50nmol/L) was used, above which subjects were categorized as vitamin D sufficient and below which subjects were classified as deficient. Negative correlations of vitamin D sufficient women were seen for $1,25(OH)_2D3$ with PBDE153 (A) and PBDE100 (B). Positive correlations of vitamin D deficient women were seen for $1,25(OH)_2D_3$ with PBDE153 (C) and ΣPBDEs (D).

Figure 2. Correlations of calcium/calmodulin-dependent protein kinase type 1 (CaMK1) with $1,25(OH)_2D_3$ (A) and polybrominated diethyl ether 153 (PBDE153) (B).
RFU: relative fluorescent units

Figure 3. A schematic to illustrate the effect of $1,25(OH)_2D_3$ on cell signaling pathways, specifically upon calcium/calmodulin-dependent protein kinases (CaMKs). The active form of vitamin D, $1,25(OH)_2D_3$, binds to the vitamin D receptor (VDR) through which it exerts its effects. The VDR affects multiple downstream cell signaling pathways, including cAMP, PIP3, PKA, PKC, MAPKs, and CaMKs.
VDR, vitamin D receptor; cAMP, cyclic adenosine monophosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; MAPKs, mitogen activated protein kinases; CaMKs, calcium/calmodulin-dependent protein kinases.

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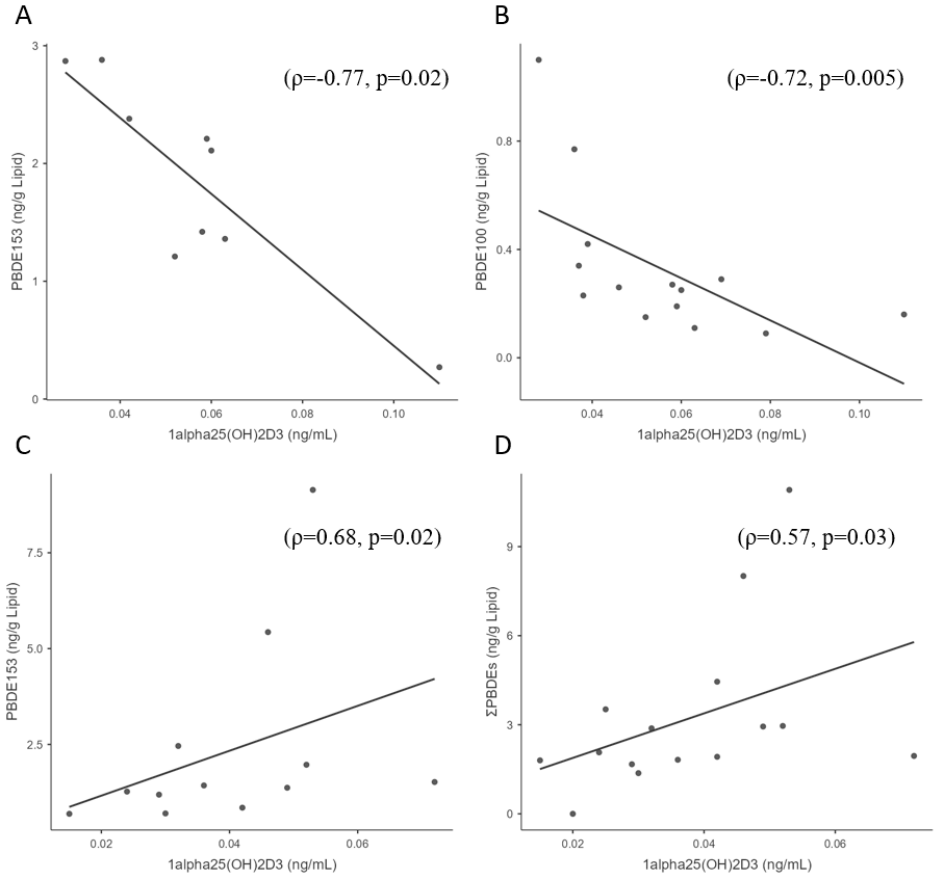

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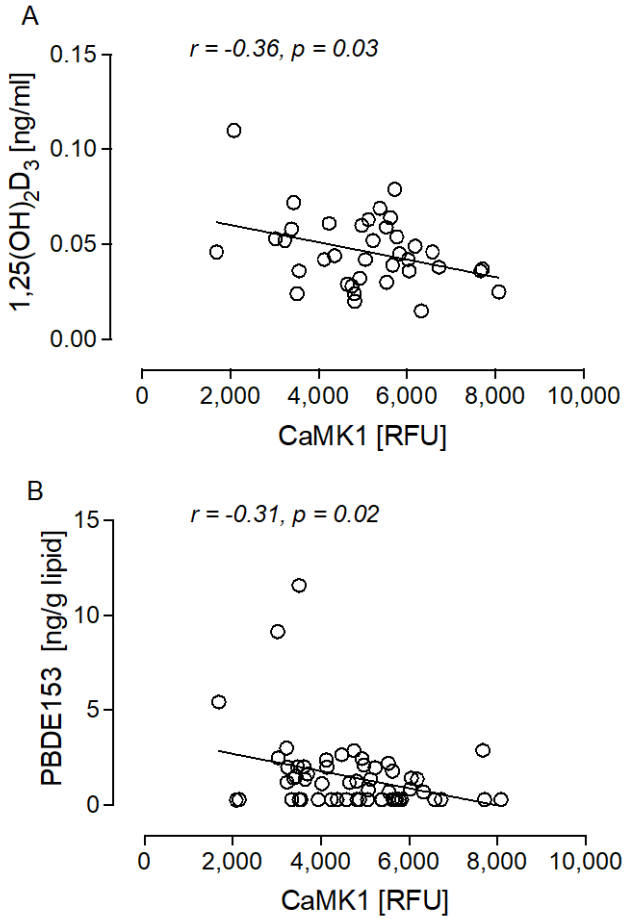

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VDR, vitamin D receptor; cAMP, cyclic adenosine monophosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; MAPKs, mitogen activated protein kinases; CaMKs, calcium/calmodulin-dependent protein kinases.