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Association of polychlorinated biphenyls with vitamin D in female subjects

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Abstract

Introduction. Polychlorinated biphenyls (PCBs) are known endocrine disrupters. A potentially causal association of PCBs with vitamin D has been reported. Higher body mass index (BMI) is associated with lower PCB levels whilst the strongest association of PCBs with BMI is in non-obese individuals. Therefore, this study examined the association of PCBs 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; BMI 25.7±3.7 kg/m^2) had seven indicator PCBs [PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180] measured using high resolution gas chromatography, with total PCB level calculated. $25(OH)D_3$ and $1,25(OH)_2D_3$ levels were determined by isotope-dilution liquid chromatography tandem mass spectrometry.

Results. In this cohort, vitamin D_3 ($25(OH)D_3$) and $1,25(OH)_2D_3$ levels were 50.7±25.3nmol/L and 0.05±0.02ng/ml, respectively. Of those, 28 had vitamin D deficiency [$25(OH)D_3$ level <20ng/ml (<50nmol/)]. Total PCBs correlated positively with total group $25(OH)D_3$ (r=0.22, p=0.04) as did PCB118 (r=0.25, p=0.03). Total PCBs did not correlate with total group $1,25(OH)_2D_3$; however, PCB180 did correlate positively with $1,25(OH)_2D_3$ (r=0.34, p=0.03) as did PCB153 (r=0.33, p<0.03), with PCB 28 correlating negatively (r=-0.29, p<0.04). In the vitamin D deficient subgroup, total PCBs, PCB153 and PCB180 positively correlated with $25(OH)D_3$ (p<0.05).

Multilinear regression analysis indicated all associations could be accounted for by BMI.

Conclusion. Though certain PCBs associated with $25(OH)D_3$ and $1,25(OH)_2D_3$, all associations could be accounted for by BMI. This study therefore indicates that the deleterious effects from PCB accumulation are not mediated by effects on $25(OH)D_3$ or $1,25(OH)_2D_3$.

Introduction

Polychlorinated biphenyls (PCBs) are organic pollutants that persist in the environment due to their resistance to biotransformation and high lipophilicity (1). Dietary consumption is the main route of exposure in humans (2) and they are classified as endocrine disrupters due to their observed thyroidogenic, estrogenic and antiandrogenic actions (3). Vitamin D deficiency is a global health issue associated with a range of negative health outcomes, including osteoporosis, cancer, cardiovascular disease, autoimmune diseases and increased mortality (4, 5).

Vitamin D_3 ($25(OH)D_3$:cholecalciferol) is endogenously produced by UV-B irradiation of 7-dehydrocholesterol and subsequently hydroxylated to $25(OH)D_3$ by multiple 25-hydroxylases in the liver (6). $25(OH)D_3$ is converted to its active metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) primarily in the kidneys by 1-alpha hydroxylase. Greenland sledgedogs fed a high PCB diet from minke whale blubber showed altered vitamin D levels suggesting a causal effect of PCB on vitamin D levels (7). Though PCBs showed little association with vitamin D in pilot whales (8), decreased levels of active $1,25(OH)_2D_3$ were found in rats after exposure to PCBs (9). In pregnant women, there was a negative trend of PCB association with $25(OH)D_3$ (10). Higher body mass index (BMI) is associated with lower PCB levels (11), with the strongest associations with BMI being in normal weight/ non-obese individuals. Therefore, this study was undertaken to look at the association of PCBs with $25(OH)D_3$ and $1,25(OH)_2D_3$ in a group of non-obese women prior to them undergoing *in vitro* fertilization (IVF).

Methods

Patient recruitment. Participants were sequentially recruited without knowledge of their vitamin D status from the Hull IVF Unit, UK, following ethical approval from The Yorkshire

and The Humber NRES ethical committee, UK (approval number 02/03/043). All gave written informed consent. Prior to IVF, 58 non-obese Caucasian women underwent venesection; fasting blood samples were taken at Day 21 during the luteal phase of the menstrual cycle prior to commencement of IVF treatment. Study participants had no other condition or illness and were required to be medication-free for nine months preceding study enrollment.

Polychlorinated biphenyl measurement. Samples were analyzed for 7 indicator PCBs [PCB28, PCB52, PCB101, PCB118 (a dioxin-like PCB), PCB138, PCB153 and PCB180], as previously described (12). Briefly, PCBs were determined using high resolution gas chromatography (Thermofisher TRACE 1300) coupled with high resolution mass spectrometry (HRGC/HRMS, Thermofisher DFS) with quality assurance checks using previously described methods (13). A sum PCB (∑PCB) variable was calculated by adding the molar concentrations of the PCB congeners analyzed.

Vitamin D3 and biochemical parameters

Biochemical and hormonal parameters were measured as previously detailed (12). Isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to determine vitamin D levels (14) with a $25(OH)D_3$ cut off of 20ng/ml (50nmol/l) to define vitamin D deficiency. In brief, vitamin D metabolites (1,25(OH)2D3 and 25(OH)D3 labeled internal standards (d6-25(OH)D3 an d6-1,25(OH)2D3) were simultaneously 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 Hypersil Gold C18 column (150x2.1mm; 1.9µ) at flow rate 0.2 ml/min, operated in Electrospray Ionisation

(ESI) positive mode and analysed by multiple reaction monitoring (MRM) method. The limit of quantification (LOQ) for 1,25(OH)2D was 10 pg/mL and 25(OH)D3 was 0.5ng/mL.

Statistics. No previous studies were available to perform a power analysis, therefore this pilot study was designed according to Birkett and Day (15). Statistical analysis was carried out using Prism version 9.5.0 (Graphpad, San Diego, USA).

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 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 50.7±25.3 nmol/L and 0.05±0.02 ng/ml, respectively.

Of the 58 women recruited, 28 had a $25(OH)D_3$ level less than 20ng/ml (50nmol/). Levels of PCBs, $25(OH)D_3$ and $1,25(OH)_2D_3$ are shown in Table 1. PCB28, PCB52 and PCB101 had detection frequencies of 26%, 7% and 45%, respectively whilst PCB118, PCB138, PCB153 and PCB180 had 100% detection frequency.

Whole group correlations. Total PCBs correlated positively with total group $25(OH)D_3$ ($r=0.22$, $p=0.04$) as did PCB118 ($r=0.25$, $p=0.03$) (Figure 1 A, B) though no correlation was seen for PCB101, PCB52, PCB153, PCB28, PCB180 or PCB138.

Total PCBs did not correlate with total group $1,25(OH)_2D_3$ (p=ns) (Figure 1C). However, PCB180 did correlate positively with total group $1,25(OH)_2D_3$ ($r=0.34$, $p=0.03$) as did PCB153 ($r=0.33$, $p<0.03$), with PCB 28 correlating negatively ($r= -0.29$, $p<0.04$) (Figure 1 D-F); there was no correlation for PCB101, PCB138, PCB118 and PCB52.

Subset analysis of women with and without vitamin D3 deficiency. When the subset of women who were 25(OH)D_3 deficient (<20ng/ml) was analyzed, total PCBs, PCB153 and PCB180 positively correlated with 25(OH)D_3 ($p<0.05$) (Figure 1 G-I); there were no correlations with 1,25$(OH)_2D_3$ in this subset ($p>0.05$).

Subset correlations. When the subset of women who were 25(OH)D_3 sufficient (>20ng/ml) was analyzed, neither total or individual PCBs correlated with 25(OH)D_3 or 1,25$(OH)_2D_3$ ($p>0.05$).

Correlations with body mass index. Total PCBs and whole group 25(OH)D_3 and 1,25$(OH)_2D_3$ were not associated with BMI ($p>0.05$). When BMI was accounted for by multiple linear regression, then those significant associations between total PCBs and PCB118 with whole group 25(OH)D_3, and between total PCBs, PCB28, PCB153 and PCB180 with whole group 1,25$(OH)_2D_3$, became non-significant ($p>0.05$). When BMI was adjusted for in the 25(OH)D_3 deficient group, then total PCB, PCB153 and PCB118 no longer significantly correlated with 25(OH)D_3.

Discussion

These data show that certain PCBs were apparently associated with both 25(OH)D_3 and its active metabolite 1,25$(OH)_2D_3$, in accord with the sledgedog study (7), without adjusting for BMI. Higher BMIs are reported to be associated with lower serum PCB levels due to sequestering in liquid rich adipose tissue (11), and with lower 25(OH)D_3 and 1,25$(OH)_2D_3$ levels (16, 17). In addition, obesity is reported to exacerbate vitamin D deficiency through decreased bioavailability due to deposition of vitamin D in the body fat compartments (18). In this cohort of non-obese women, PCB levels did not correlate with BMI, in contrast to a US population (11), nor did 25(OH)D_3 and 1,25$(OH)_2D_3$. However, when BMI was accounted

for in regression analysis, PCBs were not related to whole group $25(OH)D_3$ and $1,25(OH)_2D_3$ levels, or with $25(OH)D_3$ deficient subjects. BMI is reported to modify associations between dietary intake and serum PCB levels (11) and in this study was shown to alter the association between PCBs and vitamin D levels. This further highlights the confounding effects of BMI in epidemiological studies and the need for such study populations to be BMI matched, as is the case in this study. The lack of association between BMI and $25(OH)D_3$ and $1,25(OH)_2D_3$ levels is not surprising given that none of the subjects in this cohort were obese. The lack of association between PCBs and $25(OH)D_3$ and $1,25(OH)_2D_3$ levels when BMI was considered is consistent with the whale data (8). If there were to be an association between vitamin D and the PCBs, it would have likely manifest with the highly chlorinated congeners that reflect long term contamination (PCB118, PCB138, PCB153 and PCB180), rather than with PCB28, a low chlorinated volatile PCB that is degraded relatively fast and thus reflects acute contamination (19). The reported negative trend for PCB to be associated with vitamin D ($25(OH)D_3$) in pregnancy may have been confounded by weight change (10) and the decreased levels of $25(OH)D_3$ reported in rats may have reflected the dosage of PCBs given (9).

A multitude of factors affect vitamin D levels in humans. These factors include lifestyle, smoking, alcohol consumption, exercise, diet, sun exposure, season, atmospheric components, clothing, sunscreen use and skin pigmentation, as well as age, obesity and the incidence of several chronic illnesses as has been highlighted in a recent report in Brazilian women (20). Whilst it was not possible to control for all these factors in the women included in this study, we did seek to mitigate as many confounders as possible. The women included were of similar age and BMI; all were Caucasian living in the same geographical area of the United Kingdom and all were non-smokers. As all of these women were to undergo IVF, then they all had to stop all alcohol 3 months prior to this study. To circumvent the well-

recognized seasonal fluctuations in vitamin D metabolite levels, vitamin D sampling was performed in this study during the period between March to September, to ensure that vitamin D targets would be achieved with just 9 minutes of sunlight exposure daily in Northern England (21). Vitamin D supplement consumption was an exclusion criterion of the study.

Strengths of this study include the state-of-the-art measurement of the PCBs and vitamin D ($25(OH)D_3$ and $1,25(OH)_2D_3$). Limitations include the small numbers of subjects. That all subjects were Caucasian females could be considered a limitation, as these findings may therefore not be generalizable to male subjects or those of differing ethnicities, but in this case it is also a strength as it avoids the confounding effect of ethnicity as noted above.

In conclusion, our findings show that PCB levels were not associated with $25(OH)D_3$ levels once BMI was accounted for, indicating that the deleterious effects from PCB accumulation are not mediated by effects on $25(OH)D_3$ or its active metabolite $1,25(OH)_2D_3$.

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 polychlorinated biphenyl 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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References

1. Grimm FA, Hu D, Kania-Korwel I, Lehmler HJ, Ludewig G, Hornbuckle KC, et al. Metabolism and metabolites of polychlorinated biphenyls. Crit Rev Toxicol. 2015;45(3):245-72.
2. Guo W, Pan B, Sakkiah S, Yavas G, Ge W, Zou W, et al. Persistent Organic Pollutants in Food: Contamination Sources, Health Effects and Detection Methods. Int J Environ Res Public Health. 2019;16(22).
3. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015;36(6):E1-e150.
4. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2014(1):Cd007470.
5. Osorio Landa HK, Perez Diaz I, Laguna Barcenas SDC, Lopez Navarro JM, Abella Roa MF, Corral Orozco M, et al. Association of serum vitamin D levels with chronic disease and mortality. Nutricion hospitalaria. 2020.
6. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol. 2014;21(3):319-29.
7. Sonne C, Kirkegaard M, Jakobsen J, Jenssen BM, Letcher RJ, Dietz R. Altered vitamin D status in liver tissue and blood plasma from Greenland sledge dogs (Canis familiaris) dietary exposed to organohalogen contaminated minke whale (Balaenoptera acuterostrata) blubber. Ecotoxicol Environ Saf. 2014;104:403-8.
8. Hoydal KS, Ciesielski TM, Borrell A, Wasik A, Letcher RJ, Dam M, et al. Relationships between concentrations of selected organohalogen contaminants and thyroid hormones and vitamins A, E and D in Faroese pilot whales. Environmental research. 2016;148:386-400.
9. Lilienthal H, Fastabend A, Hany J, Kaya H, Roth-Härer A, Dunemann L, et al. Reduced levels of 1,25-dihydroxyvitamin D(3) in rat dams and offspring after exposure to a reconstituted PCB mixture. Toxicological sciences : an official journal of the Society of Toxicology. 2000;57(2):292-301.

10. Morales E, Gascon M, Martinez D, Casas M, Ballester F, Rodríguez-Bernal CL, et al. Associations between blood persistent organic pollutants and 25-hydroxyvitamin D3 in pregnancy. Environment international. 2013;57-58:34-41.
11. Lan T, Liu B, Bao W, Thorne PS. BMI modifies the association between dietary intake and serum levels of PCBs. Environment international. 2021;156:106626.
12. Brennan E, Kumar N, Drage DS, Cunningham TK, Sathyapalan T, Mueller JF, et al. A case-control study of polychlorinated biphenyl association with metabolic and hormonal outcomes in polycystic ovary syndrome. J Environ Sci Health C Toxicol Carcinog. 2022;40(1):86-105.
13. Wang X, Banks APW, He C, Drage DS, Gallen CL, Li Y, et al. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls and legacy and current pesticides in indoor environment in Australia - occurrence, sources and exposure risks. Sci Total Environ. 2019;693:133588.
14. Javed Z, Papageorgiou M, Deshmukh H, Kilpatrick ES, Mann V, Corless L, et al. A Randomized, Controlled Trial of Vitamin D Supplementation on Cardiovascular Risk Factors, Hormones, and Liver Markers in Women with Polycystic Ovary Syndrome. Nutrients. 2019;11(1).
15. Birkett MA, Day SJ. Internal pilot studies for estimating sample size. Statistics in Medicine. 1994;13(23-24):2455-63.
16. Hahn S, Haselhorst U, Tan S, Quadbeck B, Schmidt M, Roesler S, et al. Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association. 2006;114(10):577-83.
17. Konradsen S, Ag H, Lindberg F, Hexeberg S, Jorde R. Serum 1,25-dihydroxy vitamin D is inversely associated with body mass index. European journal of nutrition. 2008;47(2):87-91.
18. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. The American journal of clinical nutrition. 2000;72(3):690-3.
19. Polychlorinated biphenyls [Internet]. Available from: https://www.umweltprobenbank.de/en/documents/profiles/analytes/10062.
20. Santana KVS, Oliver SL, Mendes MM, Lanham-New S, Charlton KE, Ribeiro H. Association between vitamin D status and lifestyle factors in Brazilian women: Implications of Sun Exposure Levels, Diet, and Health. EClinicalMedicine. 2022;47:101400.
21. Webb AR, Kazantzidis A, Kift RC, Farrar MD, Wilkinson J, Rhodes LE. Meeting Vitamin D Requirements in White Caucasians at UK Latitudes: Providing a Choice. Nutrients. 2018;10(4).

290 **Table 1.** Demographics of the 58 female subjects in the population studied.
291

	Female subjects (n=58)	
	Mean	SD
Age (years)	31.9	4.6
BMI (kg/m^2)	25.7	3.7
CRP (mg/L)	2.6	2.5
TSH (mU/L)	2.4	2.2
Free-T3 (pmol/L)	4.8	0.7
Free-T4 (pmol/L)	11.3	1.8
$25(OH)D_3$ (nmol/l)	50.7	25.3
$1,25(OH)_2D_3$ (ng/ml)	0.05	0.02
PCB28 (ng/g Lipid)	2.6	2.2
PCB52 (ng/g Lipid)	6.7	11.1
PCB101 (ng/g Lipid)	2.7	0.6
PCB118 (ng/g Lipid)	5.6	2.1
PCB138 (ng/g Lipid)	12.0	5.7
PCB153 (ng/g Lipid)	15.7	8.4
PCB180 (ng/g Lipid)	13.5	6.3
∑PCBs (ng/g Lipid)	49.2	21.3

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293 *BMI: Body mass index; CRP: C reactive protein; Free-T3: Free Triiodothyronine; Free-T4: Free Thyroxine;*
294 *PCB: Polychlorinated biphenyl*

Figure 1. Correlations of PCBs with vitamin D_3 ($25(OH)D_3$) and $1,25(OH)_2D_3$. Correlations of total PCBs (A) and PCB118 (B) with vitamin D_3 ($25(OH)D_3$). Correlations of total PCBs (C), PCB153 (D), PCB180 (E) and PCB28 (F) with $1,25(OH)_2D_3$. Correlations in the subset of women who were vitamin D_3 ($25(OH)D_3$) deficient with total PCBs (G), PCB153 (H) and PCB180 (I).