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Plasma polychlorinated biphenyl concentrations and immune function in postmenopausal women ☆

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ABSTRACT

Background: Polychlorinated biphenyl (PCB) exposure has been associated with non-Hodgkin lymphoma in several studies, and the immune system is a potential mediator.
Objectives: We analyzed associations of plasma PCBs with immune function measures. We hypothesized that higher plasma PCB concentrations are associated with lower immune function cross-sectionally, and that increases in PCB concentrations over a one year period are associated with decreases in immune function.
Methods: Plasma PCB concentrations and immune function [natural killer (NK) cell cytotoxicity and PHA-induced T-lymphocyte proliferation (PHA-TLP)] were measured at baseline and one year in 109 postmenopausal overweight women participating in an exercise intervention study in the Seattle, Washington (USA) area. Mixed models, with adjustment for body mass index and other potential confounders, were used to estimate associations of PCBs with immune function cross-sectionally and longitudinally.
Results: Associations of PCBs with immune function measures differed across groups of PCBs (*e.g.*, medium- and high-chlorinated and dioxin-like [mono-*ortho*-substituted]) and by the time frame for the comparison (cross-sectional *vs.* longitudinal). Higher concentrations of medium- and high-chlorinated PCBs were associated with higher PHA-TLP cross-sectionally but not longitudinally. The mean decrease in 0.5 μg/mL PHA-TLP/50.0 pmol/g-lipid increase in dioxin-like PCBs over one year was 51.6 (95% confidence interval 2.7, 100.5; $P=0.039$). There was no association between plasma PCBs and NK cytotoxicity.
Conclusions: These results do not provide strong evidence of impaired cellular immunity from PCB exposure. Larger longitudinal studies with greater variability in PCB exposures are needed to further examine temporal associations of PCBs with immune function.

Abbreviations: CDC, Centers for Disease Control and Prevention; Con A, Concanavalin A; CV, coefficient of variation; FHCRC, Fred Hutchinson Cancer Research Center; IMEX, Immune Function and Exercise; LOD, limit of detection; LP, lymphocyte proliferation; NK, natural killer; NHL, non-Hodgkin lymphoma; PBMCs, peripheral blood mononuclear cells; PCB, polychlorinated biphenyl; PHA, phytohemagglutinin; PHA-TLP, PHA-induced T-lymphocyte proliferation; UW, University of Washington

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1. Introduction

Polychlorinated biphenyls (PCBs) continue to be found in human tissues despite the introduction of manufacturing bans in the United States and other countries decades ago (CDC, 2009; Dallaire et al., 2002). Organochlorine pollutants, which include PCBs and organochlorine pesticides/pesticide metabolites, are lipid soluble compounds that do not break down easily in the environment or in humans. The main route of human exposure to organochlorines is through the diet, and these compounds concentrate as they move up the food chain. Circulating blood organochlorine concentrations are regulated by homeostasis between adipose tissue storage and blood lipid levels (Brown and Lawton, 1984).

Exposure to organochlorines has been implicated as a risk factor for non-Hodgkin lymphoma (NHL) (Ballschmiter and Zell, 1980; Hardell et al., 1996; Rothman et al., 1997; Colt et al., 2005, 2009; DeRoos et al., 2005; Engel et al., 2007; Spinelli et al., 2007; Bertrand et al., 2010; Ng et al., 2010), and immune suppression is among the proposed mechanisms for this increased risk. An association between organochlorines and immunosuppressive effects, including decreases in natural killer (NK) cell cytotoxicity and T-lymphocyte proliferation (TLP), has been observed *in vitro* (Daniel et al., 2001; Hammond et al., 2005), in animal studies (Exon et al., 1985; Talcott et al., 1985; Ross et al., 1996; Beckman et al., 2003; Beineke et al., 2005; Mori et al., 2006; Sormo et al., 2009), and in human studies (Lü and Wu 1985; Svensson et al., 1994; Leijs et al., 2009), although associations are not entirely consistent. Toxic effects and potential mechanisms of action of PCBs and related organochlorine pesticides/pesticide by-products appear to vary by structural characteristics. PCB congeners with mono-*ortho*-substituted (dioxin-like) structures exert toxicity through binding to the aryl hydrocarbon receptor, while different mechanisms may exist for other organochlorines (CDC, 2009; Duffy and Zelikoff, 2006; Lyche et al., 2006; Ng et al., 2010). Several studies of NHL found the strongest associations with PCB 180, a moderately chlorinated PCB, compared to other PCB congeners (Bertrand et al., 2010; DeRoos et al., 2005; Spinelli et al., 2007), indicating possible differences in biologic effects between individual, non-dioxin-like congeners that may vary by degree of chlorination. Few studies have examined the association of organochlorines with immune effects in humans (Lü and Wu, 1985; Svensson et al., 1994; Leijs et al., 2009), and no study that we are aware of has reported on the effect of within-person changes in organochlorines with within-person changes in immune function.

We investigated the association between plasma PCB concentrations and immune function in a study of postmenopausal, overweight and obese women living in the Seattle, Washington metropolitan area. We focused on three summed measures of plasma PCBs: dioxin-like (mono-ortho substituted), high-chlorinated (8 or more chlorines), and medium-chlorinated (5–8 chlorines) PCBs (CDC, 2009; Ng et al., 2010). Cellular immune function was evaluated by TLP (lower levels are believed to reflect less effective immune responses) and NK cell cytotoxicity (lower levels may predict risk of future adverse health events) (Levy et al., 1991; Mizutani et al., 1996; Vedhara et al., 1999; Imai et al., 2000; Albers et al., 2005). We hypothesized that (1) higher plasma PCB concentrations are associated with lower baseline immune function cross-sectionally; and (2) increases in PCB concentrations over time are associated with decreases in immune parameters. We based our hypotheses that higher-chlorinated and dioxin-like PCBs, in particular, have immune effects on existing studies of the relationship between PCBs and immune measures (Daniel et al., 2001; Leijs et al., 2009) and between PCBs and NHL (Bertrand et al., 2010; DeRoos et al., 2005; Spinelli et al., 2007).

2. Materials and Methods

2.1. Study population

The study included participants in a previously conducted exercise intervention trial and ancillary study of immune function in sedentary and overweight/obese postmenopausal women. The Physical Activity for Total Health study, conducted at the Fred Hutchinson Cancer Research Center (FHCRC), was a randomized controlled trial comparing the effects of a one year moderate-intensity aerobic exercise intervention, with goal of 225 min/week in a combined facility and home program, *vs.* a stretching control program on sex hormone concentrations (as biomarkers of breast cancer risk) (McTiernan et al., 1999). As previously described, participants were selected to maximize the possible effects of exercise on endogenous sex hormones and to avoid other factors known to affect sex hormones (Irwin et al., 2003). Women were enrolled into the Physical Activity for Total Health study between 1998 and 2000. One hundred and seventy three women from the Seattle, Washington area participated in the study and were evaluated at baseline, three months, and one year. The ancillary study of Immune Function and Exercise (IMEX) was conducted among 115 participants of the Physical Activity for Total Health study that met additional IMEX selection criteria (Shade et al., 2004).

The present study included the IMEX subset of the Physical Activity for Total Health study participants. We excluded participants at baseline or one year who were missing all primary outcome or primary exposure values or total lipid measures necessary to compute lipid-adjusted plasma organochlorine concentrations (Phillips et al., 1989; Schisterman et al., 2005). Six of the 115 IMEX participants were missing these values at baseline, and 17 participants (including three that were lost to follow-up) were missing these values at one year. A sensitivity analysis excluding observations with any missing covariate data in any of the analyses yielded similar results.

2.2. Questionnaires

Information on demographics and smoking history was collected *via* self-administered questionnaire. Questionnaire data also included body weight history, medical history, health habits, medication use, and dietary intake over the past three months. Dietary intake and alcohol consumption over the past three months were assessed using a 120-item food frequency questionnaire designed and validated at the FHCRC (Patterson et al., 1999).

2.3. Body composition measures

Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using a balance-beam scale and a stadiometer at corresponding clinic visits. Both measurements were taken in duplicate and averaged. Body mass index (BMI) was calculated as (weight[kg]/height[m]^2).

2.4. Blood draw

Blood samples were collected for previously described studies conducted in the same population as our study population (Shade et al., 2004; Troen et al., 2006; Campbell et al., 2008). Blood draws took place at the UW Department of Laboratory Medicine. Fasting blood samples were taken between 7:30 and 8:30 AM. Samples were processed within one hour of collection.

2.5. Immune function measures

Assays of cellular immunity were conducted by the UW Department of Laboratory Medicine on blood samples from the baseline and one year IMEX visits. NK cytotoxicity was measured in fresh NK cells using a flow-cytometric assay. NK cells were isolated from peripheral blood mononuclear cells (PBMCs) by the Ficoll-Hypaque separation. Cells were washed and diluted to a mononuclear cell concentration of 7.7×10^6 cells/mL. K562 target cells were washed in the log phase of growth twice and incubated with label 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO; Live/Dead cytotoxicity kit no. L7010; Molecular Probes, Eugene, OR). Cells were incubated, washed, and re-suspended to a concentration of 1×10^6 cells/mL and then filtered through a 35-μm strainer. Culture-suspended NK cells were diluted to four effector-to-target cell (E:T) ratios of 50:1, 25:1, 12.5:1, and 6.25:1, pelleted, and incubated. Propidium iodide was added to a final concentration of 0.03 mg/mL, and cells were transferred to a polypropylene tube for flow cytometric analysis to identify dead cells. The percentage of dead target cells among a total DiO-identified target cells was used as the measure of NK cytotoxicity.

T-lymphocyte proliferation was assessed using cryopreserved PBMCs with ^{3}H-thymidine incorporation in response to the mitogen phytohemagglutinin (PHA), as previously described (Boynton et al., 2007; Campbell et al., 2008). Cells were prepared by the Ficoll-Hypaque separation and frozen in 30% fetal calf serum, 60% RPMI medium, and 10% dimethyl sulfoxide (Gibco, Gaithersburg, MD). For the

^{3}H-thymidine incorporation, PBMCs were incubated with PHA of 0.1 and 0.5 μg/mL in five replicates each. After incubation for 3 days at 37 °C, cells were pulsed for 24 h with ^{3}H-thymidine, harvested, and counted with a β-counter. The PHA-stimulated TLP (PHA-TLP) index was expressed as counts per minute of stimulated cells divided by counts per minute of unstimulated cells.

2.6. Polychlorinated biphenyl measures

Plasma samples available from 111 IMEX participants were analyzed for PCBs at the Persistent Organic Pollutants Biomonitoring Laboratory of the Centers for Disease Control and Prevention (CDC) in Atlanta, GA. Measurements of 36 PCB congeners [International Union of Pure and Applied Chemistry [IUPAC] scheme (Ballschmiter and Zell 1980) nos.: 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 105, 110, 118, 128, 138/158, 146, 149, 151, 153,156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189 194, 195, 196/203, 199, 206, and 209] were conducted by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (Sjödin et al., 2004). Measurements were conducted in samples from the baseline and one year IMEX visits. The plasma sample amount available for the assays ranged from 0.23 g to 0.84 g with a median of 0.38 g. The analytic results were reported as wet-weight concentrations (pg/g plasma) and lipid-standardized concentrations (using previously measured triglyceride and total cholesterol values from the same blood draw) in ng/g-lipid (Phillips et al., 1989; Mohanka et al., 2006).

We limited our study to the 15 PCB congeners detected in >80% of the samples. Repeatability of measurements was excellent, with coefficients of variation (CVs) between blinded quality control replicate samples ranging from 2.4 to 11.2 for these analytes. The number of samples with measured values above the limit of detection (LOD) and the average detection limits for these PCBs have been previously described (DeRoos et al., 2012) and are shown for the PCBs assessed in our primary analyses in Appendix A, Table 1. We imputed values below the LOD with a value equal to the LOD divided by two (DeRoos et al., 2012).

2.7. Statistical analysis

Statistical analyses were performed using Stata 10 (StataCorp, College Station, TX). We used mixed models to investigate the association of PCB concentrations with immune function measures, adjusting for potential confounding factors. We chose primary exposures *a priori* to be lipid-standardized summed dioxin-like PCBs (PCBs 105, 118, and 156), high-chlorinated PCBs (PCBs 194, 196/203, 199, 206, and 209), and medium-chlorinated PCBs (PCBs 138/158, 146, 153, 156, 170, 180, 183, and 187). In a secondary analysis, we examined lipid-standardized summed low-chlorinated PCBs (PCBs 74, 99, 105, and 118). The summed values were obtained by adding the molar concentrations of individual PCBs in each group.

Our primary outcomes were NK cytotoxicity and PHA-TLP, chosen based on associations with PCBs reported in previous studies. We considered these to be independent outcomes. Only NK cytotoxicity % based on effector–target ratios of 12.5:1 and 25.1 were analyzed in this study, as these dilutions were considered to have the greatest reproducibility and were in the linear range (Shade et al., 2004).

To investigate associations between each summed PCB exposure and immune function outcome both cross-sectionally at baseline and longitudinally from baseline to one year of follow-up, we used random-intercept mixed models of the form

$$\boldsymbol{Y_{ij}} = \boldsymbol{\beta_{0i}} + \boldsymbol{\beta_1}\,\boldsymbol{PCB_{i0}} + \boldsymbol{\beta_2}\,\boldsymbol{TIME_{ij}}(\boldsymbol{PCB_{ij}} - \boldsymbol{PCB_{i0}}) + \boldsymbol{\Sigma_1 \hat{s}\alpha_p}\,\boldsymbol{COV_{i0p}} + \boldsymbol{\Sigma_1 \hat{s}\gamma_p}\,\boldsymbol{TIME_{ij}}(\boldsymbol{COV_{ijp}} - \boldsymbol{COV_{i0p}}) + \boldsymbol{\varepsilon_{ij}},$$

where $\boldsymbol{\beta_{0i}} = \boldsymbol{\beta_0} + \boldsymbol{\delta_{0i}}$, and $\boldsymbol{Y_{ij}}$ = immune function for subject i at time j; $\boldsymbol{PCB_{ij}}$ = PCB level for subject i at time j; $\boldsymbol{COV_{ijp}}$ = the pth covariate for subject i at time j; $\boldsymbol{TIME_{ij}}$ = time (years) from baseline visit for subject i at time j ($TIME_{i0}=0$ at baseline; $TIME_{i1}=1$ at one year); $\boldsymbol{\delta_{0i}}$ = residual variation in initial status; and $\boldsymbol{\varepsilon_{ij}}$ = measurement error associated with $\boldsymbol{Y_{ij}}$. Mixed models were fit *via* restricted maximum likelihood using STATA *xtmixed*. At baseline ($TIME=0$), $\boldsymbol{Y_{i0}} = \boldsymbol{\beta_{0i}} + \boldsymbol{\beta_1}\,\boldsymbol{PCB_{i0}} + \boldsymbol{\Sigma_1}\,\boldsymbol{\hat{s}\alpha_p}\,\boldsymbol{COV_{i0p}} + \boldsymbol{\varepsilon_{i0}}$, and $\boldsymbol{\beta_1}$ represents the adjusted baseline cross-sectional estimate of the mean change in immune function measure per unit difference in PCB concentration. This cross-sectional effect estimate was scaled to reflect the magnitude of variation in PCB concentrations and is reported as the mean change in immune function measure per 50 pmol/g-lipid difference in PCB concentration. Between baseline ($TIME=0$) and one year of follow-up ($TIME=1$), $\boldsymbol{Y_{i1}} - \boldsymbol{Y_{i0}} = \boldsymbol{\beta_2}(\boldsymbol{PCB_{i1}} - \boldsymbol{PCB_{i0}}) + \boldsymbol{\Sigma_1 \hat{s}\gamma_p}(\boldsymbol{COV_{i1p}} - \boldsymbol{COV_{i0p}}) + \boldsymbol{\varepsilon_{i1}} - \boldsymbol{\varepsilon_{i0}}$, and $\boldsymbol{\beta_2}$ represents the adjusted estimate of the mean change in immune function measure difference from baseline to one year per unit change in PCB concentration difference from baseline to one year. This longitudinal effect estimate was also scaled to reflect the magnitude of variation in change in PCB concentrations and is reported as the mean change in immune function measure difference from baseline to one year per 50 pmol/g-lipid change in PCB concentration difference from baseline to one year. To evaluate the consistency of our results, we examined in secondary analyses the association between exposures and outcomes cross-sectionally using generalized estimating equations (GEE) with exchangeable working correlations and robust variance estimators.

We selected variables *a priori* for adjustment based on previously described confounders (Johnson et al., 1990; Nieman et al., 1999; Deutch et al., 2003, 2007; Romeo et al., 2007) and biological plausibility. Variables included baseline age (years), race (white non-Hispanic; other), baseline education status (high school or less; vocational school, some college, or associate degree; bachelor degree; master degree or doctorate), baseline smoking status (never; former), blood draw season (winter, spring, summer, and fall), alcohol use (<1, 1–5, and >5 g/day), BMI, and fish intake (<1.5, 1.5–2.4, and >2.5 servings/week). We adjusted for BMI because relationships of excess adiposity with impaired immune function (Marti et al., 2001) and lower plasma concentrations of most PCBs (DeRoos et al., 2012) have been described. We also adjusted for the Physical Activity for Total Health study intervention group (one year moderate intensity aerobic exercise *vs.* stretching control).

3. Results

3.1. Study population

Baseline characteristics of the 109 women in our study are shown in Table 1. Participants had a mean age of 60.6 years and were mostly highly educated (41% with a bachelor's degree or higher) and white (89%). All participants were non-smokers, per inclusion criteria of the parent study, and approximately half were former smokers. Alcohol intake was generally low (<1 g/day for 53% of participants). Forty five percent of participants reported eating less than 1.5 servings of fish per week. Forty seven percent were obese (BMI ≥ 30). There was a very modest change in BMI over one year (mean change −0.2 kg/m^2 in the exercise group, and 0.2 kg/m^2 in the stretching group).

Median (interquartile range) dioxin-like, high-, and medium-chlorinated PCB concentrations were 87.4 (60.4, 134.9), 83.9 (58.1, 109.0), and 407.3 (324.9, 536.9) pmol/g-lipid, respectively, at baseline and 93.9 (69.1, 135.9), 86.4 (66.5, 108.8), and 440.6 (360.9, 545.7) pmol/g-lipid at one year. Older participants were more likely than younger participants to have higher high- and medium-chlorinated plasma PCB concentrations. Changes in PCB concentrations between baseline and one year are described in Table 1. There was little variability in PCB concentrations over time; the median (interquartile range) change in PCB concentrations between baseline and one year was 1.4 (−8.6, 10.1), −0.3 (−8.9, 9.9), and 7.5 (−31.4, 47.9) pmol/g-lipid for dioxin-like, high-, and medium-chlorinated PCBs, respectively. Immune function measure distributions in the study population at baseline and one year are shown in Appendix A, Table 2. There was no significant difference in the mean (standard deviation) absolute number of T cells (CD3+, CD45+) between baseline [1302 (363)] and one year [1344 (412)] or in the percent of T cells between baseline [71 (6)] and one year [71 (7)].

3.2. Cross-sectional associations

Baseline cross-sectional associations of plasma PCB concentrations with immune function obtained *via* mixed models are shown in Table 2. There were positive cross-sectional associations of baseline PCB concentrations with PHA-TLP indices, although confidence intervals were very wide. The mean change in PHA-TLP measure per 50.0 pmol/g-lipid increase in PCB concentration was 4.0 (95% CI 0.6, 7.4) and 26.3 (95% CI 3.4, 49.2) for medium-chlorinated and high-chlorinated PCBs, respectively, with 0.5 μg/mL PHA-TLP, and 1.7 (95% CI 0.1, 3.3) for medium-chlorinated PCBs with 0.1 μg/mL PHA-TLP. There was no significant association of PCB levels with NK cytotoxicity. Cross-sectional GEE models, which included observations from baseline and one year, yielded similar results (not shown).

3.3. Longitudinal associations

Longitudinal associations of plasma PCB concentrations with immune function obtained *via* mixed models are shown in Table 2.

Table 1
Baseline and change (baseline to one year) in plasma polychlorinated biphenyl concentrations (pmol/g-lipid) by participant characteristics.

Characteristic		Baseline			Baseline to one year			
	Baseline *n* (%)	Dioxin-like PCBs[a], median (IQR)	High-chlorinated PCBs[b], median (IQR)	Medium-chlorinated PCBs[c], median (IQR)	One year *n* (%)	Change in dioxin-like PCBs[a], median (IQR)	Change in high-chlorinated PCBs[b], median (IQR)	Change in medium-chlorinated PCBs[c], median (IQR)
Total	109 (100%)				98 (100%)			
Group								
Stretching	60 (55.0)	82.6 (57.8, 134.9)	84.2 (59.8, 107.9)	403.5 (313.4, 529.5)	55 (56.1)	2.0 (−8.7, 8.4)	−2.2 (−9.1, 7.7)	10.6 (−40.0, 48.7)
Exercise	49 (45.0)	91.1 (68.4, 140.3)	82.8 (57.8, 114.7)	408.7 (345.9, 561.7)	43 (43.9)	0.0 (−6.1, 13.1)	3.7 (−8.2, 12.0)	4.0 (−24.1, 42.6)
Baseline age (years)								
50–55	32 (29.4)	79.9 (52.8, 99.3)	56.9 (45.3, 91.7)	369.0 (270.8, 466.9)	27 (27.6)	2.0 (−9.8, 7.3)	−1.4 (−7.6, 7.7)	4.0 (−37.7, 42.6)
56–60	31 (28.4)	76.4 (53.9, 127.9)	69.2 (49.4, 99.7)	378.4 (294.7, 535.3)	25 (25.5)	4.4 (−7.5, 17.0)	1.8 (−7.4, 11.8)	13.0 (−30.5, 63.2)
61–65	16 (14.7)	96.6 (71.7, 131.9)	84.2 (73.4, 106.5)	438.2 (372.7, 574.8)	15 (15.3)	−4.9 (−10.5, 8.0)	−5.1 (−8.2, 3.2)	−11.4 (−40.6, 40.2)
66–70	14 (12.8)	92.6 (60.4, 164.6)	105.4 (88.6, 125.1)	465.7 (395.6, 660.9)	15 (15.3)	−2.2 (−6.1, 8.0)	−10.4 (−17.2, 5.0)	−12.6 (−33.9, 15.5)
71–75	16 (14.7)	105.0 (84.5, 169.5)	134.4 (94.5, 162.9)	551.8 (402.2, 772.6)	16 (16.3)	4.9 (−5.2, 10.4)	6.5 (−2.2, 10.3)	40.3 (−24.1, 80.2)
Race								
White non-Hispanic	96 (88.9)	88.4 (60.0, 134.9)	84.7 (60.3, 111.3)	404.2 (322.7, 536.6)	86 (88.7)	0.4 (−8.6, 10.1)	−0.3 (−9.0, 8.9)	5.4 (−30.9, 42.2)
Other	12 (11.1)	84.8 (58.0, 117.4)	61.7 (44.3, 88.3)	427.7 (328.0, 546.5)	11 (11.3)	5.2 (−3.7, 12.2)	−0.1 (−6.2, 23.0)	48.7 (−16.7, 91.6)
Education								
High school or less	16 (14.7)	113.7 (62.5, 169.5)	91.9 (70.4, 128.0)	438.2 (375.8, 632.8)	17 (17.3)	−4.0 (−9.6, 6.6)	2.1 (−5.6, 10.4)	−12.3 (−30.8, 53.1)
Vocational school, some college, or associate's degree	48 (44.0)	90.1 (63.0, 134.9)	75.4 (51.3, 102.2)	388.6 (290.7, 531.4)	43 (43.9)	2.0 (−7.3, 10.1)	−0.6 (−8.7, 8.8)	16.9 (−29.3, 42.2)
Bachelor's degree	25 (22.9)	86.8 (62.3, 139.3)	97.7 (65.0, 116.7)	466.8 (361.2, 605.4)	22 (22.4)	3.2 (−8.6, 11.3)	−4.2 (−15.3, 2.8)	−12.6 (−37.5, 31.3)
Master's degree or doctorate	20 (18.3)	80.0 (55.7, 97.7)	78.0 (58.1, 109.5)	389.1 (302.0, 417.5)	16 (16.3)	3.8 (−9.4, 17.1)	7.5 (−0.2, 12.4)	17.8 (−24.9, 58.6)
Season of blood draw								
Winter	23 (21.1)	99.4 (60.4, 152.5)	80.3 (51.5, 119.7)	397.1 (304.4, 527.6)	22 (22.9)	−2.3 (−9.4, 6.1)	−2.0 (−10.3, 8.2)	−11.6 (−32.0, 15.5)
Spring	41 (37.6)	82.9 (53.9, 114.2)	70.6 (59.3, 102.5)	389.1 (306.3, 479.7	37 (38.5)	5.4 (−7.3, 12.6)	4.1 (−7.2, 10.2)	29.0 (−32.7, 63.3)
Summer	21 (19.3)	89.2 (60.4, 110.8)	84.4 (55.7, 116.7)	420.3 (320.4, 605.4	17 (17.7)	0.5 (−7.5, 9.3)	−3.3 (−12.3, 5.0)	10.2 (−34.7, 54.0)
Fall	24 (22.0)	102.1 (61.3, 165.8)	93.8 (73.7, 111.3)	478.1 (357.3, 566.3)	20 (20.8)	−2.1 (−10.5, 3.5)	0.7 (−7.6, 10.4)	−4.0 (−30.6, 31.3)
Smoking status								
Never smoker	54 (49.5)	86.4 (63.9, 143.8)	81.5 (56.0, 109.0)	400.0 (320.4, 580.3)	51 (52.0)	−0.6 (−9.9, 13.1)	−1.8 (−8.9, 7.6)	4.9 (−43.3, 52.0)
Former smoker	55 (50.5)	89.3 (58.1, 129.0)	84.4 (61.4, 109.5)	420.3 (324.9, 527.6)	47 (48.0)	1.7 (−6.1, 8.0)	−0.2 (−8.2, 11.0)	9.4 (−29.3, 42.2)
Alcohol intake								
< 1 g/day	58 (53.2)	89.7 (58.1, 149.0)	82.7 (56.0, 113.1)	407.6 (294.7, 567.5)	46 (49.5)	0.9 (−5.3, 10.1)	−0.3 (−8.7, 11.0)	13.2 (−29.9, 45.5)
1–5 g/day	24 (22.0)	96.2 (64.2, 127.3)	76.0 (56.9, 98.7)	392.3 (303.7, 504.9)	28 (30.1)	1.8 (−7.5, 8.6)	1.3 (−11.3, 10.1)	5.1 (−30.9, 55.8)
> 5 g/day	27 (24.8)	82.3 (62.3, 114.2)	92.2 (60.3, 113.1)	426.8 (355.9, 536.9)	19 (20.4)	3.6 (−9.8, 13.1)	3.2 (−7.7, 8.2)	8.1 (−33.9, 52.0)
Fish intake								
< 1.5/week	49 (45.4)	93.0 (58.1, 134.9)	70.6 (59.3, 100.4)	395.6 (320.4, 498.2)	33 (37.5)	2.9 (−5.7, 12.6)	−1.3 (−8.8, 10.2)	2.9 (−31.8, 42.6)
1.5–2.4/week	26 (24.1)	87.1 (59.1, 109.7)	100.6 (56.8, 119.7)	398.2 (294.7, 536.3)	23 (26.1)	1.4 (−3.9, 15.0)	2.8 (−3.5, 11.0)	19.8 (−6.9, 59.2)
> 2.5/week	33 (30.6)	89.3 (68.4, 139.3)	86.5 (69.2, 132.6)	449.3 (375.6, 618.1)	32 (36.4)	−4.4 (−11.4, 9.7)	0.7 (−10.3, 8.8)	10.6 (−64.4, 47.0)
Body mass index (kg/m^2)								
24 to < 30	58 (53.2)	84.5 (59.1, 126.8)	96.5 (65.0, 124.0)	440.9 (352.8, 567.5)	53 (54.6)	2.3 (−5.6, 13.9)	3.9 (−8.2, 11.2)	16.3 (−27.5, 58.6)
30–34	36 (33.0)	102.5 (65.2, 147.5)	77.9 (48.9, 102.2)	398.0 (291.5, 535.8)	33 (34.0)	−2.2 (−9.8, 7.3)	−1.7 (−10.0, 8.9)	−1.9 (−43.3, 40.2)
≥ 35	15 (13.8)	82.9 (57.4, 143.8)	49.8 (45.0, 75.0)	369.7 (273.3, 406.0)	11 (11.3)	0.0 (−19.5, 6.1)	−3.1 (−8.2, 1.7)	−20.0 (−45.0, 42.2)

Interquartile range (IQR); polychlorinated biphenyl (PCB).

[a] PCBs 105, 118, and 156.
[b] PCBs 194, 196/203, 199, 206, and 209.
[c] PCBs 138/158, 146, 153, 156, 170, 180, 183, and 187.

Table 2
Multivariable adjusted[a] effect estimates[b,c] (95% confidence intervals) from mixed models of associations of plasma polychlorinated biphenyl concentrations with immune function.

PCB exposure	Effect estimate	Immune function							
		NK cytotoxicity %				PHA-stimulated lymphocyte proliferation index[d]			
		n	25:1[e]	*n*	12.5:1[e]	*n*	0.1 μg/mL	*n*	0.5 μg/mL
Dioxin-like[f]		186		186		182		182	
	Cross-sectional (β_1)		1.0 (−0.1, 2.1)		1.0 (0.0, 1.9)		1.8 (−1.3, 4.8)		3.2 (−3.3, 9.7)
	Longitudinal (β_2)		0.7 (−5.3, 6.7)		3.2 (−2.2, 8.5)		−19.2 (−41.8, 3.5)		−51.6 (−100.5, −2.7)
Medium-chlorinated[g]		185		185		181		181	
	Cross-sectional (β_1)		0.2 (−0.3, 0.8)		0.2 (−0.3, 0.8)		1.7 (0.1, 3.3)		4.0 (0.6, 7.4)
	Longitudinal (β_2)		−1.0 (−2.8, 0.8)		−0.4 (−2.0, 1.2)		−2.6 (−9.2, 3.9)		−4.6 (−18.9, 9.7)
High-chlorinated[h]		185		185		181		181	
	Cross-sectional (β_1)		−0.3 (−4.1, 3.6)		0.2 (−3.3, 3.7)		7.3 (−3.7, 18.2)		26.3 (3.4, 49.2)
	Longitudinal (β_2)		−4.9 (−11.4, 1.6)		−3.1 (−9.1, 2.9)		−5.1 (−29.2, 18.9)		−3.0 (−55.1, 49.1)
Low-chlorinated[i]		186		186		182		182	
	Cross-sectional (β_1)		0.7 (0.0, 1.4)		0.7 (0.0, 1.3)		1.1 (−0.9, 3.2)		2.0 (−2.4, 6.4)
	Longitudinal (β_2)		0.5 (−2.8, 3.8)		1.9 (−1.0, 4.8)		−9.5 (−22.1, 3.1)		−29.0 (−56.1, −1.9)

Natural killer (NK); polychlorinated biphenyl (PCB); phytohemagglutinin (PHA).

[a] Adjusted for age, race, education, intervention group, smoking status, blood draw season, alcohol intake, fish intake, and body mass index.

[b] Cross-sectional effect estimate (β_1) represents the mean change in immune function measure per 50 pmol/g-lipid difference in PCB concentration.

[c] Longitudinal effect estimate (β_2) represents the mean change in immune function measure difference per 50 pmol/g-lipid change in PCB concentration difference from baseline to one year.

[d] PHA=counts per minute of simulated cells/counts per minute of unstimulated cells.

[e] Effector-to-target ratio.

[f] PCBs 105, 118, and 156.

[g] PCBs 138/158, 146, 153, 156, 170, 180, 183, and 187.

[h] PCBs 194, 196/203, 199, 206, 209.

[i] PCBs 74, 99, 105, and 118.

In general, increases in PCB levels from baseline to one year were associated with decreases in PHA-TLP indices. Although very imprecise, the mean decrease in 0.5 μg/mL PHA-TLP per 50.0 pmol/g-lipid increase in dioxin-like PCBs over one year was 51.6 (95% confidence interval 2.7, 100.5; $P=0.039$). The mean decrease in 0.5 μg/mL PHA-TLP per 50.0 pmol/g-lipid increase in low-chlorinated PCBs over one year was 29.0 (95% confidence interval 1.9, 56.1; $P=0.036$). There was no significant association of PCB level differences over time with NK cytotoxicity % differences (Table 2).

4. Discussion

This is the first study, to our knowledge, to report on associations of plasma PCB concentrations and functional assays of cellular immunity over time. Cross-sectionally, we observed positive associations of PCBs with PHA-TLP, particularly for medium- and high-chlorinated PCBs. We observed a negative association of differences in 0.5 μg/mL PHA-TLP index with differences in dioxin-like and low-chlorinated PCBs over one year. These results must be interpreted with caution given the modest size of the study, the small variability in PCB concentrations over time, and the relatively short duration of the study.

Although counter to our hypothesis, our findings of cross-sectional associations of higher concentrations of medium- and high-chlorinated PCBs with higher PHA-TLP are consistent with previous findings in patients exposed to high levels of PCBs at Yu-Cheng (Lü and Wu, 1985). Phytohemagglutinin-TLP in these patients was noted to be significantly elevated one and three years after cessation of high-level exposure, compared to control subjects, and it was hypothesized that these findings reflected a rebound phenomenon resulting from sub-lethal toxic doses of PCBs (Lü and Wu, 1985). However, compared to levels in our study, PCB levels were several orders of magnitude higher in the Lü and Wu (1985) study.

Our study is not directly comparable to other studies that have used different methods to assess immunity in individuals with lower-level PCB exposures. Leijs et al. (2009) reported a significant negative correlation between current serum levels of dioxin-like PCBs and concentrations of segmental neutrophils in the blood/serum, but immune function was not assessed. Svensson et al. (1994) reported lower absolute numbers of NK cells, but no significant difference in Concanavalin A (Con A)-induced LP, in high- *vs.* non-consumers of fish. High and non-consumers had median wet-weight levels of PCB 118 of 500 and 200 pg/g, respectively (136 pg/g in our study).

The generally smaller cross-sectional effect estimates of dioxin-like, compared to non-dioxin-like, PCBs with PHA-TLP observed in our study are consistent with previous studies. Mori et al. (2006) reported that changes in Con A-induced LP were mostly explained by non-dioxin-like PCBs, particularly medium-chlorinated PCBs 138 and 180, rather than dioxin-like (specifically, coplanar) PCBs in certain marine mammals. Differential effects of dioxin-like and non-dioxin-like PCBs on immune function may reflect different mechanisms of toxicity.

Although variability in exposures was extremely small and effect estimates imprecise in longitudinal analyses, increases in PCB levels from baseline to one year were generally associated with decreases in PHA-TLP. The higher longitudinal effect sizes for dioxin-like compared to non-dioxin-like (medium and high-chlorinated) PCBs may again reflect differences in immunotoxic mechanisms. A similar pattern in effect estimates was observed for dioxin-like and low-chlorinated PCBs. This may be explained by the overlap in PCBs in these two groups; PCBs 105 and 118 are both dioxin-like and low-chlorinated congeners. Although these findings need to first be confirmed in larger longitudinal studies with greater variability in PCB exposures, declines in LP may have public health implications, for example in the elderly, who are also more likely to have had higher PCB exposures. Lymphocyte proliferation has been reported to correlate inversely with mortality in elderly patients and patients with HIV (Albers et al., 2005).

In addition, a reduction in LP may result in impaired tumor immunity by reducing the accumulation of tumor-specific T cells (Datta and Sarvetnick, 2009).

Effect sizes of NK cytotoxicity with PCB levels were generally small. NK cells are involved in the innate immune response and can destroy virally infected cells and tumor cells if activated (Friberg et al., 1996), but the relationship between *in vivo* NK function and NK cytotoxicity assays is unclear. Further, findings of the relationship between PCBs and NK function have not been entirely consistent. Omara et al. (1998) found no evidence of decreased NK cytotoxicity with *in vitro* exposure of rat leukocytes to low levels of PCB mixtures, but dosing of rats with higher levels of PCBs has been reported to cause decreases in NK cytotoxicity (Exon et al., 1985; Talcott et al., 1985).

Strengths of our study include the use of functional assays for characterizing immune status, the strict criteria for blood draws, and the quantitative exposure assessment using plasma PCB concentrations. Polychlorinated biphenyl measures were consistent with previous reports. Baseline plasma PCB concentrations in our study, and unadjusted trends of increasing PCB levels with age, were comparable to those reported in the National Health and Nutrition Examination Survey (NHANES) (CDC, 2009; Patterson et al., 2009).

Our study also has important limitations. First, there was very little variability in PCB concentrations over the one-year time frame of the study. PCB levels are expected to be relatively stable over short time periods in the absence of significant exogenous or endogenous exposures (Carpenter, 2006), and the modest weight change in the Physical Activity for Total Health trial participants contributed to the lack of substantial variability seen in PCB levels. Observation and interpretation of longitudinal changes in immune function are difficult with such small variability in PCB concentrations over time, as the possibility of chance findings exists. We limited the number of comparisons (*e.g.* exposure and immune function outcomes) by identifying *a priori* primary exposure and outcome measures in order to minimize the possibility of chance findings. However, longitudinal studies with greater changes in PCB concentrations are needed to confirm these findings. Second, we used summed PCB measures rather than isolated congener measures. An argument for this approach is that organochlorine mixtures are more commonly present in humans, and our findings may therefore be more biologically relevant than for individual congeners. Third, we adjusted for fish intake over the past three months but not over the longer term. A previous study in the same population reported an association between fish intake over the past three months and a summed PCB measure (DeRoos et al., 2012). However, it is possible that residual confounding by dietary factors or dietary intake over time periods other than those addressed in our study is present.

The extent to which our findings may generalize to populations other than postmenopausal, overweight/obese women, and in other settings, is unknown. It is also unknown whether the NK cytotoxicity assay used in our study is sufficiently sensitive to detect mildly impaired NK function, and PHA-TLP may have been affected by cryopreservation of cells. Future studies should incorporate additional methods, such as assays involving CD107a degranulation and stimulation with anti-CD3/CD28/CD49, to assess NK cell and T-lymphocyte function, respectively.

5. Conclusions

In this study, higher concentrations of medium- and high-chlorinated PCBs were associated with higher PHA-TLP cross-sectionally, and an increase in dioxin-like PCB concentrations was associated with a decrease in PHA-TLP over one year. Larger longitudinal studies with greater variability in PCB exposures are needed to confirm these findings and to further examine temporal associations of PCBs with immune function. Such studies may help elucidate whether negative associations of differences in LP with differences in dioxin-like PCBs over time are relevant to the development of cancers such as NHL.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.03.011.

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