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Higher circulating plasma polychlorinated biphenyls (PCBs) in fit and lean children: The European youth heart study

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ABSTRACT

Background: Lipophilic compounds such as polychlorinated biphenyls (PCBs) are primarily stored in adipose tissue, but exercise-induced lipolysis is able to release PCBs from the adipose tissue into the circulation. The plasma concentration, distribution and metabolism of PCBs can thus vary much among individuals due to inter-human variations in lifestyle behavior and pharmacokinetics.
Objectives: We examined the observational relationship of circulating plasma PCB concentrations with cardiorespiratory fitness, engagement in vigorous physical activity and fat mass in a healthy Danish child population.
Methods: Data on ΣPCB (PCB138, PCB153 and PCB180), cardiorespiratory fitness, skinfold thickness and objectively measured physical activity of 509 children derived from the Danish sub-study of The European Youth Heart Study.
Results: Higher fitness and greater leanness were associated with elevated plasma ΣPCB in both boys and girls. The associations were independent of each other and persisted after controlling for socio-economic status and duration of breastfeeding. We observed an almost three-fold increase in plasma ΣPCB level in the most fit/least fat children relative to the least fit/most fat children. The association between fatness and ΣPCB was strongest for boys as girls, and especially pubertal girls, displayed lower decrease in plasma ΣPCB with higher fat mass.
Discussion: Our findings suggest that increased lipolysis stimulates the release of PCBs into the vasculature. The consequence is higher plasma levels of PCB in very fit and lean subjects. This scenario is likely to cause negative confounding in epidemiological observations of PCB and cardio-metabolic health. At the same time adipose tissue may play a dual role in promoting adverse health and providing a relatively safe place to store PCB.

1. Introduction

Polychlorinated biphenyls (PCBs) have been classified as endocrine disruptors with the ability to cause obesogenic effects in the human body through mimicking, antagonizing or modifying natural hormonal activity (Perkins et al. 2016; Zeliger 2014). However, epidemiological studies have not always been able to detect an association between PCBs and health outcomes or the association has been reversed (Lee et al. 2014). The review by Lee et al. (2014) on chlorinated persistent organic pollutants, obesity and type 2 diabetes (T2D) thus found fifteen studies investigating PCBs and T2D, whereof nine studies (60%) displayed a positive association, five studies (33.3%) displayed no association and one study's results were highly depending on congener. The same review found twenty-four unique studies investigating PCBs and obesity, whereof 24% displayed a positive association, 31% displayed an inverse association and 45% displayed no association (Lee et al. 2014). This divergence distorts the general presumption of PCBs acting obesogenic and stresses the complexity of the human metabolism and individual pharmacokinetics.

Even though the production and use of PCBs were banned decades

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ago, the chemicals still persist in the environment as PCBs bioaccumulate in the food chain (Goralczyk and Majcher 2019). In humans, the most common route of exposure is thus via food intake with a high fat content such as meat and fish (Sipos et al. 2012). Drinking water is a negligible exposure source, whereas non-dioxin-like PCBs are highly present in human milk, hence nursing infants are estimated to absorb 96–97% of higher chlorinated biphenyls (like PCB138, PCB153 and PCB180) from breast milk (McLachlan 1993). Nursing, transplacental and dietary exposure in combination with long half-lives of higher chlorinated biphenyls thus serve as prolonged exposure to PCBs from as early as prenatal life, which have deteriorating consequences for population health.

As PCBs are lipophilic compounds with high affinity for lipid-rich tissues, they accumulate particularly in adipose tissue (Matthews and Dedrick 1984). However, research on levels of circulating PCB following gastric bypass surgery or restricted dieting has showed marked release of PCB from adipocytes to the vascular network as a result of drastic weight loss (Dirinck et al. 2015; Kim et al. 2011). This increased "PCB burden" in the circulation may easily be taken up by cells and induce cellular and systemic toxicity. The phenomenon has been proposed as one explanation for "the obesity paradox" suggesting that obesity is protective by providing a storage compartment for lipophilic compounds, thereby reducing their interaction with more sensitive tissues (Baillie-Hamilton 2002).

Meanwhile, adipose tissue lipolysis is stimulated by engagement in habitual physical activity. Light- to moderate-intensity results in a 5–10-fold increase in fat oxidation above resting state (Krogh and Lindhard 1920). Children, specifically, oxidize more fat than adults at rest (Kostyak et al. 2007) and during exercise (Foricher et al. 2003). Correspondingly, unfit subjects have decreased adipocyte triglyceride turnover and lipolytic activity relative to fit subjects (Horowitz and Klein 2000; Ryden et al. 2013). We therefore suspect that people who exercise regularly or otherwise have a high physical activity level release more PCB in the circulation relative to sedentary individuals because the transition between lipid uptake, storage and release occurs with higher rate and frequency. As opposed to normal-weight individuals, overweight subjects have impaired lipolysis in adipocytes reflecting their current BMI status (Ryden et al. 2013). Counter-intuitively, overweight may thus be protective against circulatory toxicity, because the majority of PCB is stored in adipose tissue, while only little is metabolized or released into the vasculature. Although studies have shown a decrease in PCB body burden with drastic weight loss, it remains unclear whether the decrease is due to elimination or simply redistribution in different nonfat compartments (Kim et al. 2011).

Therefore, this study aims to examine the relationship of circulating plasma PCB concentrations with cardiorespiratory fitness, engagement in vigorous physical activity, and fat mass in a healthy Danish child population. We hypothesize that plasma PCB concentrations are elevated in fit and lean children. If our hypothesis is verified, it may explain some of the divergence in results from studying the obesogenic effect of PCBs.

2. Materials and methods

2.1. Study population

Data derived from the first wave of The European Youth Heart Study (EYHS). The Danish sub-study, which is a prospective cohort study, was founded in Denmark in 1997 by enrolling a random sample of third grade students (age 9–10 years) from twenty-five public schools within the Municipality of Odense. Since then the EYHS has been deployed in Norway, Portugal, Estonia, and Iceland with further endorsement from other European countries. In this study 509 Danish children were eligible due to valid plasma samples out of a total of 589 participating and 771 enrolled children. Written informed consent was obtained from all parents of participating children prior to examination. The study was approved by The Regional Scientific Ethical Committees for Southern Denmark and carried out in accordance with The Code of Ethics of the World Medical Association.

2.2. PCB

A fasting blood sample was collected from the antecubital vein of participants in the morning. Samples were immediately centrifuged and subsequently cryopreserved to −80 degrees. The stored plasma samples were analyzed for eight different congeners of PCB (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB156, PCB180) by liquid chromatography-mass spectrometry at the Department of Environmental Medicine at the University of Southern Denmark (for a detailed description; (Jensen et al. 2014)). However, only three indicator congeners PCB138, PCB153 and PCB180 were included in this study because the sum of their concentrations has been found to account for an average of 50% of PCB exposure from dietary intake. Therefore, it has been suggested to multiply the sum of their concentrations with a factor 2 to estimate the total PCB body burden (Kraft et al. 2017). Since PCBs were manufactured in diverse industrial mixtures e.g. Aroclor® rather than as single individual compounds, it makes further sense to investigate PCB exposure as a sum of the most abundant congeners (Matthews and Dedrick 1984). All PCBs were lipid adjusted to account for fasting state before blood collection. The limit of detection (LOD) was 0.005 μg/g lipid. Values under LOD were imputed to LOD/square root(2) = 0.0035 μg/g lipid for seven individuals. One child had values under LOD for PCB138, PCB153 and PCB180, whereas the remaining six children had values under LOD for PCB180 only.

2.3. Fitness and fatness

Skinfold thickness was assessed with a Harpenden caliper at the biceps, triceps, subscapular, and suprailiac site and presented in millimeters total to express the child's amount of subcutaneous fat. The child's cardiorespiratory fitness was measured indirectly during an incremental ergometer bicycle test, which has previously been validated in children showing high correlation (r = 0.93) between maximal power output and VO_2max (Riddoch et al. 2005). The workload was set to 20 W for children weighting less than 30 kg and 25 W for children weighting 30 kg or more. The workload was increased every third minute until exhaustion. The criteria for a valid test was HR ≥ 185 bpm or subjective observation from the researcher that the child could not continue. Cardiorespiratory fitness was presented as the maximal power output relative to the child's body weight (watt/kg). Physical activity behavior was tracked with waist-worn MTI Actigraph accelerometers. Activity was averaged and stored over 60-seconds epochs for 4 consecutive days, including 2 weekdays and 2 weekend days (for a detailed description of the EYHS protocol; (Riddoch et al. 2005). To meet the physical activity criteria, children should present ≥10 h of recorded daily activity on a minimum of three days. Physical activity was expressed as counts per minute (cpm) and minutes in vigorous intensity defined by bouts of 60 s with activity output > 6000 cpm. An a priori approach in defining cut offs of minutes in vigorous intensity was taken based on the literature that classifies vigorous intensity as activity above ~5000–6000 cpm reflecting running (Trost et al. 2011). We also had to acknowledge the distribution of our study population's physical activity level in respect of statistical power. Since children's activity reflects play more than exercise training, the activity pattern is intermittent with frequent but short bouts of vigorous physical activity. More than five minutes of summed vigorous activity thus indicate a highly active child because it reflects continuous running.

2.4. Co-variates

BMI was calculated from the child's body weight divided by the

child's height squared (kg/m^2). Weight and height were assessed by standard anthropometric procedures. Information on maternal education and duration of breastfeeding was gathered form a parental questionnaire. The mother's highest completed educational level comprised three categories; low educational level (primary and secondary school), medium educational level (vocational education) and high educational level (short/medium/high further education). Duration of breastfeeding was divided into four options; < 1 month, 1–3 months, 4–6 months and > 6 months. Puberty was assessed by a trained researcher of same sex as the study participant using the Tanner scale with pictograms. Puberty was defined based on pubic hair and genital development for boys and pubic hair and breast development for girls. Tanner stage 1 referred to a prepubertal child with no signs of secondary sex characteristics. Tanner stages > 1 indicated that puberty had started.

2.5. Statistics

Difference in mean age, height, weight, BMI, fitness and activity counts per minute between girls and boys was tested with Student's *t*-test. Difference between sexes for categorical variables (pubertal status, maternal education and duration of breastfeeding) was tested with Pearson's chi^2 test. Sex difference in mean skinfold thickness and accelerometer wear time was tested with Wilcoxon rank-sum test because of a positively skewed distribution of data. To further accommodate the positively skewed distribution of plasma ΣPCB, skinfold thickness and accelerometer wear time, we presented these data in median with interquartile range (IQR). Plasma ΣPCB and skinfold thickness were also transformed using the natural logarithm to meet the criteria of normal-distribution in the multiple linear regression models (Table 4). To examine the concentration of ΣPCB in subgroups, we presented crude median levels of ΣPCB (μg/g lipid) across sex, BMI status, fitness level and vigorous physical activity level (Table 2). To further expand on the difference in ΣPCB concentrations across fatness and fitness level, we stratified the study participants into tertiles of fatness and fitness. The tertiles were standardized according to sex as girls represented lower fitness level and higher amount of fat relative to boys (Table 3). We compared the median ΣPCB level of the leanest and most fit children with the least lean and least fit children by linear combinations of coefficients after a linear regression model.

The regression models presented in Table 4 were built up by an iterative process where co-variates were added consecutively. Included co-variates were related to either exposure (age and sex), outcome (breastfeeding) or both exposure and outcome (fatness, fitness and maternal education). Thus, model (1) was adjusted for sex and age, model (2) was adjusted for sex, age and fatness/fitness and model (3) was adjusted for sex, age, fatness/fitness, maternal education and duration of breastfeeding. The adjustment for fatness or fitness depended on the explanatory variable being studied, hence associations of fitness or vigorous physical activity on concentrations of plasma ΣPCB were adjusted for fatness. Likewise, associations between fatness and ΣPCB were adjusted for fitness. Since plasma ΣPCB and fatness (skinfold thickness) were log-transformed, we performed a back-transformation of the beta-coefficients. The beta-coefficients thus represented percent change in ΣPCB concentration as a result of (1) a one-watt per kg increase in fitness, (2) a 10% increase in fatness or (3) an upgrade in physical activity level by one stratum. As a supplement to the beta-coefficients, we appended standardized beta-coefficients to investigate the strength of the associations. We also examined PCB138, PCB153 and PCB180 separately in the regression models to test if the same trend as for ΣPCB was evident for the three most abundant PCBs (Supplemental Material).

Interaction by sex and pubertal status among girls was tested in the linear regression models of fitness and fatness on ΣPCB while adjusting for age (model 1). A post hoc regression analysis elucidating the relationship between fatness and ΣPCB levels stratified by sex (Table 5) and pubertal status among girls (Table 6) were thus performed.

Data were analyzed using Stata IC 16.0 (StataCorp, College Station, Texas, USA) with a significance level of 0.01 (two-sided). Power calculations for the main statistical tests were estimated to 1.0 indicating that our sample size was sufficient in both reduced and full models.

Table 2
Median plasma concentrations of ΣPCB stratified by sex, BMI status, fitness level and amount of vigorous physical activity (n = 509).

	ΣPCB (ng/g lipid)
Sex	
Girls (n = 267)	0.18 (0.12–0.30)
Boys (n = 242)	0.20 (0.13–0.33)
BMI	
Underweight (n = 25)	0.33 (0.22–0.52)
Normal-weight (n = 406)	0.20 (0.13–0.33)
Overweight (n = 53)	0.13 (0.09–0.19)
Fitness (sex-specific tertiles)	
Tertile I (n = 167)	0.13 (0.08–0.20)
Tertile II (n = 167)	0.20 (0.13–0.32)
Tertile III (n = 167)	0.27 (0.17–0.40)
Vigorous physical activity	
< 5 min per day (n = 87)	0.17 (0.11–0.30)
> 5 and < 15 min per day (n = 94)	0.21 (0.12–0.34)
≥15 min per day (n = 148)	0.21 (0.14–0.33)

Median (IQR).
Note: ΣPCB = (PCB138 + PCB153 + PCB180)*2.
Note: Overweight comprising obesity.

Table 1
Descriptive characteristics of the study participants (n = 509).

	N =	Girls	N =	Boys	p-value[a]
Age (years)	267	9.6 (0.4)	242	9.7 (0.4)	< 0.01
Height (cm)	267	138.7 (6.4)	242	139.5 (6.3)	0.15
Weight (kg)	267	33.4 (6.5)	242	33.9 (6.4)	0.36
BMI (kg/m^2)	267	17.3 (2.5)	242	17.3 (2.4)	0.78
Skinfold thickness (mm)[b]	262	33.3 (26.4–46.9)	233	28.8 (22.6–38.8)	< 0.01
Fitness (watt/kg)	261	2.77 (0.54)	240	3.16 (0.59)	< 0.01
Accelerometer wear time (hours/day)[b]	173	12.8 (10.0–14.0)	156	12.4 (9.7–14.7)	0.09
Activity counts per minute (cpm)	173	608.3 (211.8)	156	726.7 (242.4)	< 0.01
Puberty (prepubertal/pubertal)	267	182/85	238	238/0	< 0.01
Maternal education (low/medium/high)[c]	255	82/69/104	221	75/68/78	0.34
Months of breastfeeding (< 1, 1–3, 4–6, > 6)	263	17/74/78/94	230	18/68/71/73	0.37

Mean ± SD.
[a] Difference between girls and boys.
[b] Median (IQR).
[c] Maternal education; low = primary/secondary school, medium = vocational education, high = short/medium/high further education.

Table 3
Median plasma concentrations of ΣPCB (ng/g lipid) over sex-adjusted fitness and fatness tertiles (n = 487).

	Fitness (watt/kg)					
Fatness (mm)	Least fit		Average		Most fit	
Least fat	n = 18	0.21 (0.14–0.28)	n = 54	0.30 (0.18–0.46)	n = 91	0.29 (0.20–0.44)
Average	n = 39	0.16 (0.12–0.21)	n = 65	0.20 (0.14–0.28)	n = 55	0.25 (0.16–0.36)
Most fat	n = 105	0.11 (0.07–0.18)	n = 42	0.16 (0.10–0.24)	n = 18	0.21 (0.15–0.32)

Median (IQR).
Note: ΣPCB = (PCB138 + PCB153 + PCB180) * 2.

3. Results

Descriptive characteristics of the study participants are shown in Table 1. Boys were slightly older and more lean, fit and active compared to girls. On the other hand, a third of the girls displayed signs of onset of puberty, whereas all boys were at the prepubertal stage. Table 2 provides a descriptive overview of the median concentrations of plasma ΣPCB when stratified by sex, BMI status, cardiorespiratory fitness and physical activity level. There was a tendency towards higher plasma ΣPCB with lower BMI, higher fitness and more vigorous physical activity. Although, this tendency was not tested statistically due to subgroups with very low and uneven numbers (e.g. underweight or overweight according to BMI). Thus, we wanted to elaborate these observations by examining the ΣPCB concentration in combined strata of fitness and fatness. From tabulation of a 3x3 contingency table divided into tertiles of fatness (least/average/most) and fitness (least/average/most), results showed a markedly higher plasma ΣPCB level among the most fit/least fat children compared to the least fit/most fat children (Table 3). Children in the highest strata of fitness and lowest strata of fatness thus had 2.64-fold higher circulating plasma ΣPCB relative to children in the highest strata of fatness and lowest strata of fitness (0.20 ng/g lipid, 95% CI 0.15;0.25, p-value < 0.01).

Results from multiple linear regressions showed higher circulating plasma ΣPCB with greater leanness and higher fitness level independent of each other (Table 4). The beta-coefficients represented percentual change in plasma ΣPCB concentration as a result of a one-unit increase in fitness (watt/kg), a 10% increase in fatness (mm) or an upgrade in physical activity level by one stratum. In regard to fitness, a one-unit increase in watt/kg equaling 36% increase in power output for girls (33.4 W) and 32% for boys (33.9 W) was associated with 76% and 45% higher levels of circulating plasma ΣPCB levels unadjusted and adjusted for fat mass respectively. In regard to fatness, a 10% increase in subcutaneous fat mass in girls equaling 3.3 mm and in boys 2.9 mm was associated with 7% and 4% lower circulating plasma ΣPCB levels when not adjusted and adjusted for fitness. Model 1 showed the highest correlation (β) between both fitness and fatness on plasma ΣPCB level. The associations remained statistically significant after controlling for further co-variates, although the strength of the correlations generally attenuated with increasing co-variates. Thus, model 3 shows the independent associations between fitness/fatness and plasma ΣPCB concentrations while controlling for the remaining explanatory variable fatness/fitness, maternal education and duration of breastfeeding. We did not observe a relationship between vigorous physical activity and plasma ΣPCB concentration (Table 4).

To test if the observed findings were evident for each of the three most abundant PCB congeners, we reran the regression analysis separately for PCB138, PCB153 and PCB180. The results appeared similar to the results with ΣPCB (Supplemental Material). Although, we found the most chlorinated congener with six chlor-atoms (PCB180) more strongly associated with fitness and fatness than less chlorinated congeners with five chlor-atoms (PCB138 and PCB153).

As we discovered an interaction by sex in the relationship between fatness and plasma ΣPCB concentration (Boys −2.7%, −5.0; −0.2, p-value 0.03), we performed the multiple linear regression models separately for girls and boys (Table 5). The association was consequently strongest for boys, hence girls experienced lower decrease in plasma ΣPCB concentration with increasing fat mass relative to boys. We sought to examine the sex discrepancy by running the regression models stratified by pubertal status as we found an interaction by pubertal status among girls (Pubertal 4.1%, 0.01; 8.2, p-value 0.05). Prepubertal girls displayed a greater decrease in plasma ΣPCB concentration with increasing fat mass relative to pubertal girls (Table 6).

4. Discussion

The results verify our hypothesis that lean and fit children are exposed to higher levels of circulating plasma PCBs compared to children with greater fat mass and poorer cardiorespiratory fitness. We observed an almost three-fold increase in plasma ΣPCB level among the most fit/least fat children relative to the least fit/most fat children. Both fatness and fitness were independently associated with higher circulating plasma ΣPCB, also when controlling for maternal education and breastfeeding. Even though the estimates attenuated after adjustment, indicating that a part of the variation in PCB level could be explained by behavior supported by socioeconomic status (e.g. fish consumption) and longer duration of breastfeeding, the PCB level still increased 24%

Table 4
Percentual change in plasma concentrations of ΣPCB (ng/g lipid) with increase in fitness, fatness and vigorous physical activity.

	Fitness				Fatness				Vigorous physical activity			
	N	% (95% CI)[a]	β[b]	p-value	N	% (95% CI)[c]	β[b]	p-value	N	% (95% CI)[a]	β[b]	p-value
Model 1	501	76.2 (60.4, 93.5)	0.50	< 0.01	495	−7.0 (−8.1, −5.8)	−0.47	< 0.01	329	4.3 (−4.3, 13.7)	0.05	0.34
Model 2	487	45.2 (29.8, 62.4)	0.33	< 0.01	487	−4.4 (−5.8, −3.1)	−0.30	< 0.01	324	3.6 (−4.5, 11.7)	0.04	0.42
Model 3	387	24.7 (12.6, 38.2)	0.20	< 0.01	387	−5.0 (−6.2, −3.8)	−0.35	< 0.01	256	0.5 (−6.4, 8.0)	0.01	0.88

Note: Model 1–3 describes an iterative process where co-variates are added consecutively. Thus, model 1 is adjusted for sex and age. Model 2 is adjusted for sex, age and fatness/fitness. Model 3 is adjusted for sex, age, fatness/fitness, maternal education (low/medium/high) and duration of breastfeeding (< 1 month, 1–3 months, 4–6 months, > 6 months).
Note: ΣPCB = (PCB138 + PCB153 + PCB180) * 2.
[a] Beta-coefficients represent percent change in dependent variable due to a one-unit increase in independent variable.
[b] β standardized beta-coefficient.
[c] Beta-coefficients represent percent change in dependent variable due to 10% increase in independent variable.

Table 5
Percentual change in plasma concentrations of ΣPCB (ng/g lipid) per 10% increase in fatness stratified by sex.

	Girls				Boys			
	N	% (95% CI)[a]	β[b]	p-value	N	% (95% CI)[a]	β[b]	p-value
Model 1	262	−5.8 (−7.4, −4.2)	−0.40	< 0.01	233	−8.3 (−9.9, −6.6)	−0.54	< 0.01
Model 2	256	−3.3 (−5.2, −1.4)	−0.22	< 0.01	231	−5.7 (−7.7, −3.7)	−0.37	< 0.01
Model 3	207	−4.1 (−5.8, −2.4)	−0.30	< 0.01	180	−6.1 (−7.8, −4.4)	−0.42	< 0.01

Note: Model 1–3 describes an iterative process where co-variates are added consecutively. Thus, model 1 is adjusted for sex and age. Model 2 is adjusted for sex, age and fitness. Model 3 is adjusted for sex, age, fitness, maternal education (low/medium/high) and duration of breastfeeding (< 1 month, 1–3 months, 4–6 months, > 6 months).
ΣPCB = (PCB138 + PCB153 + PCB180) * 2.
[a] Beta-coefficients represent percent change in dependent variable due to 10% increase in independent variable.
[b] β standardized beta-coefficient.

with one-watt/kg bodyweight increase in cardiorespiratory fitness. Meanwhile the strength of the association between fatness and PCB increased with adjustment as evidence of negative confounding. However, we suspect higher lipolytic activity among the very fit and lean children may have contributed to the higher exposure levels as physical activity and exercise stimulate fat mobilization from adipose tissue. During and after muscle work free fatty acid and glycerol are released from fat droplets of the adipocyte into the blood to restore lipid depots in the muscle cell (Polak et al. 2008). In this process fatty acids are likely to carry PCB molecules into the circulation, where they bind to transport protein albumin.

We did not find evidence for a similar association with vigorous physical activity, despite differences in median concentrations of PCB between less active (< 5 min of vigorous intensity per day) and highly active children (≥5 min of vigorous intensity per day). However, physical activity behavior is complex as it reflects everything from sedentary activity (e.g. TV viewing and crafting) to sports participation. Indeed, our study population was very active, which hindered the opportunity of setting the cut point lower as it would leave too few cases in the analysis. Moreover, certain activities were highly underestimated or even unregistered, which caused discordance between the actual energy expenditure and the recorded activity. Weight-bearing activities such as strength training and uphill running and cycling were underestimated as the accelerometer detects moving of center of mass and not force. Since the instrument was not water resistant, activities like swimming were unregistered leaving potential for information bias. Although, cardiorespiratory fitness ought to encompass somewhat physical activity as cardiorespiratory fitness is build up by vigorous physical activity in combination with genetic predisposition (Bouchard et al. 2011). In that regard fitness can work as a proxy for long-term engagement in habitual physical activity without being prone to misclassification as a few days of accelerometer assessment.

The association between fatness and ΣPCB was moderated by sex. In both sexes the concentration of PCBs in the circulation decreased with increasing fat mass, but the decrease was greater for boys than girls. This gender difference could be driven by the third of the girls, who had started puberty as they displayed an equally lower decrease in plasma ΣPCB concentration with increasing fat mass relative to prepubertal girls. It could be differences in fat metabolism and pubertal status compared with boys that contributed to less circulatory detoxification by increasing fat mass in girls. Since the onset of puberty marks a distinct change in human biology and sets the sexes further apart, the partitioning of lipophilic compounds may differ between males and females from this point. As an example, women have smaller lean mass compartment and larger fat mass compartment than men from puberty and throughout adult life (O'Sullivan 2009). At the same time, women store fat more efficiently than men; it has been hypothesized that estrogen is partly responsible for the reduced fatty acid oxidation postprandially and immediately after exercise in women (Wu and O'Sullivan, 2011). With this knowledge, fat mass should otherwise be an offset against plasma PCB accumulation for women in particular. However, in the review by Lee et al. (2014) half of the studies investigating PCBs and obesity also reported gender specific results indicating a general sex discrepancy.

With the verification of our hypothesis we might be able to explain part of the divergence in results from epidemiological observations of the health effects of PCBs. When fitness and leanness act as negative confounders they are able to strengthen or in extreme cases even reverse the association between PCB and an adverse health outcome when appropriately adjusted for. If not adjusted for, the estimate will be biased towards the null hypothesis. An example of negative confounding can be described as follows; because fitness is inversely related to lower risk of many adverse health outcomes e.g. T2D (inverse relationship (−)) (Knowler et al. 2002; Kodama et al. 2009; Tarp et al. 2019; Thomas and Naughton, 2006), and PCBs were positively related to fitness in our study (positive relationship (+)) and to T2D according to the literature (positive relationship (+)) (Lee et al. 2011; Vasiliu et al. 2006), the association of plasma PCB with T2D risk with no adjustment for fitness is expected to be either biased towards the null or in an extreme case in reverse direction of the true association. Previous findings from the European Youth Heart Study reported an inverse relationship between ΣPCB and markers of glucose metabolism. The study found a reduction in fasting insulin and insulin resistance with increasing ΣPCB exposure, which the authors sought to explain by favorable health traits among the highest exposed children, who presented lower BMI, higher fitness, were breastfed longer and had

Table 6
Percentual change in plasma concentrations of ΣPCB (ng/g lipid) per 10% increase in fatness stratified by pubertal status in girls.

	Prepubertal				Pubertal			
	N	% (95% CI)[a]	β[b]	p-value	N	% (95% CI)[a]	β[b]	p-value
Model 1	179	−6.9 (-9.3, −4.5)	−0.38	< 0.01	83	−3.2 (-5.9, −0.5)	−0.25	0.02
Model 2	175	−4.1 (-6.8, −1.4)	−0.22	< 0.01	81	−1.6 (-5.1, 2.0)	−0.12	0.37
Model 3	139	−5.0 (-7.4, −2.6)	−0.30	< 0.01	68	−1.9 (-5.1, −1.5)	−0.14	0.26

Note: adjusted for age.
ΣPCB = (PCB138 + PCB153 + PCB180) * 2.
[a] Beta-coefficients represent percent change in dependent variable due to 10% increase in independent variable.
[b] β standardized beta-coefficient.

mothers with higher education and lower BMI (Jensen et al. 2014). Although the adjustment for these factors did not circumvent nor attenuate the inverse relationship. In the light of our hypothesis, a higher fitness level is likely to have improved the insulin sensitivity while also having stimulated to the release of PCB in the vasculature, hence the highest exposed children displayed better function of insulin as a result of higher cardiorespiratory fitness compared to less exposed children with lower fitness. In that regard, uncontrolled or residual confounding may also be accountable for the 33.3% of studies reporting no association between PCB and T2D and 45% of studies reporting no association between PCB and obesity (Lee et al. 2014). Although most of the studies investigating PCB and T2D included a marker of adiposity as a co-variate, only one study controlled for self-reported physical activity (Wu et al. 2013). Of all the studies reporting no association between PCB and obesity, only one study controlled for self-reported exercise (Lee et al. 2012). However, self-report holds inherent biases due to recall, misclassification and overestimation of positive health behaviors. To add more complexity to the association between lipophilic compounds and adverse health outcomes, fitness and leanness may act as mediators or moderators apart from acting as confounders. A study on aerobic fitness and neurocognitive function thus reported a moderating effect of prenatal methylmercury (Oulhote et al. 2017). Hence, higher aerobic fitness was associated with improved short-term memory and processing speed, but higher levels of methylmercury seemed to attenuate this relationship. In the light of our hypothesis, methylmercury could also mediate the association. If the fitness-induced increment in lipolytic activity results in greater release of lipophilic compounds into the circulation and less retention in lipid-rich tissue, it promotes higher measured exposure levels in the blood with higher fitness level. Previously, Pelletier et al. have compared the total plasma organochlorine concentration (including PCB138, PCB153 and PCB180) of endurance athletes, lean sedentary, and obese individuals (Pelletier et al. 2002). Generally, they found higher concentrations in obese individuals and lowest concentrations in athletes, but low numbers and obvious differences in participant characteristics (e.g. age) circumscribed the study. Moreover, total organochlorines comprised 11 chlorinated pesticides, 14 PCB congeners in addition to a commercial mixture of PCBs (Aroclor 1260), whereof only 4–27% of the compounds differed significantly in concentration across study groups (e.g. PCB138 was higher in obese versus athletes but no difference was observed with PCB153 or PCB180).

5. Strengths and limitations

A considerable strength to this study is the time of exposure as the manufacturing and use of PCBs were more recent in 1997 than today. Even though PCB levels were lower than among 7-year-old Faorese children examined in 1997 (Grandjean et al. 2001), they exceeded those of 12–19-year-old adolescents from NHANES 2001–2002 (Nichols et al. 2007). In our cohort, 64% of the study participants were breastfed for at least 4 months, which was the same as the national level with six out of ten mothers who breastfed their children for 5 months or more in 1986 (Vestermark et al. 1991). Since our study population was born in 1988 of mothers born in the 1950s and 1960s, where PCBs were still available on the market, nursing may have been a significant route of exposure.

Another strength is the completeness of data. As we applied complete-case analysis, we depended on high compliance. Of all children with a valid plasma sample analyzed for PCBs (n = 509), 98% had performed a valid fitness test, 97% had complete measure of skinfold thickness, 65% had objective data on physical activity level and 94% had information on mother's education. The techniques used to detect and measure the PCB concentrations further benefitted from being sensitive with only few samples under LOD. The physiological tests were also highly reliable as they were objective and standardized; cardiorespiratory fitness was assessed on an ergometer bicycle, where others have used less reliable methods (e.g. shuttle run test), physical activity was assessed with accelerometry instead of questionnaire or observation, and body fatness was estimated from skinfold thickness and not BMI.

However, there are some limitations to the study worth mentioning. The cross-sectional design prevents us from making causal deductions, hence the observed associations may be spurious. Measurement error may account for the insignificant associations between physical activity and PCB due to crude segmentation of minutes in vigorous activity. Unknown confounding from socioeconomic status other than maternal education may explain why the fit and lean children, whose mothers' were better educated, had higher plasma PCB concentrations. Although, after controlling for maternal education and duration of breastfeeding the inverse relationship maintained, thus the inverse relationship was not likely to be due to socioeconomic factors entirely. Instead, the considerable difference in plasma PCB between most fit/least fat and least fit/most fat children (also reflected by the size of the standardized beta-coefficients) could suggest a substantial degree of negative confounding in an analysis of plasma PCB and health outcomes when unaccounted for. In addition, adjusting for maternal education may not entirely control for differences in fish consumption and it is possible that fit and lean children have consumed more fish containing PCB compared with unfit and less lean children, which also could explain part of the relationship between fitness, fatness, and PCB. In future studies we recommend collecting information on individual diet and calorie consumption since PCB exposure beyond the breast-feeding period attributes to diet. Furthermore, fit individuals are likely to have higher energy consumption relative to unfit individuals due to higher energy expenditure. In the view of our study, the fit and lean children may have consumed more calories and more food containing PCBs (e.g. fish, meat and dairy products) than less fit and more fat children. However, we assume that without any influence of higher lipolytic activity, dietary fat from consumption of extra calories and more contaminated food would accumulate in lipid-rich tissue instead of in plasma.

Comparing our findings with studies among adults, it is important to keep in mind that differences in results could be attributable to genuine differences in physiology between children and adults. Another limitation is the lack of data on PCB exposure in adipose tissue. Hypothetical examples relying on animal or in silico models have been proposed (Lutz et al. 1977; Verner et al. 2008), but the exact distribution and concentration ratios between tissues remain uncertain among humans. Although, it has been proposed that the exposure of each tissue is proportional to the respective tissue/blood ratios and the concentration in the major tissue depots e.g. adipose tissue (Matthews and Dedrick 1984). According to a hypothetical pharmacokinetic model though, overweight subjects are measured with lower levels of PCB in plasma and adipose tissue relative to normal-weight subjects given similar mass absorption (Wolff and Anderson 1999). In theory, this is because overweight subjects as opposed to lean subjects (1) have larger amounts of PCB stored in adipose tissue relative to plasma due to PCB's higher affinity for lipid-rich tissue rather than the water-soluble plasma and (2) dilute the concentration of PCB by a larger adipose reservoir. Hypothetically, our findings could be biased due to overweight children having lower plasma PCB concentrations not as a result of lower lipolytic activity but as a result of sequestering PCBs to adipose tissue. However, as fitness was independently associated with plasma PCBs, that hypothesis is not likely to explain the whole relationship. Preferably, we would have PCB concentrations from both plasma and adipose tissue during or immediately after a prolonged bout of exercise and at rest to gain further insight into the role of exercise influencing the transport of PCB from adipose tissue to the circulation. The study would also have benefitted from a higher participant number allowing for statistical modelling of a three-dimensional interaction with fitness, fatness and ΣPCB. Such a model would be appropriate in future studies to elucidate the potential synergies between cardiorespiratory fitness,

body fatness and lipophilic compounds.

Counterintuitively, we found an inverse relationship between markers of good health and PCBs displaying higher exposure levels among the fittest and leanest children. Fitness and leanness are valuable and requisite for maintenance of health and prevention of disease related to obesity and physical inactivity. If fitness and leanness cause higher circulating PCB levels and more frequent re-distribution of PCB in tissues such as the liver and skeletal muscle, thereby possibly intoxicating these tissues in addition to the vasculature, it may leave otherwise healthy populations disadvantaged and susceptible. In that way adipose tissue may provide a relatively safe place to store PCB as suggested by Lee et al. (2014). Hypothetically, intoxication of the circulation may partially explain why some people regain weight after a weight-reduction program (Greenway 2015) or why some people are non-responders to health-enhancing exercise programs (Pickering and Kiely 2019).

6. Conclusions

Our investigations suggest that fitness and leanness may act as negative confounders in observational studies examining the toxic effects of PCB. Future investigations of PCB and other lipophilic compounds in relation to health outcomes, are encouraged to carefully consider the body composition and fitness level of their study population as proxies for their lipolytic activity, because fitness and leanness may work as an intensifier of the circulatory toxification. To gain insight into the interaction between PCB (or other lipophilic compounds) and short- and long-term lipolysis from exercise or calorie restriction, an experimental study with manipulation of either exercise or energy intake would be valuable, including adipose tissue biopsies and blood samples to elucidate the distribution and metabolism of PCB with increased lipolytic activity and its effect on health outcomes such as insulin sensitivity.

CRediT authorship contribution statement

Sidsel L. Domazet: Formal analysis, Writing - original draft, Visualization. **Anders Grøntved:** Data curation, Writing - review & editing, Visualization. **Tina K. Jensen:** Writing - review & editing, Funding acquisition. **Niels Wedderkopp:** Investigation, Data curation, Visualization. **Lars B. Andersen:** Conceptualization, Methodology, Data curation, Writing - review & editing.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

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