Influence of the circulating lipids component and options for assessing the link between internal exposure to POPs and health: a compared approach applied to endometriosis

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Introduction

Endometriosis is an estrogen-dependent gynaecological disease whose pathogenesis has been attributed to multiple factors including retrograde menstruation, (epi)genetic dysregulation, immune and/or inflammatory dysfunction. Although the aetiology remains still elusive[1], the environmental factor was suspected to play a role in this disease. Persistent organic pollutants (POPs) are in particular a matter of concern because bioaccumulate in the environment and are stored in body fat, and they therefore have continued potential for adverse health effects. However, results from existing epidemiological studies are still non-convergent as far as the association between POP exposure and endometriosis is concerned. Actually, several challenges appear when one attempt to establish a conceptual causal structure to evaluate the associations between organochlorine compounds (OCs) exposure markers and endometriosis considering the complex interrelationships between exposure, related metabolic effects, and pathological outcomes. On the one hand, circulating lipid profile [2] and adiposity has been reported to be altered among those women with endometriosis, being low amounts of adipose tissue associated with endometriosis [3]. On the other hand, the exposure of adipose tissue to OCs may cause a bimodal disruption of lipid balance, by increasing the adipogenic differentiation and lipogenesis (i.e. obesogenic chemicals), or conversely, enhancing lipolysis. For instance, mice exposed to the dioxin TCDD exhibited decreases in serum lipid levels consistent with AhR-mediated enhancement of dietary fat distribution to the liver [4]. Overall, the formulation of causals models on the associations between POPs and endometriosis becomes especially complex when the disruption of lipid metabolism is actively considered, questioning the extended use of lipid normalization of biomarkers of exposure.

Hence, in this study we aimed to compare different approaches to use circulating biomarkers of exposure to selected OCs on the exploration of associations with endometriosis using real data from a preliminary case-control study performed in "Pays de la Loire", a north-west region of France.

Materials and methods

Population was enrolled as part of a case–control study performed in the Region Pays-de-Loire between 2013 and 2015 on a French population [5]. Case individuals (n=48) were 18 to 45 years old, with surgical diagnosis of DIE first based on clinical examination Controls (n=26) were adult women of similar age and BMI, consulting for other benign gynecological conditions, without any clinical symptoms like chronic pelvic pain, dysmenorrhea, dyspareunia or history of infertility, precluding the diagnosis of endometriosis (tubal ligation, surgery for genital prolapse, ovarian cystectomy). A serum sample (20 mL) was collected the day before surgery.

Chemical analysis

From the initial large array of biomarkers (around 70 measured substances), we have selected several chemicals to illustrate different patterns of association with lipids and endometriosis, including dioxins such us HxCDF (1.2.3.6.7.8-HxCDF) and OCDF, PCBS (PCB 77, PCB 189) or flame retardants (PBDE 183, PBB 153). The

measurements were performed by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) on double sector instruments after electron impact ionization[5]. The lipid content for serum samples was determined with enzymatic kits (Biolabo,Maizy, France) permitting to determine the concentrations of phospholipids (PL), triglycerides (TG), total cholesterol (TC) and free cholesterol (FC). Total serum lipids (TL) were estimated using the formula: TL=1.677*(CT-CL)+CL+TG+PL [6].

Statistical analysis

We used directed acyclic graphs (DAGs) to develop different causal scenarios relating the circulating levels of chemicals, the circulating lipids and the presence of endometriosis, considering some major confounders (Figure 1A-C). The chemical concentrations were first log-transformed and then rescaled by their standard deviations so that OR could be interpreted per 1-SD change in the log-transformed chemical concentration. Univariate linear models were used to evaluate linear associations between biomarkers and total serum lipids. The associations between biomarkers and endometriosis were measured with odds ratios (OR) and their 95% confidence intervals (CI), calculated by unconditional univariate and multivariate logistic regression. Based on the published literature [7-10], we defined four main approaches to deal with circulating levels of lipophilic pollutants included in health risk models (Table 1, Models 1-4). Furthermore, we extended the set of models including the confounding variables, body mass index (BMI, kg/m^2 and age (years) in the crude equations (Table 1, Models 5-8). All the statistical analyses were performed using R (v.3.3.1.) software.

Results and discussion

Descriptive analysis showed significant differences in term of serum lipid content between cases and controls, with lower values for the former group, while on-significant differences were observed for the BMI (Figure 1D-E). The preliminary exploration of linear correlations between circulating biomarker levels and serum lipids revealed a differential trend by using raw versus normalized biomarkers in the linear models. Overall, the use of raw biomarkers (reported to wet weight) resulted in positive association for all chemicals, while negative associations were observed when applying the lipid normalization (reported to lipid weight). In the former case the regression coefficients were statistically significant for TCDD (β=1.89; p = 0.002), OCDF (β=1.29; p = 0.04), PCB 81 (β=1.23; p = 0.01) and PCB 189 (β=1.05 p = 0.006), while the normalized biomarkers with significant coefficients were HxCDF (β= –0.36; p = 0.002), OCDF (β= –0.28; p = 0.015), PCB 77 (β= –0.59; p = <0.001), PBB 153 (β= –0.33; p = 0.009). This differential pattern was previously observed by Porta et al. (2009) who explored the partial correlations between organochlorine compounds (both crude and normalized) and circulating lipids from patients with pancreatic ductal adenocarcinoma.

The main results computed using the eight different models tested are summarized in Table 2. Five out of 9 chemicals showed statistically significant associations for some of the eight models tested. The overall trend was that normalized models resulted in the highest estimates for both, crude and covariate adjusted models. This effect modification, commonly transformed odds ratios below 1 (raw models) to estimates above the unit (normalized models), being especially notorious the case of PCB 77 where the effect size was modulated from OR(95%CI) of 0.8 (0.5-1.4) to 2.7 (1.4-6.2), when compared Model 1 and 2, respectively. Beyond the modification of direction and magnitude of the effect sizes, it is also noteworthy the impact over the precision of estimates and relative quality. On this regard, the 2-stage models exhibited the narrower CIs, while the lipid-adjusted raw models (3 and 7), the largest. The comparison of magnitude and direction of effect size between models point out an underlying effect of lipids on the causal framework between OCs and endometriosis, stressing the need for more comprehensive and integrative exploration of the causal structure.

The use of circulating biomarkers of lipophilic compounds has been previously discussed because the complex underlying relationship between their concentrations with lipids under pathophysiological conditions. In this study we have demonstrated empirically the large influence of the method used to analyse the associations between organochlorine pollutants, serum lipids and endometriosis using real data. Future research is required to better understand the role of lipids and OCs in the pathophysiology of endometriosis.

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Figure 1. Directed acyclic graphs (DAGs) for three different scenarios (A-C) to depict the associations between serum organochlorine compounds (OCs), serum lipids and endometriosis (ENDO). Box-plots illustrating the levels of total lipids (D) and body mass index (E), for cases and controls (* p<0.001).**

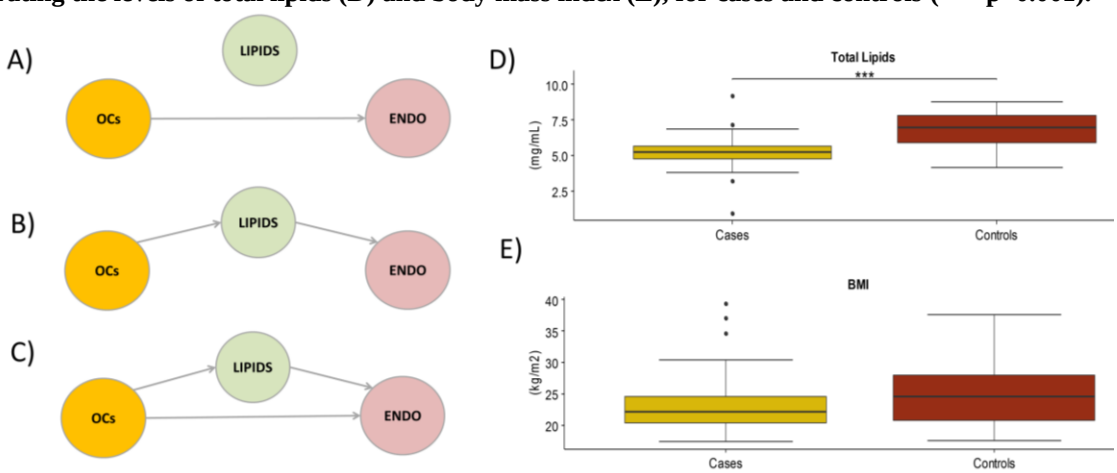

Table 1. Models used to analyse the associations between OCs, lipids and endometriosis. (X_1) raw concentration of serum biomarkers; (TL) total serum lipids; (R) residuals; (C) confounding variables.

Model 1. R. Raw model (biomarkers in wet weight). $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log(X_1)$	**Model 5. R-CA.** Raw model (biomarkers in wet weight) adjusted by confounders. $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log(X_1) + \beta_2 C_2$
Model 2. N. Lipid*normalized (raw serum biomarkers divided by the serum concentration of total lipids). $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log\left(\frac{X_1}{TL}\right)$	**Model 6. N-CA.** Lipid normalized (biomarkers in lipid weight) adjusted by confounders. $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log\left(\frac{X_1}{TL}\right) + \beta_2 C_2$
Model 3. RLA. Raw model adjusted by the lipid levels. $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log(X_1) + \beta_2 TL$	**Model 7. RLA-CA.** Raw model adjusted by the lipid levels and confounders. $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log(X_1) + \beta_2 TL + +\beta_2 C_2$
Model 4. 2S. Two-stage model. $\log(TL) = \beta_o + \beta_1 \log(X_1) + R$ $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log(X_1) + \beta_2 R$	**Model 8. 2S-CA.** Two-stage model adjusted by confounders. $\log(TL) = \beta_o + \beta_1 \log(X_1) + R$ $ln\left(\frac{p_i}{1-p_i}\right) = \beta_o + \beta_1 \log(X_1) + \beta_2 R + \beta_3 C_3$

Table 2. Associations between biomarker of exposure of organochlorine compounds and endometriosis, odds ratios and 95% confidence interval (OR 95%CI), estimated from the 8 different models. *p <0.05; **p <0.01; ***p<0.001.

	HxCDF *OR (95%CI)*	**OCDF** *OR (95%CI)*	**PCB 77** *OR (95%CI)*	**PCB 189** *OR (95%CI)*	**PBDE 183** *OR (95%CI)*	**PBB 153** *OR (95%CI)*
Model 1	0.96 (0.56-1.64)	0.66 (0.36-1.14)	0.82 (0.48-1.40)	0.65 (0.34-1.13)	0.70 (0.40-1.20)	0.57 (0.30-0.96)
Model 2	1.75 (1.01-3.19)	1.31 (0.77-2.27)	**2.72**(1.37-6.22)**	1.01 (0.58-1.70)	1.09 (0.63-1.91)	0.89 (0.51-1.61)
Model 3	1.16 (0.62-2.28)	0.89 (0.43-1.79)	1.27 (0.60-2.79)	1.10 (0.53-2.21)	0.91 (0.45-1.79)	0.58 (0.28-1.09)
Model 4	0.99 (0.91-1.08)	0.93 (0.85-1.01)	0.96 (0.88-1.05)	0.93 (0.85-1.01)	0.99 (0.90-1.08)	**0.88**(0.81-0.96)**
Model 5	0.9 (0.0-16.5)	0.76 (0.40-1.36)	0.90 (0.51-1.59)	**0.14** (0.03-0.48)**	0.83 (0.46-1.47)	0.59 (0.29-1.03)
Model 6	1.8(1.0-3.4)	1.47 (0.84-2.70)	**2.72* (1.36-6.32)**	0.65 (0.23-1.52)	1.27(0.71-2.33)	0.89 (0.50-1.66)
Model 7	1.2(0.0-3.9)	0.98 (0.43-2.13)	1.23 (0.57-2.78)	0.29 (0.06-1.11)	1.07 (0.51-2.32)	0.53 (0.23-1.01)
Model 8	0.9(0.6-1.4)	0.95 (0.87-1.04)	0.98 (0.90-1.06)	**0.82** (0.71-0.95)**	1.01(0.92-1.11)	**0.88**(0.81-0.97)**