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Increased levels of dioxin-like substances in adipose tissue in patients with deep infiltrating endometriosis

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STUDY QUESTION: Are the levels of biologically active and the most toxic dioxin-like substances in adipose tissue of patients with deep infiltrating endometriosis (DIE) higher than in a control group without endometriosis?

SUMMARY ANSWER: DIE patients have higher levels of dioxins and polychlorinated biphenyls (PCBs) in adipose tissue compared with controls without endometriosis.

WHAT IS KNOWN ALREADY: Some studies have investigated the levels of dioxin-like substances, in serum samples, in patients with endometriosis, with inconsistent results.

STUDY DESIGN, SIZE, DURATION: Case–control study including two groups of patients. The study group (DIE group) consisted of 30 patients undergoing laparoscopic surgery because of DIE. In all patients, an extensive preoperative work-up was performed including clinical exploration, magnetic resonance imaging (MRI) and transvaginal sonography. All patients with DIE underwent a confirmatory histological study for DIE after surgery. The non-endometriosis control group (control group), included the next consecutive patient undergoing laparoscopic surgery in our center due to adnexal benign gynecological disease (ovarian or tubal procedures other than endometriosis) after each DIE patient, and who did not present any type of endometriosis.

PARTICIPANTS/MATERIALS, SETTING, METHODS: During the surgical procedure 1–2 g of adipose tissue from the omentum were obtained. Dioxin-like substances were analyzed in adipose tissue in DIE patients and controls without endometriosis.

MAIN RESULTS AND THE ROLE OF CHANCE: The total toxic equivalence and concentrations of both dioxins and PCBs were significantly higher in patients with DIE in comparison with the control group ($P < 0.05$), mainly due to the significantly higher values of the two most toxic dioxins (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [2,3,7,8-TCDD] and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin [1,2,3,7,8-PeCDD]) ($P < 0.01$ for each compound). The levels of furan 2,3,4,7,8-PeCDF were statistically higher in the DIE group compared with controls. Only four congeners of PCBs had toxic equivalence values and concentrations that were statistically higher in patients with DIE, but these included the most toxic and carcinogenic PCB-126 (PCB-114 $P < 0.05$; PCB-156 $P < 0.05$; PCB-189 $P = 0.04$; PCB-126 $P < 0.01$).

LIMITATIONS, REASONS FOR CAUTION: Since few patients were recruited, the study is only exploratory. Our results need to be confirmed in larger and more heterogeneous population studies since environmental and even genetic factors involved in determining dioxins and PCBs widely vary in different countries. Furthermore, the strict eligibility criteria used may preclude generalization of the results to other populations and the surgery-based sampling frame may induce a selection bias. Finally, adipose tissue was obtained only from the omentum, and not from other adipose tissue of the body.

WIDER IMPLICATIONS OF THE FINDINGS: Our results suggest a potential role of dioxin-like substances in the pathogenesis of DIE. Further studies are warranted to confirm our findings.

STUDY FUNDING/COMPETING INTEREST(S): None.

TRIAL REGISTRATION NUMBER: Not applicable.

Key words: endometriosis / deep infiltrating endometriosis / dioxins / dioxin-like substances / adipose tissue

Introduction

Endometriosis is a gynecologic disorder defined by the presence of endometrium outside the uterine endometrial cavity, most commonly in the pelvis. It affects 8–10% of women of reproductive age. It can cause pain and infertility and is a major problem for women, health systems, and society.

At present there is increasing interest and awareness of deep infiltrating endometriosis (DIE), which is a particularly severe form of endometriosis that penetrates >5 mm under the peritoneal surface (Nisolle and Donnez, 1997) and whose diagnosis and treatment are especially complex and challenging (Chapron *et al.*, 1999; Varol *et al.*, 2003; Carmona *et al.*, 2009; Vercellini *et al.*, 2009). DIE is composed of glands and scarce stroma, surrounded by hyperplastic smooth muscle cells and causes severe clinical symptoms (Cornillie *et al.*, 1990; Fauconnier *et al.*, 2002). This disease can invade the pelvic organs and is strongly associated with pelvic pain, dyschezia, dysmenorrhea, dyspareunia and poor quality of life (Fauconnier and Chapron, 2005).

The pathogenesis of endometriosis and DIE is currently under debate in the literature and roles for both environmental (Carmona *et al.*, 2013) and genetic factors (Treloar *et al.*, 2005) have been proposed. Since the 90s, several studies have examined the relationship between environmental exposures, such as dioxins, and endometriosis.

Dioxin-like substances, which compose polychlorinated dioxins (PCDD/F) and biphenyls (PCBs), are halogenated aromatic hydrocarbons and ubiquitous environmental pollutants that are chemically stable and lipid soluble. They mainly enter the body through food, and because they are highly lipophilic accumulate in tissues with a high fat content (Van den Berg *et al.*, 2006). Because they are deposited in fat, the levels of dioxins increase with age in humans and decrease after delivery and breastfeeding (Uemura *et al.*, 2008). Exposure results in a variety of toxic effects in experimental animals and humans including immunologic, neurochemical, neurotoxic, carcinogenic and endocrine changes (Safe, 1990; Huff *et al.*, 1991; Arisawa *et al.*, 2005; Van den Berg *et al.*, 2006).

The biological potency of dioxin-like substances refers to the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), to which a toxic equivalency factor (TEF) of 1 is assigned (Safe, 1990; Van den Berg *et al.*, 2006). Thus, the concentration of these chemicals in adipose and blood is typically expressed in toxic equivalent units (TEQ), which is the product of the concentration of an individual compound and its corresponding TEF (TEQ = concentration × TEF). This approach provides a figure which is useful for comparative purposes (Safe, 1990; Van den Berg *et al.*, 2006).

Some studies have investigated the levels of dioxin-like substances in serum samples in patients with endometriosis but with inconsistent results. Thus, while a number of investigations have found no statistically significant relationship between endometriosis and serum levels of dioxin-like substances (Mayani *et al.*, 1997; Lebel *et al.*, 1998; Pauwels *et al.*, 2001; Fierens *et al.*, 2003; Tsukino *et al.*, 2005; Hoffman *et al.*, 2007; Niskar *et al.*, 2009; Trabert *et al.*, 2010; Cai *et al.*, 2011), other studies have shown that the risk of endometriosis was significantly higher in women with increased serum concentrations of dioxins and PCBs (Heilier *et al.*, 2004, 2005; Louis *et al.*, 2005; Porpora *et al.*, 2006, 2009; Reddy *et al.*, 2006; Gennings *et al.*, 2010; Simsa *et al.*, 2010). The reasons for this discrepancy among previous studies may include a number of different causes such as different control populations and cases, different approaches for the analysis of dioxins and PCBs or the analysis of different congenerers of dioxins and PCBs. Moreover, to be able to detect low concentrations of dioxin-like substances in serum samples a large volume of serum is usually necessary, which is often difficult to obtain in clinical studies (Kitamura *et al.*, 2001, 2005). To the best of our knowledge, only one previous study has analyzed levels of PCBs in adipose tissue from endometriosis patients. It was able to show an association with endometriosis for only for one among the ten organochlorine pollutants analyzed (Buck Louis *et al.*., 2012). We do not know of any study that has investigated levels of dioxins in DIE patients focusing on adipose tissue as a biological indicator to assess the possible participation of xenobiotics in the pathogenesis of this illness.

This is the first study to provide the levels of biologically active and the most toxic dioxins and PCBs in adipose tissue of patients with DIE compared with an appropriate control group.

Materials and Methods

Study design and subjects

For the specific purpose of this study, patients were recruited from a gynecology department of a tertiary referral university hospital located in the Autonomous Community of Catalonia in Spain. Patients were referred to our center for benign adnexal pathology or suspicion of endometriosis. Two groups of patients were included in this case–control study. The study group (DIE group) consisted of thirty patients undergoing laparoscopic surgery because of DIE. In all patients, an extensive preoperative work-up was performed including clinical examination, magnetic resonance imaging (MRI) and transvaginal sonography, and the diagnosis of DIE was highly suspected before surgery. In addition, all patients with DIE underwent a confirmatory histological study for DIE after surgery.

The non-endometriosis control group (control group), included the next consecutive patient undergoing laparoscopic surgery in our center due to benign adnexal gynecological diseases (ovarian or tubal procedures other than endometriosis) after each DIE patient, and who did not present with any type of endometriosis but fulfilled the inclusion and exclusion criteria reported below. Among the controls, conditions warranting surgery were as follows: unilateral ovarian cystectomy, 21 patients (serous cystoadenoma, 10 patients; dermoid cyst, 6 patients; mucinous cystoadenoma, 3 patients; simple functional cyst, 2 patients); bilateral ovarian cystectomy, 3 patients (dermoid cyst, 2 patients; serous cystoadenoma, 1 patient); unilateral salpingectomy, 4 patients (unilateral hydrosalpinx, 4 patients); and bilateral salpingectomy, 2 patients (bilateral hydrosalpinx, 2 patients). No patient in this non-endometriosis control group had a history of pelvic surgery, and there

was no suspicion of endometriosis according to the anamnesis, physical examination, and transvaginal sonography carried out before surgery.

The inclusion criteria were: age between 18 and 40 years and having a normal body mass index (BMI) between 18.5 and 25 kg/m^2. The exclusion criteria were: history of cancer, suspected malignancy, previous abdominal surgery, autoimmune diseases, and any other chronic condition, previous pregnancies, previous breastfeeding, change of body weight >5 kg in the last 5 years. The inclusion and exclusion criteria applied to both cases and controls. The inclusion and exclusion criteria reported above were selected in order to avoid bias when comparing the study and control groups due to individual factors potentially associated with the increased body burden of dioxin-like substances (Nelson *et al.*, 2005; Sasamoto *et al.*, 2006; Uemura *et al.*, 2008; Axelrad *et al.*, 2009). Figure 1 shows the flow chart of inclusion and drop out of patients included in the study. Among 70 eligible patients asked to participate, four (three and one patients in control and study groups, respectively) refused to participate.

All women were Caucasian, had been living in the same area for at least 10 years and reported a mixed diet including meat and fish and no occupational exposure to dioxins or related compounds. A well-structured clinical anamnesis and routine physical examination were carried out in all the patients in order to appropriately collect the individual patient characteristics. All patients gave informed consent to participate in the study, which was approved by the hospital Ethics Committee. Operative laparoscopy was performed as previously reported (Carmona *et al.*, 2009, 2011).

Sample collection and storage

During the surgical procedure 1–2 g of adipose tissue from the omentum were obtained. Adipose tissues were placed into a glass vial on ice and frozen at −20°C, always within 30 min of being excised and were stored at the same temperature until analysis. Although patients were sampled during fasting, this is necessary for measurements of dioxin-like compounds in serum but not in adipose tissue. However, patients were fasting at the time of being studied because they were sampled on the day of surgery.

Dioxin and PCB analyses

Seventeen dioxins (7 polychlorinated-dibenzo-dioxins [PCDDs] and 10 polychlorinated-dibenzo-furans [PCDFs]) and 12 PCBs were analyzed in the present study. They are all known to bind a specific aryl hydrogen receptor, which mediates most of their toxic effects (Safe, 1990; Birnbaum, 1994; World Health Organization, 1997; Mimura and Fujii-Kuriyama, 2003; Van den Berg *et al.*, 2006; Heilier *et al.*, 2008).

The analytical method was previously described elsewhere (Zubero *et al.*, 2009). Briefly, adipose tissue samples (~0.5 g) were spiked with a mixture of ^{13}C labeled dioxin and PCB standards. Fat soluble substances were then extracted by reflux with toluene:cyclohexane (1:1).

Adipose tissue extracts were washed using multilayer silica, basic alumina and carbon adsorbents. PCBs were then eluted from the multilayer silica-alumina column using hexane:dichloromethane (9:1), while PCDD/Fs were eluted from the basic alumina column with hexane:dichloromethane

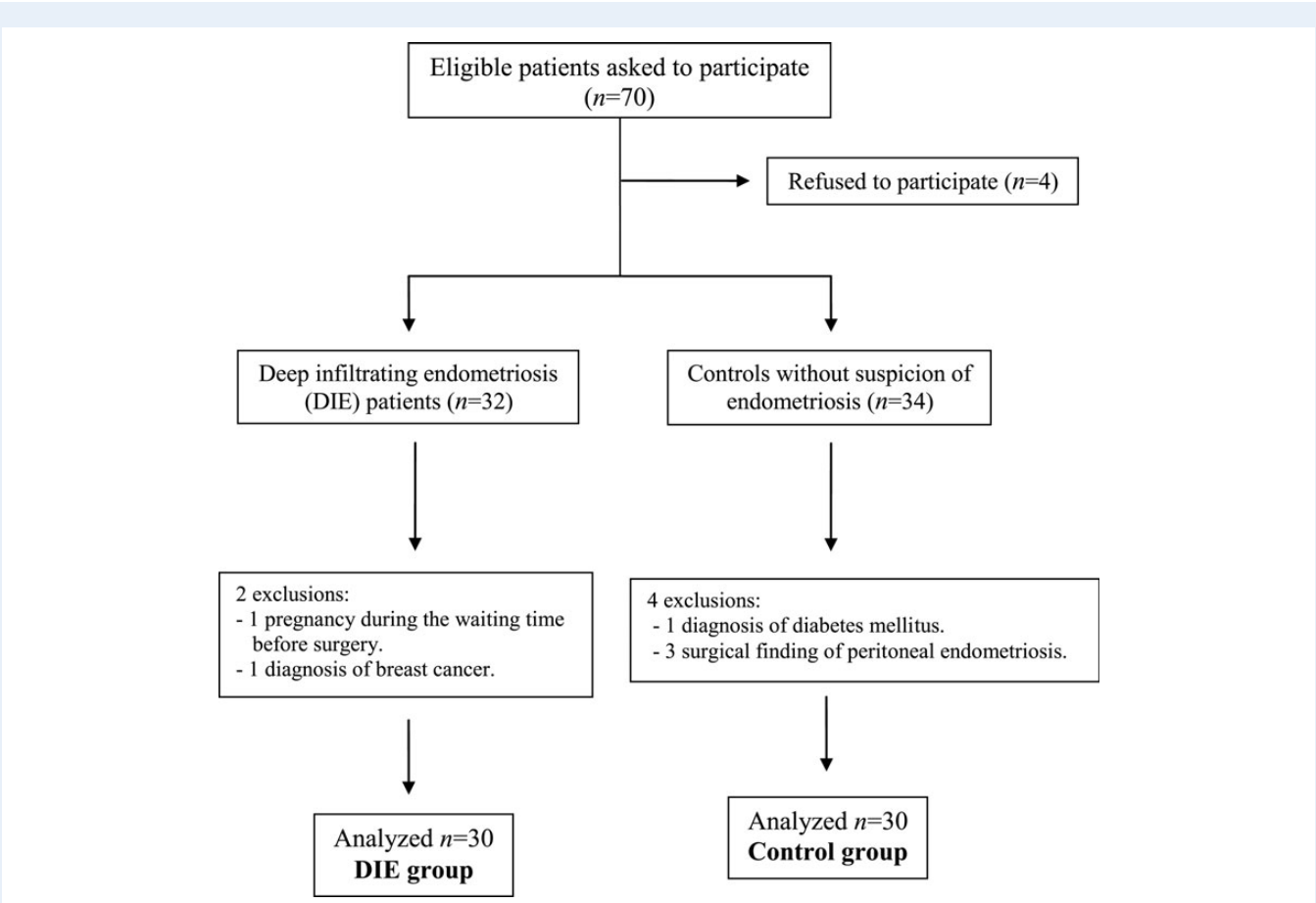

Figure 1 Flow chart of inclusion and drop-out of patients included in this study.

(1:1). The PCDD/Fs fraction was purified using a carbon column (Carbopack C 80/100 mesh, Supelco, Bellefonte, PA, USA) and dioxins were eluted using toluene. Finally, the extracts were concentrated by rotary evaporation, transferred into vials and concentrated to dryness by a gentle stream of nitrogen. To evaluate the recovery rates, final extracts were reconstructed in a known amount of a mixture of labeled $^{13}C_{12}$-PCDD/Fs (EPA-1613ISS, Wellington Laboratories, Inc., Guelph, Canada), $^{13}C_{12}$-DL-PCBs (WP-ISS, Wellington Laboratories, Inc., Guelph, Canada).

Instrumental analysis was based on the use of high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC–HRMS). Analyses were performed on a Trace GC Ultra gas chromatograph coupled with a DFS high-resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) at 10 000 resolving power (10% valley definition). Gas chromatographic separation was performed on a fused silica column DB-5ms GC column (J&W Scientific, CA, USA) for PCDD/PCDFs and DL-PCBs. Quantification was carried out by the isotopic dilution method. Relative response factors were measured for each individual compound by the analysis of six different calibration solutions for PCDDs/PCDFs and DL-PCBs (Abad *et al.*, 2000). The results were expressed as TEQ values using WHO-TEQ (Safe, 1990; Van den Berg *et al.*, 2006).

Dioxin and PCB concentrations in adipose tissue were measured in batches of 10 samples, including 8 patient samples, 1 quality control and 1 blank analysis. In this respect, it is important to note that the statistical analysis took the batch number into account and that, cases and controls were evenly distributed in each batch. Quality assessment and quality control (QA/QC) measures, including certified reference materials were applied. The uncertainty values for the whole method expressed in TEQs is 17% and the intrasample coefficient of variation for dioxins and PCBs is 12.5%.

Table I Limit of quantification (LOQ) of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) (RSD = relative standard deviation; TEQ = toxic equivalence).

Compound	LOQ fat pg TEQ/g (%RSD intra-assay, % RSD inter-assay)	Number of samples below LOQ—Deep Invasive Endometriosis Group	Number of samples below LOQ—Control Group
PCDDs			
2,3,7,8-TCDD	0.22 (11, 21)	0	0
1,2,3,7,8-PeCDD	0.83 (11, 17)	0	0
1,2,3,4,7,8-HxCDD	0.04 (10, 22)	0	0
1,2,3,6,7,8-HxCDD	0.17 (12, 23)	0	0
1,2,3,7,8,9-HxCDD	0.04 (11, 23)	0	0
1,2,3,4,6,7,8-HpCDD	0.03 (11, 22)	0	0
OCDD	0.01 (16, 25)	0	0
PCDFs			
2,3,7,8-TCDF	0.02 (13, 17)	0	0
1,2,3,7,8-PeCDF	0.01 (14, 24)	1	0
2,3,4,7,8-PeCDF	0.55 (13, 23)	0	0
1,2,3,4,7,8-HxCDF	0.08 (14, 24)	0	0
1,2,3,6,7,8-HxCDF	0.07 (14, 24)	0	0
2,3,4,6,7,8-HxCDF	0.03 (16, 22)	0	0
1,2,3,7,8,9-HxCDF	0.00 (15, 25)	1	1
1,2,3,4,6,7,8-HpCDF	0.01 (15, 23)	0	0
1,2,3,4,7,8,9-HpCDF	0.001 (16, 23)	1	1
OCDF	0.0003 (15, 25)	0	0
Mono-ortho PCBs			
PCB-105	0.01 (10, 20)	1	1
PCB-114	0.002 (15, 19)	0	0
PCB-118	0.04 (11, 19)	0	0
PCB-123	0.001 (13, 18)	0	0
PCB-156	0.01 (10, 18)	0	0
PCB-157	0.003 (12, 18)	0	0
PCB-167	0.004 (13, 20)	0	0
PCB-189	0.002 (11, 18)	0	0
Non-ortho PCBs			
PCB-77	0.0002 (10, 18)	0	0
PCB-81	0.0005 (12, 17)	0	0
PCB-126	0.62 (11, 17)	0	0
PCB-169	0.17 (11, 19)	0	0

The latter value is related to the mean %RSD intra-assay in Table I, where the limit of quantification for each chemical measurement in adipose tissue is given. A series of control blanks was analyzed to assess laboratory cross-contamination.

Statistical analyses

Data analysis was performed with the SPSS 18.0 software. The chi-square and the Fisher exact tests were used for comparison of categoric variables. The Mann–Whitney test was used for comparison of continuous variables. Logistic regression was used to adjust the dioxin-like body burden for age, smoking habit and BMI and to estimate the odds ratio (OR) and corresponding 95% CI for each chemical. Results are presented as median (interquartile range). $P < 0.05$ was considered to be statistically significant.

Table II Clinical characteristics of the two study groups.

Parameter	DIE group ($n = 30$)	Control group ($n = 30$)	P
Age (years)[a]	32.5 ± 3.8	31.1 ± 4.9	NS
BMI (kg/m^2)[a]	22.3 ± 2.4	21.9 ± 2.3	NS
Smoker[b]			
Never	23 (76.7)	26 (86.7)	NS
Past	5 (16.7)	1 (3.3)	
Current	2 (6.6)	3 (10)	

DIE group = patients with deep infiltrating endometriosis;
Control group = non-endometriosis controls.
BMI = body mass index.
NS ($P > 0.05$; no statistical differences).
[a]Values are mean ± SD.
[b]Values are *n* (%).

Results

The baseline clinical characteristics of patients were similar in both groups (Table II). All patients recruited were Caucasian and nulliparous. All the patients in the DIE group had rectovaginal endometriosis and some had other types of DIE: sigmoid endometriosis, three patients; bladder endometriosis, three patients; ureteral endometriosis, two patients. Eleven patients had ovarian endometriomas.

Only seven values (0.4% of 1740 analyses) were under the limit of quantification (see Table I) and, as previously reported (Dinse *et al.*, 2014; Park *et al.*, 2010), these cases were excluded from the statistical analyses. The TEQ of dioxins and PCBs in both groups are shown in Tables III and IV. The concentrations of dioxins and PCBs in the DIE and control groups are shown in Tables V and VI. The total concentration for both dioxins and PCBs in adipose tissue was significantly higher in the DIE group as compared with the control group ($P < 0.05$) (Tables V and VI) (Total Dioxins in pg/g lipid DIE group, Median (quartiles): 6.90 (5.6–7.4)); control group: 6.10 (3.63–6.76) (Total PCBs in pg/g lipid DIE group: 4.64 (2.95–6.03); control group: 3.60 (1.36–2.77)). The two most toxic dioxins showed significantly increased TEQ values (TEQ

Table III Toxic equivalence (TEQ) of the dioxins and furans analyzed in adipose tissue samples in the two groups and odds ratios (OR) and 95% CIs for the relationship between each dioxin or furan and deep infiltrating endometriosis (DIE).

	TEQ pg/g lipid Median (interquartile range)		OR (95% CI)	P*
	DIE group ($n = 30$)	Control group ($n = 30$)		
Dioxins				
2,3,7,8-TCDD	0.70 (0.53, 0.76)	0.40 (0.32, 0.64)	1.41 (1.12–2.10)	<0.01
1,2,3,7,8-PeCDD	2.41 (2.12, 2.89)	1.67 (1.11, 2.53)	1.82 (1.36–7.14)	<0.01
1,2,3,4,7,8-HxCDD	0.15 (0.11, 0.18)	0.12 (0.05, 0.17)	1.07 (0.24–1.98)	NS
1,2,3,6,7,8-HxCDD	0.99 (0.80, 1.33)	0.84 (0.37, 1.33)	2.07 (0.64–5.4)	NS
1,2,3,7,8,9-HxCDD	0.14 (0.10, 0.16)	0.11 (0.06, 0.18)	1.26 (0.14–2.10)	NS
1,2,3,4,6,7,8-HpCDD	0.08 (0.07, 0.19)	0.10 (0.07, 0.17)	0.94 (0.62–1.87)	NS
OCDD	0.02 (0.02, 0.03)	0.02 (0.01, 0.37)	0.99 (0.95–1.03)	NS
Furans				
2,3,7,8-TCDF	0.03 (0.03, 0.05)	0.04 (0.035, 0.062)	0.86 (0.36–2.15)	NS
1,2,3,7,8-PeCDF	0.007 (0.004, 0.01)	0.009 (0.006, 0.011)	0.91 (0.63–1.84)	NS
2,3,4,7,8-PeCDF	1.55 (1.28, 1.85)	1.18 (0.84, 1.56)	2.13 (1.97–6.42)	<0.01
1,2,3,4,7,8-HxCDF	0.20 (0.13, 0.23)	0.15 (0.10, 0.17)	1.36 (0.27–3.12)	NS
1,2,3,6,7,8-HxCDF	0.16 (0.12, 0.23)	0.14 (0.11, 0.17)	1.09 (0.16–2.01)	NS
2,3,4,6,7,8-HxCDF	0.07 (0.05, 0.10)	0.07 (0.05, 0.10)	1.05 (0.16–1.94)	NS
1,2,3,7,8,9-HxCDF	0.01 (0.01, 0.03)	0.02 (0.01, 0.03)	0.89 (0.39–1.14)	NS
1,2,3,4,6,7,8-HpCDF	0.02 (0.01, 0.04)	0.02 (0.01, 0.02)	1.02 (0.64–1.92)	NS
1,2,3,4,7,8,9-HpCDF	0.002 (0.001, 0.004)	0.003 (0.002, 0.004)	0.96 (0.53–2.07)	NS
OCDF	0.0003 (0.0002, 0.0005)	0.0005 (0.0003, 0.0009)	0.84 (0.17–1.43)	NS

OR (95% CI): odds ratio and 95% confidence interval for the association with deep infiltrating endometriosis.
*Mann–Whitney test. NS ($P > 0.05$; no statistical differences).

Table IV Toxic equivalence (TEQ) of the polychlorinated biphenyls (PCBs) analyzed in adipose tissue samples in the two groups and odds ratios (OR) and 95% CIs for the relationship between each PCB and deep infiltrating endometriosis (DIE).

	TEQ pg/g lipid Median (interquartile range)		OR (95% CI)	P*
	DIE group (n = 30)	Control group (n = 30)		
Mono-ortho PCBs				
PCB-105	0.03 (0.027, 0.058)	0.03 (0.018, 0.57)	0.92 (0.60–1.73)	NS
PCB-114	0.01 (0.008, 0.014)	0.006 (0.003, 0.013)	2.47 (1.24–5.64)	<0.05
PCB-118	0.17 (0.12, 0.25)	0.11 (0.09, 0.28)	1.16 (0.55–2.14)	NS
PCB-123	0.002 (0.002, 0.003)	0.002 (0.001, 0.003)	1.02 (0.46–1.84)	NS
PCB-156	0.10 (0.075, 0.20)	0.07 (0.03, 0.13)	1.84 (1.12–3.08)	<0.05
PCB-157	0.02 (0.010, 0.040)	0.01 (0.007, 0.002)	1.44 (0.74–2.26)	NS
PCB-167	0.03 (0.20, 0.065)	0.02 (0.010, 0.045)	1.35 (0.22–2.01)	NS
PCB-189	0.02 (0.014, 0.04)	0.01 (0.003, 0.028)	1.52 (1.09–3.15)	0.04
Non-ortho PCBs				
PCB-77	0.0009 (0.0005, 0.002)	0.001 (0.001, 0.004)	0.64 (0.17–1.19)	NS
PCB-81	0.0009 (0.0007, 0.001)	0.002 (0.001, 0.003)	0.56 (0.23–1.32)	NS
PCB-126	3.10 (2.9, 4.7)	2.45 (1.41, 4.57)	1.62 (1.21–2.17)	<0.01
PCB-169	0.98 (0.69, 1.57)	0.56 (0.32, 1.05)	1.54 (0.82–1.97)	NS

OR (95% CI): odds ratio and 95% confidence interval for the association with deep infiltrating endometriosis.
*Mann–Whitney test. NS ($P > 0.05$; no statistical differences).

Table V Concentration of the dioxins and furans (pg/g lipid) analyzed and their total concentration in adipose tissue samples in the two groups and odds ratios (OR) and 95% CIs for the relationship between each dioxin or furan and deep infiltrating endometriosis (DIE).

	DIE group (n = 30)	Control group (n = 30)	OR (95% CI)	P*
Dioxins				
2,3,7,8-TCDD	0.70 (0.54, 0.76)	0.40 (0.32, 0.64)	1.41 (1.12–2.10)	<0.01
1,2,3,7,8-PeCDD	2.41 (2.12, 2.89)	1.67 (1.11, 2.53)	1.82 (1.36–7.14)	<0.01
1,2,3,4,7,8-HxCDD	1.45 (1.01, 1.80)	1.23 (0.52, 1.70)	1.16 (0.72–2.08)	NS
1,2,3,6,7,8-HxCDD	9.20 (8.00, 13.1)	8.40 (3.72, 13.37)	1.03 (0.86–1.77)	NS
1,2,3,7,8,9-HxCDD	1.21 (0.91, 1.58)	1.10 (0.61, 1.79)	1.11 (0.55–1.91)	NS
1,2,3,4,6,7,8-HpCDD	7.80 (6.56, 18.22)	10.22 (7.28, 16.97)	0.67 (0.24–1.47)	NS
OCDD	68.10 (59.45, 91.00)	61.20 (39 70 112 72)	1.21 (0.63–1.92)	NS
Furans				
2,3,7,8-TCDF	0.34 (0.29, 0.47)	0.42 (0.35, 0.62)	0.62 (0.24–1.27)	NS
1,2,3,7,8-PeCDF	0.23 (0.12, 0.37)	0.30 (0.21, 0.40)	0.81 (0.31–1.43)	NS
2,3,4,7,8-PeCDF	4.98 (4.06, 6.12)	3.95 (2.81, 5.21)	1.94 (1.27–5.16)	<0.01
1,2,3,4,7,8-HxCDF	1.75 (1.28, 2.18)	1.50 (1.04, 1.73)	1.09 (0.91–1.49)	NS
1,2,3,6,7,8-HxCDF	1.44 (1.18, 2.18)	1.40 (1.08, 1.78)	1.10 (0.87–1.36)	NS
2,3,4,6,7,8-HxCDF	0.62 (0.48, 0.99)	0.74 (0.47, 0.99)	0.91 (0.46–1.59)	NS
1,2,3,7,8,9-HxCDF	0.11 (0.069, 0.27)	0.18 (0.068, 0.26)	0.62 (0.24–1.16)	NS
1,2,3,4,6,7,8-HpCDF	1.78 (1.14, 3.01)	1.83 (1.11, 2.81)	0.94 (0.63–1.71)	NS
1,2,3,4,7,8,9-HpCDF	0.15 (0.12, 0.33)	0.28 (0.20, 0.39)	0.59 (0.23–1.11)	NS
OCDF	0.87 (0.61, 1.57)	0.65 (0.47, 0.87)	1.19 (0.72–1.47)	NS
Dioxins + Furans	6.90 (5.6, 7.4)	6.10 (3.63, 6.76)	1.72 (1.16–3.15)	<0.05

OR (95% CI): odds ratio and 95% confidence interval for the association with deep infiltrating endometriosis.
*Mann–Whitney test. NS ($P > 0.05$; no statistical differences).

Table VI Concentrations of the polychlorinated biphenyls (PCBs) analyzed and total concentration of PCBs in adipose tissue samples in the two groups and odds ratios (OR) and 95% CIs for the relationship between each PCB and deep infiltrating endometriosis (DIE).

	DIE group ($n = 30$)	Control group ($n = 30$)	OR (95% CI)	P*
Mono-ortho PCBs				
PCB-105	1162.00 (874.00, 1675.75)	1037.50 (597.75, 1929.25)	1.46 (0.64–2.95)	NS
PCB-114	325.00 (257.75, 482.00)	202.50 (105.25, 445.25)	2.24 (1.41–7.31)	<0.05
PCB-118	4695.00 (4039.00, 7956.25)	3861.50 (3185.50, 9394.00)	1.97 (0.84–3.15)	NS
PCB-123	65.60 (53.07, 110.30)	63.2 (40.60, 115.85)	1.04 (0.66–1.47)	NS
PCB-156	3152.00 (2490.25, 6585.00)	2609.00 (1016.00, 4463.25)	3.26 (1.98–6.15)	<0.05
PCB-157	615.00 (481.25, 1107.75)	429.00 (225.25, 904.75)	1.42 (0.74–2.01)	NS
PCB-167	1035.00 (721.25, 1830.00)	783.00 (471.25, 1553.00)	1.16 (0.83–1.73)	NS
PCB-189	662.00 (497.75, 1253.50)	509.00 (111.95, 951.00)	1.67 (1.16–2.10)	0.04
Non-ortho PCBs				
PCB-77	8.50 (4.77, 15.9)	17.45 (10.75, 42.35)	0.71 (0.25–1.16)	NS
PCB-81	2.80 (2.12, 4.50)	6.45 (3.72, 11.75)	0.54 (0.16–1.09)	NS
PCB-126	29.30 (19.45, 41.67)	24.50 (14.12, 45.75)	1.89 (1.21–2.25)	<0.01
PCB-169	31.90 (23.45, 49.22)	18.65 (11.02, 35.05)	1.91 (0.84–3.02)	NS
PCBs	4.64 (2.95, 6.03)	3.60 (2.47, 6.08)	1.97 (1.36–2.77)	0.01

OR (95% CI): odds ratio and 95% confidence interval for the association with deep infiltrating endometriosis.
*Mann–Whitney test. NS ($P > 0.05$; no statistical differences).

median (quartiles) in pg/g lipid 2,3,7,8-TCDD DIE group: 0.70 (0.53–0.76); control group: 0.40 (0.32–0.64); TEQ median (quartiles) in pg/g lipid 1,2,3,7,8-PeCDD DIE group: 2.41 (2.12–2.89); control group: 1.67 (1.11–2.53) ($P < 0.01$ for each compound)) (Table III). The levels of furan 2,3,4,7,8-PeCDF were statistically higher in the DIE group compared with controls. The individual TEQs of only four PCBs were statistically higher in patients in the DIE group than in those in the control group, including the most toxic and carcinogenic PCB-126 (Table IV) (TEQ in pg/g lipid median (quartiles) PCB-114: DIE group: 0.01 (0.008–0.014); control group: 0.006 (0.003–0.57) ($P < 0.05$); PCB-156: DIE group: 0.10 (0.075–0.20); control group: 0.07 (0.03–0.13) ($P < 0.05$); PCB-189: DIE group: 0.02 (0.014–0.04); control group: 0.01 (0.02) ($P = 0.04$); PCB-126: DIE group: 3.10 (2.9–4.7); control group: 2.45 (1.41–4.57) ($P < 0.01$)). Logistic regression analysis with adjustment for age, smoking habit and BMI showed no differences in dioxin-like chemical body burden.

Discussion

Our study shows, for the first time, that the total concentration of dioxins and PCBs in adipose tissue is higher in patients with DIE compared with controls without endometriosis. The TEQ values and concentrations of the most toxic dioxins (2,3,7,8-TCDD and 1,2,3,7,8-PeCDD) and the most toxic PCB (PCB-126) were statistically higher in patients with DIE.

The possible role of exposure to environmental chemicals as a co-causal factor in the etiology of endometriosis has been the subject of scientific debate over the last 20 years with contradictory results (Rier and Foster, 2003; Heilier *et al.*, 2008; Guo *et al.*, 2009; Bruner-Tran and Osteen, 2010; Carmona *et al.*, 2013). This discrepancy among previous studies may be explained by the different control populations and cases included (i.e. patients with different types and severity of endometriosis), different methods for assessment of the dioxins and PCBs used, as well as the analysis of different dioxins and PCBs. Moreover, most studies have focused on assessing the association between dioxins and/or PCBs and peritoneal endometriosis (Heilier *et al.*, 2008). Besides other forms of endometriosis, deep endometriotic nodules of the recto-vaginal septum are considered as a distinct clinical entity with a specific histopathogenesis (Nisolle and Donnez, 1997). Interestingly, Heilier *et al.* (2004, 2005) observed a significantly increased risk for this specific form of the disease associated with PCBs and dioxin-like compound concentrations in serum. This association was not statistically significant for peritoneal endometriosis, suggesting that in humans organochlorines might preferably induce the deep endometriotic form of the disease. Other studies have not considered these different forms of endometriosis when investigating the dioxin-like body burden (Mayani *et al.*, 1997; Lebel *et al.*, 1998; Pauwels *et al.*, 2001; Fierens *et al.*, 2003; Louis *et al.*, 2005; Tsukino *et al.*, 2005; Porpora *et al.*, 2006; Reddy *et al.*, 2006; Hoffman *et al.*, 2007; Niskar *et al.*, 2009; Porpora *et al.*, 2009; Gennings *et al.*, 2010; Simsa *et al.*, 2010; Trabert *et al.*, 2010; Cai *et al.*, 2011). Therefore, no additional information is available in this regard.

Despite the dioxin-like body burden increasing with age and BMI (Uemura *et al.*, 2008; Caspersen *et al.*, 2013), no differences were found in dioxin-like body burden after adjustment for age and BMI in this study. This may be explained, at least in part by the small sample size of our study and the relatively narrow range of age of the 60 patients included (24–39 years old), with as many as 42 (70%) being between 28 and 35 years of age. The present study has several strengths. First, we included only patients with surgically confirmed DIE and controls who

had undergone laparoscopy for other benign illnesses, confirming the absence of all forms of endometriosis (Heilier et al., 2008). Second, all patients came from the same geographic area, and therefore, cases and controls were similar regarding their exposure to dioxin-like substances (Koppen et al., 2002). Third, to our knowledge this investigation was the first to analyze dioxins and PCBs in adipose tissue in patients with DIE. Only one previous study has investigated concentrations of PCBs but not dioxins in adipose tissue in endometriosis patients. However, data regarding DIE patients were not provided (Buck Louis et al., 2012). In previous studies exposure to dioxins and PCBs was assessed by means of serum concentrations. Since dioxin-like substances are lipophilic and the serum lipid content is extremely low (about 0.5% fat), when differences in terms of concentrations are expected to be very low, discrimination between patients and controls becomes a challenge. This scenario becomes much more critical when considering difficulties to analyze clinical serum samples related to the large volume of samples usually needed, a fact previously stressed by others (Kitamura et al., 2001, 2005). Furthermore, dioxins and PCBs accumulate in tissues with a high fat content and, therefore, adipose tissue seems to be more representative of the body burden of dioxin and dioxin-like substances, and is considered to be the preferred indicator of biological human exposure (Aronson et al., 2000; Allam and Lucena, 2001; Quintana et al., 2004; Whitcomb et al., 2005). Finally, the present study included nulliparous patients, who had not previously breastfed and were comparable in terms of age, thereby making the groups comparable and avoiding these possible biases. This is important considering that it has been demonstrated that parturition or lactation can be an efficient way to lose dioxins, resulting in decreased maternal levels of TCDDs (Nelson et al., 2005; Sasamoto et al., 2006). Moreover, the body burden of dioxins and PCBs generally increases with age (Uemura et al., 2008; Axelrad et al., 2009). Again, most previous studies did not take into account patient age, parity or history of breastfeeding.

The current study also has some limitations. First, the sample size was decided arbitrarily but in keeping with previous studies investigating dioxins in patients with DIE (Heilier et al., 2004, 2005). Sample size planning for a clinical study is generally based on an estimate from prior information and performed to ensure the ability to detect a difference in outcome (Daya, 2006; Röhring et al., 2010). The scientific community has appropriately accepted that only systematic reviews and meta-analyses combining high-quality evidence from many trials yield robust answers. Individual trials are best viewed as providing important information that contributes to the larger body of evidence (Guyatt et al., 2008). Being the first report on the TEQ values in adipose tissue in patients with DIE, the present study lacked previous information on the subject at study planning. Thus, the number of patients recruited was small from a biometric point of view, allowing the study to only be exploratory in nature, but it is intended to stimulate future larger, adequately powered investigations to address this issue and provide a larger basis for eventual meta-analysis to help clarify the value of the approach investigated. On the other hand, our results need to be confirmed in larger heterogeneous population studies since environmental and even genetic factors involved in determining dioxins and PCBs vary widely in different countries (Kogevinas, 2001). In addition, the lack of generalization to other populations given the strict eligibility criteria, and the potential for selection bias given the use of a surgery-based sampling frame are also limitations. A selection bias is possibly based on the selection of controls, since women undergoing surgery for other conditions may not represent the frequency of dioxin-like chemical concentrations in the underlying population that gave rise to the cases in this study (Upson et al., 2013a,b, 2014). Another potential limitation in this study is that socioeconomic and lifestyle factors potentially associated with dioxin-like body burden were not available. However, according to data in the literature, this relationship has not been well established (Uemura et al., 2008; Humblet et al., 2010; Caspersen et al., 2013). Therefore, our findings should be confirmed in larger population studies, including patients and controls representative of different geographic areas and without any other gynecological processes. To the best of our knowledge, no previous study has reported the potential differences in dioxin and PCB levels among different adipose tissue compartments.

In conclusion, this study shows that DIE patients have higher TEQ values of dioxins and PCBs in adipose tissue compared with controls without DIE, thus, suggesting a potential role of dioxin-like substances in the pathogenesis of this illness. Further studies are warranted to confirm our findings.

Authors' roles

M.A.M.-Z.: contributed to the study design, recruitment of patients, sample extraction, results analysis and manuscript drafting and critical discussion. L.M.: performed sample analysis, results analysis and contributed to manuscript drafting. J.P.: contributed to the study design, sample analysis and results analysis. E.A.: contributed to the study design, sample analysis, results analysis and manuscript drafting and critical discussion. J.L.C.: contributed to the recruitment of patients, sample extraction and manuscript preparation. B.v.B.: contributed to the study design, results analysis and critical discussion. M.T.G.: contributed to the study design, results analysis and manuscript preparation and critical discussion. J.B.: contributed to the study design, results analysis, manuscript preparation and critical discussion. F.C.: contributed to the study design, results analysis, manuscript preparation and critical discussion.

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Conflict of interest

None declared.

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