Inflammatory and Cardiometabolic Risk on Obesity: Role of Environmental Xenoestrogens

Diana Teixeira, Diogo Pestana, Cristina Santos, Luísa Correia-Sá, Cláudia Marques, Sónia Norberto, Manuela Meireles, Ana Faria, Ricardo Silva, Gil Faria, Carla Sá, Paula Freitas, António Taveira-Gomes, Valentina Domingues, Cristina Delerue-Matos, Conceição Calhau, and Rosário Monteiro

Departments of Biochemistry (D.T., D.P., S.N., M.M., A.F., R.S., C. Sá, C.C., R.M.), Health Information and Decision Science (C.San.), Endocrinology, Diabetes and Metabolism (P.F.), S. João Hospital General Surgery, Pedro Hispano Hospital (A.T.G.), Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal; Center for Research in Health Technologies and Information Systems (D.P., C.San., G.F., C.C.), 4200-450 Porto, Portugal; Rede de Química e Tecnologia - Instituto Superior de Engenharia (L.C., V.D., C.D.M), Instituto Politécnico do Porto, 4200-072 Porto, Portugal; Faculty of Nutrition and Food Sciences (A.F.), University of Porto, 4200-465 Porto, Portugal; Rede de Química e Tecnologia, Laboratório Associado de Química Verde (A.F.), Faculdade de Ciências, Universidade do Porto, 4169-009 Porto, Portugal; and Department of General Surgery, Oporto Hospital Center (G.F.), 4050-313 Porto, Portugal

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Context: Some chemicals used in consumer products or manufacturing (eg, plastics, pesticides) have estrogenic activities; these xenoestrogens (XEs) may affect immune responses and have recently emerged as a new risk factors for obesity and cardiovascular disease. However, the extent and impact on health of chronic exposure of the general population to XEs are still unknown.

Objective: The objective of the study was to investigate the levels of XEs in plasma and adipose tissue (AT) depots in a sample of pre- and postmenopausal obese women undergoing bariatric surgery and their cardiometabolic impact in an obese state.

Design and Participants: We evaluated XE levels in plasma and visceral and subcutaneous AT samples of Portuguese obese (body mass index $\geq$ 35 kg/m^2) women undergoing bariatric surgery. Association with metabolic parameters and 10-year cardiovascular disease risk was assessed, according to menopausal status (73 pre- and 48 postmenopausal). Levels of XEs were determined by gas chromatography with electron-capture detection. Anthropometric and biochemical data were collected prior to surgery. Adipocyte size was determined on tissue sections obtained during surgery.

Results: Our data show that XEs are pervasive in this obese population. Distribution of individual and concentration of total XEs differed between plasma, visceral AT, and subcutaneous AT, and the pattern of accumulation was different between pre- and postmenopausal women. Significant associations between XE levels and metabolic and inflammatory parameters were found. In premenopausal women, XEs in plasma seem to be a predictor of 10-year cardiovascular disease risk.

Conclusions: Our findings point toward a different distribution of XE between plasma and AT in pre- and postmenopausal women, and reveal the association between XEs on the development of metabolic abnormalities in obese premenopausal women. **(*J Clin Endocrinol Metab* 100: 1792–1801, 2015)**

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Abbreviations: AT, adipose tissue; BMI, body mass index; CVD, cardiovascular disease; HbA1c, glycated hemoglobin; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment value for insulin resistance; HOMA-2B, homeostasis model assessment value for β-cell function; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MCP1, monocyte chemotatic protein-1; *p,p'*-DDE, dichlorodiphenyldichloroethylene; R_S, Spearman correlation coefficient; scAT, subcutaneous AT; vAT, visceral AT; XE, xenoestrogen; ΣXE, sum of plasma XEs.

Polychlorinated pesticides, widespread in the environment, such as metabolites of dichlorodiphenyltrichloroethane, α-, β- and γ-hexachlorocyclohexane (HCH), as well as hexachlorobenzene (HCB) and aldrin, have estrogenic properties, being called environmental estrogens [xenoestrogens (XEs)] (1).

Environmental estrogens have several unique features that distinguish them from other common chemicals. Due to their long half-life and lipophilicity, they accumulate in adipose tissue (AT) and move within the body bound to lipids (2). Furthermore, humans are exposed to XEs as chemical mixtures due to the coexistence in the environment, food webs, and long-term retention in AT, resulting in virtually everyone in modern society having some XE exposure (3, 4).

Moreover, there is accumulating evidence of potential impacts of XE exposure on human health that might be mediated by a variety of mechanisms, including endocrine disruption locally at the AT and in components of the human immune system (5). Additionally, XE bioaccumulation in AT, a dynamic organ involved in the integrative network that maintains global energy homeostasis, may result in high local concentrations and lead to metabolic disruption in adipocytes (6). On the other hand, it is recognized that chronic low-grade inflammation and an activation of the immune system are involved in the pathogenesis of obesity-related comorbidities and the AT is an important site of inflammation in presence of obesity (7).

Although recent epidemiological evidence has linked environmental chemicals with obesity, insulin resistance, and cardiovascular disease (8–10), few studies link XEs with the complications arising with obesity when it is already installed. In this regard, we have recently reported interesting positive associations between AT concentrations of persistent organic pollutants, some of them with xenoestrogenic activity, and metabolic abnormalities among a sample of obese patients undergoing bariatric surgery. The associations were stronger with chemicals present in visceral AT (vAT). Furthermore, it was highlighted that these anthropogenic chemicals favored dysmetabolism despite the presence of obesity, proposing a shift on the focus to their dysmetabolic, and not only the obesogenic, effect (11).

The reduction of circulatory estrogens is a key factor in the onset of cardiovascular disease (CVD) during the menopausal period. Disturbances of this endocrine signal lead to the development of metabolic syndrome and a higher CVD risk in women, associated with predominant abdominal fat accumulation, even if in premenopausal women (12). However, the relationship between XEs in the presence/absence of these hormones and the cardiometabolic profile of obese women during menopause is not clear.

Moreover, few studies have reported correlations between plasma and AT concentrations of certain XEs and metabolic traits according to menopausal status. Thus, the present study was performed to investigate the levels of XEs in plasma and AT depots in a sample of pre- and postmenopausal obese women undergoing bariatric surgery and their cardiometabolic impact in an obese state.

Materials and Methods

Participants

The study involved 121 obese women (73 premenopausal and 48 postmenopausal as classified after evaluation by clinical endocrinology) with an age range of 19–61 years undergoing bariatric surgery (gastric banding or Roux-en-Y) at the General Surgery Department, S. João Hospital (Porto, Portugal), who were recruited between January 2010 and June 2011. Patients met the criteria for obesity surgery according to the latest criteria of the country's Department of Health. This investigation was conducted according to the Declaration of Helsinki, approved by the hospital's ethics committee. All participants provided written informed consent. Sociodemographic characteristics, anthropometric characteristics, clinical history, lifestyle factors, parity, and occupation were collected from the Medical Support System of S. João Hospital.

Clinical and biological parameters

Anthropometrics, adiposity-related markers, and various clinical variables were measured at baseline. Body mass index (BMI; kilograms per square meter) was calculated from the measured body weight and height. Analysis of blood samples collected after an overnight fast was performed in the Department of Clinical Pathology of S. João Hospital. Routine serum chemistries were measured using conventional methods with an Olympus AU5400 automated clinical chemistry analyzer (Beckman-Coulter, Izasa). Biological parameters evaluated included fasting blood glucose and insulin, lipid profile [total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides] and high-sensitivity C-reactive protein (hsCRP). LDL cholesterol was calculated according to Friedewald's equation (13). The homeostasis model assessment value for insulin resistance (HOMA-IR) and β-cell function (HOMA-2B) were calculated as described previously (14). Lastly, we used the Framingham risk score to estimate the 10-year CVD risk (a person's chance of developing CVD in the next 10 years) of these individuals (15).

Assessment of XE concentrations in plasma and adipose tissue

XEs were quantified in plasma samples stored at −80°C collected prior to surgery. The samples were analyzed by a previously published methodology (16), with some modifications, involving solid-phase extraction and a final determination using a gas chromatograph coupled to an electron capture detector. A total of five XEs were measured: aldrin (purchased from Pestanal; Fluka), *p,p′*-dichlorodiphenyldichloroethylene [dichloro-

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diphenyldichloroethylene (*p,p′*-DDE); purchased from Chem Service], HCB (purchased from Pestanal Riedel-de Haën), Σ-hexachlorocyclohexane (sum of α-HCH, β-HCH, and δ-HCH, purchased from Sigma-Aldrich), and lindane (purchased from Pestanal Riedel-de Haën). The recovery percentages of samples spiked with a mixture of XEs were higher than 80%. Agreement in the retention time in the sample and in the reference standard was also required to confirm a positive result. Results were expressed as nanograms per milliliter of plasma and represent a mean of three individual injections of the same sample. XE concentrations were expressed in wet-weight levels because lipid-standardized model produces large biases and appears poorly suited for investigations in regard to cardiometabolic risk (17).

In AT samples [scAT and vAT collected during bariatric surgery], XEs analysis was performed according to the method described by Fernandes et al (18).

Cytokine ELISA

IL-6, IL-10, and monocyte chemotatic protein-1 (MCP1) concentrations in plasma were measured using, respectively, LEGEND MAX human IL-6, IL-10, and MCP1 ELISA kits (BioLegend Inc), according to the manufacturer's instructions.

Statistical analyses

Statistical analyses were performed using SPSS (22.0 version statistical software; IBM Corp). Data were described as frequencies and median (minimum, maximum). A Mann-Whitney test was used to compare clinical and biological characteristics and median of XEs between pre- and postmenopausal women. Friedman tests were used to compare XE levels in plasma, vAT, and scAT, both in pre- and postmenopausal women. The strength of the association between XE concentrations in plasma and AT and various parameters was estimated by Spearman correlation coefficients (R_S). To evaluate contribution of the different variables to plasma concentration of MCP1 (log transformed) or 10-year CVD risk (log transformed), we performed a linear regression analysis. The independent variables were included in the models as a priori knowledge, ie, all the possible covariates with a significant correlation with a dependent variable. A multivariable linear model were adjusted with age, vAT adipocyte area, and sum of plasma XEs (ΣXEs) as independent variables and MCP1 (log transformed) as the dependent variable. Other linear models were adjusted with the number of years of obesity evolution, plasma MCP1 levels, and ΣXEs in plasma as the independent variable and 10-year CVD risk (log transformed) as the dependent variable. All tests were two tailed and $P < .05$ was regarded as significant.

Results

Clinical and biological parameters

The comparison of biological and clinical characteristics between pre- and postmenopausal women is shown in Table 1. Postmenopausal women were obese for more years (37.0 vs 53.0 years, $P < .001$) than premenopausal women. Regarding anthropometric parameters, there was no difference in BMI and waist to hip ratio between the two groups. vAT (4242.7 vs 3834.9 μm^2, $P = .037$) and scAT (6625.4 vs 6134.9 μm^2, $P = .036$) adipocyte area was higher in postmenopausal women. With respect to glucose metabolism, postmenopausal women presented higher fasting glucose (97.0 vs 91.0 mg/dL, $P < .001$) and glycated hemoglobin (HbA1c) (5.8% vs 5.6%, $P < .001$) than premenopausal women. There was no significant difference in HOMA-IR, but HOMA-2B was lower in postmenopausal women (116.5% vs 156.6%, $P = .003$). We found no significant difference in plasma lipid profile or in either systolic or diastolic blood pressure between the two groups. Regarding inflammatory markers, there were no differences in IL-6, IL-10, MCP1, and hsCRP. As expected, the 10-year CVD risk was significantly higher in postmenopausal women (13.3% vs 3.7%, $P < .001$) (Table 1).

XEs in plasma

Among the selected XEs, in premenopausal women, the most frequently detected compound in plasma was ΣHCH (sum of α-HCH, β-HCH, and δ-HCH), present in 79.5% of the samples, followed by HCB, found in 72.6% of the samples. On the contrary, aldrin could be measured only in 6.8% of the samples. In postmenopausal women, the most frequently detected compound found in plasma was HCB, present in 87.5% of the samples, followed by ΣHCH, found in 52.1% of the samples. HCH lindane and *p,p′*-DDE were measured in 18.8% of the samples obtained from postmenopausal women (Table 2). *p,p′*-DDE was not found in the plasma of the premenopausal women and aldrin in postmenopausal women. However, when the Mann-Whitney test was used to compare pre- and postmenopausal women, the median of HCH lindane level (23.61 vs 5.81 ng/mL, $P = .041$) was significantly higher in premenopausal women (Table 2). Median concentrations of the sum of all XE (ΣXEs) present in plasma did not differ significantly between the pre- and postmenopausal women (41.43 vs 35.71 ng/mL, $P = .306$), and for both groups of women, HCB accounted for more than half of the XE plasma burden.

XEs in vAT and scAT

We examined the presence of the five studied XEs both in vAT and scAT samples in pre- and postmenopausal women (Table 2). In premenopausal women, the percentage of samples with detectable concentrations of XEs was 90.4% with median values of 106.72 (0.28, 687.18) ng/g of fat in vAT and 94.5% with median values of 61.62 (1.44, 368.06) ng/g of fat in scAT. In postmenopausal women, the percentage of samples with detectable concentrations of XEs was 97.9% with median values of 141.62 (2.51, 601.32) ng/g of fat in vAT and 97.6% with median values of 161.52 (0.41, 557.17) ng/g of fat in scAT. The most frequently detected XEs, the ΣHCH ap-

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Table 1. Clinical and Biological Characteristics of the Patients

Parameters	Premenopausal Women		Postmenopausal Women		*P* Value
	n	Median (Minimum, Maximum)	n	Median (Minimum, Maximum)	
Age, y	73	37.0 (19.0, 59.0)	48	53.0 (36, 62)	<.001[a]
Obesity evolution, y	73	16.0 (5.0, 33.0)	48	24.0 (4.0, 49.0)	.005[a]
Anthropometric and morphometric measurements					
BMI, kg/m^2	73	44.1 (36.8, 56.2)	48	44.6 (36.0, 60.0)	.560
Waist to hip ratio	60	0.85 (0.75, 1.14)	37	0.90 (0.77, 1.08)	.552
vAT adipocyte area, μm^2	70	3834.9 (2001.9, 7220.4)	47	4242.7 (2512.2, 6486.9)	.037[a]
scAT adipocyte area, μm^2	73	6134.9 (2693.3, 10 294.2)	46	6625.4 (3511.6, 11 000.1)	.036[a]
Plasma lipid profile					
Total cholesterol, mg/dL	73	199.0 (114.0, 300.0)	48	210.5 (118.0, 357.0)	.117
Total triglycerides, mg/dL	73	120.0 (53.0, 261.0)	48	114.0 (57.0, 274.0)	.609
HDL cholesterol, mg/dL	73	51.0 (31.0, 81.0)	48	54.0 (32.0, 97.0)	.061
LDL cholesterol, mg/dL	73	131.0 (58.0, 206.0)	48	136.0 (62.0, 290.0)	.324
Glucose homeostasis					
Fasting glycemia, mg/dL	73	91.0 (73.0, 153.0)	48	97.0 (75.0, 297.0)	<.001[a]
HbA1c, %	71	5.6 (4.8, 6.9)	45	5.8 (5.2, 10.3)	<.001[a]
HOMA-IR	70	2.2 (0.1, 7.8)	36	1.8 (0.4, 8.4)	.354
HOMA-2B, %	70	156.6 (13.3, 370.7)	36	116.5 (42.5, 265.9)	.003[a]
Blood pressure					
Systolic blood pressure, mm Hg	58	130.0 (100.0, 171.0)	37	140.0 (109.0, 184.0)	.098
Diastolic blood pressure, mm Hg	58	80.0 (60.0, 120.0)	37	80.0 (63.0, 155.0)	.230
Inflammatory parameters					
IL-6, pg/mL	53	32.7 (4.3, 129.5)	45	30.9 (7.5, 99.0)	.695
IL-10, pg/mL	64	4.8 (0.3, 104.0)	45	3.4 (0.1, 117.4)	.267
IL-6 to IL-10 ratio	52	5.17 (0.47, 317.96)	42	7.60 (0.44, 213.13)	.076
MCP1, pg/mL	67	113.8 (17.0, 792.7)	48	106.1 (20.0, 417.7)	.628
Other parameters					
hsCRP, mg/L	45	3.4 (0.3, 37.0)	29	4.2 (0.3, 45.0)	.373
Estradiol, pg/mL	22	59.4 (15.0, 193.0)	32	26.5 (10.0, 110.0)	.030[a]
Monocytes, %	66	6.0 (0.4, 9.8)	44	6.1 (0.2, 10.2)	.707
10-Year CVD risk, %	51	3.7 (0.3, 27.8)	37	13.3 (1.9, 34.7)	<.001[a]

Ten-year CVD risk was calculated according to D'Agostino et al (15).

[a] Statistical analysis was performed with a Mann-Whitney test: $P < .05$.

peared in 90.4% of vAT and scAT in premenopausal women, also being detected in 95.8% of both vAT and scAT samples in postmenopausal women. Regarding the percentage of XE detection in vAT, HCH lindane was found in 28.8% vs 22.9%, HCB in 5.5% vs 8.3%, aldrin in 15.1% vs 29.2%, and *p,p′*-DDE in 23.3% vs 33.3% of vAT samples from pre- and postmenopausal women, respectively. On the other hand, when comparing the absolute concentration in each AT depot, we verified that the medians of the XEs in vAT were not different between the two groups. Concerning scAT, HCH lindane was found in 23.3% vs 17.0%, HCB was found in 5.5% vs 8.3%, aldrin in 15.1% vs 29.2%, and *p,p′*-DDE in 32.9% vs 43.8% of the samples for pre- and postmenopausal women, respectively. Concentrations of ΣHCH (42.24 vs 98.51 ng/g of fat, $P = .018$), *p,p′*-DDE (1.55 vs 6.21 ng/g of fat, $P = .003$), and ΣXEs (61.62 vs 161.52 ng/g of fat, $P = .020$) were higher in the scAT of postmenopausal women. In this AT depot, ΣHCH was the compound that contributed most in most samples to the total scAT burden of XEs. Finally, in premenopausal women the concentration of XEs in vAT was significantly higher compared with scAT (106.72 vs 61.62 ng/g of fat, $P = .002$). The plasma concentration of XEs was lower than that found in vAT and scAT in pre- and postmenopausal women.

Association between XEs in plasma and AT

Paired-sample comparisons was made among the three evaluated compartments (XEs levels in plasma and the two ATs) both in premenopausal and postmenopausal women (see Supplemental Table 1). In both pre- and postmenopausal women, there was a different distribution of XEs among the three analyzed locations. Although premenopausal women had higher XE accumulation in vAT followed by scAT and plasma, the levels of XEs in vAT and scAT from postmenopausal women were similar, with much lower plasma levels. The levels of XEs in both AT depots were positively correlated in pre- ($R_S = 0.488$, $P < .01$) and postmenopausal women ($R_S = 0.545$, $P < .01$). In premenopausal women, XEs levels in vAT were positively correlated with plasma levels ($R_S = 0.642$, $P < .01$), but plasma levels were not correlated with scAT XEs. In

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Table 2. XE Levels in Plasma (Nanograms per Milliliter) and in Both vAT and scAT (Nanograms per Grams of Fat) of the 121 Patients

	%[a]	Premenopausal Women	%[a]	Postmenopausal Women	*P* Value
Plasma					
ΣHCH	79.5	16.20 (2.98, 57.83)	52.1	14.18 (4.41, 26.38)	.336
HCH lindane	16.4	23.61 (2.17, 55.53)	18.8	5.81 (0.46, 33.06)	.041[b]
HCB	72.6	31.52 (21.56, 95.80)	87.5	29.19 (20.75, 58.76)	.195
Aldrin	6.8	0.12 (0.04, 0.38)		<LD	
p,p′-DDE		<LD	18.8	7.10 (4.61, 26.64)	
ΣXEs	93.2	41.43 (2.17, 209.16)	97.9	35.71 (2.80, 101.64)	.306
vAT					
ΣHCH	90.4	103.69 (0.01, 485.67)	95.8	99.57 (1.94, 399.48)	.947
HCH lindane	28.8	15.19 (0.59, 87.44)	22.9	33.96 (5.19, 338.39)	.051
HCB	5.5	87.10 (84.91, 104.00)	8.3	60.67 (0.33, 149.23)	.343
Aldrin	15.1	11.41 (0.47, 48.87)	29.2	6.79 (0.57, 55.72)	.267
p,p′-DDE	23.3	24.83 (1.26, 257.52)	33.3	15.20 (2.02, 289.51)	.873
ΣXEs	90.4	106.72 (0.28, 687.18)	97.9	141.62 (2.51, 601.32)	.442
scAT					
ΣHCH	90.4	42.24 (1.29, 330.30)	95.8	98.51 (0.34, 458.94)	.018[b]
HCH lindane	23.3	30.89 (6.47, 262.81)	17.0	44.77 (24.92, 271.86)	.157
HCB	5.5	104.46 (92.79, 121.77)	8.3	77.85 (14.72, 160.30)	.343
Aldrin	12.3	23.53 (2.17, 112.90)	22.9	9.00 (1.76, 88.85)	.175
p,p′-DDE	32.9	1.55 (0.19, 30.14)	43.8	6.21 (0.30, 308.03)	.003[b]
ΣXEs	94.5	61.62 (1.44, 368.06)	97.6	161.52 (0.41, 557.17)	.020[b]

All data are medians (minimum, maximum).

[a] Percentage of the total number of positive samples.

[b] Values are presented as median (minimum, maximum) ($P < .05$, statistical analysis with Mann-Whitney test).

contrast, in postmenopausal women plasma XEs levels were positively correlated with XEs levels present in either AT compartment (Table 3). The strongest association observed both in pre- and postmenopausal women was that between vAT and plasma XE levels.

XE levels were associated with metabolic dysfunction

The associations between XE levels and the patients' clinical and biochemical parameters were also evaluated. We show correlations between the two most frequently detected XEs in each compartment and biological parameters (see Supplemental Tables 2–4). In premenopausal women, the vAT ΣXEs concentration was significantly and positively correlated with HbA1c and the count of plasma monocytes and inversely correlated with plasma IL-10. However, no significant correlation was found between scAT ΣXEs and the different parameters. Plasma ΣXEs were positively correlated with age, vAT adipocyte area, plasma IL-10, MCP1, and 10-year CVD risk and negatively correlated with HOMA-2B. In postmenopausal women, the only significant correlations found were a positive association between vAT ΣXEs and age and a negative association between plasma ΣXEs and the IL-6 to IL-10 ratio (Table 4).

Plasma XE levels were associated with inflammation and 10-year CVD risk in premenopausal women

In premenopausal women, the concentration of MCP1 was positively correlated with plasma ΣXEs (Table 4). In the linear regression model analysis, only ΣXEs plasma levels ($\beta = 0.006$, $P = .018$) remained statistically associated with plasma MCP1, even when adjusted for age and vAT adipocyte area (Table 5), highlighting the fact that XEs are independent risk factors for inflammation. On the other hand, the 10-year CVD risk correlated positively with ΣXEs plasma levels in premenopausal women (Table

Table 3. Correlation and Comparison Between Levels of XEs Present Simultaneously in Both vAT (Nanograms per Gram of Fat) and scAT (Nanograms per Gram of Fat) and Plasma (Nanograms per Milliliter)

	%[a]	Premenopausal Women	%[a]	Postmenopausal Women
ΣXEs, vAT vs scAT	86.3	0.488[b]	95.8	0.545[b]
ΣXEs , vAT vs plasma	83.6	0.642[b]	95.8	0.625[b]
ΣXEs , scAT vs plasma	87.7	−0.084	95.8	0.416[b]

[a] Percentage of the total number of positive samples in both vAT and scAT.

[b] Statistical analysis with Spearman's correlation ($P < .01$).

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Table 4. Correlation of Clinical and Biological Characteristics With XE Levels in vAT and scAT (Nanograms per Gram of Fat) and Plasma (Nanograms per Milliliter) of Premenopausal and Postmenopausal Women

	Premenopausal Women			Postmenopausal Women		
Parameters	**vAT XEs**	**scAT XEs**	**Plasma XEs**	**vAT XEs**	**scAT XEs**	**Plasma XEs**
Age, y	0.077	0.102	0.368[a]	0.351[b]	0.074	−0.052
Obesity evolution, y	0.131	−0.097	0.172	−0.012	−0.046	0.219
Anthropometric and morphometric measurements						
BMI, kg/m^2	0.115	0.027	0.001	−0.055	0.106	−0.016
Waist to hip ratio	−0.105	−0.043	0.076	0.026	−0.070	−0.158
vAT adipocyte area, μm^2	−0.014	0.109	0.278[b]	−0.264	−0.208	0.059
scAT adipocyte area, μm^2	0.000	0.201	−0.109	0.047	−0.045	0.138
Plasma lipid profile						
Total cholesterol, mg/dL	0.035	0.091	0.052	−0.024	0.017	0.115
Total triglycerides, mg/dL	0.041	0.004	0.019	0.107	0.206	0.221
HDL cholesterol, mg/dL	−0.035	0.111	−0.174	0.051	0.082	−0.039
LDL cholesterol, mg/dL	0.101	0.148	0.083	−0.060	−0.023	0.156
Glucose homeostasis						
Fasting glycemia, mg/dL	−0.066	0.048	0.074	0.134	0.186	−0.129
HbA1c, %	0.252[b]	0.232	0.085	0.116	0.154	0.037
HOMA-IR	−0.057	0.121	−0.234	−0.01	0.243	0.164
HOMA-2B, %	−0.050	0.096	−0.250[b]	−0.173	0.097	0.162
Blood pressure						
Systolic blood pressure, mm Hg	0.136	0.175	0.165	0.071	0.127	−0.031
Diastolic blood pressure, mm Hg	0.103	0.118	0.255	−0.159	0.166	0.116
Inflammatory parameters						
IL-6, pg/mL	−0.058	−0.152	0.172	−0.138	−0.015	−0.030
IL-10, pg/mL	−0.279[b]	0.017	0.328[b]	0.108	0.131	0.291
IL-6 to IL-10 ratio	0.259	−0.062	−0.131	−0.138	−0.158	−0.365[b]
MCP1, pg/mL	−0.170	−0.004	0.285[b]	−0.053	0.039	0.008
Other parameters						
hsCRP, mg/L	−0.228	−0.158	−0.231	−0.047	−0.019	0.005
Estradiol, pg/mL	0.122	0.022	−0.339	−0.179	−0.311	−0.232
Monocytes, %	0.360[a]	−0.096	−0.054	0.220	0.058	0.014
10-Year CVD risk, %	0.211	0.098	0.363[b]	0.185	0.155	−0.142

Ten-year CVD risk was calculated according to D'Agostino et al (12).

[a] $P < .01$ (statistical analysis with Spearman's correlation).

[b] $P < .05$ (statistical analysis with Spearman's correlation).

4). In the multivariate linear model, ΣXEs plasma levels were also significantly associated with 10-year CVD risk ($\beta = 0.012$, $P = .009$), even when adjusted for the time of obesity and MCP1. A significant interaction ($\beta = -0.001$, $P = 0.006$) between MCP1 and the time of obesity seems to occur, even after adjustment for ΣXEs plasma levels (Table 6) in premenopausal women. Considering postmenopausal women in multivariate models, no independent variables were significantly associated with MCP1 or with 10-year CVD risk (Table 4). Considering the 44 premenopausal woman included in the linear regression

Table 5. Coefficients From Linear Regression Model With Ln (MCP1) as Dependent Variable[a]

	Premenopausal Women		Postmenopausal Women	
	β	***P* Value**	***β***	***P* Value**
ΣXEs in plasma, ng/mL	0.006	**.012**	0.000	.966
Age	−0.017	.108	−0.012	.451
vAT adipocyte	0.000	.845	0.000	.870

β-values from a linear regression model are reported. Significant *P* values are shown in bold.

[a] Adjusted for all variables present in table.

Table 6. Coefficients From Linear Regression Model With Ln (10 Year CVD Risk) as Dependent Variable[a]

	Premenopausal Women		Postmenopausal Women	
	β	***P* Value**	***β***	***P* Value**
ΣXEs in plasma, ng/mL	0.012	**.009**	−0.005	.384
Obesity evolution, y	0.014	**.016**	0.000	.982
MCP1, pg/mL	0.135	**<.001**	0.017	.517
Obesity evolution, y, MCP1, pg/mL[a]	−0.001	**.006**	0.000	.615

β-Values from linear regression model is reported. Significant *P* values are shown in bold.

[a] Adjusted for all variables present in table.

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model with Ln 10-year CVD risk as dependent variable, we studied the power of testing the predictor ΣXEs in plasma (nanograms per milliliter), in the presence of two other predictors (obesity evolution, MCP1). The power of the test of a regression coefficient depends on the error SD, the SD of the predictor itself, and the multiple correlation between that predictor and other predictors in the model (related to the variance inflation factor). These parameters were estimated based on the sample enrolled. For an α of .05 and a power of 0.80, a sample size of n = 44 will detect a regression coefficient of 0.013. Considering the 36 postmenopausal woman included in the linear regression model with Ln 10-year CVD risk as a dependent variable, we studied the power of testing the predictor ΣXEs in plasma (nanograms per milliliter), in the presence of two other predictors (obesity evolution and MCP1). With the parameters estimated with the sample enrolled, for an α of .05 and a power of 0.80, a sample size of n = 36 will detect a regression coefficient of 0.015.

Discussion

In the present study, we carried out a novel and comprehensive assessment in pre- and postmenopausal women of the distribution and putative effects of several XEs that are known to be preferentially accumulated in the AT. We show that several environmental estrogens such as ΣHCH, HCH lindane, HCB, aldrin, and *p,p′*-DDE can be currently detected in biological samples obtained from obese patients, although their use has long been banned. Analysis of the association of health-related outcomes with XEs in the different biological samples was considered superior to the analysis of individual compounds because essentially all humans are exposed to many different XEs at the same time (3, 4), although perhaps in different relative concentrations.

A previous work from our group (11) demonstrated that persistent organic pollutants, some of which have xenoestrogenic activity, accumulated preferentially in vAT when women were considered regardless of menopausal status. In the present study, we were able to demonstrate that the distribution of XEs changes with menopausal status. For the compounds analyzed here, we can observe that this is true for premenopausal women but not postmenopausal women whose XE levels in scAT are similar to those in vAT and significantly higher than those found in the scAT of premenopausal women. On the other hand, this observation has important practical implications because it suggests that scAT, despite being an easily accessible compartment for determination of contamination levels, is not representative of the XE distribution in deeper AT depots, in accordance with different studies (19, 20). Plasma levels of XEs also did not reflect total levels accumulated in other compartments, highlighting that using plasma XE levels to estimate exposure may be misleading as well. Apart from being lower than AT levels, the type of compound present in plasma does not mimic the pattern accumulated in AT, possibly due to differences in solubility and toxicokinetics, resulting in different potential toxicities.

In premenopausal women there is a significant positive correlation between XEs in vAT and plasma but not with XEs in scAT. This association makes sense in that vAT adipocytes are metabolically more active and more sensitive to lipolysis than scAT adipocytes (21). On the other hand, plasma XEs correlated both with vAT and scAT XEs in postmenopausal women, suggesting that the increased turnover of the AT after menopause, including higher lipolysis rate due to decreased estrogens (22), may contribute to the concentration of XEs in scAT. Indeed, this pattern change may be a consequence of a changing hormonal milieu because of the menopausal transition, but it may also be modulated by the presence of XEs. Nevertheless, it seems to accompany a dysfunctional AT in this subgroup of women.

We observed that although older, postmenopausal women present higher metabolic dysfunction, as confirmed by our results, we found that XEs are positively associated with markers of impaired metabolism and with CVD risk in younger, premenopausal women. This suggests that the effect of these compounds may be independent of the decline of metabolic function that occurs with age.

Fat accumulated in vAT, in comparison with scAT, is known to positively correlate with metabolic complications, such as CVD, hypertension, and type 2 diabetes (23). Indeed, because XEs in vAT of premenopausal women were found to be more tightly associated with markers of worst metabolic profile compared with XEs in scAT, namely glucose homeostasis and inflammatory parameters, this might suggest that the presence of these compounds in vAT contributes to local toxicity and dysfunction and potentially favors the metabolic complications associated with obesity (6). Therefore, our study highlights the contribution of chronic internal exposure of obese subjects to XEs as possible additional factors leading to AT dysfunction.

In metabolically unhealthy obesity, adipocyte expansion occurs through hypertrophy with overproduction and secretion of signals that recruit immune cells, namely macrophages, leading to increased circulatory proinflammatory (eg, IL-6 and MCP1) and decreased antiinflammatory (IL-10) factors that contribute to local AT inflammation and to systemic low-grade inflammation (24, 25).

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Indeed, plasma XEs were correlated with increased vAT adipocyte area, a known marker of adipocyte dysfunction (26, 27).

Additionally, it has been proposed that XEs in AT can be involved in inflammatory activation/perpetuation, a critical condition for metabolically unhealthy AT (28). In accordance, we have found that in premenopausal women the presence of XEs in vAT and plasma may contribute to a more proinflammatory status. Plasma XEs correlated positively with MCP1 levels, which is compatible with higher production by activated cells in the AT. In fact, the mesenteric AT is a major producer of MCP1, which can modulate macrophage trafficking and activation during obesity-related inflammation (29). Furthermore, proinflammatory chemokines such as MCP1 are highly expressed by hypertrophic adipocytes accelerating migration and homing of bone marrow-derived monocytes/macrophages to the AT (30). It is worth highlighting that in the linear regression model, adjustment for age and vAT adipocyte area did not modify the association between plasma XEs and MCP1 levels.

Once again, the increase in vAT XEs is correlated with the increase in the number of monocytes in circulation, a possible intermediate step to their migration to the AT. This reinforces the hypothesis that XE exposure, through its effect on the immune system, may contribute to the high rate of metabolic disorders (31). The negative association between vAT XEs and plasma IL-10 levels adds to this evidence, although an opposite association was observed with plasma XEs.

In addition to inflammatory activation, dysfunctional AT also displays insulin resistance (32). In the same line, we have observed a positive association between vAT XEs and HbA1c. We also observed a negative relationship between plasma XEs and HOMA-2B. Accordingly, several researches provide evidence on possible actions of XEs on β-cell function and insulin resistance (33, 34). An observational study of Greenland Inuits highly exposed to environmental pollutants showed associations of some XEs with HOMA-2B, but not with HOMA-IR (35), indicating that the primary mechanism by which XEs increase the risk of type 2 diabetes is by modulating β-cell function. Furthermore, the association of plasma, but not vAT, XEs with this marker highlights the fact that different compounds are present in plasma and AT and that plasma XEs have many possible targets for toxicity.

Another interesting fact is that XE concentration is not BMI dependent. This is an important result because it demonstrates that body weight per se is not the primary factor explaining plasma XE concentration (36). Plasma XE levels may potentially be affected by a number of factors including the degree and source of exposure, the time since exposure occurred, genetic differences among individuals in rates of metabolism (37), and the number of pregnancies and breast-feeding practices (38).

Our results support the hypothesis that XEs increase systemic inflammation/AT dysfunction in premenopausal women, which may aggravate metabolic status and induce target organ damage. Indeed, plasma XEs helped predict CVD risk, even after adjustment for plasma MCP1 and the time of obesity. In addition, these results indicate that XE exposure or release from AT and inflammation may be independent factors for CVD risk, suggesting that increased plasma XEs may at least partially account for the inflammation and risk elicited by obesity in premenopausal women with the same time of obesity.

As mentioned earlier, the compounds evaluated in this study have been highlighted as endocrine disruptors interfering with estrogens, although also possessing other biological activities (39). However, their capacity to interfere with estrogens' actions is complex, given that these compounds may behave differently, often with opposing actions, if in the presence of absence of the natural hormone and/or depending on its concentration (40). This possibly underlines the fact that most of the associations of XEs with markers of metabolic deterioration observed herein were in the premenopausal women subgroup, leading to the speculation that the effect of XEs is more relevant when in the presence of preserved estrogen endocrine signaling.

Our findings also suggest that XEs and not obesity alone may contribute to increase CVD risk and inflammation, especially in premenopausal women, and thus, these chemicals may have a potential role in the later development of cardiometabolic disease in obese women. Moreover, the associations observed are compatible with the endocrine disruptor character of these compounds by compromising the dynamic protective physiological function of estrogens, leading to the release and redistribution of these pollutants. We also provide new insights into the profile and kinetics of XEs and their putative pathogenic effects according to menopausal status. This is in line with the observation that the relationship between 10-year CVD risk and plasma concentrations of MCP1 tended to be weakened with the time of obesity evolution in premenopausal women.

We acknowledge that the present study is not without limitations: 1) the patients were at the end of the line of obesity treatment, which limits the generalization of our findings to the overall population, and 2) there were multiple comparisons made in this study; however, because the present study is an exploratory study, we believe that it is not needed to include the correction for multiple comparisons, and thus, the significant results found in this

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study should be verified in further confirmatory studies (34). Nonetheless, our study has significant assets regarding the large sample size, the characterization of environmental exposures in three tissue compartments (plasma, vAT, and scAT) in obese women undergoing bariatric surgery, and the analysis of the results according to menopausal status, exploring a differential link to the metabolic abnormalities and cardiovascular risk.

Importantly, there is a need to extend the knowledge of the mechanisms of action of XEs, which may alter metabolic function, which will open novel directions for the prevention, and treatment of metabolic disease. The question of whether XEs could serve as biomarkers representing a novel tool to predict cardiometabolic risk remains to be fully answered, but if these findings are reproducible in different populations, it means that as early as possible, any effort to reduce exposure to XEs would be necessary to decrease the social burden of cardiometabolic disease.

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Address all correspondence and requests for reprints to: Diana Teixeira, BSc, Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal. E-mail: dianamst@med.up.pt.

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