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Associations of accumulated selected persistent organic pollutants in adipose tissue with insulin sensitivity and risk of incident type-2 diabetes

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ABSTRACT

Continuous exposure to low doses of persistent organic pollutant (POPs), such as those occurring in the general population, might contribute to the burden of type 2 diabetes mellitus (T2DM). However, evidences from longitudinal studies are scarce. We aimed to explore the associations of accumulated POP exposure with the development of T2DM by means of 1) longitudinal associations with the 16-year incidence of the disease, and 2) complementary cross-sectional analyses with markers of glucose homeostasis at recruitment. Organochlorine pesticide and polychlorinated biphenyl (PCB) concentrations were analyzed in adipose tissue samples and incident T2DM cases were retrieved from clinical records. Homeostatic model assessment values of insulin sensitivity/resistance and β-cell function at recruitment were calculated. Linear and Cox-regression models were performed. In individuals with normal weight/overweight (n = 293), we observed positive dose–response relationships between the studied POPs and T2DM risk, particularly for hexachlorobenzene (HCB) [hazard ratio (HR): 3.96 for 4th quartile versus 1st quartile (Q1); confidence interval (CI) 95%: 0.79, 19.71]. PCB-180 showed a positive but seemingly non-linear association with T2DM risk [HR of 3er quartile (Q3) versus Q1: 6.48; CI 95%: 0.82, 51.29]. Unadjustment for body mass index considerably increased the magnitude of the associations. In the cross-sectional study (n = 180), HCB and PCB-180 were inversely associated with insulin sensitivity and positively associated with insulin resistance parameters. Our results suggest that a higher burden of specific POPs in adipose tissue may disrupt glucose homeostasis, possibly contributing to increase T2DM risk, especially in non-obese adults.

1. Introduction

According to the International Diabetes Federation, around 463 million adults in the world were affected by diabetes in 2019 (with future projections of a 51% increase by 2045), of which type 2 diabetes mellitus (T2DM) accounted for 90% of the diagnoses (Saeedi et al., 2019). The differences in prevalence of T2DM between regions cannot be fully explained by well-established risk factors such as population ageing, obesity trends, dietary patterns or lifestyles (Stöckl et al., 2016; Tamayo et al., 2014). Thereby, it has been suggested that external environmental factors, including exposure to some persistent organic pollutants (POPs), could contribute to the burden of T2DM (Misra and

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Misra, 2020).

POPs are a heterogeneous group of lipophilic chemicals with a high resistance to chemical degradation. These properties induce a high potential of bioaccumulation in living organisms, particularly in fatty tissues, and biomagnification up the food chain (González-Casanova et al., 2020). Long-term POP exposure, even at low doses, is suspected to cause a number of negative effects in human health and the environment (Thompson and Darwish, 2019). POPs include a variety of chemicals, such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans. Although strict legal restrictions have been implemented over the last decades on their production and use, their ubiquity, persistence and the residual use in certain countries have maintained their relevance in public health (Vijgen et al., 2018). Long-term POP exposure in the general population occurs mainly through diet, although other routes such as air inhalation and skin contact also contribute to internal levels (Mrema et al., 2013).

Previous review articles (Jaacks and Staimez, 2015; Lee et al., 2018; Magliano et al., 2014; Ngwa et al., 2015; Taylor et al., 2013) and meta-analyses (Song et al., 2016; Wu et al., 2013) support the associations of OCP and PCB concentrations with T2DM. Despite this, there are still several knowledge gaps: 1) the causal link between POPs and T2DM remains unclear, partly because of the cross-sectional design of most previous studies (Jaacks and Staimez, 2015; Ngwa et al., 2015; Taylor et al., 2013); 2) the existing longitudinal studies have mainly focused on the association between POPs and T2DM through accidental or occupational exposures, which are not applicable to the general population (Ngwa et al., 2015); and 3) recent studies have suggested a non-linear dose–response association between PCBs and OCPs with T2DM (Lee et al., 2018; Magliano et al., 2014), which lead to a statistical challenge for modelling the associations with U shape or inverted U. Moreover, previous longitudinal studies have used plasma or serum to measure the exposure of selected POPs (Han et al., 2020; Tornevi et al., 2019; Vasiliu et al., 2006; Wolf et al., 2019). Up to our knowledge, no longitudinal study with this aim has used adipose tissue as a source of POP exposure, which indeed is considered the most adequate matrix for assessing long-term exposure to mixtures of these lipophilic compounds (Mustieles and Arrebola, 2020).

Obesity is an important risk factor for T2DM development and progression, as well as for a greater bioaccumulation of POPs due to increased adipose tissue (Jackson et al., 2017). However, the role of obesity in influencing the relationship between levels of specific POPs and T2DM remains unclear. Current bibliography remains controversial, suggesting a potential modification effect with no clear direction (Han et al., 2020; Tornevi et al., 2019; Vasiliu et al., 2006; Wolf et al., 2019), since it might probably vary among different populations. Taking into account the growing obesity prevalence in virtually all countries (Chooi et al., 2019), the study of this variable in the analysis of POPs-T2DM associations warrants further research.

The present work is framed within previous efforts to assess the metabolic disrupting potential of chronic exposure to environmental contaminants in GraMo adult cohort (Arrebola et al., 2013b, 2015a; Artacho-Cordón et al., 2016; Mustieles et al., 2017). In fact, previous prospective research in the cohort have evidenced an increase in hypertension risk and metabolic syndrome components in relation to the exposure to OCPs and PCBs (Arrebola et al., 2015a; Mustieles et al., 2017). Thereby, the objective of this study was to assess the potential contribution of long-term exposure to a selection of POPs on the development of T2DM by combining two approaches: 1) a longitudinal analysis exploring associations with the 16-year T2DM incidence, and 2) a cross-sectional analysis focused on the associations with markers of insulin sensitivity/resistance and beta-cell function at recruitment.

2. Materials and methods

2.1. Study design and population

The present study population was a subsample of the GraMo cohort, extensively described elsewhere (Arrebola et al., 2013b, 2015a; Artacho-Cordón et al., 2016; Mustieles et al., 2017). Briefly, patients undergoing non-cancer-related surgery (41% hernias, 21% gallbladder diseases, 12% varicose veins and 26% other conditions) were recruited from July 2003 to June 2004 in two public hospitals in the province of Granada (San Cecilio University Hospital in Granada city and Santa Ana Hospital in Motril), both located in the south of Spain. Inclusion criteria were: age over 16 years, absence of cancer, absence of hormone therapy at recruitment, and at least 10-year residence in the hospitals reference area. Of the 409 subjects who were invited, 387 (94.6%) accepted to participate. For the present study, we excluded 15 participants with T2DM diagnosis at recruitment. After the follow-up performed in September-October 2019, the longitudinal analysis was conducted in 372 participants who provided adipose tissue and had no T2DM at recruitment. The cross-sectional analysis was performed in a subcohort of 226 individuals who provided blood in addition to adipose tissue samples. There was no loss of participants over the follow-up since no patient moved from the original place of residence (Fig. 1).

All participants included in the study signed an informed consent and ethical approval was given by the Ethics Committee of Granada (Comité de Ética de la Investigación Provincial de Granada, 8/2016).

2.2. POP analyses

Samples of 5–10 g of adipose tissue were intra-operatively collected and immediately coded and stored at − 80 °C until chemical analysis. Sample preparation and purification was based on an adaptation of solid–liquid extraction procedure, and chemical analyses were performed by means of Gas-Chromatography couppled Mass Spectrometry

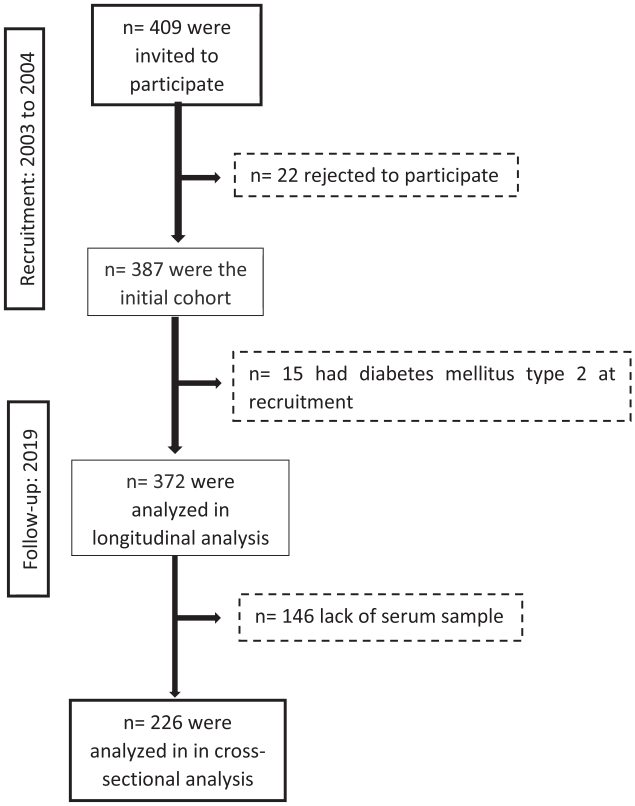

Fig. 1. Participant's flowchart.

in tandem mode (Moreno Frías et al., 2004; Rivas et al., 2001). More details on the specific parameters used in GraMo cohort have been described elsewhere (Arrebola et al., 2013b, 2013a, 2010, 2009). The limit of detection (LOD) was set at 0.01 μg/L for all POPs under study. Chromatographic concentrations below the limit of detection were assigned a random value between 0 and the LOD. Residues of the following OCPs were quantified: *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE, the main metabolite of the pesticide dichloro diphenyl trichloroethane), hexachlorobenzene (HCB), dicofol, α- and β-hexachlorocyclohexane (α- and β-HCH, respectively), and the PCB congeners − 138,−153 and − 180. POPs were *a priori* selected on the basis of the reported high prevalence in human populations as well as their suspected health implications (Guo et al., 2019; Lee et al., 2018). POP recovery from adipose tissue samples was studied to assess the extraction efficiency of the method and ranged from 90 to 98%. Lipid content in adipose tissue samples was quantified gravimetrically as reported by Rivas et al. (Rivas et al., 2001). Normalized POP concentrations were expressed in nanograms per gram of lipid (ng/g lipid).

2.3. Glucose homeostasis markers

Biomarkers of glucose homeostasis (i.e., serum glucose, immunoreactive insulin and C-peptide) were analyzed in blood samples collected at recruitment under 12-h fasting conditions. Glucose was analyzed by means of a validated enzymatic method by using a Cobas c311 bioanalyzer (Roche) (Wu, 2006). Insulin and C-peptide were quantified using validated *in vitro* immunological tests performed on a Cobas e-411 bioanalyzer (Roche) (Clark, 1999; Sapin, 2003).

The Homeostasis Model Assessment (HOMA) is a validated index of glucose homeostasis, which estimates steady state pancreatic beta cell function (%B) and insulin sensitivity (%IS), as percentages of a normal reference population. Based on previous works, Levy and colleagues updated the HOMA model (HOMA2), taking into account variations in hepatic and peripheral glucose resistance, increases in the insulin secretion curve for plasma glucose concentrations above 180 mg/dL, and the contribution of circulating proinsulin (Levy et al., 1998). This model was recalibrated to also provide %B and %S values of 100% in normal young adults when using currently available assays for insulin, specific insulin or C-peptide (Levy et al., 1998). The HOMA2 Calculator v.2.2.3 allowed to determine HOMA-%B, HOMA-%IS and HOMA-IR (insulin resistance), the latter being the reciprocal or inverse of %IS (100/%IS) values (https://www.dtu.ox.ac.uk/homacalculator/). Fasting serum glucose and insulin levels were used to calculate HOMA-values [HOMA-IS (I), HOMA-β (I), HOMA-IR (I)]. We also calculated the same parameters using glucose and C-peptide levels [HOMA-IS (CP), HOMA-β (CP), HOMA-IR (CP)], to assess coherence and reduce potential bias (Wallace et al., 2004).

2.4. Diabetes and covariate assessment

Between September and October 2019, data from new cases of diabetes were retrieved from the DIRAYA clinical records and the medical prescriptions databases. DIRAYA, developed in 1997 and implemented in 2003, was designed to facilitate clinical procedures and to assist clinical and epidemiological research. The system integrates all clinical information for each user of the regional public health system, who receives a unique ID code, including primary and specialized care, with data on all diagnostic tests performed and pharmacological treatments received. With this updated revision including additional health databases, we refined T2DM diagnosis of those previously classified as diabetic based on self-reported information and/or point fasting glucose levels in routine laboratory tests (Arrebola et al., 2013b). A participant was considered as prevalent (at recruitment) or incident type-2 diabetic when: 1) T2DM diagnosis had been registered in his/her DIRAYA records, and/or 2) regular prescription of antidiabetics had been registered in the prescriptions database. Inconsistencies between the two datasets were further elucidated by performing a thorough review of the individual clinical history sheets, consultation reports, laboratory data and other medical reports.

Face-to-face interviews were conducted by trained personnel at the time of recruitment to collect information about sociodemographic characteristics, lifestyles and health status. Body mass index (BMI) was calculated from self-reported weight and height from each participant, and expressed as kg/m^2. Any level of daily tobacco (≥1 cig/day) and weekly alcohol (≥1 drink/week) was considered to classify participants as smokers and alcohol consumers respectively. Residence in the city of Granada at the time of the surgery was considered "urban" and residence in the area of Motril was considered "semi-rural".

2.5. Statistical analysis

Descriptive analyses of POP concentrations included medians, 25th and 75th percentiles. Percentages over LOD were used to the description of dicofol and α-HCH due to their low detection frequency of samples above the LOD. The magnitude of associations between POP concentrations and the 16-year diabetes incidence was evaluated by estimating Cox-regression models with time-to-events as the time variable, calculating hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). Estimations of time-to-events were based on the dates of recruitment, diagnosis, and end of follow-up (15-October-2019). Data on deceased participants over follow-up (18%) or on those reaching the end of follow-up without the event of interest were censored and only their disease-free time was considered in the analyses. A test for the proportional hazard assumption was performed with satisfactory results as part of the diagnostics of the models, using the cox.zph function implemented in the R survival package (Therneau, 2021). This function correlates the scaled Schoenfeld residuals with the Kaplan-Meier estimate of the survival function.

The functional form of the associations (i.e. linearity) was evaluated using generalized additive models (GAM) with gamlss package (Rigby and Stasinopoulos, 2005). POP concentrations were log-transformed in order to reduce the skewness of the distributions. Concentrations were also categorized in quartiles, considering quartile 1 (Q1) as the reference category.

In our study, the following scenarios were considered in the conceptual framework of the hypothetical associations between POPs and markers of glucose homeostasis and T2DM (Supplementary Figure S1 and Fig. 2, respectively): 1) BMI, smoking, alcohol consumption, sex and

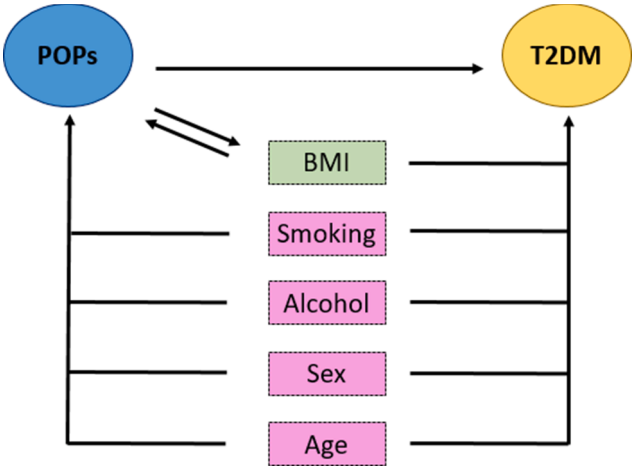

Fig. 2. Conceptual framework of longitudinal analysis. Smoking, alcohol, sex, body mass index (BMI), and age are variables meet the criteria of potential confounding factors in the association between persistent organic pollutants (POPs) and type 2 diabetes mellitus (T2DM). BMI could be also an intermediate variable in that association.

age as confounders; and 2) BMI as part of the causal pathway between POPs and markers of glucose homeostasis and T2DM (Kolb and Martin, 2017; Mustieles et al., 2017; Petrie et al., 2018; Wood et al., 2016).

The cross-sectional analysis of the associations between adipose tissue OCP and PCB concentrations and markers of glucose homeostasis was assessed by means of multivariable linear regression models. As part of the model diagnostics, multicollinearity between independent variables was assessed by calculating generalized standard-error inflation factors, while homoscedasticity was tested by plotting residual against fitted values. The normality of errors was evaluated through normal QQ plots with 95% confidence intervals.

Models were adjusted for *a priori* identified potential predictors of the exposure or the disease, other variables whose inclusion produced changes > 10% in beta coefficients, and relevant confounders reported in previous studies, i.e., age, sex (male/female), residence (urban/semi-rural), education (primary schooling not completed/primary or higher), BMI, smoking habit (non-smoker/ex-smoker/smoker) and alcohol consumption (consumer/non-consumer).

On the basis of previous evidences of potential modifying effect of obesity on the associations of targeted POPs and cardiometabolic outcomes in GraMo cohort (Arrebola et al., 2013b, 2015a), we tested the interaction by entering the product term BMI*POP concentrations in each model. In addition, we performed sex-stratified analyses, since sex differences in the burden of T2DM and a higher susceptibility to PCBs and OCPs for women have been reported previously (Huebschmann et al., 2019; Wolf et al., 2019).

R statistical computing environment v 4.0.3 (R Core Team, 2020), www.r-project.org/, was used for data analyses. The interpretation of results was not based solely on p-values and statistical significance, but also on the magnitude of effect estimates, internal consistency, previous evidence on biological plausibility as well as coherence with the previous epidemiological literature (Amrhein et al., 2019). In this regard, we have compared the results across different levels of statistical modelling, integrating analyses with predictors as continuous variables but also in percentiles and GAMs in order to relax the linearity assumption.

3. Results

Table 1 summarizes the characteristics of the study population and the distribution of adipose tissue targeted POP concentrations at enrollment. After excluding the participants with T2DM diagnosis at recruitment (n = 15), the final study sample size was 372. There was a homogenous distribution by sex (51% were women) and by residence (52% lived in Motril). The mean age ± standard deviation (SD) was 50.3 ± 17.1 years and 28% of the participants had incomplete primary studies. Regarding lifestyle habits, 33% were smokers, 52% were considered alcohol consumers, and 64% were overweight or obese. 52 participants (14%) were newly diagnosed of T2DM during the follow-up (see Supplementary Figure S2) with a median of time until the event or censure of 185.5 months (Q1: 157.0, Q3: 192.6 months). With respect to POP concentrations, the detection percentages were: 100% for *p,p'*,*p*-DDE, 90% for HCB, 84% for β-HCH, 86% for PCB-138, 92% for PCB-153 and 90% for PCB-180. β-HCH showed the lowest dispersion in their levels (25th: <LOD and 75th: 21.0 ng/g lipid) while *p,p'*-DDE presented the highest dispersion (25th: 32.4 and 75th: 205.5 ng/g lipid). Dicofol and α-HCH were found over the LOD in 20% and 21% participants, respectively. An extensive description of OCP and PCB concentrations in the GraMo cohort as well as their predictors have been reported elsewhere (Arrebola et al., 2013a, 2010, 2009). The distribution of all the above-mentioned variables was similar in the subcohort where glucose homeostasis was analyzed.

Associations of OCPs and PCBs, as continuous variables and in quartiles (Q), with the incidence of T2DM are summarized in Table 2 and GAM models in Supplementary Figure S3. For the whole study population, the HRs were close to the null value and no clear trends were found when entering the variables in quartiles.

Table 1
Characteristics of the study population.

Variable	16-year follow-up cohort (n = 372) Longitudinal analysis			Subcohort[a] (n = 226) Cross-sectional analysis		
Sex, n (%)						
Men	181 (48.7)			115 (50.9)		
Women	191 (51.3)			111 (49.1)		
Age, mean ± standard deviation (SD)	50.3 ± 17.1			49.3 ± 17.0		
Education, n (%)						
Incomplete primary	103 (27.7)			68 (30.1)		
Primary	168 (45.2)			97 (42.9)		
Secondary or higher	101 (27.2)			61 (27.0)		
Residence, n (%)						
Urban (Granada)	177 (47.6)			65 (28.8)		
Semi rural (Motril)	195 (52.4)			161 (71.2)		
Smoking status, n (%)						
NoNo	154 (41.4)			94 (41.6)		
Ex-smokerEx-smoker	95 (25.5)			52 (23.0)		
Smoker	123 (33.1)			80 (35.4)		
Alcohol consumption (yes), n (%)	195 (52.4)			120 (53.1)		
Incident type 2 diabetes mellitus, n (%)	52 (14.0)			–		
Body mass index, mean ± SD	27.3 ± 5.3			27.2 ± 5.5		
Normal weight (<25 kg/m^2)	135 (36.3)			80 (35.4)		
Overweight (25-≤ 30 kg/m^2)	158 (42.5)			100 (44.2)		
Obesity (>30 kg/m^2)	79 (21.2)			46 (20.4)		
Variable				25th	50th	75th
Glucose, mg/dl				73.9	86.3	112.7
Insuline, U/ml				5.6	7.8	12.6
C-peptide, ng/ml				2.1	2.7	3.6
HOMA-IS (CP)[b]				38.0	51.0	66.7
HOMA-β (CP)[b]				101.8	163.4	227.2
HOMA-IR (CP)[b]				1.5	2.0	2.6
HOMA-IS (I)[c]				59.8	99.3	143.3
HOMA-β (I)[c]				66.8	102.4	147.3
HOMA-IR (I)[c]				0.7	1.0	1.7
Variable, ng/g lipid	25th	50th	75th	25th	50th	75th
p,p'-DDE[d]	32.4	88.1	205.5	32.8	88.1	205.8
HCB[e]	4.9	13.8	39.1	4.8	13.1	37.1
β-HCH[f]	< LOD*	10.4	21.0	3.7	10.3	20.5
PCB-138[g]	30.3	81.9	135.2	26.0	82.4	141.2
PCB-153[g]	126.5	213.8	357.1	131.9	220.5	364.5
PCB-180[g]	102.0	177.9	282.3	101.6	183.7	290.8
Variable	n (%)				n (%)	
Dicofol (>LOD*)	73 (19.6)				60 (26.5)	
α-HCH[h] (>LOD*)	78 (21.0)				61 (27.0)	

[a] : participants with available serum samples for POPs and HOMA.
[b] : Calculated using glucose and C-peptide levels (CP).
[c] : Calculated using fasting serum glucose and insulin levels (I).
[d] : *p-ṕ*-Dichlorodiphenyldichloroethylene.
[e] : Hexachlorobenzene.
[f] : β-Hexachlorocyclohexane; *LOD: limit of detection.
[g] : Polychlorinated Biphenyls 138, 153 and 180.
[h] : α- Hexachlorocyclohexane.

Significant interactions were found between BMI and β-HCH (p-value = 0.02), PCB-153 (p-value = 0.04) and HCB (p-value = 0.03); for *p,p'*,*p*-DDE and PCB-180 the p-value of interaction was 0.07 and for PCB-138 was 0.17. All interaction terms showed the same direction, so that the effect of the studied POPs decreased at higher BMI levels (data not shown). Therefore, a sensitivity analysis was performed by excluding the obese population (≥30 kg/m^2). Despite the lack of statistical significance at a 95% level, probably due to the limited sample size, we observed suggestive positive dose–response associations of *p,p'*,*p*-DDE, HCB, PCB-138, and PCB-153 with T2DM risk in those individuals with normal weight or overweight (n = 293), which were particularly strong in the case of HCB (HR: 3.96 for Q4 versus Q1; CI 95%: 0.79, 19.71)

Table 2
Associations of adipose tissue POP concentrations with the 16-year incidence of type 2 diabetes mellitus among all participants (n = 372).

	Cox regression			Cox regression by quartiles (Q)	
	HR (95% CI)	p value	Quartile (Q) (min–max)	HR (95% CI)	p value
p,p'-DDE[a]	0.93 (0.72, 1.20)	0.57	Q1 (1.0–32.3)	1.00 (ref.)	
			Q2 (32.8–88.0)	0.70 (0.29, 1.72)	0.44
			Q3 (88.3–205.7)	0.77 (0.33, 1.79)	0.55
			Q4 (208.7–2331.4)	0.78 (0.32, 1.87)	0.58
HCB[b]	1.03 (0.82, 1.30)	0.77	Q1 (0.0–4.9)	1.00 (ref.)	
			Q2 (5.0–13.7)	1.78 (0.67, 4.74)	0.25
			Q3 (13.8–39.1)	1.41 (0.51, 3.90)	0.50
			Q4 (39.5–395.3)	1.87 (0.63, 5.59)	0.26
PCB-138[c]	0.92 (0.79, 1.09)	0.35	Q1 (0.0–30.2)	1.00 (ref.)	
			Q2 (30.4–81.8)	0.89 (0.36, 2.19)	0.80
			Q3 (82.1–135.4)	0.64 (0.25, 1.59)	0.33
			Q4 (135.5–564.1)	0.70 (0.29, 1.78)	0.48
PCB-153[c]	0.92 (0.75, 1.14)	0.47	Q1 (0.1–126.5)	1.00 (ref.)	
			Q2 (127.5–216.0)	1.58 (0.63, 3.98)	0.33
			Q3 (217.7–357.9)	1.07 (0.42, 2.73)	0.89
			Q4 (358.3–1519.5)	0.93 (0.35, 2.44)	0.88
PCB-180[c]	0.92 (0.77, 1.11)	0.40	Q1 (0.0–101.9)	1.00 (ref.)	
			Q2 (102.2–177.8)	1.33 (0.54, 3.22)	0.53
			Q3 (178.1–282.3)	1.04 (0.43, 2.50)	0.93
			Q4 (287.9–1363.2)	0.86 (0.35, 2.16)	0.76
β-HCH[d]	1.02 (0.83, 1.26)	0.81	Q1 (0.0–3.6)	1.00 (ref.)	
			Q2 (3.7–10.4)	1.31 (0.54, 3.20)	0.55
			Q3 (10.4–21.1)	1.08 (0.44, 2.66)	0.86
			Q4 (21.3–211.8)	0.62 (0.22, 1.72)	0.36
Dicofol	1.13 (0.56, 2.25)	0.73	–	–	–
α-HCH[e]	0.58 (0.26, 1.30)	0.18	–	–	–

Note: models adjusted for sex, age, education, residence, smoking, alcohol, body mass index. All POP concentrations were expressed in ng/g lipid, except dicofol and α-HCH: > limit detection vs. < limit detection.
[a] : *p-ṕ*-Dichlorodiphenyldichloroethylene.
[b] : Hexachlorobenzene.
[c] : Polychlorinated Biphenyls -138, -153 and -180.
[d] : β-Hexachlorocyclohexane.
[e] : α- Hexachlorocyclohexane.

Table 3
Associations of adipose tissue POP concentrations with the 16-year incidence of type 2 diabetes mellitus in normal weight/overweight participants (n = 293).

	Cox regression			Cox regression by quartiles (Q)	
	HR (95% CI)	p value	Quartile (Q) (min–max)	HR (95% CI)	p value
p,p',p-DDE[a]	1.21 (0.86, 1.70)	0.27	Q1 (1.0–28.8)	1.00 (ref.)	
			Q2 (30.3–71.9)	0.76 (0.18, 3.13)	0.71
			Q3 (73.1–171.9)	1.37 (0.41, 4.58)	0.60
			Q4 (172.6–2331.4)	1.50 (0.45, 5.02)	0.51
HCB[b]	1.16 (0.87, 1.55)	0.30	Q1 (0.0–4.0)	1.00 (ref.)	
			Q2 (4.1–10.5)	3.18 (0.63, 15.97)	0.16
			Q3 (10.5–33.3)	3.35 (0.70, 15.97)	0.13
			Q4 (33.8–288.2)	3.96 (0.79, 19.71)	0.09
PCB-138[c]	1.06 (0.84, 1.34)	0.63	Q1 (0.0–22.6)	1.00 (ref.)	
			Q2 (23.2–72.9)	0.64 (0.15, 2.75)	0.55
			Q3 (73.3–134.4)	1.22 (0.37, 4.08)	0.74
			Q4 (135.5–564.1)	1.46 (0.44, 4.88)	0.54
PCB-153[c]	1.27 (0.85, 1.90)	0.24	Q1 (0.1–115.8)	1.00 (ref.)	
			Q2 (115.9–206.5)	2.23 (0.43, 11.49)	0.34
			Q3 (206.6–361.3)	2.82 (0.58, 13.69)	0.20
			Q4 (361.5–1519.5)	3.16 (0.67, 14.74)	0.14
PCB-180[c]	1.11 (0.82, 1.51)	0.49	Q1 (0.0–92.1)	1.00 (ref.)	
			Q2 (93.4–176.1)	4.59 (0.55, 38.33)	0.16
			Q3 (176.4–300.7)	6.48 (0.82, 51.29)	0.08
			Q4 (305.5–1363.2)	4.86 (0.60, 39.01)	0.14
β-HCH[d]	1.14 (0.86, 1.49)	0.36	Q1 (0.0–2.5)	1.00 (ref.)	
			Q2 (2.5–9.3)	1.56 (0.43, 5.58)	0.50
			Q3 (9.4–18.3)	1.34 (0.40, 4.45)	0.61
			Q4 (18.6–211.8)	1.19 (0.33, 4.24)	0.78
Dicofol	1.57 (0.64, 3.83)	0.32	–	–	–
α-HCH[e]	0.93 (0.34, 2.51)	0.89	–	–	–

Note: models adjusted for sex, age, education, residence, smoking, alcohol, body mass index. All POP concentrations were expressed in ng/g lipid, except dicofol and α-HCH: > limit detection vs. < limit detection.
[a] : *p-ṕ*-Dichlorodiphenyldichloroethylene.
[b] : Hexachlorobenzene.
[c] : Polychlorinated Biphenyls -138, -153 and -180.
[d] : β-Hexachlorocyclohexane.
[e] : α- Hexachlorocyclohexane

(Table 3 and Fig. 3). Furthermore, PCB-180 showed a positive but seemingly non-linear association with the risk of T2DM (HR of Q3 versus Q1: 6.48; CI 95%: 0.82, 51.29).

Sex was not found to be an important modifier of the previous associations, neither in the stratified analyses nor as an interaction term, showing the latter all the p-values of interaction > 0.25.

Considering that BMI might also be in the causal pathway between selected POPs and T2DM development (Fig. 2), and in order to avoid model overfitting, we repeated the Cox-regression models without adjustment for this variable (Table 4). The direction of the associations did not substantially change in comparison to the fully-adjusted models presented in Table 3, but the magnitude of associations was substantially higher.

In the complementary cross-sectional analyses of targeted POPs

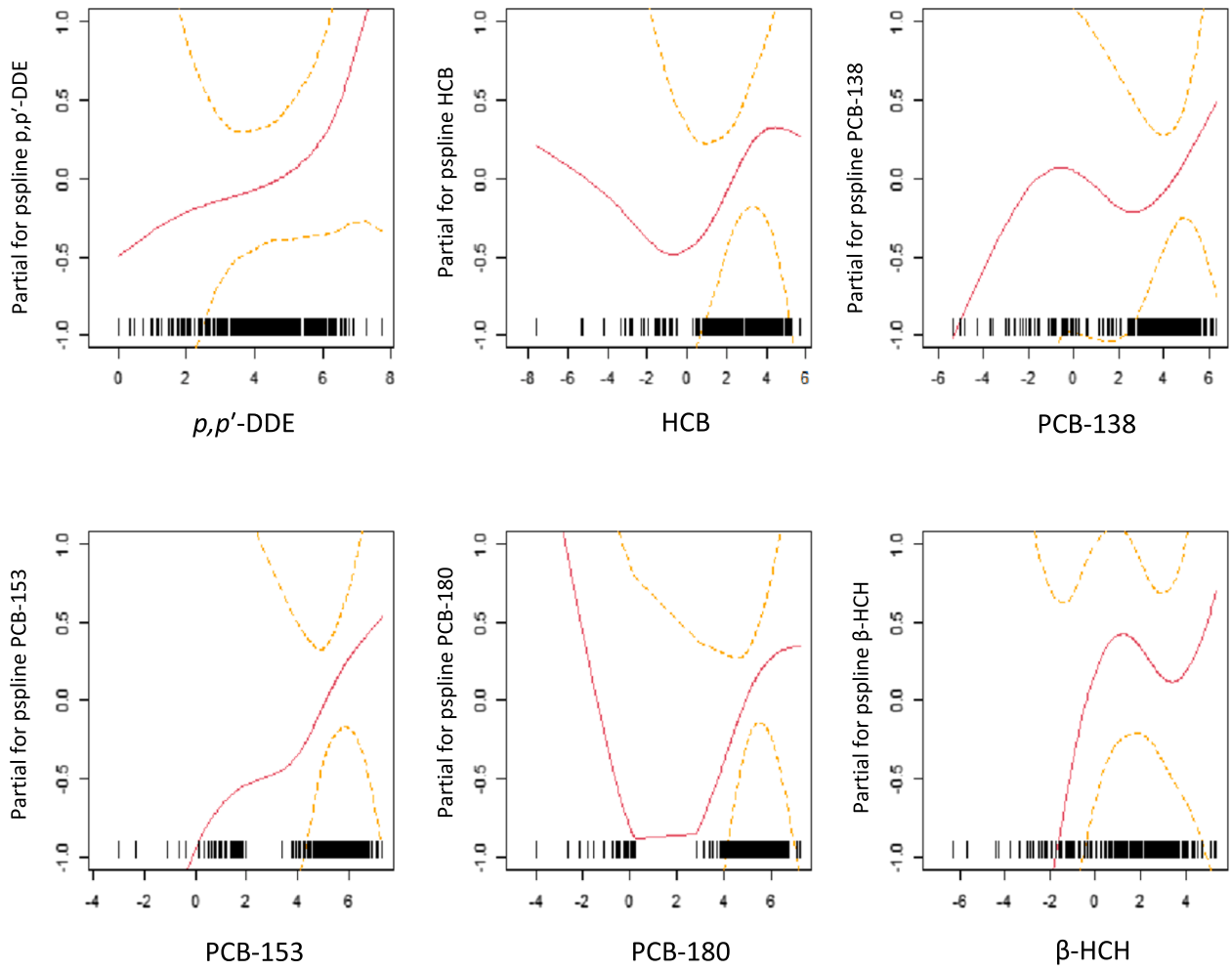

Fig. 3. Generalized additive models for the association between selected POPs and T2DM among normal weight/overweight participants (n = 293). Note: models adjusted for sex, age, education, residence, smoking, alcohol, body mass index. All POP concentrations were log-transformed. The degrees of freedom were 2.5 for all models. *p,p'*-DDE: Dichlorodiphenyldichloroethylene; HCB: Hexachlorobenzene; PCB: Polychlorinated Biphenyls 138, 153 and 180; β-HCH: β-Hexachlorocyclohexane.

versus glucose homeostasis markers (Fig. 4, Fig. 5), HCB and PCB-180 were inversely associated with insulin sensitivity parameters. These associations were particularly strong for HOMA-IS (CP) parameters, and maintained a similar shape than those observed in the longitudinal analysis with incidence of T2DM (Fig. 4). HCB and PCB-180 were also positively associated with insulin resistance. In general, HCB and PCB-180 showed inverted U-shape-like relationships with beta cell function parameters (Fig. 5). These trends in the different parameters were similar for the rest of POPs analysed (Supplementary Tables S1-S3).

4. Discussion

Our results suggested that accumulated exposure to certain POPs might contribute to the development of a sustained subclinical reduction in insulin sensitivity that may contribute to an increased T2DM risk in the long-term, and that obesity could be a modifier in the causal association. To the best of our knowledge, this is the first prospective study analyzing the association between adipose tissue PCB and OCP concentrations and the risk of T2DM, confirming our previous cross-sectional findings (Arrebola et al., 2015b, 2013b). It is noteworthy that the mixed longitudinal and complementary cross-sectional approach in the present study reinforces causality of the associations and minimizes potential reverse causality issues.

POPs have been linked to disturbances in the homeostatic balance of adipose tissue leading to inflammatory and oxidative effects (La Merrill et al., 2013; Lee et al., 2017). This local inflammatory scenario alters the role of adipose tissue in glucose homeostasis and induces the release of free fatty acids, adipokines and inflammatory proteins to systemic circulation, reaching peripheral tissues and promoting an early subclinical metabolic damage that might be evident in the long-term (Mustieles and Arrebola, 2020). In addition, the chronification of the POP-induced low-grade inflammation could disrupt the normal metabolic function of cells responsible for insulin action (Mostafalou, 2016). Although our HOMA results should be carefully interpreted because of the cross-sectional design, they suggest a potential POP-induced peripheral insulin resistance for the pollutants analyzed here. Conversely, Clair et al. reported an inverse association between serum POP exposure and insulin resistance in a cross-sectional study (Clair et al., 2018). Several factors might account for these paradoxical findings, including the different biological matrices used to estimate the exposure and the relatively increased POP exposure levels of the referred cohort. Interestingly, a recent review article provided evidence supporting that exposure to POPs increases the risk of developing insulin resistance (Kim et al., 2019). Thus, and taking into account that data from HOMA could reflect more the insulin secretion than pancreatic beta cell "health" (Wallace et al., 2004), the observed positive associations between lower levels of HCB and PCB-180 and beta-cell function parameters may be interpreted as an insulin overproduction compensatory mechanism at the beginning of the damage, which might eventually reach a certain plateau at higher POP concentrations. These subclinical effects, if prolonged in time, might

Table 4
Associations of adipose tissue POP concentrations with the 16-year incidence of type 2 diabetes mellitus in normal weight/overweight participants (n = 293).

	Cox regression			Cox regression by quartiles (Q)	
	HR (95% CI)	p value	Quartile (Q) (min–max)	HR (95% CI)	p value
p,p',p-DDE[a]	1.27 (0.90, 1.78)	0.17	Q1 (1.0–28.8)	1.00 (ref.)	
			Q2 (30.3–71.9)	0.89 (0.22, 3.66)	0.88
			Q3 (73.1–171.9)	1.61 (0.48, 5.41)	0.44
			Q4 (172.6–2331.4)	1.83 (0.54, 6.18)	0.33
HCB[b]	1.22 (0.91, 1.62)	0.18	Q1 (0.0–4.0)	1.00 (ref.)	
			Q2 (4.1–10.5)	3.21 (0.64, 16.07)	0.16
			Q3 (10.5–33.3)	4.13 (0.87, 19.56)	0.07
			Q4 (33.8–288.2)	4.88 (0.97, 24.43)	0.05
PCB-138[c]	1.09 (0.85, 1.39)	0.49	Q1 (0.0–22.6)	1.00 (ref.)	
			Q2 (23.2–72.9)	0.77 (0.18, 3.28)	0.72
			Q3 (73.3–134.4)	1.46 (0.44, 4.82)	0.54
			Q4 (135.5–564.1)	1.82 (0.55, 6.04)	0.33
PCB-153[c]	1.30 (0.86, 1.97)	0.21	Q1 (0.1–115.8)	1.00 (ref.)	
			Q2 (115.9–206.5)	2.17 (0.42, 11.17)	0.35
			Q3 (206.6–361.3)	2.93 (0.60, 14.21)	0.18
			Q4 (361.5–1519.5)	3.39 (0.72, 15.85)	0.12
PCB-180[c]	1.13 (0.83, 1.52)	0.44	Q1 (0.0–92.1)	1.00 (ref.)	
			Q2 (93.4–176.1)	5.03 (0.60, 41.97)	0.14
			Q3 (176.4–300.7)	6.89 (0.87, 54.44)	0.07
			Q4 (305.5–1363.2)	5.21 (0.64, 42.10)	0.12
β-HCH[d]	1.19 (0.90, 1.56)	0.22	Q1 (0.0–2.5)	1.00 (ref.)	
			Q2 (2.5–9.3)	1.61 (0.44, 5.88)	0.47
			Q3 (9.4–18.3)	1.63 (0.49, 5.43)	0.42
			Q4 (18.6–211.8)	1.40 (0.39, 5.07)	0.61
Dicofol	1.54 (0.63, 3.76)	0.34	–	–	–
α-HCH[e]	0.97 (0.36, 2.65)	0.96	–	–	–

Note: models adjusted for sex, age, education, residence, smoking, alcohol. All POP concentrations were expressed in ng/g lipid, except dicofol and α-HCH: > limit detection vs. < limit detection.
[a] : *p-ṕ*-Dichlorodiphenyldichloroethylene.
[b] : Hexachlorobenzene.
[c] : Polychlorinated Biphenyls -138, -153 and -180.
[d] : β-Hexachlorocyclohexane.
[e] : α- Hexachlorocyclohexane.

eventually lead to T2DM development, which supports our longitudinal findings.

Noteworthy, associations were more evident in non-obese individuals. Nowadays there is still no consensus in the literature about the role of obesity in the association between certain POPs and T2DM (Han et al., 2020; Tornevi et al., 2019; Vasiliu et al., 2006; Wolf et al., 2019), probably due to the complex dynamic of POPs between adipose tissue and blood. Obesity is considered a critical risk factor for T2DM, and obese individuals frequently present additional risk factors such as unhealthy lifestyles (e.g., sedentary behaviour, high sugar consumption…) and/or other comorbidities (e.g., cardiovascular disease, hypertension…) (Blüher, 2019). These factors could "mask" the effect of POPs. Our results might also be explained by a potential sequestration of POPs in adipose tissue of obese individuals. This storage of POPs could minimize the circulatory levels and, therefore, their harmful effect on other organs (Jackson et al., 2017; Lee et al., 2017), including those target tissues of insulin and glucose metabolism, e.g., liver, pancreas or skeletal muscles. This "protective" effect is plausible even though this storage might increase POPs half-life in the long term (Lee et al., 2018). This phenomenon of dilution and sequestration of POPs in adipose tissue would be in agreement with the so-called "obesity paradox" (Cheung et al., 2017; Lee et al., 2017). However, it is important to keep in mind that we used BMI to classify the participants. Despite being a widely use indicator of the amount of body fat, BMI does not allow to differentiate between the location or function of adipose tissue, the most relevant aspect in the association between adiposity and chronic diseases (Goyal et al., 2014). Therefore, our research warrants further confirmation with other adiposity indices.

In a previous cross-sectional analysis of this cohort focused on the prevalence of clinical T2DM at recruitment, we evidenced a positive association with β-HCH among all participants and with *p,p',p*-DDE in the non-obese individuals (Arrebola et al., 2013b). In general, these findings are congruent with those in the present longitudinal study, although the magnitude of the associations here was markedly attenuated. Being diet, occupational setting and BMI the main predictors of concentrations of PCBs and OCPs in this cohort (Arrebola et al., 2013a, 2010, 2009), the mentioned attenuattion may be partially explained by possible uncontrolled changes over the long follow-up time in these variables which could affected the risk estimates, as it has been described previously (Tornevi et al., 2019). In addition, previous researches have warned about the potential overestimation when the risk is calculated using odds ratios instead of relative risks (Davies et al., 1998). Similarly to what was shown in our cross-sectional study and by others (Arrebola et al., 2013b; Wolf et al., 2019), our current results suggest a likely-linear relationship with PCB-138, PCB-153 and HCB, but also an apparently non-linear association with PCB-180 in non-obese participants, while in obese individuals the associations are seemingly more complex.

BMI-unadjustment yielded stronger model coefficients. Considering a scenario in which it is an intermediate factor, controlling for this variable would likely lead to over-adjustment and obscure the ability to detect a potential true association. On the other hand, without this adjustment, we do not control for the effect of this important risk factor, strongly associated with adipose tissue PCB and OCP concentrations and T2DM risk (Donat-Vargas et al., 2014).

Strengths of the present study include the use of adipose tissue for exposure characterization, which is considered the most adequate estimator of long-term exposure to POPs (reviewed by: Mustieles and Arrebola, 2020). In addition, the combination of a cross-sectional analysis focused on early markers as glucose homeostasis, with a longitudinal approach based on a long follow-up of clinical events reinforces the causality of the associations investigated shedding light on potential mechanisms of action. Regarding HOMA values, the use of two measures (C-peptide and insulin) to determine beta-cell function and insulin sensitivity, can help to reduce bias (Wallace et al., 2004).

This study also has potential limitations to take into account in the interpretation of findings: i) our results may have been compromised by the relatively small sample size and lack of statistical power to detect significant associations. Nevertheless, sample size was enough to detect trends that might be reproduced in larger populations; ii) although we have analyzed potential confounders, we cannot rule out the influence of third uncontrolled variables, such as physical exercise and/or changes in covariates during follow-up. We cannot either ignore the possible

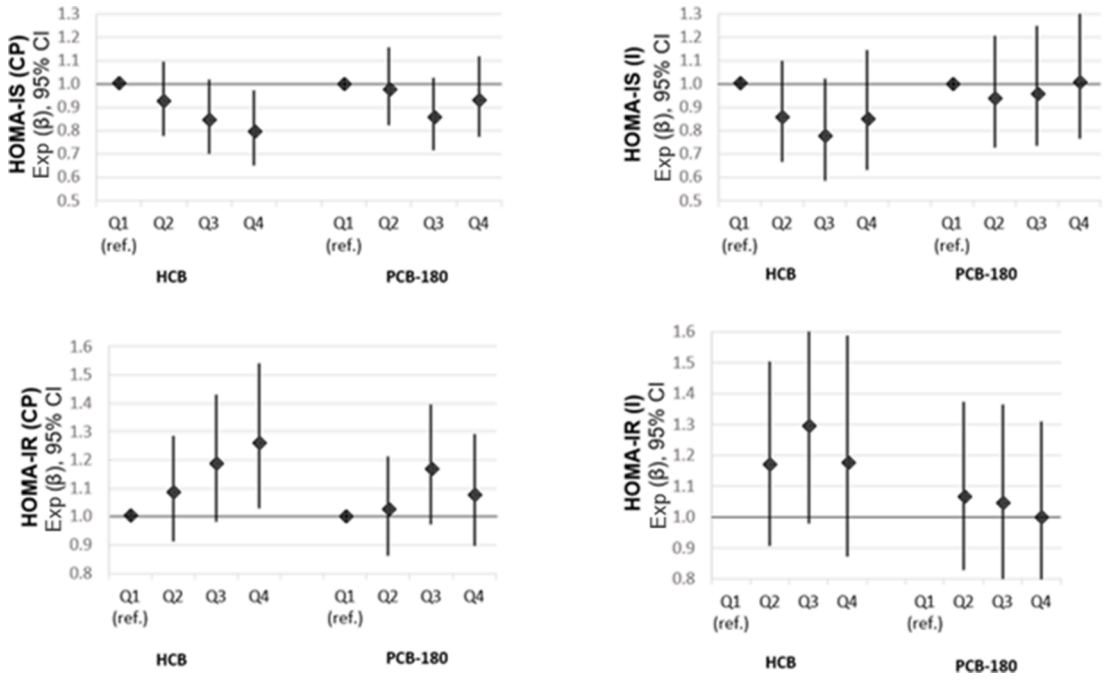

Fig. 4. Associations of quartiles of adipose tissue Hexachlorobenzene (HCB) and Polychlorinated Biphenyl -180 (PCB-180) concentrations with insulin sensitivity (HOMA-IS)/resistance (HOMA-IR) in normal weight/overweight participants (n = 180). Models adjusted for sex, age, education, residence, smoking, alcohol, body mass index. The calculation was performed using fasting serum glucose and insulin levels (I) and using glucose and C-peptide levels (CP). Q: quartile; ref: reference category; 95% CI: confidence interval.

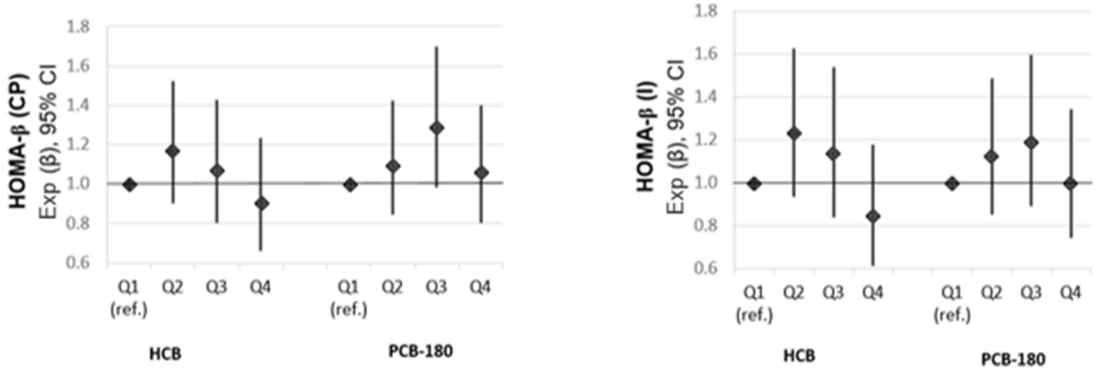

Fig. 5. Associations of quartiles of adipose tissue Hexachlorobenzene (HCB) and Polychlorinated Biphenyl -180 (PCB-180) concentrations with beta-cell function (HOMA-β) in normal weight/overweight participants (n = 180). Models adjusted for sex, age, education, residence, smoking, alcohol, body mass index. The calculation was done performed fasting serum glucose and insulin levels (I) and using glucose and C-peptide levels (CP). Q: quartile; ref: reference category; 95% CI: confidence interval.

interference from other unmeasured co-exposures to other contaminants in the observed associations; iii) the hospital-based setting of this study could limit the generalization of results to the general population. However, the incidence of T2DM found in our study is similar to that recently described in a nation-wide population based cohort from Spain with 7.5 years of follow-up (Rojo-Martínez et al., 2020); iv) BMI was derived from self-reported weight and height, that could have led to certain non-differential misclassification bias, which might move the estimates towards the null; v) we did not stratify obese subjects according to metabolic health status; vi) the analyzed pollutants might correlate among them but also with other unmeasured exposures. The ascertainment of individual and global effects represents an important challenge for current environmental epidemiology, and a priority for further analysis in the GraMo cohort.

In conclusion, our results evidenced that historical exposure to specific OCPs and PCBs might be a risk factor for insulin resistance and T2DM, particularly HCB and PCB-180, and that obesity is a potential effect modifier in the associations found. Despite the numerous studies carried out so far on this issue, the complexity of this relationship still needs to be further confirmed in future studies.

CRediT authorship contribution statement

Rocío Barrios-Rodríguez: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Francisco M. Pérez-Carrascosa:** Data curation, Investigation, Validation, Writing - review & editing. **Celia Gómez-Peña:** Data curation, Investigation, Validation, Writing - review & editing. **Vicente Mustieles:** Data curation, Investigation, Methodology, Writing - review & editing. **Inmaculada Salcedo-Bellido:** Conceptualization, Formal analysis, Methodology, Writing - review & editing. **Pilar Requena:** Methodology, Investigation. **Piedad Martín-Olmedo:** Investigation, Project

administration. **José Juan Jiménez-Moleón:** Conceptualization, Formal analysis, Writing - review & editing. **Juan Pedro Arrebola:** Conceptualization, Investigation, Methodology, Formal analysis, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106607.

References

Amrhein, V., Greenland, S., McShane, B., 2019. Scientists rise up against statistical significance. Nature 567, 305–307. https://doi.org/10.1038/d41586-019-00857-9.

Arrebola, J.P., Fernández, M.F., Martin-Olmedo, P., Bonde, J.P., Martín-Rodriguez, J.L., Expósito, J., Rubio-Domínguez, A., Olea, N., 2015a. Historical exposure to persistent organic pollutants and risk of incident hypertension. Environ Res 138, 217–223. https://doi.org/10.1016/j.envres.2015.02.018.

Arrebola, J.P., Fernández, M.F., Olea, N., Ramos, R., Martin-Olmedo, P., 2013a. Human exposure to p, p'-dichlorodiphenyldichloroethylene (p, p'-DDE) in urban and semi-rural areas in southeast Spain: a gender perspective. Sci Total Environ 458–460, 209–216. https://doi.org/10.1016/j.scitotenv.2013.04.001.

Arrebola, J.P., Fernandez, M.F., Porta, M., Rosell, J., de la Ossa, R.M., Olea, N., Martin-Olmedo, P., 2010. Multivariate models to predict human adipose tissue PCB concentrations in Southern Spain. Environ Int 36, 705–713. https://doi.org/10.1016/j.envint.2010.05.004.

Arrebola, J.P., González-Jiménez, A., Fornieles-González, C., Artacho-Cordón, F., Olea, N., Escobar-Jiménez, F., Fernández-Soto, M.L., 2015b. Relationship between serum concentrations of persistent organic pollutants and markers of insulin resistance in a cohort of women with a history of gestational diabetes mellitus. Environ Res 136, 435–440. https://doi.org/10.1016/j.envres.2014.11.007.

Arrebola, J.P., Martin-Olmedo, P., Fernandez, M.F., Sanchez-Cantalejo, E., Jimenez-Rios, J.A., Torne, P., Porta, M., Olea, N., 2009. Predictors of concentrations of hexachlorobenzene in human adipose tissue: a multivariate analysis by gender in Southern Spain. Environ Int 35, 27–32. https://doi.org/10.1016/j.envint.2008.05.009.

Arrebola, J.P., Pumarega, J., Gasull, M., Fernandez, M.F., Martin-Olmedo, P., Molina-Molina, J.M., Fernández-Rodríguez, M., Porta, M., Olea, N., 2013b. Adipose tissue concentrations of persistent organic pollutants and prevalence of type 2 diabetes in adults from Southern Spain. Environ Res 122, 31–37. https://doi.org/10.1016/j.envres.2012.12.001.

Artacho-Cordón, F., León, J., Sáenz, J.M., Fernández, M.F., Martin-Olmedo, P., Olea, N., Arrebola, J.P., 2016. Contribution of Persistent Organic Pollutant Exposure to the Adipose Tissue Oxidative Microenvironment in an Adult Cohort: A Multipollutant Approach. Environ Sci Technol 50, 13529–13538. https://doi.org/10.1021/acs.est.6b03783.

Blüher, M., 2019. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 15, 288–298. https://doi.org/10.1038/s41574-019-0176-8.

Cheung, Y.-M., Joham, A., Marks, S., Teede, H., 2017. The obesity paradox: an endocrine perspective. Intern Med J 47, 727–733. https://doi.org/10.1111/imj.13257.

Chooi, Y.C., Ding, C., Magkos, F., 2019. The epidemiology of obesity. Metab Clin Exp 92, 6–10. https://doi.org/10.1016/j.metabol.2018.09.005.

Clair, H.B., Pinkston, C.M., Rai, S.N., Pavuk, M., Dutton, N.D., Brock, G.N., Prough, R.A., Falkner, K.C., McClain, C.J., Cave, M.C., 2018. Liver Disease in a Residential Cohort With Elevated Polychlorinated Biphenyl Exposures. Toxicol Sci 164, 39–49. https://doi.org/10.1093/toxsci/kfy076.

Clark, P.M., 1999. Assays for insulin, proinsulin(s) and C-peptide. Ann Clin Biochem 36 (Pt 5), 541–564. https://doi.org/10.1177/000456329903600501.

Davies, H.T.O., Crombie, I.K., Tavakoli, M., 1998. When can odds ratios mislead? BMJ 316, 989–991.

Donat-Vargas, C., Gea, A., Sayon-Orea, C., Carlos, S., Martinez-Gonzalez, M.A., Bes-Rastrollo, M., 2014. Association between dietary intakes of PCBs and the risk of obesity: the SUN project. J Epidemiol Community Health 68, 834–841. https://doi.org/10.1136/jech-2013-203752.

González-Casanova, J.E., Pertuz-Cruz, S.L., Caicedo-Ortega, N.H., Rojas-Gomez, D.M., 2020. Adipogenesis Regulation and Endocrine Disruptors: Emerging Insights in Obesity. Biomed Res Int 2020, 7453786. https://doi.org/10.1155/2020/7453786.

Goyal, A., Nimmakayala, K.R., Zonszein, J., 2014. Is there a paradox in obesity? Cardiol Rev 22, 163–170. https://doi.org/10.1097/CRD.0000000000000004.

Guo, W., Pan, B., Sakkiah, S., Yavas, G., Ge, W., Zou, W., Tong, W., Hong, H., 2019. Persistent Organic Pollutants in Food: Contamination Sources, Health Effects and Detection Methods. Int J Environ Res Public Health 16. https://doi.org/10.3390/ijerph16224361.

Han, X., Meng, L., Li, Y., Li, A., Turyk, M.E., Yang, R., Wang, P., Xiao, K., Zhao, J., Zhang, J., Zhang, Q., Jiang, G., 2020. Associations between the exposure to persistent organic pollutants and type 2 diabetes in East China: A case-control study. Chemosphere 241, 125030. https://doi.org/10.1016/j.chemosphere.2019.125030.

Huebschmann, A.G., Huxley, R.R., Kohrt, W.M., Zeitler, P., Regensteiner, J.G., Reusch, J.E.B., 2019. Sex differences in the burden of type 2 diabetes and cardiovascular risk across the life course. Diabetologia 62, 1761–1772. https://doi.org/10.1007/s00125-019-4939-5.

Jaacks, L.M., Staimez, L.R., 2015. Association of persistent organic pollutants and non-persistent pesticides with diabetes and diabetes-related health outcomes in Asia: A systematic review. Environ Int 76, 57–70. https://doi.org/10.1016/j.envint.2014.12.001.

Jackson, E., Shoemaker, R., Larian, N., Cassis, L., 2017. Adipose Tissue as a Site of Toxin Accumulation. Compr Physiol 7, 1085–1135. https://doi.org/10.1002/cphy.c160038.

Kim, Y.A., Park, J.B., Woo, M.S., Lee, S.Y., Kim, H.Y., Yoo, Y.H., 2019. Persistent Organic Pollutant-Mediated Insulin Resistance. Int J Environ Res Public Health 16. https://doi.org/10.3390/ijerph16030448.

Kolb, H., Martin, S., 2017. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. BMC Med 15, 131. https://doi.org/10.1186/s12916-017-0901-x.

La Merrill, M., Emond, C., Kim, M.J., Antignac, J.-P., Le Bizec, B., Clément, K., Birnbaum, L.S., Barouki, R., 2013. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ Health Perspect 121, 162–169. https://doi.org/10.1289/ehp.1205485.

Lee, Y.-M., Jacobs, D.R., Lee, D.-H., 2018. Persistent Organic Pollutants and Type 2 Diabetes: A Critical Review of Review Articles. Front Endocrinol (Lausanne) 9, 712. https://doi.org/10.3389/fendo.2018.00712.

Lee, Y.-M., Kim, K.-S., Jacobs, D.R., Lee, D.-H., 2017. Persistent organic pollutants in adipose tissue should be considered in obesity research. Obes Rev 18, 129–139. https://doi.org/10.1111/obr.12481.

Levy, J.C., Matthews, D.R., Hermans, M.P., 1998. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 21, 2191–2192. https://doi.org/10.2337/diacare.21.12.2191.

Magliano, D.J., Loh, V.H.Y., Harding, J.L., Botton, J., Shaw, J.E., 2014. Persistent organic pollutants and diabetes: a review of the epidemiological evidence. Diabetes Metab 40, 1–14. https://doi.org/10.1016/j.diabet.2013.09.006.

Misra, B.B., Misra, A., 2020. The chemical exposome of type 2 diabetes mellitus: Opportunities and challenges in the omics era. Diabetes Metab Syndr 14, 23–38. https://doi.org/10.1016/j.dsx.2019.12.001.

Moreno Frías, M., Jiménez Torres, M., Garrido Frenich, A., Martínez Vidal, J.L., Olea-Serrano, F., Olea, N., 2004. Determination of organochlorine compounds in human biological samples by GC-MS/MS. Biomed Chromatogr 18, 102–111. https://doi.org/10.1002/bmc.300.

Mostafalou, S., 2016. Persistent Organic Pollutants and Concern Over the Link with Insulin Resistance Related Metabolic Diseases. Rev Environ Contam Toxicol 238, 69–89. https://doi.org/10.1007/398_2015_5001.

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88. https://doi.org/10.1016/j.tox.2012.11.015.

Mustieles, V., Arrebola, J.P., 2020. How polluted is your fat? What the study of adipose tissue can contribute to environmental epidemiology. J Epidemiol Community Health 74, 401–407. https://doi.org/10.1136/jech-2019-213181.

Mustieles, V., Fernández, M.F., Martin-Olmedo, P., González-Alzaga, B., Fontalba-Navas, A., Hauser, R., Olea, N., Arrebola, J.P., 2017. Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. Environ Int 104, 48–57. https://doi.org/10.1016/j.envint.2017.04.002.

Ngwa, E.N., Kengne, A.-P., Tiedeu-Atogho, B., Mofo-Mato, E.-P., Sobngwi, E., 2015. Persistent organic pollutants as risk factors for type 2 diabetes. Diabetol Metab Syndr 7, 41. https://doi.org/10.1186/s13098-015-0031-6.

Petrie, J.R., Guzik, T.J., Touyz, R.M., 2018. Diabetes, Hypertension, and Cardiovascular Disease: Clinical Insights and Vascular Mechanisms. Can J Cardiol 34, 575–584. https://doi.org/10.1016/j.cjca.2017.12.005.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [WWW Document]. URL https://www.R-project.org/.

Rigby, R.A., Stasinopoulos, D.M., 2005. Generalized additive models for location, scale and shape. Journal of the Royal Statistical Society: Series C (Applied Statistics) 54, 507–554. https://doi.org/10.1111/j.1467-9876.2005.00510.x.

Rivas, A., Fernandez, M.F., Cerrillo, I., Ibarluzea, J., Olea-Serrano, M.F., Pedraza, V., Olea, N., 2001. Human exposure to endocrine disrupters: standardisation of a marker of estrogenic exposure in adipose tissue. APMIS 109, 185–197. https://doi.org/10.1034/j.1600-0463.2001.090302.x.

Rojo-Martínez, G., Valdés, S., Soriguer, F., Vendrell, J., Urrutia, I., Pérez, V., Ortega, E., Ocón, P., Montanya, E., Menéndez, E., Lago-Sampedro, A., González-Frutos, T., Gomis, R., Goday, A., García-Serrano, S., García-Escobar, E., Galán-García, J.L., Castell, C., Badía-Guillén, R., Aguilera-Venegas, G., Girbés, J., Gaztambide, S., Franch-Nadal, J., Delgado, E., Chaves, F.J., Castaño, L., Calle-Pascual, A., 2020. Incidence of diabetes mellitus in Spain as results of the nation-wide cohort di@bet.es study. Sci Rep 10, 2765. https://doi.org/10.1038/s41598-020-59643-7.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Bright, D., Williams, R., IDF Diabetes Atlas Committee, 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract 157, 107843. https://doi.org/10.1016/j.diabres.2019.107843.

Sapin, R., 2003. Insulin assays: previously known and new analytical features. Clin. Lab. 49, 113–121.

Song, Yan, Chou, E.L., Baecker, A., You, N.-C.Y., Song, Yiqing, Sun, Q., Liu, S., 2016. Endocrine-disrupting chemicals, risk of type 2 diabetes, and diabetes-related metabolic traits: A systematic review and meta-analysis. J Diabetes 8, 516–532. https://doi.org/10.1111/1753-0407.12325.

Stöckl, D., Rückert-Eheberg, I.-M., Heier, M., Peters, A., Schipf, S., Krabbe, C., Völzke, H., Tamayo, T., Rathmann, W., Meisinger, C., 2016. Regional Variability of Lifestyle Factors and Hypertension with Prediabetes and Newly Diagnosed Type 2 Diabetes Mellitus: The Population-Based KORA-F4 and SHIP-TREND Studies in Germany. PLoS ONE 11, e0156736. https://doi.org/10.1371/journal.pone.0156736.

Tamayo, T., Schipf, S., Meisinger, C., Schunk, M., Maier, W., Herder, C., Roden, M., Nauck, M., Peters, A., Völzke, H., Rathmann, W., 2014. Regional differences of undiagnosed type 2 diabetes and prediabetes prevalence are not explained by known risk factors. PLoS ONE 9, e113154. https://doi.org/10.1371/journal.pone.0113154.

Taylor, K.W., Novak, R.F., Anderson, H.A., Birnbaum, L.S., Blystone, C., Devito, M., Jacobs, D., Köhrle, J., Lee, D.-H., Rylander, L., Rignell-Hydbom, A., Tornero-Velez, R., Turyk, M.E., Boyles, A.L., Thayer, K.A., Lind, L., 2013. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review. Environ Health Perspect 121, 774–783. https://doi.org/10.1289/ehp.1205502.

Therneau, T., 2021. A Package for Survival Analysis in R. R package version 3.2-10. https://CRAN.R-project.org/package=survival.

Thompson, L.A., Darwish, W.S., 2019. Environmental Chemical Contaminants in Food: Review of a Global Problem. J Toxicol 2019, 2345283. https://doi.org/10.1155/2019/2345283.

Tornevi, A., Sommar, J., Rantakokko, P., Åkesson, A., Donat-Vargas, C., Kiviranta, H., Rolandsson, O., Rylander, L., Wennberg, M., Bergdahl, I.A., 2019. Chlorinated persistent organic pollutants and type 2 diabetes - A population-based study with pre- and post- diagnostic plasma samples. Environ Res 174, 35–45. https://doi.org/10.1016/j.envres.2019.04.017.

Vasiliu, O., Cameron, L., Gardiner, J., Deguire, P., Karmaus, W., 2006. Polybrominated biphenyls, polychlorinated biphenyls, body weight, and incidence of adult-onset diabetes mellitus. Epidemiology 17, 352–359. https://doi.org/10.1097/01.ede.0000220553.84350.c5.

Vijgen, J., Weber, R., Lichtensteiger, W., Schlumpf, M., 2018. The legacy of pesticides and POPs stockpiles-a threat to health and the environment. Environ Sci Pollut Res Int 25, 31793–31798. https://doi.org/10.1007/s11356-018-3188-3.

Wallace, T.M., Levy, J.C., Matthews, D.R., 2004. Use and abuse of HOMA modeling. Diabetes Care 27, 1487–1495. https://doi.org/10.2337/diacare.27.6.1487.

Wolf, K., Bongaerts, B.W.C., Schneider, Alexandra, Huth, C., Meisinger, C., Peters, A., Schneider, Andrea, Wittsiepe, J., Schramm, K.-W., Greiser, K.H., Hartwig, S., Kluttig, A., Rathmann, W., 2019. Persistent organic pollutants and the incidence of type 2 diabetes in the CARLA and KORA cohort studies. Environ Int 129, 221–228. https://doi.org/10.1016/j.envint.2019.05.030.

Wood, S.A., Xu, F., Armitage, J.M., Wania, F., 2016. Unravelling the Relationship between Body Mass Index and Polychlorinated Biphenyl Concentrations Using a Mechanistic Model. Environ Sci Technol 50, 10055–10064. https://doi.org/10.1021/acs.est.6b01961.

Wu, A., 2006. Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders Co, Philadelphia.

Wu, H., Bertrand, K.A., Choi, A.L., Hu, F.B., Laden, F., Grandjean, P., Sun, Q., 2013. Persistent organic pollutants and type 2 diabetes: a prospective analysis in the nurses' health study and meta-analysis. Environ Health Perspect 121, 153–161. https://doi.org/10.1289/ehp.1205248.