Associations of persistent organic pollutants in human adipose tissue with retinoid levels and their relevance to the redox microenvironment

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ABSTRACT

Humans are exposed to a myriad of chemical substances in both occupational and environmental settings. Persistent organic pollutants (POPs) have drawn attention for their adverse effects including cancer and endocrine disruption. Herein, the objectives were 1) to describe serum and adipose tissue retinol levels, along with serum retinol binding protein 4 (RBP4) concentrations, and 2) to assess the associations of adipose tissue POP levels with these retinoid parameters, as well as their potential interaction with the previously-observed POP-related disruption of redox microenvironment. Retinol was measured in both serum and adipose tissue along with RBP4 levels in serum samples of 236 participants of the GraMo adult cohort. Associations were explored by multivariable linear regression analyses and Weighted Quantile Sum regression. Polychlorinated biphenyls (PCBs) 180, 153 and 138 were related to decreased adipose tissue retinol levels and increased serum RBP4/retinol ratio. Dicofol concentrations > limit of detection were associated with decreased retinol levels in serum and adipose tissue. Additionally, increased adipose tissue retinol levels were linked to an attenuation in previously-reported associations of adipose tissue PCB-153 with *in situ* superoxide dismutase activity. Our results revealed a suggestive link between retinoids, PCBs and redox microenvironment, potentially relevant for both mechanistic and public health purposes.

1. Introduction

Persistent organic pollutants (POPs), including organochlorine pesticides, polyhalogenated dibenzo-*p*-dioxins and furans and polyhalogenated biphenyls, among others, are persistent, bio-accumulative and toxic chemicals which distribute ubiquitously throughout the environment as complex mixtures and bio-magnify in the food chain (WHO UNEP, 2001). Organochlorine pesticides are comprised of a numerous group of chemicals which include dichlorodiphenyltrichloroethane (*p,p′*-DDT), its metabolite dichlorodiphenyldichloroethylene (*p,p′*-DDE), dicofol which is structurally-related to *p,p′*-DDT, hexachlorocyclohexane (HCH) and hexachlorobenzene (HCB).

Polychlorinated biphenyls (PCBs) were widely used as technical mixtures due to their low electrical conductance, fire resistance, resistance to thermal breakdown and chemical inertness. Among their 209 possible congeners, 12 of them show non-*ortho* or mono-*ortho* chlorine substitution allowing for a coplanar structure capable of activating the aryl hydrocarbon receptor (AhR) and thus they are known as dioxin-like PCBs (DL PCBs). The remaining congeners are known as non-dioxin-like PCBs (NDL PCBs). The presence of PCB contamination is estimated by

using indicator PCBs, i.e. the DL PCB-118 and the following 6 NDL congeners: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180 (FAO/WHO, 2016).

POPs can be frequently detected in human biological samples such as serum, breast milk and adipose tissue, and they are subject to biomonitoring (Fång et al., 2015; Porta et al., 2008; van den Berg et al., 2017; Vasseur and Cossu-Leguille, 2006). Noteworthy, intense research evidenced a potential contribution of POP exposure to the development of obesity, diabetes, cardiovascular diseases, metabolic syndrome, immune system alterations, cancer, as well as reproductive and developmental disorders (Deierlein et al., 2017; Gore et al., 2015; Heindel et al., 2017; La Merrill et al., 2013; Nadal et al., 2017). As human populations are exposed to complex mixtures of environmental contaminants, a broad number of mechanisms of action might operate simultaneously which explains the extensive individual variability of the aforementioned diseases. Nevertheless, many POPs along with other environmental contaminants cooperate on the induction of the oxidative stress affecting different organs and systems (Limón-Pacheco and Gonsebatt, 2009; Perkins et al., 2016). Frequently, POPs exert those adverse effects by interfering with the actions of hormones for which they are also considered to be endocrine-disrupting chemicals (EDCs). Although EDCs are known to alter oestrogen, androgen and thyroid signalling systems and steroidogenesis, other pathways might be disturbed as well, which might explain the deleterious effects of POPs on human health (OECD, 2012).

Under the current need for the identification of EDCs, the retinoic acid (RA) pathway has been considered to be a potential target (Grignard et al., 2020; OECD, 2012). The retinoid system is involved in vision (Kiser et al., 2014), metabolism (Blaner, 2019), functioning of the cardiovascular (D'Aniello and Waxman, 2015) and immune (Rühl, 2007) systems and development (Piersma et al., 2017), among others, so its disruption might anticipate adverse consequences to the organism. In fact, retinoid-related endpoints have been highlighted in adverse outcome pathways (Baker et al., 2020; Tonk et al., 2015) and they have been proposed for enhancing testing methods for developmental and reproductive toxicology (Nilsson, 2020), as different EDCs were able to interact with such a pathway (Esteban et al., 2019; Nilsson and Hakansson, 2002; Nishikawa et al., 2004; Novak et al., 2008; Shmarakov, 2015; Shmarakov et al., 2017). As previously reviewed (Blaner, 2019; Chelstowska et al., 2016; Kedishvili, 2013; Rhinn and Dolle, 2012; Rodriguez-Concepcion et al., 2018; Senoo et al., 2017; Tanumihardjo et al., 2016; Theodosiou et al., 2010), the retinoid system is dependent on the uptake, transport, metabolism, storage and signalling of retinoids and their precursors. Briefly, an adequate diet provides carotenes from plants, which are precursors of retinoids, as well as retinol (REOH) and retinyl esters from animals. In the cells, REOH is a pivotal element in the balance between storage and bioactivation of retinoids. REOH can be stored as such in the adipose tissue, whereas it is esterified into retinyl esters in the liver. Furthermore, REOH can be oxidized into functional RAs which activate retinoid receptors, i.e. retinoic acid receptor (RAR) and retinoic X receptor (RXR) (Blomhoff and Blomhoff, 2006). Additionally, RXR form heterodimers with other receptors such as the peroxisome proliferator-activated receptor (PPAR), constitutive androstane receptor (CAR) and thyroid hormone receptor, among others (Evans and Mangelsdorf, 2014).

Besides that, REOH is distributed in the circulation from the liver to peripheral tissues bound to the REOH binding protein (RBP4), by forming a complex with transthyretin (TTR) which prevents RBP4 glomerular filtration in the kidney (Blaner, 1989; Newcomer and Ong, 2000; Noy, 2000). With regard to RBP4 as an adipokine, following the publications by Barbara Kahn and colleagues (Graham et al., 2006; Yang et al., 2005), several studies reported associations with cardiovascular diseases, adiposity, non-alcoholic fatty liver disease, insulin resistance, type-2 diabetes, and components of the metabolic syndrome, though there are conflicting results among clinical studies (Blaner, 2019; Kotnik et al., 2011; Olsen and Blomhoff, 2020; Zabetian-Targhi et al., 2015).

Nonetheless, there is a lack of studies addressing the potential effects of human exposure to POPs on the adipose tissue retinoid system homeostasis. Since research in the GraMo cohort, studied herein, previously showed associations between POP exposure and a number of chronic diseases (Arrebola et al, 2013b, 2014a, 2014b, 2015; Mustieles et al., 2017), in which the retinoid system might play an important role, the aims of the current work were 1) to describe retinoid levels in serum and adipose tissue, along with RBP4 levels in serum from the GraMo cohort samples, and 2) to evaluate the associations between biomarkers of retinoid status and POP adipose tissue concentrations, and their effect modification (statistical interaction) with *in situ* oxidative stress markers in adipose tissue.

2. Materials and methods

2.1. Study population: GraMo cohort

This research work is part of a larger, ongoing prospective study (GraMo cohort) that aims to investigate the role of various environmental pollutants on the development of chronic conditions. The cohort was intraoperatively recruited in 2003–2004 from two public hospitals in the province of Granada, Southern Spain, i.e. San Cecilio University Hospital in the city of Granada (240,000 inhabitants) and Santa Ana Hospital in the town of Motril (60,000 inhabitants). Adipose tissue and serum samples were collected from different anatomical sampling regions during routine surgery: waist (46.1%), anterior abdominal wall (40.3%), and limbs (13.6%) and they were immediately coded and stored at −80 °C until chemical analysis. Socio-demographic and lifestyle data were collected at baseline with a validated questionnaire administered face-to-face by trained field staff. The details of the GraMo cohort, including methods of recruitment and biological sample collection, have been widely reported elsewhere (Arrebola et al, 2009, 2013a, 2014a, 2015; Artacho-Cordon et al., 2016). Initially, 409 participants were recruited out of whom 387 (94.6%) provided adipose tissue samples for POP analyses. From these, 183 and 170 provided, respectively, adequate adipose tissue and serum samples for retinoid system analyses. Overall, no relevant differences in their general characteristics were observed between participants included in the current study and the rest of the cohort participants (data not shown).

All participants signed the informed consent forms and the study was approved by the Biomedical Research Ethics Committee of Granada (Comité de Ética de la Investigación Provincial de Granada, 8/2016).

2.2. Retinoid observations

2.2.1. Retinol determination in serum and adipose tissue

Retinol (REOH) (purity > 95%) and retinyl acetate (REAC) (purity > 90%) were purchased from Sigma-Aldrich (Madrid, Spain). All the other reagents and chemicals used were of High-Performance Liquid Chromatography (HPLC) or analytical grade. REOH was analysed by HPLC (Agilent 1100 series, Agilent, CA, USA) as previously described (Mahiout et al., 2017; Schmidt et al., 2003). Briefly, protein precipitation was accomplished by adding 1.6 mL of isopropanol to either 50 µL of serum or 50 mg of adipose tissue homogenized in 300 µL of water. REAC was added as an internal standard for the determination of REOH. Retinoids were extracted from the organic phase after adding 3.2 mL of chloroform. Sample clean-up was performed by solid-phase extraction using an aminopropyl phase (Agilent SampliQ amino, Agilent, CA, USA). The analytes were eluted by using chloroform-isopropanol 2:1 (v/v). The solvent was evaporated, the dried residue reconstituted and finally transferred to HPLC micro-vials. Separation was achieved on a Poroshell 120 EC-C18 column (Agilent, CA, USA) and detection performed at 325 nm for REOH and REAC.

2.2.2. Quantification of RBP4 levels in serum

RBP4 levels in sera were determined by using the enzyme-linked

immunosorbent assay kit BMS2199 (Invitrogen, Life Technologies, Carlsbad, CA, USA). Briefly, samples were diluted at 1:40,000 with sample diluent. A lyophilized human RBP4 standard was reconstituted at 8 ng/mL and then 7 standards were prepared between 0.063 and 4 ng/mL by serial dilutions with sample diluent. Samples were analysed in duplicates according to kit instructions. The colour development was monitored at 620 nm and the absorbance was recorded at 450 nm by using a Beckman Coulter 34 CE0C microplate reader (Palo Alto, CA, USA). The quantification of RBP4 levels was considered acceptable when absorbance of sample duplicates was within 20% of the mean value. Levels of RBP4 in ng/mL were calculated by using the package *polynom* in R software version 3.5.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). Conversion into nmol/mL units was performed by using the molecular weight value of about 21000 g/mol (Colantuoni et al., 1983). Hence, the RBP4/REOH ratio was calculated by dividing RBP4 by REOH levels both expressed in nmol/mL. Glass vacuum tubes for serum separation without anticoagulants were used to collect blood samples so that additive-related artifacts on RBP4 levels were avoided (Graham et al., 2007).

2.3. POP chemical analyses in adipose tissue

Sample analysis and purification procedures were conducted as previously described (Frias et al., 2004; Rivas et al., 2001). A selection of POPs were quantified by high-resolution gas chromatography coupled with a mass spectrometry detector in tandem mode, as detailed elsewhere (Arrebola et al, 2009, 2010, 2013a). We analysed concentrations of p,p′-DDE, the main metabolite of the pesticide p,p′-DDT, HCB, dicofol, α- and β- HCH, and PCB congeners −138, −153 and −180. Chromatographic concentrations below the limit of detection (LOD) were assigned a random value between 0 and the LOD. Lipid content in adipose tissue samples was quantified gravimetrically as previously reported (Rivas et al., 2001). Lipid-basis POP concentrations were calculated and expressed in nanograms per gram of lipid (ng/g lipid).

2.4. Analysis of oxidative stress markers in adipose tissue

The oxidative stress biomarkers were analysed using commercially available kits (Enzo Life Sciences, Inc., Farmingdale, NY, USA) in an automatic microplate reader (TRIAD MRX II series, Dynex Technologies Inc., Chantilly, Virginia, USA), as previously described (Leon et al., 2019). We analysed total superoxide dismutase (SOD) activity (isoenzymes SOD1 [cytosolic Cu/Zn SOD], SOD2 [mitochondrial Mn SOD, and SOD3 [extracellular Cu/Zn SOD]), heme oxygenase-1 levels, glutathione peroxidase and glutathione reductase activities, glutathione and glutathione disulfide levels, and thiobarbituric acid reactive substance-lipid peroxidation (TBARS) in adipose tissue.

2.5. Statistical analysis

Retinol and RBP4 levels were described by using medians, along with 25th and 75th percentiles. The RBP4/REOH ratio was calculated and included in the statistical analysis following previous publications (Aeberli et al., 2007; Erikstrup et al., 2009; Krzyzanowska et al., 2008; Mills et al., 2008; Ortega-Senovilla et al., 2019). The shapes of the potential associations of POP concentrations with retinol and RBP4 levels were visually evaluated through generalized additive models. The associations were further explored by means of multivariable linear regression analyses. The models were adjusted for age, body mass index (BMI), sex, education, residence, and consumption of alcohol, fruit, vegetables, meat, dairy products, fish, and milk. Adjustment for dietary intake was based on previous evidences supporting a key role of diet on both POP concentrations (Arrebola et al., 2018) and retinoid levels (Tanumihardjo et al., 2016). The following categories were used in the covariates: women and men for sex; urban and semi-urban for residence; no studies and primary or higher for education; non-smoker and former

smoker or smoker for smoking habit; non-habitual consumer and habitual consumer for alcohol consumption; 1 portion/week and ≥2 portions/week for fruit consumption; 1 portion/week; 2 portions/week, > 2 portions/week for vegetable consumption; 1 portion/week, 2 portion/week and >2 portions/week for meat consumption; ≤ 1 portion/-day and >2 portions/day for dairy product consumption; 1 portion/week, 2 portions/week and >2 portions/week for fish consumption. Independent and dependent variables were log-transformed in order to reduce the skewed distributions. Therefore, β coefficients are also presented as exp(β). Considering their high frequency of detected samples (>80%), all independent and dependent were considered both as continuous and in quartiles, with the exception of dicofol and α-HCH concentrations, that were dichotomized (>LOD/< LOD) because of the low rate of detection. Based on previous results (Artacho-Cordon et al., 2016), the interaction between POPs and the elements of the retinoid system on the oxidative microenvironment was studied by entering their product term in the equation.

The combined effect of POPs on the retinol and RBP4 levels in adipose tissue was assessed by using weighted quantile sum regression analyses (WQS), which calculates a weighted index from the individual associations of the exposure variables with each oxidative stress marker, and in which each chemical has a specific weight. We further explored the associations between each WQS index and its corresponding outcome using multivariate linear regression. It has been demonstrated that WQS regression is a highly accurate method to address collinearity and high-dimensionality (Carrico et al., 2015). Statistical tests were run in R version 3.5.2 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria), and gWQS package v2.0.1 for the calculations of WQS index (Renzetti et al., 2020).

3. Results

3.1. Description of the study population

The main sociodemographic, lifestyle and clinical characteristics of the study population are summarized in Table 1. Dietary characteristics and POP concentrations in adipose tissue are provided as Supplementary material in Tables S1-2. An extensive characterization of GraMo cohort has been described elsewhere (Arrebola et al, 2009, 2010, 2012, 2013a). REOH levels in both serum and adipose tissue, and RBP4 levels in serum, as well as the RBP4/REOH ratio in serum are summarized in Table 2.

Table 1
Sociodemographic, lifestyle and clinical characteristics of the GraMo cohort.

Observation	Subset	
	Adipose tissue	Serum
Count	N = 183	N = 170
Sex		
Women	85 (46.4%)	89 (52.4%)
Men	98 (53.6%)	81 (47.6%)
Residence		
Urban	89 (48.6%)	63 (37.1%)
Semi-rural	94 (51.4%)	107 (62.9%)
Education		
No studies	53 (29.0%)	47 (27.6%)
Primary or higher	130 (71.0%)	123 (72.4%)
Smoking habit		
Non-smoker	72 (39.3%)	77 (45.3%)
Former smoker	54 (29.5%)	36 (21.2%)
Smoker	57 (31.1%)	57 (33.5%)
Alcohol consumption		
Non-habitual consumer	92 (50.3%)	79 (46.5%)
Habitual consumer	91 (49.7%)	91 (53.5%)
Adipose tissue origin		
Hernia	89 (48.6%)	–
Gall Bladder	28 (15.3%)	–
Varicose Veins	8 (4.4%)	–
Other	58 (31.7%)	–

3.2. Multivariable analyses

With the exception of β-HCH, all the studied POPs showed negative beta coefficients for serum REOH, that were only marginally significant for dicofol, so that those individuals with detectable dicofol concentrations showed lower levels of REOH in serum [exp(β) 0.703, p = 0.051] (Table 3).

On the contrary, those participants within the fourth quartile of the β-HCH concentrations showed increased REOH levels in serum compared with those in the first quartile (Fig. 1A).

We did not find evidence of any significant association between POPs and RBP4 levels in serum (Supplementary material, Table S3).

In addition, all POPs showed positive coefficients in the models for serum RBP4/REOH ratios that were only significant for PCB-180 [exp(β) 1.058, p = 0.040] and marginally significant for PCB-153 [exp(β) 1.058, p = 0.069)] (Table 3). Additionally, significantly increased serum RBP4/REOH ratios were found in the fourth quartile of PCB-138, -153 and −180 concentrations compared with those in the first quartile (Fig. 1B–D).

On the other hand, adipose tissue concentrations of PCB-138 [exp(β) 0.931, p = 0.008], PCB-153 [exp(β) 0.887, p = 0.001], PCB-180 [exp(β) 0.895, p = 0.001] and dicofol [exp(β) 0.762, p = 0.016] were inversely associated with REOH concentrations in adipose tissue (Table 4). We did not find evidence of any significant association between organochlorine pesticide concentrations (p,p'-DDE, HCB, α-HCH and β-HCH) and REOH levels in adipose tissue (Table 4).

On the basis of the relatively increased number of significant associations in the models for adipose tissue REOH levels, we performed WQS analyses in order to ascertain a potential mixture effect. Such analyses revealed a significant positive mixture contribution of the three PCB congeners on adipose tissue REOH levels (β = −0.12; p = 0.02), with relative contributions of 52% (PCB-180), 30% (PCB-153), and 18% (PCB-138) to the overall WQS index.

As PCBs levels were positively associated with oxidative stress markers such as increased SOD levels in the GraMo cohort (Artacho-Cordon et al., 2016), herein the interactions between retinol levels in adipose tissue and each of the concentrations of PCB-138, -153 and −180 on the induction of SOD levels were evaluated by entering their product term in the model equations. In this regard, we found a marginally significant interaction between PCB-153 and adipose tissue retinol levels (p = 0.057, data not shown) so that higher retinol levels in adipose tissue were related to a lower effect of the aforementioned PCBs on the increase of SOD levels (Fig. 2).

Assuming that diet is the main source for POP exposure, the inclusion of dietary habits as covariates might cause an over-adjustment of the models. Therefore, we performed sensitivity analyses by removing dietary habits from the models, with no relevant modification in the magnitude or significance of the associations found (data not shown in tables).

Table 2
Concentrations of retinoid parameters in serum and adipose tissue of the GraMo cohort.

Observation	Subset	
	Adipose tissue	Serum
REOH (nmol/g; nmol/mL)		
Median (Q1, Q3)	1.03 (0.68, 1.40)	1.96 (1.48, 2.53)
Max.	5.21	4.75
RBP4 (nmol/mL)		
Median (Q1, Q3)	–	1.56 (1.13, 2.22)
Max.	–	4.36
RBP4/REOH Ratio		
Median (Q1, Q3)	–	0.82 (0.59, 1.04)
Max.	–	8.52

Data are expressed as median and 1st and 3rd quartiles (Q1, Q3).

Table 3
Individual multivariable linear regression models for the association between each POP and retinoid parameters in serum.

	β	SE	exp(β)	p-value
Retinol				
p,p'-DDE	−0.016	0.031	0.984	0.594
HCB	−0.010	0.022	0.990	0.667
β-HCH	0.012	0.021	1.012	0.566
PCB-138	−0.005	0.016	0.995	0.759
PCB-153	−0.003	0.049	0.997	0.950
PCB-180	−0.004	0.023	0.996	0.869
Dicofol	***−0.353***	***0.177***	***0.703***	***0.051***
α-HCH	−0.117	0.239	0.889	0.625
RBP4/REOH ratio				
p,p'-DDE	0.007	0.035	1.007	0.839
HCB	0.025	0.025	1.025	0.334
β-HCH	0.010	0.028	1.010	0.731
PCB-138	0.028	0.018	1.029	0.128
PCB-153	***0.056***	***0.031***	***1.058***	***0.069***
PCB-180	**0.057**	**0.027**	**1.058**	**0.040**
Dicofol	0.037	0.087	1.038	0.669
α-HCH	0.070	0.113	1.072	0.536

SE, standard error. Models were adjusted for age, BMI, sex, education, residence, and consumption of alcohol, fruit, vegetable, meat, dairy products, fish, and milk. POP concentrations in adipose tissue were entered in ng/g lipid, with the exception of those of dicofol and α-HCH which were entered as categories higher or lower than the limit of detection. Bold fonts highlight associations with p-values <0.05. Bold and italics fonts highlight associations with p-values <0.10.

4. Discussion

In the present exploratory observational study, we found suggestive evidences of potential PCB and dicofol-induced responses of key elements within the retinoid system known to be involved in the homeostasis of healthy organisms (Blaner, 2019; Blomhoff and Blomhoff, 2006; D'Aniello and Waxman, 2015; Piersma et al., 2017; Rhinn and Dolle, 2012; Tanumihardjo et al., 2016; Theodosiou et al., 2010) (Fig. 3). This is one of the very first studies showing the effects of POP exposure on the human retinoid system in adipose tissue, which is considered to be the best biological sample to assess the cumulative exposure to POPs (Mustieles and Arrebola, 2020).

One of the mechanisms by which PCBs could alter retinoid homeostasis is related with the distribution of retinoids in the circulation in an AhR-independent manner, and hence of possible relevance for non-dioxin-like compounds such as the PCBs −180, −153 and −138, while CYP induction is involved in the generation of their hydroxylated metabolites (Dhakal et al., 2018) (Fig. 3). In fact, it has been reported that hydroxylated PCBs show affinity towards TTR (Brouwer et al., 1988; Brouwer and Vandenberg, 1986; Dhakal et al., 2018; Gutleb et al., 2010; Miller et al., 2009; Routti et al., 2019; Zoeller, 2007), as well as other ubiquitous contaminants, including hydroxylated polybrominated diphenyl ether (PBDEs) (Hamers et al., 2020; Meerts et al., 2000), since they share structural and toxicological similarities (Miller et al., 2009; Zoeller, 2007). Such interaction between environmental pollutants and TTR caused the displacement of RBP from the TTR-RBP complex and hence the reduction of both RBP and REOH levels in blood by increased glomerular clearance (Brouwer et al., 1988; Brouwer and Vandenberg, 1986; Ellis-Hutchings et al, 2006, 2009) (Fig. 3). Additionally, female zebrafish exposed to an environmentally relevant concentration of the PBDE technical mixture DE-71 showed increased RBP4 levels in serum together with an overall reduction of the hepatic stores of retinoids (Chen et al., 2012). Besides that, it was suggested that dicofol, an acaricide used at relatively high doses which is structurally similar to p,p'-DDT, binds TTRs (Ishihara et al., 2003). Such perturbation of the TTR-RBP complex together with the mobilization of hepatic retinoid stores explained, at least in part, changes in serum REOH levels found in experimental animals treated with commercial mixtures of PCBs such as
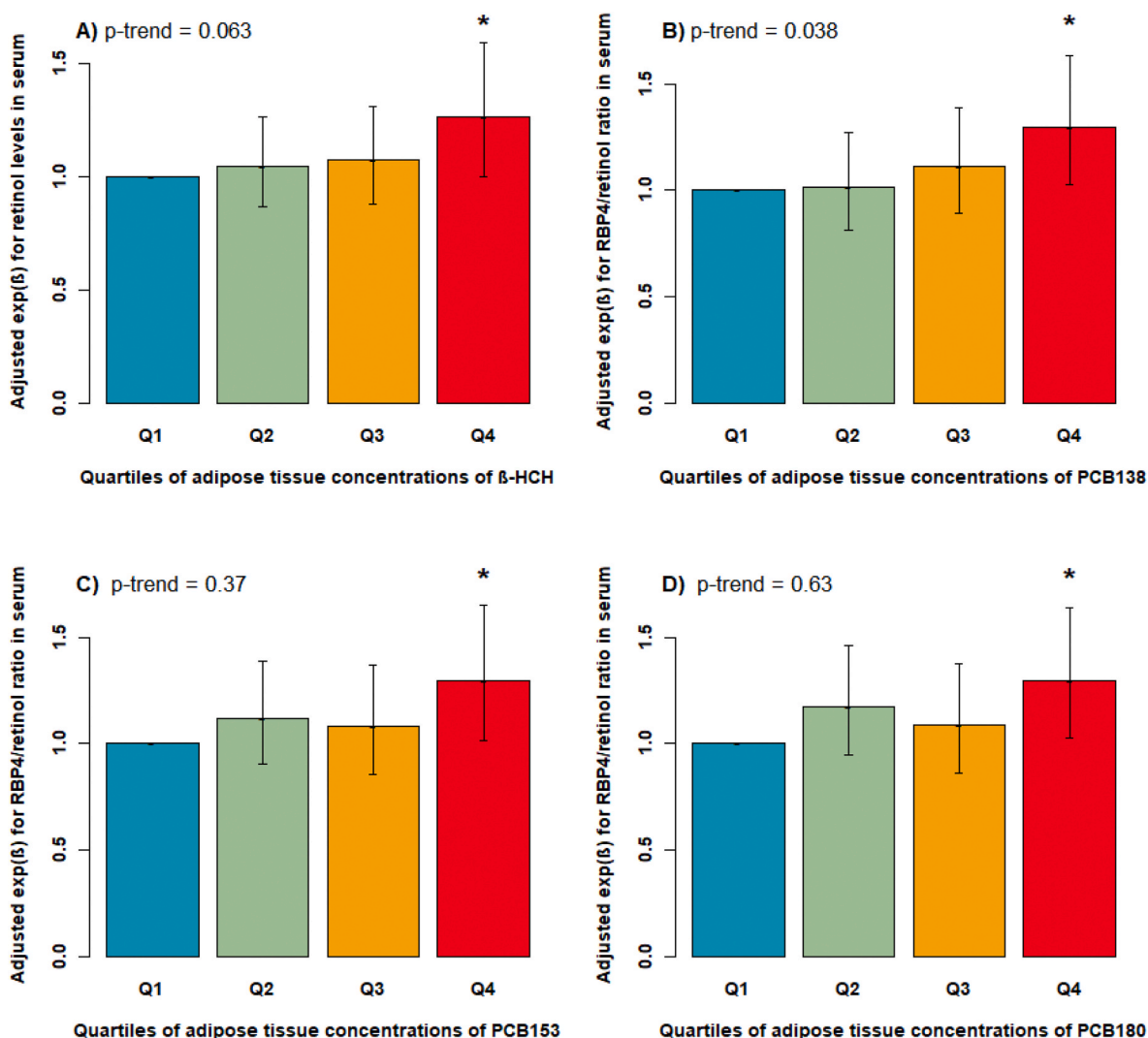
Fig. 1. Adjusted exp(β) for the associations between quartiles of adipose tissue POP concentrations and retinoid measurements. The error bars represent the 95% confidence interval of exp(β). The linear regression models were adjusted for age, BMI, sex, education, residence, and consumption of alcohol, fruit, vegetable, meat, dairy products, fish, and milk. p-trend, p-value of the linear trend. * p-value < 0.05. Q1 was always used as the reference category.

Table 4
Individual multivariable linear regression models for the association between each POP and retinol in adipose tissue.

	β	SE	exp(β)	p-value
p,p'-DDE	−0.003	0.043	0.997	0.950
HCB	−0.014	0.040	0.986	0.727
β-HCH	−0.037	0.032	0.963	0.249
PCB-138	**−0.071**	**0.026**	**0.931**	**0.008**
PCB-153	**−0.120**	**0.036**	**0.887**	**0.001**
PCB-180	**−0.111**	**0.034**	**0.895**	**0.001**
Dicofol	−0.272	0.112	0.762	0.016
α-HCH	−0.086	0.130	0.918	0.509

SE, standard error. Models were adjusted for age, BMI, sex, education, residence, and consumption of alcohol, fruit, vegetable, meat, dairy products, fish, and milk. POP concentrations in adipose tissue were entered in ng/g lipid, with the exception of those of dicofol and α-HCH which were entered as categories higher or lower than the limit of detection. Bold fonts highlight associations with p-values <0.05.

Aroclor 1254 (Esteban et al., 2014), ultrapure PCB-180 (Viluksela et al., 2014), among others (Nilsson and Hakansson, 2002; Novak et al., 2008; Shmarakov, 2015).

The importance of REOH determination in adipose tissue, as carried out herein, stems from the correlation between retinoid levels in adipose tissue and those in the liver (Sheftel et al., 2019), the latter being regarded as the gold standard biomarker for vitamin A status assessment (Tanumihardjo, 2011; Tanumihardjo et al., 2016). Additionally, adipose tissue plays a pivotal role in the development of an increasing number of chronic conditions of highly public health concern, such as obesity, diabetes, cardiovascular diseases and cancer (Mustieles et al., 2017). Interestingly, several POPs are believed to be involved in the aetiology of such conditions (Gore et al., 2015; La Merrill et al., 2013; Lee et al., 2017; Nadal et al., 2017). Thus, the toxicological relevance of our findings relies on the fact that REOH is a precursor of RA involved in the homeostasis of adipose tissue (Blaner, 2019; Bonet et al, 2012, 2015, 2020; Evans and Mangelsdorf, 2014; Saeed et al., 2018) (Fig. 3) for which it might be an early indicator of adverse effects, which might be apparent later in time and/or at higher exposures to POPs.

Currently there is a lack of studies assessing the potential effects of POPs such as PCBs and dicofol on the retinoid system in adipose tissue of human populations. A previous study reported a positive association between p,p'-DDE and REOH in adipose tissue of women from five different cities, though its biological implications were unclear (Sanz-Gallardo et al., 1999). Thus, in our study we intend to assess the association between REOH levels in adipose tissue and PCB exposure (Fig. 3), as those compounds are known to reduce retinoid stores
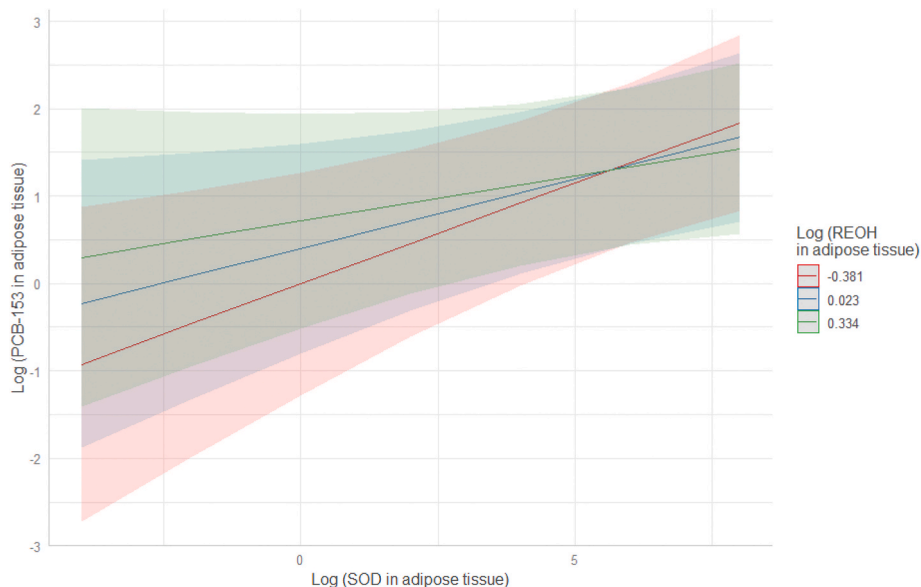
Fig. 2. Association of log-PCB-153 concentrations and log-SOD concentrations at three percentiles of log-retinol in adipose tissue (25th, 50th, 75th). SOD, superoxide dismutase; REOH, retinol levels in adipose tissue.
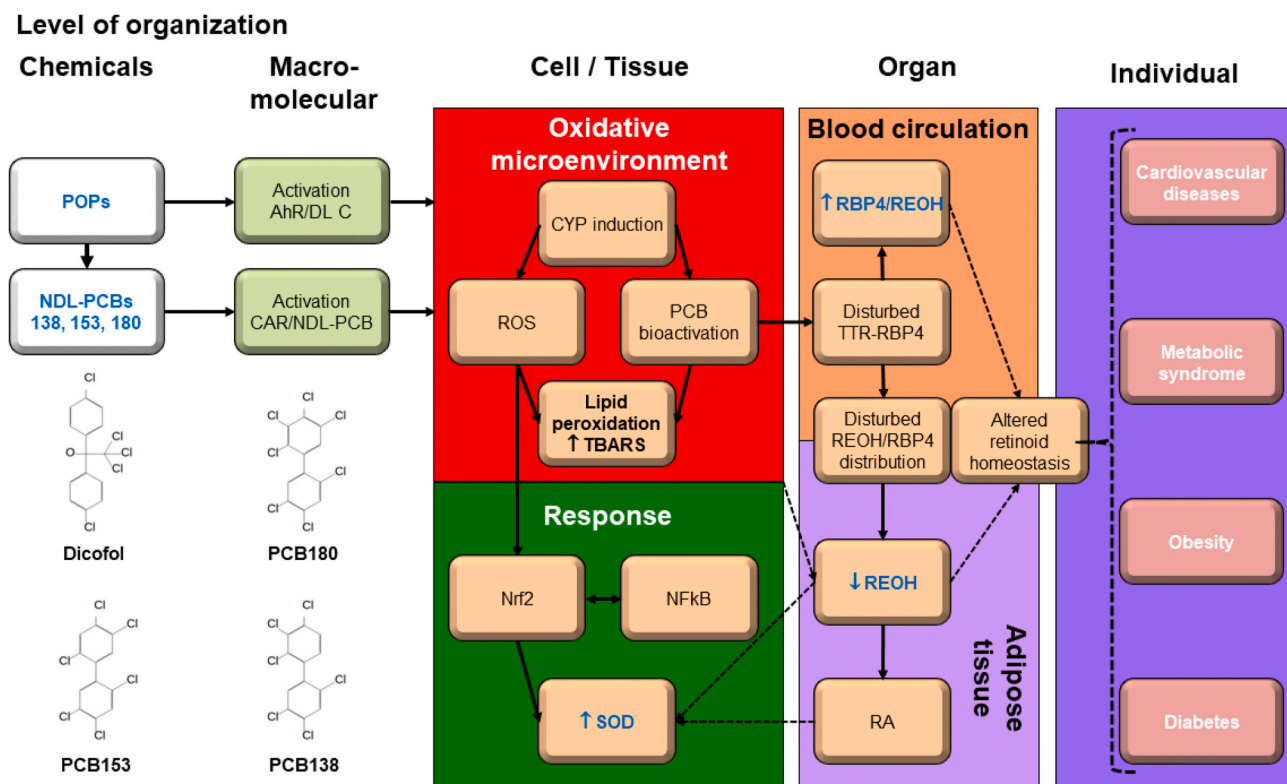

Fig. 3. Hypothesized pathway and conceptual framework for the associations of adipose tissue persistent organic pollutants with retinoid observations and chronic diseases found within the GraMo cohort. Blue font, results acquired and/or statistically analysed in the current work; white font, chronic diseases, in which the retinoid system might be involved; solid arrow, accepted relationships between events; dashed arrows, hypothesized relationships between events under study; AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; CYP, Cytochrome P450; DL C, dioxin-like compounds; NDL PCB, non-dioxin-like polychlorinated biphenyl; NFkB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-like 2; PCB, polychlorinated biphenyl; POP, persistent organic pollutant; RA, retinoic acid; RBP4, retinol binding protein 4; REOH, retinol; ROS, reactive oxygen species; SOD, superoxide dismutase, TBARS, thiobarbituric acid reactive substance-lipid peroxidation; TTR, transthyretin. This exploratory observational study reports preliminary findings, which facilitate potential mechanistic views on key elements of the retinoid system, the exposure to POPs and an oxidative stress microenvironment. Future research is needed to consider those interactions as conclusive and causal. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Nilsson and Hakansson, 2002; Novak et al., 2008; Roos et al., 2011; Shmarakov, 2015; Shmarakov et al., 2019; Viluksela et al., 2014). Additionally, an experimental mixture of 27 contaminants including PCBs, organochlorine pesticides, and methylmercury (at similar exposure levels than those found in the blood of the Canadian Arctic Inuit population) caused a reduction of the hepatic retinoid stores in the rat offspring (Elabbas et al., 2014). Noteworthy, the retinoid stores in adipose tissue are also mobilized to maintain retinoid homeostasis in the organism (Blaner, 2019). While it has long been known that DL PCBs decrease hepatic REOH and retinyl ester levels by an AhR-dependent mechanism (Mahiout et al., 2017; Nilsson and Hakansson, 2002; Novak et al., 2008), NDL PCB-153 disturbed the retinoid system in a CAR-dependent one, as it was found in C57BL/6 male mice (Shmarakov et al., 2019). Thus, this might explain the association between the levels of PCBs −180, −153 and −138 with the above-mentioned REOH reduction in adipose tissue observed herein (Fig. 3). Noteworthy, REOH as a precursor of functional RAs (de Lera et al., 2016; Krężel et al., 2019) might also be involved in the activation of the RXR-CAR heterodimer, as well as in the activation of other RXR heterodimers including those with farnesoid X receptor, thyroid hormone receptor and vitamin D receptor (Evans and Mangelsdorf, 2014), which also causes a consumption of functional retinoids (Shmarakov, 2015).

In any case, human populations are frequently exposed to mixtures of POPs including both DL and NDL PCBs. Moreover, PCBs −180, −153 and −138 are included within the PCB indicators used to estimate the body burden of PCBs (FAO/WHO, 2016). Thus, the activation of both AhR and CAR might be anticipated in general populations with the consequent induction of the corresponding CYP isoforms (Al-Salman and Plant, 2012; Chen and Liu, 2019; Shmarakov et al., 2019). Such inductions have been associated to the generation of reactive intermediate PCB metabolites, including PCB arene oxides and quinones, and resultant generation of reactive oxygen species (Dhakal et al., 2018; Klopcic and Dolenc, 2019) (Fig. 3). Additionally, CYP activities release hydrogen peroxide and superoxide radical anion by uncoupling reactions (Denisov et al., 2005; Veith and Moorthy, 2018). In fact, the activation of CAR induced the nuclear factor erythroid 2-like 2 (Nrf2) (Rooney et al., 2019), which triggers the response of antioxidant enzymes such as SOD involved in the biotransformation of superoxide radical anion into hydrogen peroxide (Limón-Pacheco and Gonsebatt, 2009). Furthermore, dicofol was suggested as a source of reactive oxygen species in red blood cells (Ahmad and Ahmad, 2017). For all the above, both CYP-mediated bioactivation of PCBs and reactive oxygen species generated by different sources contribute, at least in part, to the development of the oxidative stress microenvironment observed in the adipose tissue, and hence, of a number of health conditions (Artacho-Cordon et al., 2016; Mustieles and Arrebola, 2020) (Fig. 3). Additionally, POP exposure has been associated with the translocation of the nuclear factor kappa B (NFkB) into the nucleus for the expression of a number of genes involved in inflammatory processes thus aggravating the oxidative stress (Peinado et al., 2020).

Exposure to environmental levels of POPs such as β-HCH, dicofol, and PCBs −180, −153 and −138, was previously associated to the generation of an oxidative stress microenvironment and the response of adipose tissue SOD activity in GraMo cohort (Artacho-Cordon et al., 2016). The robustness of the association was strengthened with the degree of chlorination. Remarkably, the heptachlorobiphenyl PCB-180, and the two hexachlorobiphenyls PCBs −153 and −138, show half-lives measured in years with high bioaccumulation potential due to their slow biotransformation being precursors of lower chlorinated and reactive intermediate oxidized PCBs (Dhakal et al., 2018; FAO/WHO, 2016). In the current work, those GraMo participants with higher REOH storages showed lower SOD activities in adipose tissue (Fig. 3). Thus, the current study expands on the observations found previously (Artacho-Cordon et al., 2016), involving key elements of the retinoid system in the adipose tissue. Such observation on the relationship among REOH, SOD and PCB levels in adipose tissue might be compatible with a protective effect

in cases with an enriched anti-oxidant environment. Along with ascorbate and tocopherol, well known protective factors against reactive oxygen species, carotenes located in cell membranes quench liposoluble radicals (Limón-Pacheco and Gonsebatt, 2009). Additionally, β-carotene-15,15′-dioxygenase converts carotenes into retinal, which in turn can be reduced to REOH or oxidized to RA. Then, retinoid system plays a role in such protective microenvironment involving RXR-RAR and RXR-PPAR heterodimers for the regulation of gene expression (Balmer and Blomhoff, 2002) involving Nrf2 and NFkB pathways (Bohn, 2019; Bonet et al., 2020; Kaulmann and Bohn, 2014; Rodriguez-Concepcion et al., 2018; Theodosiou et al., 2010).

In the present study, adipose tissue samples were collected from different anatomical locations, which might induce certain variability in the measurements of retinoid parameters, which would result in a non-differential bias. Nevertheless, there was a correlation in the REOH levels between epididymal and retroperitoneal fat of male Mongolian Gerbils (Sheftel et al., 2019) and the sampling site of breast adipose tissue did not affect REOH concentrations (Rautalahti et al., 1990). Furthermore, socio-demographic, lifestyle, and dietary determinants influence retinoid levels (Moran et al., 2018). Particularly, a borderline significant association was found with alcohol consumption. As alcohol affects the endocrine activity of adipose tissue and liver (Blaner et al., 2017) and both organs are involved in the retinoid system homeostasis, alcohol consumption must be controlled to assess perturbations on the retinoid system (Clugston and Blaner, 2012; Gyamfi and Wan, 2010; Kim et al., 2015; Napoli, 2011), as we did herein. With regard to RBP4 determination, single nucleotide polymorphisms, renal dysfunction in diabetic patients and shortcomings in methodology might affect RBP4 levels (Blaner, 2019; Kotnik et al., 2011). In any case, not only western blotting but also commercial ELISA kits provided correlations between those diseases and RBP4 serum levels (Erikstrup et al., 2009; Gavi et al., 2007; Graham et al., 2006; Mills et al., 2008; Reinehr et al., 2008; Yao-Borengasser et al., 2007). Although a potential loss of sensitivity due to the use of RBP4 ELISA kits might underestimate the associations, they have been considered reliable for the determination of RBP4 (Erhardt et al., 2004).

The selection of POPs in this study was based on the following criteria: 1) they are frequently detected in human populations (Arrebola et al, 2009, 2010, 2013a) and 2) we found associations of these chemicals with oxidative stress and a number of health conditions potentially related to retinoid levels (Arrebola et al, 2013b, 2014a, 2014b, 2015; Artacho-Cordon et al., 2016; Mustieles et al., 2017).

In our study, we cannot rule out the influence of other environmental factors not accounted for, such as other co-exposures or undetected dietary patters. Furthermore, considering the cross-sectional design of the current research, we cannot rule out reversed-causality and feedback regulation issues, that would hamper the validity of our results. However, these issues would only affect (if so) to retinoid and oxidative stress markers, since it is unlikely that these effect biomarkers would modify POP concentrations in our population. In this regard, we believe that these issues would represent a non-differential error, but not cause false positive associations. Therefore, our study has yielded robust and suggestive associations that warrant further confirmation in longitudinal studies. In this regard, GraMo cohort provides a unique opportunity to study subclinical effects of lipophilic chemicals in adipose tissue. Indeed, the cohort has been extensively characterized in terms of POP exposure and its association with cancer, obesity, diabetes, arterial hypertension, among others, some of which might involve endocrine disruption (Arrebola et al, 2013b, 2014a, 2014b, 2015; Mustieles et al., 2017).

Overall, our results suggest that exposure to POPs at environmental levels in an adult cohort from Southern Spain might disturb the distribution and bioavailability of retinoids on the basis of increased serum RBP4/retinol ratio, indicative of metabolic disease, and the reduction of retinol stores, a precursor of all-*trans*-retinoic acid involved in the regulation of gene expression through the activation of RXR-RAR, in the adipose tissue. In addition, adipose tissue retinol levels were found to

represent a likely protective factor by ameliorating the SOD induction positively associated with PCBs −138, −153 and −180 concentrations. Considering the relevance of the retinoid system in homeostasis, our findings might have important health implications at a population level.

Ethics in publishing

Ethics committee

The study was approved by the Biomedical Research Ethics Committee of Granada (Comité de Ética de la Investigación Provincial de Granada, 8/2016).

Credit author statement

Suylen Galbán Velázquez: Investigation, Writing - Original Draft, Visualization; Javier Esteban: Conceptualization, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Funding acquisition; Gonca Çakmak: Investigation, Writing - Original Draft, Writing - Review & Editing, Supervision; Francisco Artacho-Cordón: Investigation, Writing - Review & Editing; Josefa León: Investigation, Writing - Review & Editing; José Barril: Investigation, Writing - Review & Editing, Funding acquisition; Fernando Vela-Soria: Investigation, Writing - Review & Editing; Piedad Martin-Olmedo: Investigation, Writing - Review & Editing; Mariana F Fernandez: Investigation, Writing - Review & Editing; María de la Cruz Pellín: Investigation, Writing - Review & Editing, Funding acquisition; Juan P Arrebola: Conceptualization, Investigation, Resources, Writing - Review & Editing, Visualization, Project administration, Funding acquisition.

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.envres.2021.110764.

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