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Associations of accumulated exposure to persistent organic pollutants with serum lipids and obesity in an adult cohort from Southern Spain

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ABSTRACT

The aim of this research was to study the association of the accumulated human exposure to persistent organic pollutants with serum lipid levels and obesity, in a cohort of 298 adults.

In the multivariable analyses, HCB concentrations evidenced a significant quadratic association with levels of total cholesterol, HDL, LDL, and total serum lipids. Likewise, PCBs 138 and 180 were associated with triglycerides and total serum lipids, and PCB 153 with LDL. HCB, p,p′-DDE, and β-HCH showed quadratic associations with BMI. All quadratic models showed a positive trend at low exposure levels, while the slope decreased or even became negative at higher exposure levels. Additionally, PCB 138 was positively associated with BMI but in a linear manner. Our results suggest a potential relationship between historical POP exposure and serum lipids/obesity, which followed a non-linear pattern in most cases.

1. Introduction

Cardiovascular diseases (CVDs) are among the most frequent causes of adult mortality in developed countries (Goncharov et al., 2008). In Spain, cardiac and cerebrovascular diseases were, respectively, the 2nd and 3rd causes of death in 2010 (Regidor and Gutiérrez-Fisac, 2013). Main risk factors for CVD include high-fat diet, stress, smoking, genetic susceptibility, obesity, dyslipidemia, diabetes, or hypertension (Meigs, 2000; Lee et al., 2011; Goncharov et al., 2008). Thus, researchers have proposed a cluster of pathophysiological conditions, closely related to CVD, called the "metabolic syndrome", which includes dyslipidemia, obesity, hypertension, and insulin resistance and affects 20–30% of the European population (Branca et al., 2007; Shen et al., 1970).

The prevalence of obesity has tripled over the past few decades in Europe, reaching epidemic proportions (Branca et al., 2007). Obesity is usually recognized as the result of increased calorie intake and decreased energy expenditure, with a genetic contribution that has been estimated as 20–84% (Sharpe and Drake, 2013). However, there is increasing evidence that environmental factors play a major role in this condition (McAllister et al., 2009). It is well documented that adipose tissue is an endocrine organ that actively participates in the regulation of metabolism (Kershaw and Flier, 2004), and that the accumulation and mobilization of lipids in adipose tissue is highly influenced by hormonal signaling (Grun and Blumberg, 2009b).

Persistent organic pollutants (POPs) are a wide group of highly lipophilic environmental pollutants that tend to accumulate and biomagnify in food chains, resulting in the considerable exposure of living organisms (UNEP, 2003). POPs include organochlorine pesticides, which have long been widely used in agriculture and public health as highly effective pest control agents (UNEP, 2003). POPs also comprise polychlorinated biphenyls (PCBs), used worldwide in numerous industrial and commercial applications. POPs have been detected in virtually all human populations and environmental matrices, and diet (especially fatty food) has been reported to be

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the main route for human exposure (Brauner et al., 2011). Once absorbed, POPs are mainly stored in the adipose tissue, where they can be released or persist for decades (Yu et al., 2011).

There is scientific evidence that human exposure to some POPs might be involved in lipid homeostasis disorders, and several action mechanisms have been proposed at different levels, including the down-regulation of insulin-induced gene-1 (Insig-1) and Lpin1, two master regulators of lipid homeostasis (Ruzzin et al., 2010). Additionally, dioxin- and non-dioxin-like PCBs have been reported to induce P450 enzymes, causing an over-reactive liver that synthesizes increased levels of cholesterol and triglycerides (Goncharov et al., 2008). Moreover, PCBs can induce oxidative stress in endothelial cells by stimulating inflammatory processes (Choi et al., 2003). Many POPs are also suspected of acting as obesogens, i.e., capable of altering lipid accumulation and adipogenesis (Grun and Blumberg, 2007) and therefore promoting obesity and obesity-related disorders (Sharpe and Drake, 2013). Besides the aforementioned lipid metabolism impairments, other reported mechanisms of action include the enhancement of adipocyte hyperplasia, disruption of energy balance controlling systems, and interaction with the nervous system (Grun and Blumberg, 2009a; Newbold et al., 2009; Heindel and vom Saal, 2009). It has also been suggested that lipophilic chemicals, such as POPs, may facilitate absorption across membranes of hydrophilic endocrine disruptors that would not otherwise be absorbed, which is also potentially related to alterations in lipid levels (Zeliger, 2003, 2013).

Some epidemiological evidence has also been reported on the disruptive potential of POPs in serum lipid homeostasis. Goncharov et al. (2008) found that high serum PCB levels were associated with elevated serum lipids and CVD risk in a native American population, while others have also described these associations in subpopulations highly exposed to PCBs (Chase et al., 1982; Baker et al., 1980; Kreiss et al., 1981; Tokunaga and Kataoka, 2003). Furthermore, Lee et al. (2011) described significant associations between low POP exposure levels and various lipid metabolism parameters (e.g., triglycerides and HDL) in a sample of non-diabetic individuals.

Nevertheless, the health effects of human exposure to chronic low doses of POPs remain poorly understood (Porta et al., 2008) and paradoxical results have been reported, with larger effects from low than high doses in some cases (Hennig et al., 2002).

The study of the potential influence of POP exposure on lipid homeostasis is of special concern, because it has been suggested that POP-mediated elevation in serum lipids might be related to the increased incidence of cardiovascular disease observed in individuals with elevated exposure to PCBs and organochlorine pesticides (Aminov et al., 2013). Therefore, the aim of the present research was to study the relationship of the accumulation of POPs in adipose tissue with serum lipid levels and obesity in a cohort of adults from Southern Spain.

2. Material and methods

2.1. Study area and population

The study population and epidemiological design were extensively described elsewhere (Arrebola et al., 2013a, 2013b, 2009, 2010). In summary, adipose tissue/serum samples and data were collected between July 2003 and June 2004 in two areas of Granada province (Southern Spain) that are approximately 70 km apart (from center to center): a densely populated urban area, corresponding to the city of Granada and metropolitan suburbs (economy based on the service sector and light industry, with high levels of traffic-related air pollution); and a semi-rural area, corresponding to the town of Motril and surroundings (small towns and villages on the Mediterranean coast, with intensive agricultural activity, including greenhouse cultivation). Participants were recruited from among subjects undergoing non-cancer-related surgery (47% inguinal hernia or abdominal surgery; 17% gall bladder surgery, 12% varicose vein surgery, and 24% other surgery) at San Cecilio University Hospital in Granada and Santa Ana Hospital in Motril. Surgical treatment made it ethically and practically feasible to obtain adipose tissue samples. Inclusion criteria were: age over 16 years, absence of hormone-related disease or cancer, no hormone therapy, and residence in one of the study areas for ≥10 yrs. Out of the 409 eligible individuals meeting the inclusion criteria and invited to participate, 387 (94.6%) accepted and were included in the study, but 89 individuals were dropped because of missing analytical or covariate data, leaving a final sample of 298 participants (73%). All participants signed their informed consent to participate in the study, which was approved by the Ethics Committee of each hospital.

2.2. Biological samples and chemical analyses

A 5–10 g sample of adipose tissue and 10 mL of blood were collected during surgery and immediately coded and stored at −80 °C until chemical analysis. Blood samples were then centrifuged for 5 min at 2500 rpm to separate the serum. Main sources of tissue were pelvic waist (42%), front abdominal wall (39%), and limbs (13%). Samples were all collected under 12-h fasting conditions.

Before chemical analyses, adipose tissue samples were extracted and processed as described elsewhere (Martinez Vidal et al., 2002; Moreno-Frias et al., 2004). Briefly, 200 mg of adipose tissue were mechanically homogenized in the presence of n-hexane, and the solution was then purified through 200 mg alumina in a glass column and kept in test tubes at −80 °C.

POP analyses in adipose tissue extracts were performed in the laboratories of Laboratorio Analítico Bioclínico (LAB) in Almería (Spain). POP residues were quantified by high-resolution gas chromatography with a mass spectrometry detector in tandem mode, using a Saturn 2000 ion trap system (Varian, Walnut Creek, CA) and a 2 m × 0.25 mm silica capillary column (Bellefonte, PA) coupled to a Factor Four VF-5MS 30 m × 0.25-mm i.d. analytical column (Varian Inc., Walnut Creek, CA). Residues of *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE, the main metabolite of the pesticide dichlorodiphenyltrichloroethane [DDT]), hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and PCB congeners 138, 153 and 180 were quantified. Procedural blanks were extracted with the same methodology, and no sample showed detectable levels of the analytes. Inter- and intra-day variabilities were calculated by analyzing fortified samples within the same day (repeatability) and on different days (intermediate precision), respectively, always yielding a result < 20%. For the quality control, laboratory-fortified matrix samples at different concentrations were used. The limit of detection (LD) and limit of quantification were determined as the smallest amount of the analyte that gave a signal-to-noise ratio ≥ 3 and ≥10, respectively, and were set at 0.01 μg/L and 0.04 for each POP, respectively. Concentrations below the LD were assigned a random value between zero and the LD, as recommended by Antweiler and Taylor (2008).

POP concentrations were calculated by using matrix-matched calibration and were expressed in lipid basis (nanograms per gram of lipid). C13-labeled *p,p′*-DDE and 3′-fluoro-2,4,4′-trichlorobiphenyl (PCB 28F) were used as internal standards in the analyses of organochlorine pesticides and PCBs, respectively. The recovery of POPs from adipose tissue was studied to assess the extraction efficiency of the methods, spiking 10 adipose tissue samples with target analytes at an intermediate point on the calibration curve and processing them as described above. Recoveries ranged from 90 to 98%.

Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels were enzymatically quantified in 10 μL of serum from each participant. Total serum lipids were calculated using the standard short formula of Phillips et al. (1989). Total lipid content in adipose tissue was quantified gravimetrically as described by Rivas et al. (2001). BMI was used as a measure of obesity, calculated as weight/height squared (kg/m^2).

2.3. Covariates

Covariates were gathered using an *ad hoc* questionnaire, which was completed by each participant before surgery and administered face-to-face by a trained interviewer during the hospital stay. Socio-demographic characteristics included information on age, education, smoking habit, occupation, medical history, and medication.

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". A subject was considered a smoker (past or present) at any level of daily tobacco consumption (≥1 cig/day).

Subjects were classified into six occupational categories, following Goldthorpe's proposal (Regidor, 2001), and three grouped categories were formed: social classes I + II + III (non-manual workers) and IV + V (manual workers).

Questionnaires and research procedures were standardized and validated in a pilot study of 50 subjects, in which adipose tissue POP concentrations were quantified and questionnaires were completed. Based on this experience, sample collection protocols, analytical methodologies, and data collection criteria were tested and refined.

2.4. Statistical analysis

First, the shape of the relationships between individual POP concentrations and serum lipids or BMI was visually evaluated through locally weighted scatterplot smoothing (LOWESS), a non-parametric local regression method (Supplementary material). The associations were then tested by using polynomial regression