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Relationship of polychlorinated biphenyls (PCBs) with parasitism, iron homeostasis, and other health outcomes: Results from a cross-sectional study on recently arrived African immigrants

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ABSTRACT

Polychlorinated biphenyls (PCBs) are toxic and persistent chemicals produced between 1930s and 1980s, which accumulate in humans and wildlife. Although a decreasing trend of PCB levels in humans has been described in developed countries, mainly as a consequence of strict regulations and remediation plans, an inverse trend has been recently reported in people from developing countries. We had the opportunity of sampling a series of African immigrants recently arrived to the Spanish archipelago of the Canary Islands, in which high levels of PCBs have been described, and we studied the relationships between their level of contamination and health status. A total of 570 subjects who underwent a complete medical examination and a face-to-face interview were recruited for this study. Hematological and biochemical parameters (blood and urine) were determined in all participants. Serology for the diagnostic of infectious diseases was also performed, and direct identification of parasites was performed in feces, urine or blood samples when appropriate. It is remarkable that up to 26.0% of the population had intestinal parasites, and we found an inverse relationship between PCB levels and parasitism and parasitic diseases: median values of PCBs were lower in parasitized subjects than in subjects without parasites in stool (237.6 ng/g fat vs. 154.4 ng/g fat for marker PCBs, $p=0.015$) and median values of dioxin-like PCBs were lower in subjects carrying pathogen parasites than among subjects showing non-pathogen parasites in stool (0.0 ng/g fat vs. 13.1 ng/g fat, respectively; $p=0.001$). Although this inverse association had been described in some vertebrates this is the first study reporting such an association in humans. Furthermore, it has been also recently described that PCBs may disrupt iron metabolism, and we found a direct relationship between serum iron and total PCBs burden ($r=0.231$, $p=0.025$), suggesting that PCBs, although at subclinical level, could play a role on iron homeostasis. Although the role of PCBs in parasitism and in the iron metabolism needs future research, our findings may help to understand the adverse health outcomes associated to environmental exposure to PCBs and they might be used in exposed populations as indicators of subtle effects due to environmental insult.

1. Introduction

Polychlorinated biphenyls (PCBs) are halogenated chemicals that have been widely used in closed systems such as electrical transformers and capacitors, as well as in a large number of other applications (Headrick et al., 1999; Safe, 1990). Due to their fat solubility and resistance to chemical and biological degradation, these chemicals are ubiquitous contaminants with long persistence in the environment, and tend to accumulate in fatty tissues of humans and wildlife. Due to their persistence and toxicity, PCBs were banned or restricted worldwide in the late 1970s (Henriquez-Hernandez et al., 2011; Kimbrough, 1987, 1995). Despite the time that has elapsed since their ban, PCBs continue to be found in human tissues and serum from populations all over the world

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(Henriquez-Hernandez et al., 2011; Luzardo et al., 2014b), although a decreasing trend in the burden of these pollutants has been observed along the period 1970–2000 (Consonni et al., 2012; Kim and Yoon, 2014; Nost et al., 2013). However, this decline has not been uniform worldwide and high levels of these pollutants have been recently reported in certain African countries, which have not been producers or major users of PCBs. This has been shown both in environmental samples (air and soil), food, and also in humans (Adu-Kumi et al., 2012; Asante et al., 2011; Gioia et al., 2014; Luzardo et al., 2014a).

Although human exposure is mainly determined by the consumption of fatty foods from animal origin (Almeida-Gonzalez et al., 2012; Boada et al., 2014; Luzardo et al., 2012a), a direct exposure from the environment is possible. Thus, it has been proposed that in some developing countries PCBs could reach soils and waters as a result of improper management of old devices and e-waste that may originate leaks or spills (Agudo et al., 2009; Henriquez-Hernandez et al., 2011; Kimbrough, 1995; Luzardo et al., 2014a; Porta et al., 2010; Safe, 1994; Zubero et al., 2009). Even more so, it has been recently reported that PCB levels are higher in the inhabitants of developing countries with a higher degree of socioeconomic development, where there exists a quick implementation of information and communication technologies (Luzardo et al., 2014a). Much of this demand for electrical and electronic equipment is covered by imports of second-hand devices, with very short useful lives, which consequently cause the generation of large amounts of e-waste (Schmidt, 2006; UNEP, 2011). The abandonment of these electronic wastes in open fields and improper handling of the same for the recovery of valuable materials seem to be contributing to raise the levels of pollution in the area, which would pose a risk for the inhabitants of these countries (Chatterjee, 2007; Gioia et al., 2014; Luzardo et al., 2014a).

Numerous adverse effects on human's health have been associated with PCBs' exposure, and particularly to the exposure to those congeners that are similar to dioxins (Van den Berg et al., 2006). PCBs have been classified as carcinogenic to humans by the IARC (Lauby-Secretan et al., 2013) and have been linked to many health concerns involving the reproductive (Buck Louis et al., 2009), endocrine (Hagmar, 2003; Luzardo et al., 2012b), neurological (Longnecker et al., 2003), immune system (Tsuji, 2015) and cancer (Arrebola et al., 2015). Although most studies devoted to evaluate adverse health effects exerted by these pollutants in humans have been performed in series of occupationally exposed people or in subjects affected by acute intoxications (Yorifuji et al., 2013), a number of adverse health effects have been also described in general population in relation to chronic environmental exposure to PCBs (Carpenter, 2006; Vaiserman, 2014). Studies have shown that low-dose and long-term exposure to PCBs may modify a number of physiological parameters, including hematological and immunological parameters (Kumar et al., 2014a, 2014b, 2014c, 2014d; Serdar et al., 2014). It has to be highlighted that these effects on general population might not be clinically evident because they may occur at subclinical level, opening the possibility that those alterations might be indicators of subtle effects due to environmental insult.

Because we had the exceptional opportunity of conducting the health assessments of a cohort of sub-Saharan immigrants recently arrived to the European Union (EU), in which we had previously shown high levels of PCBs (Luzardo et al., 2014a), we developed this study aimed to explore the possible influence of PCBs on health status of a population that is highly environmentally exposed to these pollutants.

2. Material and methods

2.1. Study population

The study population consisted of 570 African immigrants, who were sequentially and prospectively recruited within the first two months after their arrival on the island of Gran Canaria (Canary Islands, Spain), as previously reported (Luzardo et al., 2014a). The Canary Islands are a territory of the EU in Africa (100 km off the coast of Morocco) with a socioeconomic level of development comparable to any other territory of the Union. The geographical location of this region has made the archipelago a target of irregular immigration and has favored immigrant-based studies (Carranza-Rodriguez et al., 2008; de-la-Iglesia-Inigo et al., 2013; Pardo et al., 2006) aimed to obtain information that would otherwise be very difficult to obtain due to the economic, logistic, and idiosyncratic characteristics of African countries. All of the participants were temporarily lodged in shelters as part of the general screening for imported diseases, and provided their written consent for the use of their biological samples for research. They underwent a physical examination to rule out the presence of signs or symptoms of disease. Trained nurses recorded their heights and weights, and their corresponding body mass index (BMI) was calculated. The demographic characteristics of the participants are presented in Table 1. Blood and urine samples were obtained from all of the participants, and fecal samples were also obtained for parasite analyses. As described previously (Carranza-Rodriguez et al., 2008; de-la-Iglesia-Inigo et al., 2013; Pardo et al., 2006; Sanz-Pelaez et al., 2008), hematological and biochemical parameters were determined using a Beckman Coulter Analyzer AV5800 in which a colorimetric assay is developed following the manufacturer's instructions. For serum iron, the reagents OSR6286 and OSR6186 were employed. As the contamination of serum with red cells (hemolysis) may affect the iron determination, we also used the reagent OSR62166 to evaluate hemoglobin levels and assuring that none of the samples overpassed the limit of 0.3 g/L of hemoglobin.

2.2. Data of serum PCBs

The serum levels of PCBs of the participants in this study have been previously published (Luzardo et al., 2014a). However, we considered it pertinent to briefly describe the methodology that was employed to obtain those data. An aliquot of serum from each participant was subjected to solid phase extraction using

Table 1
Demographic characteristics of the studied population ($n=570$).

Variable	n (%)	Mean ± SD
Age, years	–	26.8 ± 6.7
Gender (male)	512 (89.8)	–
BMI, kg/m^2	–	23.8 ± 3.4
Toxic habits (yes)	205 (36.0)	–
Smoker (yes)	146 (25.6)	–
Drinker (yes)	139 (24.4)	–
Region of origin		
Central – East Africa	37 (6.5)	–
North Africa	20 (3.5)	–
West Africa	513 (90.0)	–
Gross national income per capita[†]		
Low income (≤ US$1005)	197 (34.6)	438.5 ± 125.8
Upper–middle income (> US$1005)	316 (55.4)	1204.3 ± 126.1
NA	57 (10.0)	–

Abbreviations: SD, standard deviation; BMI, body mass index; NA, not available.

[†] These values are referred to the country of origin of the immigrants (UNEP, 2011; World Bank, 2013).

Chromabond® C18ec columns (Macherey-Nagel, Germany) without further purification steps. The samples were subjected to chromatographic analysis on a Trace GC Ultra coupled with a QuantumMax triple quadrupole mass spectrometer (Thermo Fisher Scientific, Palo Alto, CA) for the quantification of the analytes in addition to internal standards and surrogates. We conducted quality controls aimed to assure the validity of the measures. The recovery efficiency for the analytes was above 74% in all cases; all the individual measurements were corrected by the recovery efficiency for each analyte; all the measurements were performed in triplicate, and the values used for calculation were the mean of the three values. A factor of concentration of 20 was applied (100 μl of extract in cyclohexane from 2 ml of sample), and thus the limit of quantification (LOQ) could be set at 5 pg/mL for all the compounds. For the calculations, when the concentration of a given contaminant was below the limit of quantification (LOQ) but above the limit of detection (LOD) of the technique, the value was assumed to be ½ LOQ. Otherwise the value was considered to be 0. This methodology has been validated and widely employed by our research group (Kakuschke et al., 2010; Luzardo et al., 2009, 2013a, 2013b).

2.3. Statistical analyses

We used PASW Statistics v19.0 (SPSS Inc., Chicago, IL, USA) to manage the database of the study and to perform statistical analyses. Throughout these analyses, we express the total PCB body burden (∑PCBs) as the sum of the 18 PCBs measured (IUPAC congeners #28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189), marker PCBs body burden (∑M-PCBs) as the sum of those congeners considered as markers of environmental contamination for PCBs (IUPAC congeners #28, 52, 101, 138, 153, and 180), and dioxin-like PCBs body burden (∑DL-PCBs) as the sum of the 12DL-PCBs measured (IUPAC congeners #77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189).

Normality was examined using the one-way Kolmogorov–Smirnov test. The PCB distributions lacked normality and homoscedasticity; therefore, we used non-parametric tests. We used ANOVA test to analyze the normally distributed variables (mainly the anthropometric variables), and the Mann–Whitney and Kruskal–Wallis tests to analyze the non-normally distributed variables. Chi-squared test was employed to examine the relationships between the categorical variables. The results were reported as medians and interquartile ranges. Correlations between individual or grouped PCBs were assessed using one dependent variable and one independent variable, and Pearson coefficients were calculated by taking the square root of R square for the regression. Probability levels of less than 0.05 (two tailed) were considered statistically significant.

3. Results

A total of 570 African immigrants were included in the study. The majority of the participants were healthy young males (89.8%) with a mean age of 26.8 years old (range 15–49 years old) and a BMI of 23.8 kg/m^2. The majority of immigrants came from West African countries (90.0%) and a third of them belonged to low-income countries (Table 1). A total of 205 subjects declared to have toxic habits: 25.6% claimed to be smoker and 24.4% claimed to be a regular drinker of alcoholic beverages. As reported previously, in this series we observed a trend of increased ΣPCB levels with age (Luzardo et al., 2014a), although this trend did not reach statistical significance. We did not observe differences in PCBs serum levels in relation to gender, BMI, and toxic habits. Therefore we did not consider that these variables would affect the serum levels of this study group, and subsequently we did not perform multivariate analyses, as it is usual in this type of studies. Serum concentrations of PCBs in the whole series are contained in Table 2.

Clinically relevant data of the participants in this study are shown in Table 3. Most analytical parameters were within the normal range. We did not observe differences in analytical parameters in relation to gender, BMI, and toxic habits (data not shown). It has come to our attention that the percentage of eosinophils and the values of creatine kinase (CK) exceeded what is considered normal for healthy people (Table 3).

All the participants underwent a specific medical examination aimed to detect infectious diseases. In our series, a total of 66 subjects were positive to tuberculin skin test (11.6% of the series), 23 subjects were infected by human immunodeficiency virus (4.0% of the series) and only 9 subjects were infected by hepatitis C virus (1.6% of the series). Analysis of feces was also performed in order to check the presence of parasites and a total of 148 subjects (26.0% of the subjects) presented some kind of parasite in feces. The parasites most frequently detected were hookworms ($n=42$), *Trichuris trichiura* ($n=22$), and *Schistosoma mansoni* ($n=19$). Nevertheless, it has to be taken into account that some of the parasites detected were intestinal worms (helminthes; i.e. *Schistosoma mansoni*, *Trichuris trichura*, and hookworms), while others were unicellular organisms (protozoa; i.e. *Giardia Intestinalis*, *Entamoeba Coli*). In addition, only a number of the detected parasites induce human disease (for example, *Giardia intestinalis*, *Schistosoma mansoni*, *Trichuris trichiura*, and *Uncinaria*), while others are not pathogens for human beings (such as, *Entamoeba coli* and *Endolimax nana*). Because eosinophilia may be linked with a number of pathologies, among them parasitic diseases (Pardo et al., 2006), we explored the potential relationship between parasitism and increasing values of eosinophils. In our study, median value of eosinophils among non-parasitized subjects was significantly lower than those observed among parasitized people (4.0 vs. 6.3%, respectively; $p < 0.0001$). The presence of parasites in stool was not related to the country of origin of the participants, nor the economic development of the region of origin (data not

Table 2
Serum concentration (ng/g fat) of polychlorinated biphenyls in the whole series of African immigrants ($n=570$).

Congener	% of detection	Median (p25th–p75th)
M-PCBs		
PCB-28	84.7	20.7 (7.4–41.8)
PCB-52	38.6	0.0 (0.0–2.0)
PCB-101	17.9	0.0 (0.0–0.0)
PCB-118	27.2	0.0 (0.0–10.2)
PCB-138	58.4	10.7 (0.0–92.6)
PCB-153	78.9	80.5 (6.7–254.9)
PCB-180	84.2	52.0 (9.8–160.1)
DL-PCBs		
PCB-77	4.2	0.0 (0.0–0.0)
PCB-81	3.5	0.0 (0.0–0.0)
PCB-105	7.2	0.0 (0.0–0.0)
PCB-114	3.9	0.0 (0.0–0.0)
PCB-118	27.2	0.0 (0.0–10.2)
PCB-123	6.7	0.0 (0.0–0.0)
PCB-126	12.6	0.0 (0.0–0.0)
PCB-156	13.5	0.0 (0.0–0.0)
PCB-157	2.3	0.0 (0.0–0.0)
PCB-167	6.1	0.0 (0.0–0.0)
PCB-169	0.9	0.0 (0.0–0.0)
PCB-189	8.2	0.0 (0.0–0.0)
∑ M-PCBs	99.5	203.4 (73.7–580.7)
∑ DL-PCBs	54.9	10.2 (0.0–51.1)
∑ Total PCBs	99.5	232.1 (82.4–670.5)

Abbreviations: PCBs, polychlorinated biphenyls; M-PCBs, marker PCBs; DL-PCBs, dioxin-like PCBs.

Table 3
Blood count data of the studied population.

Variable	Whole series		Reference values*
	Mean ± SD	Median (p25th–p75th)	
Red cells			
Hemoblin (g/dL)	14.7 ± 1.5	14.9 (14.0–15.6)	13.8–17.2
Hematocrit (%)	44.0 ± 5.1	44.5 (41.8–47.0)	40.3–50.7
ESR (mm/h)	12.9 ± 15.6	8.0 (4.0–15.2)	15
MCV (fL)	85.4 ± 7.9	86.4 (81.8–90.2)	87
RDW (%)	14.1 ± 1.5	13.8 (13.2–14.6)	10.6–14.5
Platelets ($x10^9$/L)	227.7 ± 61.9	222 (184.0–267.0)	150–400
White cells			
Total leukocytes ($x10^3$/ μL)	5.9 ± 1.5	5.7 (4.8–6.8)	4.5–10
Eosinophils (%)	6.0 ± 5.0	4.3 (2.4–8.4)	1–4
Biochemical factors			
Glucose (mg/dL)	83.8 ± 9.8	83.0 (78.0–90.0)	72–145
Urea (mg/dL)	30.6 ± 7.3	30.0 (26.0–35.0)	< 40
Creatinine (mg/dL)	1.0 ± 0.2	1.0 (0.9–1.2)	< 1.3
Uric acid (mg/dL)	5.4 ± 2.2	5.3 (4.4–6.1)	3.5–7.2
Total proteins (mg/dL)	8.0 ± 0.6	7.9 (7.6–8.4)	6.0–8.3
Na (mEq/L)	138.9 ± 10.8	140.0 (138.0–141.0)	135–145
K (mEq/L)	4.4 ± 0.8	4.3 (4.1–4.6)	3.7–5.2
Cl (mEq/L)	102.2 ± 6.7	103.0 (101.0–104.0)	100–108
Mg (mg/dL)	1.9 ± 0.5	1.8 (1.7–2.0)	1.7–2.2
Ca (mg/dL)	9.4 ± 0.6	9.3 (9.0–9.7)	8.5–10.2
P (mg/dL)	4.1 ± 0.7	4.1 (3.7–4.5)	2.4–4–1
Fe (μg/dL)	82.4 ± 28.0	78.5 (62.0–98.2)	60–170
Total bilirrubin (mg/dL)	0.7 ± 0.5	0.6 (0.4–0.8)	0.3–1.9
ALT (IU/L)	39.7 ± 28.4	36.0 (27.0–46.0)	10-40
AST (IU/L)	30.2 ± 15.5	26.8 (21.7–33.1)	10-34
GGT (IU/L)	43.4 ± 37.6	35.0 (24.8–48.0)	0–51
AP (IU/L)	79.4 ± 25.8	76.9 (62.9–91.0)	44–147
CPK (μg/L)	363.6 ± 462.6	236.0 (148.0–388.0)	10–120

Abbreviations: SD, standard deviation; p25th–p75th, percentiles 25 and 75 of the distribution; ESR, erythrocyte sedimentation rate; MCV, mean corpuscular volume; RDW, red cell distribution width; g/dL, grames per deciliter; fL, femtoLiters; mEqL, milliequivalents per liter; Na, sodium; K, potassium; Cl, chlorine; Mg, magnesium; Ca, calcium; P, phosphorus; Fe, iron; IU/L, international units per liter; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; AP, alkaline phosphatase; CPK, creatine phosphokinase.
*References values are referred to males. Values obtained from MedlinePlus (available at: www.nlm.nih.gov/medlineplus).

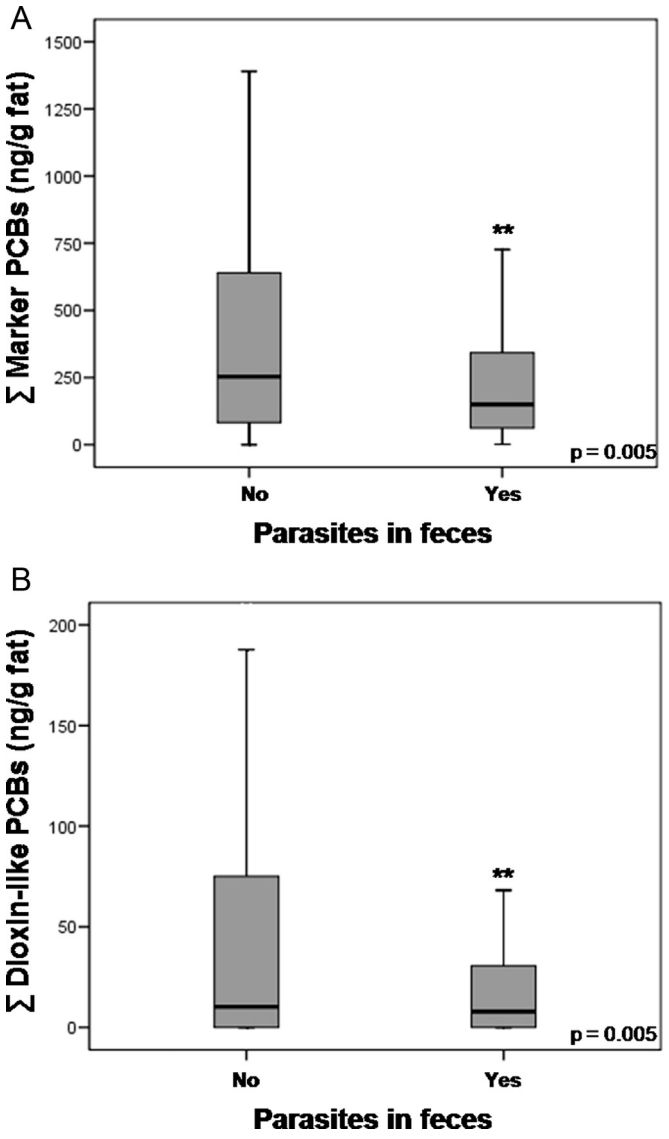

Fig. 1. Box plot showing the distribution of sum marker PCBs (A) and sum dioxin-like PCBs (B) among African immigrants with and without parasites detected in feces. The line inside the box represents the median, the bottom and top of the box are the first and third quartiles of the distribution, and the lines extending vertically from the boxes indicate the variability outside the upper and lower quartiles. Sum of marker PCBs: congeners 28, 52, 101, 118, 138, 153, and 180; sum of dioxin-like PCBs: congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189.

shown). However, it was evident an association between parasitism and serum PCB levels. As shown in Fig. 1A, we observed that immigrants showing parasites in feces had lower levels of ∑M-PCBs than those subjects without parasites. Thus, ∑M-PCBs median values in subjects without and with parasites detected in stool were 237.6 ng/g fat and 154.4 ng/g fat, respectively ($p=0.015$). In addition, when we segmented the population according their serum PCB levels (tertiles of the distribution), we found that the presence/absence of parasites varied significantly depending on the degree of exposure. Thus, while 29.3 and 37.7% of the subjects encompassed in tertiles 1 and 2 of M-PCBs showed parasites in stool, among subjects in the highest exposure group (tertile 3) this percentage decreased to 19.2% ($p=0.007$). When we analyzed the distribution of individual M-PCB congeners, this inverse trend was maintained for CBs 52, 138, and 153 (Table 4). In addition, we also found an association between levels of DL-PCBs and parasitism. Again in this case parasitized immigrants showed lower levels of ∑DL-PCBs than non-parasitized subjects, although in this case without reaching an statistically significant difference (10.2 ng/g fat vs. 7.9 ng/g fat, respectively; $p=0.068$) (Fig. 1B). Taken individually, only CBs 126 and 118 (among the DL-PCBs) maintained this inverse association with the presence of parasites in feces (Table 4). Moreover, TEQ level of CB126 (Van den Berg et al., 2006) was higher among subjects without parasites in stool than in parasitized subjects (25.1 pg/g fat vs. 1.8 pg/g fat in the percentile 95 of the distribution; $p=0.003$). Although we did not find any significant association among PCBs (individually or grouped) with specific parasites (possibly due to the limited sample size), we observed significant differences in PCB burden in

Table 4
Serum concentration (ng/g fat) of polychlorinated biphenyls among African immigrants with and without parasites in stool (values expressed as median and percentiles 25th and 75th of the distribution; the percentage of subjects (%) with detected levels of PCB congeners was included).

Congener	Parasites in feces (No, $n=391$) %	Median (p25th–p75th)	Parasites in feces (Yes, $n=148$) %	Median (p25th–p75th)	*P* value*
M-PCBs					
PCB-28	92.8	20.7 (7.4–42.9)	91.2	20.7 (7.8–41.1)	ns
PCB-52	43.5	0.0 (0.0–4.1)	33.8	0.0 (0.0–2.0)	0.016
PCB-101	18.9	0.0 (0.0–0.0)	18.9	0.0 (0.0–0.0)	ns
PCB-118	32.7	0.0 (0.0–20.5)	18.2	0.0 (0.0–0.0)	0.001
PCB-138	64.9	17.8 (0.0–96.1)	53.4	3.6 (0.0–49.8)	0.002
PCB-153	84.9	93.9 (10.1–261.6)	79.7	58.7 (3.4–140.1)	0.007
PCB-180	89.2	61.8 (9.8–178.8)	88.5	39.0 (6.5–113.0)	ns
DL-PCBs		0.0 (0.0–0.0)		0.0 (0.0–0.0)	
PCB-77	3.6	0.0 (0.0–0.0)	6.8	0.0 (0.0–0.0)	ns
PCB-81	3.1	0.0 (0.0–0.0)	5.4	0.0 (0.0–0.0)	ns
PCB-105	7.4	0.0 (0.0–0.0)	8.1	0.0 (0.0–0.0)	ns
PCB-114	4.8	0.0 (0.0–0.0)	2.0	0.0 (0.0–0.0)	ns
PCB-118	32.7	0.0 (0.0–20.5)	18.2	0.0 (0.0–0.0)	0.001
PCB-123	5.4	0.0 (0.0–0.0)	11.5	0.0 (0.0–0.0)	ns
PCB-126	16.1	0.0 (0.0–0.0)	6.1	0.0 (0.0–0.0)	0.003†
PCB-156	15.8	0.0 (0.0–0.0)	10.1	0.0 (0.0–0.0)	ns
PCB-157	2.8	0.0 (0.0–0.0)	1.3	0.0 (0.0–0.0)	ns
PCB-167	7.4	0.0 (0.0–0.0)	4.0	0.0 (0.0–0.0)	ns
PCB-169	1.0	0.0 (0.0–0.0)	0.7	0.0 (0.0–0.0)	ns
PCB-189	9.2	0.0 (0.0–0.0)	7.4	0.0 (0.0–0.0)	ns

Abbreviations: PCBs, polychlorinated biphenyls; M-PCBs, marker PCBs; DL-PCBs, dioxin-like PCBs; ns, non-significant.

† *p*5th–*p*95th=0.0–250.9 vs. 0.0–17.5, respectively.

* Kruskal–Wallis test.

relation to the pathogenicity of the parasites detected. Thus, median values of ∑DL-PCBs was lower among subjects carrying pathogen parasites than among subjects showing non-pathogen parasites (0.0 ng/g fat vs. 13.1 ng/g fat, respectively; $p=0.001$). A similar trend was observed for ∑M-PCBs (114.7 ng/g fat vs. 240.5 ng/g fat), but, in this case, the difference was not significant ($p=0.073$). Furthermore, we also observed differences in the serum levels of ∑DL-PCBs in African immigrants according to the class of parasites detected in feces. In that sense, subjects infected with helminths showed lower levels of ∑DL-PCBs than subjects infected with protozoan parasites (0.0 ng/g fat vs. 10.9 ng/g fat, respectively; $p=0.015$). We did not found differences regarding to ∑M-PCBs or PCB congeners taken individually.

Despite the fact that in the study population iron serum levels were within the range of normality, we observed a direct association between serum levels of iron and total burden of M-PCBs and DL-PCBs (bivariate correlation test, Fig. 2). No other associations with known factors that may affect serum levels of iron, such as age (Pearson correlation test, $p=0.091$), gender (Kruskal–Wallis test, $p=0.763$), or hepatic disease (ALT, AST, GGT or AP; Pearson correlation test, $p>0.05$ in all cases), were found. Serum levels of iron increased with increasing serum values of ∑M-PCBs ($r=0.231$, $p=0.025$). Such correlation came marked by the most frequently detected and abundant M-PCB congeners in the study population: CB138 (present in 58.4% of the samples) and CB 180 (present in more than 80% of the samples), which were positively associated to serum levels of iron ($r=0.261$, $p=0.011$; $r=0.213$, $p=0.039$; respectively). Similarly, the positive association of iron serum levels and DL-PCBs came marked by one of the most frequently detected congener, CB126, detected in 12.6% of the samples, and that was positively correlated to serum iron ($r=0.257$, $p=0.013$).

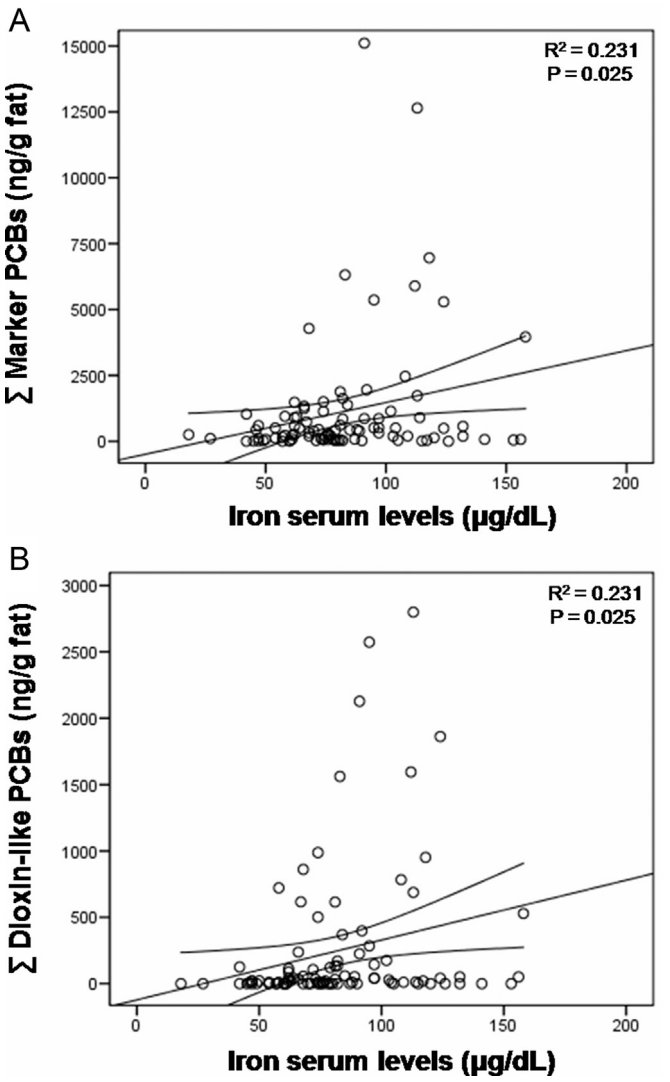

Fig. 2. Bivariate correlation analyses between sum of marker PCBs (A) and sum of dioxin-like PCBs (B) with serum levels of iron among African immigrants subjects (Pearson correlation test). Sum of marker PCBs: congeners 28, 52, 101, 118, 138, 153, and 180; sum of dioxin-like PCBs: congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189.

4. Discussion

Despite the global ban on manufacturing of PCBs dates almost four decades back, recent studies indicate that measurable quantities of these pollutants are still detected in serum samples obtained from the general population (Agudo et al., 2009; Henriquez-Hernandez et al., 2011; Porta et al., 2010; Serdar et al., 2014; Zubero et al., 2015, 2009). In any case it is noteworthy that, although many of the studies indicate a downward trend in environmental levels of PCBs, in some regions of the planet, mostly in developing countries, levels remain high, or even appear to have increased. This has been found in environmental samples (Gioia et al., 2014) and recently also in the people covered by this study, which come from Western and Central African countries (Luzardo et al., 2014a).

As already mentioned in the introduction to this article, the toxic effects to humans of exposure to PCBs have been widely documented, even in the case of environmental exposure. In this context, this population of sub-Saharan immigrants showing high levels of contamination by PCBs may be an excellent group to evaluate potential adverse health effects associated to PCBs

exposure.

Although in general terms this is a young and healthy population in which the great majority of clinical and biological magnitudes were within the normal values (Tables 1 and 3), we highlight two features of this study population, which drove our attention: the high median values of creatine kinase (CK), and the high percentage of participants with intestinal parasites (26.0%).

With regard to the values of CK is noteworthy that the median values were well above baseline values (as much as twice the upper range value). Elevated CK activity has been observed previously in healthy black people both "in vivo" (Sanz-Pelaez et al., 2008) and in forensic studies (Brewster et al., 2012), related with different muscle fiber type proportions as compared with Caucasian subjects (Ama et al., 1986). Specifically, black Africans had a higher percent type IIa muscular fiber with high CK activity that fuels highly energy demanding processes such as cardiovascular contractility, sodium pumping, and trophic responses, at a faster rate than glycolysis and oxidative phosphorylation together (Ama et al., 1986; Brewster et al., 2012).

More striking is the relationship between parasitism and levels of PCBs, which could be evaluated because this population shows a high percentage of subjects with intestinal parasites. As mentioned above, 25.6% of participants had parasites in stool, and moreover these people had higher median levels of eosinophils than non-parasitized participants, an association which has been well established in the literature (Pardo et al., 2006). As it was exposed, we found an inverse relationship between parasitism and PCB levels, when we expected the opposite since PCBs are considered immunosuppresor chemicals (Sagerup et al., 2009; Tsuji, 2015). Therefore, it may be logical to expect a higher parasite infection as a consequence of an impaired immune system with increasing PCB pollution (Sagerup et al., 2009). However, it is interesting to note that this inverse association has been previously described in certain species of vertebrates (Carreras-Aubets et al., 2012). In these species, food borne parasites as well as ectoparasites have been demonstrated to decrease with increasing levels of PCBs. However, as far as we know this is the first time that such an inverse relationship is described in humans. Two hypotheses can help to explain these results. On the one hand, it would be possible that high environmental PCB residues could disrupt the life cycle of parasites, thereby reducing their ability to survive and reach the host. It is reasonable to think in this because the free life stages of many parasites are susceptible to different types of pollutants, as described in the literature (Blanar et al., 2009; Pietrock and Marcogliese, 2003). Therefore, according to this hypothesis, those parasites whose intermediate forms (free-living forms) were sensitive to the toxic effects of exposure to high levels of PCBs would have less success in reaching the hosts (Carreras-Aubets et al., 2012). On the other hand it is also a plausible hypothesis that intestinal parasites are able to bioaccumulate in their bodies part of the PCBs of food entering the intestine (Yen Le et al., 2014). When intestinal parasites are not present, a higher concentration of PCBs would be available from food and the levels of exposure of the person would be higher. Our results showing an inverse relationship between helminths (which, unlike protozoa, strongly could disrupt their hosts' food absorption), and DL-PCBs burden reinforce such a possibility. Moreover, it has been described that parasites seem to be able to metabolize PCBs through cytochrome P-450 (Menzel et al., 2007; Schafer et al., 2009). This ability would mediate the biotransformation of certain pollutants, and therefore this would reduce the total body burden of residues in the host. This phenomenon has been described to be parasite-species dependent, but this specificity could not be observed in this study population, probably due to the limited number of subjects with each one of the specific parasites. Although it has been reported that parasites have different effects on the bioaccumulation of various pollutants (including persistent organochlorines) in the host, all the literature published in that field uses animal models (Yen Le et al., 2014). To our knowledge, this is the first time that a PCB-mediated connection between parasitic diseases and environmental pollutants is observed in human beings. On the light of the above, this relationship needs to be explored in depth in larger epidemiological studies.

Finally, the fact that the serum levels of iron were positively correlated to serum levels of PCBs is remarkable. Thus, subjects with higher levels of CBs 126, 138, and 180 also exhibited higher levels of serum iron. Iron is an essential constituent of the internal environment and an absolute element for nearly all forms of life (Tapiero et al., 2001). Iron homeostasis is complex and a tight control of the balance for iron intake, utilization and storage is essential to keep the cell homeostasis. It has been reported that there are a number of factors, such as age or sex, which may influence the serum levels of iron. However we found no statistical differences of these levels in relation to gender or age. Additionally, it is also well known that certain conditions, such as chronic inflammatory liver disease or hepatitis may influence the levels of iron, but we did not observe statistical differences of iron serum levels when we considered the liver enzymes clinically used as indicators of hepatic damage or inflammation. In this study, although iron levels were always within normal range, an association with PCB levels was observed, which is consistent with previous experimental studies Thus, it has been recently reported that PCBs interferes with iron homeostasis (Wang et al., 2013). Specifically, authors reported that CBs 77, 126, and 153 suppress hepcidin expression in vivo and in vitro at nontoxic concentrations with a subsequent increase of serum iron content (Wang et al., 2013). Hepcidin is a peptidic hormone that plays a major role in systemic iron homeostasis, and that is secreted by the liver in response to iron loading (Nemeth et al., 2004). Decreased hepcidin would lead to a tissue iron overload, whereas hepcidin overproduction would lead to the opposite: hypoferremia and anemia. In fact, it has been described in epidemiological studies that people, which are more exposed to PCBs, show higher values of iron in serum (Serdar et al., 2014). However, these subtle effects have not received adequate attention because iron values were always within the normal range. Furthermore, PCBs are considered as xenoestrogens because they can exert estrogenic activity on living organisms, including human beings (Longnecker et al., 2003). In this sense, it has been suggested that PCBs inhibit hepcidin expression through an estrogen-like mechanism (Qian et al., 2015). Thus, the effects on iron homeostasis exerted by PCBs should be encompassed in connection with the other endocrine disrupting activities carried out by these contaminants (Qian et al., 2015). As far as we know, this is one of the few epidemiological studies that reinforce those in vitro studies that point to the possibility that PCBs may disrupt iron homeostasis. Although further studies are necessary to elucidate if these effect could be clinically relevant, our result is of concern since an excess in iron availability and supply in cells increases the likelihood of gene mutation through production of free radicals and also provide a favorable environment for the growth of tumor cells (Bystrom et al., 2014; Torti and Torti, 2011).

5. Conclusions

In summary, this is one of the few studies reporting the association between burden of PCBs and health outcomes in African people. Although the series is not representative of the general population of that continent (this study population presents an important bias because the subjects enrolled in the study are mostly healthy young males), we believe that our results improve

the knowledge of the adverse health effects exerted by environmental exposure to PCBs. It must be noted that the described effects here are subtle and do not cause obvious clinical entities, so they can go unnoticed for a long time. In this sense, the potential role of PCBs on iron metabolism, and the first report on the significant association between PCBs and parasitism in humans deserve future studies and open novel research lines in the field of long-term toxicity of persistent organic pollutants.

Conflict of interest

Authors declare no conflict of interest.

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