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Original Research

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Serum concentrations of organochlorine pesticides associated with diabetes and obesity in Northern Benin (West Africa)

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ABSTRACT

Aim: Diabetes rising prevalence is of great concern in Africa because of its socio-economic impacts in a context of limited access to health care. The inappropriate use of pesticides may add to the burden of diabetes in Africa. This study was carried out in a cotton producing area of Benin in order to assess the relationship between the highest prevalence of diabetes observed in the country and organochlorine pesticide (OCP) exposure. **Methods:** This was a case–control study conducted in 2011. A sample of 106 subjects with diabetes and 106 non-diabetic controls were paired by age, gender, ethnicity, and residential area. Personal and socio-economic information, along with anthropometric measurements were collected. Blood samples were assayed for total lipids and 14 OCPs by gas-chromatography coupled with mass-spectrometry. Data were recorded for the four detectable OCPs: *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT), β-hexachlorocyclohexane (β-HCH), and trans-nonachlor. **Results:** Serum levels of all four detected OCPs were consistently higher in diabetic subjects as compared to non-diabetic controls. The odds ratio (OR) of diabetes was nearly three-fold higher when comparing the third tertile of *p,p'*-DDE and *p,p'*-DDT and β-HCH levels with the first tertile, without adjustment for potential confounders. The association remained significant for *p,p'*-DDT (OR = 2.59; 95% confidence interval (CI): 1.17-5.42) and *p,p'*-DDE (OR = 2.11; 95% CI: 1.01-4.54) after adjusting for a family history of diabetes, abdominal obesity, and wealth index or education. **Conclusion:** Our data showed that exposure to *p,p'*-DDT and *p,p'*-DDE was associated with an increased risk of diabetes. These findings have major public health implications.

KEYWORDS: Africa, obesity, organochlorine pesticides, type 2 diabetes

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INTRODUCTION

Non-communicable diseases such as type 2 diabetes are an important public health concern now a days in both developed and developing countries [1]. The highest increasing trend in diabetes prevalence in the world (109%) is expected to occur in Sub-Saharan African countries by 2035 [2-4]. Diabetes thus represents a challenge for African countries without sufficient health resources to deal with this chronic disease. The absence of community-based financial support for proper treatment is such that individuals seek health care treatment as late as possible, with ensuing complications and higher mortality rates. In these circumstances, identification and reduction of risk factors becomes important.

To address the multifactorial aspects of diabetes, research and intervention programs are intensively focusing on recognized risk factors, such as obesity and lifestyle determinants (diet, sedentary lifestyle), genetic predisposition, and physiopathological pathways. New risk determinants have been identified including stress, inflammation, micronutrient deficiencies, gut microbiota and environmental contaminants [5].

The contribution of environmental contaminants in the etiology of diabetes has been suspected for more than three decades [6]. A growing number of epidemiological and animal studies have shown associations between organic pollutant exposure and diabetes or its co-morbidities, such as obesity, metabolic syndrome, and cardiovascular diseases [5,7-12]. The chemicals of concern include organochlorine pesticides (OCPs) in particular [13-18].

In Sub-Saharan Africa, the relationship between pesticide exposure and diabetes has not yet been explored. We recently reviewed that serum levels of some OCPs in the adult African populations were high and likely due to inadequate management of pesticides, inappropriate use, high exposure during early life stage and individual susceptibility [19]. High levels of exposure to pollutants may indeed affect the offspring, exposing them to higher risks of chronic diseases, like diabetes [20-22]. Therefore, pollutants such as pesticides could contribute to the burden of diabetes in Africa, in addition to genetic predisposition, early-life malnutrition, obesity, infectious diseases and their treatment, and factors associated with the epidemiologic, demographic and nutrition transition [19].

The current study on associations between OCP exposure and diabetes was initiated in Borgou district of Benin (West Africa) where a widespread and inappropriate use of pesticides coincides with a high prevalence of diabetes [23]. In this area, we observed that levels of dichlorodiphenyltrichloroethane (DDT) compounds in diabetics were higher than in other countries [24]. In the present paper, we further explored the odds of diabetes and obesity with increasing exposure to OCPs, while accounting for potential confounders.

METHODS

The current case–control study was approved by ethical committees of University of Montreal and Benin National Ministry of Health. The study was conducted in Borgou, one of the 12 districts of Benin. It is divided into 8 municipalities, 43 districts and 310 villages. Borgou covers an area, which represents 23% of the country. The agricultural area covers 54% of the total land of the district. With a total population of 969,896 inhabitants, the area is mainly characterized by agriculture (cotton and food crops) along with cattle breeding and trade [25]. Participants with diabetes were primarily selected from the database of a concurrent prevalence survey in 4740 adults selected through a four-stage cluster sampling in the whole Borgou district. Interviews and serum sample collections for the present study were carried out from October 5 to December 30, 2011.

Fasting capillary and venous glucose at a threshold of 7 mmol/L was used to select an initial number of 65 diabetics from the prevalence survey database. An additional 64 diabetic subjects were chosen using random digit numbers generated with SPSS Software, version 18.0 (SPSS Inc., Chicago/USA, 2009). We used hospital records from the geographic areas of the diabetic subjects already enrolled in the prevalence survey to select these additional subjects. Paired control subjects (n = 129) were selected a priori from the prevalence survey population when both capillary and venous glycemic values were lower than 5.6 mmol/L. Pairing criteria were age ± 5 years, sex, ethnic group, and residence area. Figure 1 illustrates the sampling process. Capillary blood glucose was measured using "one touch ultra" glucometers (LifeScan, France). Venous glucose was determined using the glucose oxidase enzymatic method, at the Biochemistry Laboratory of the Institute of Applied Biomedical Sciences in Cotonou, Benin. Of the 258 subjects enrolled in the case-control study, 212 well-paired subjects had enough sampled serum for the determination of OCP concentrations and were therefore included in the present study.

Following shipment in dry ice, 14 OCPs were assayed at the Laboratory of Toxicology of the National Institute of Public Health of Quebec by gas-chromatography coupled with mass-spectrometry. Compounds analyzed included the four detected pesticides: β-hexachlorocyclohexane (β-HCH), trans-nonachlor, *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) and *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT). Sampling procedure, pesticide analytical methods and detection limits were described in a previous paper [24]. OCP concentrations were adjusted for total serum lipids computed with the following formula [26,27]:

Total serum lipids = 1.677 × ([total cholesterol − free cholesterol] + free cholesterol + triglycerides + phospholipids).

Lipid fractions (in g/L) were determined with a colorimetric enzymatic method at the Hospital Center of Laval University, Quebec City. Data are reported in the current paper for the four detected OCPs: *p,p'*-DDE, *p,p'*-DDT, β-HCH and trans-nonachlor.

Personal Data

A face-to-face questionnaire was administered to all study participants to document socio-economic profile, a family history of diabetes, a history of gestational diabetes (women), and alcohol and tobacco consumption. Socio-economic data pertained to education level, occupation and wealth indicators. Education was described as: (i) No formal schooling; (ii) functional literacy or primary school level; (iii) high school or university. Main occupation of the subjects was compiled into three categories: (i) Farmers, (ii) manual workers and (iii) office workers. Wealth index as an income proxy was assessed on the basis of household assets, source of energy for light and cooking, size of the household and home employees. Assets included land plots, housing features, furniture and appliances, transportation vehicle, communication equipment, and size of livestock or farm [28-30]. Assets were identified with questions requiring a yes or no answer, for instance: "Do you or any member in your household have a car?" The same question was used for the other assets: bicycle, TV, radio, cell phone, fridge, livestock, and house staff. The characteristics of the house were assessed by research assistants specifying the material used for the walls, roof, and floor. The source of energy for light and cooking as well as the source of water supply were also documented." Wealth

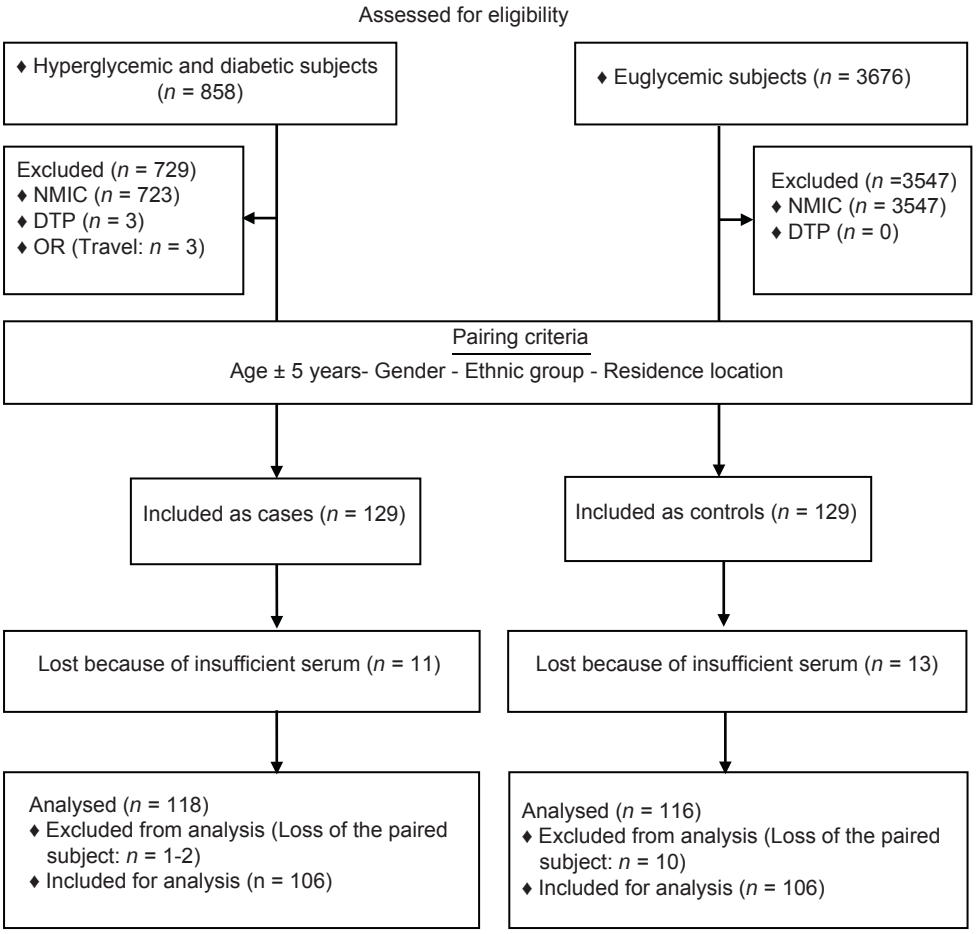

Figure 1: Sampling method. NMIC: Not meeting inclusion criteria, DTP: Declined to participate, OR: Other reasons. Adapted from: CONSORT statement diagram (http://www.consort-statement.org/downloads)

Sub-variables were weighted according to their importance; for example, scores for ownership of a bicycle, a motorcycle and a car were respectively set to 1, 2 and 4; otherwise, the score was 0. The total wealth index was split into tertiles for data analyses.

Anthropometric Data

Body weight was measured in the standing position using a SECA scale with a precision of 0.1 kg. Height was measured in the same position using a measuring board with a precision of 0.1 mm. Body mass index (BMI) was computed with the following formula: BMI = weight (kg)/height $(m)^2$. Waist circumference was measured with a tape mid-way between the iliac crest and last rib. Mean value of the two measurements was used in the analyses. Abdominal obesity was defined according to International Diabetes Federation's cut-offs: 94 cm for men and 80 cm for women [31].

Body composition was assessed with bioelectrical impedance using the weight (kg), the height (cm) and the resistance in ohms (Ω). Lean body mass (LM) was obtained with the following formula [32,33]:

- LM for men = −10.68 + 0.65 $height^2$/resistance + 0.26 weight + 0.02 resistance;
- LM for women = −9.53 + 0.69 $height^2$/resistance + 0.17 weight + 0.02 resistance.

Body fat mass (BFM) was then obtained by subtracting LM from total body weight. The percentage of body fat was considered high when above of 33% for women and 25% for men [34].

Statistical Analysis

Data analysis was performed using SPSS version 21.0 (IBM Corp., Armonk, NY/USA, 2012). Bivariate and multivariate analyses were performed to test associations between diabetes and risk factors or confounding variables (family history of diabetes, abdominal obesity, education, occupation, alcohol and tobacco consumption, and wealth index). Serum concentrations of the four OCPs were compared between diabetic cases and non-diabetic controls, which were already paired for age, gender, ethnic group and residence area. Bivariate analyses included the assessment of differences in OCP levels between these two groups using the Mann–Whitney test (OCP levels as continuous variables) and the Chi-square test (OCP levels in tertiles). Associations between OCP levels and dichotomized personal factors, such as family history of diabetes, abdominal obesity (or overall obesity or high BFM), tobacco smoking, and alcohol

consumption, were also examined using the Mann–Whitney test, while three and more category factors, such as the body weight status, wealth index, occupation and education level, were assessed with the Kruskal–Wallis test.

The associations between OCP levels and diabetes were also analyzed using multivariate logistic regression. The second and third tertiles of OCP concentrations were compared with the first tertile, after adjusting for family diabetes, abdominal obesity, and socio-economic status. Occupation, education level and the wealth index as indicators of socio-economic status were added separately to the model because they were strongly correlated. Those potential confounders were the only variables associated with OCP levels in bivariate analysis and thus considered in the multivariate analysis (alcohol consumption and tobacco not related). Abdominal obesity was selected instead of percentage body fat or overall obesity because of its consistently significant association with pesticide levels in bivariate analysis. In addition, the three obesity indicators are highly correlated, and abdominal obesity was associated with a higher risk of diabetes. Associations were considered as significant for $P < 0.05$.

The relationship between serum OCPs (second and third tertile versus first tertile) and overall obesity, percentage body fat and abdominal obesity was also assessed by logistic regression, after adjusting for sex, diabetes status, and wealth index (or education or occupation) The log-transformed values of OCP concentrations were used for all statistical analyses.

RESULTS

Sample Characteristics and Factors Associated with Diabetes

Mean age of subjects (±standard deviation) was 50.2 ± 11.2 years, without any difference between cases and controls, which confirms proper case-control pairing for this variable. Subjects aged 40-60 years represented 57.5% of the whole sample. As shown in Table 1, diabetes was more prevalent in office workers than in the other two categories of occupation ($P = 0.007$). Overall and abdominal obesity was observed in 14.6% and 48.6% of the sample, respectively. Abdominal obesity and the family history of diabetes were both more frequent among diabetic cases than non-diabetic controls ($P < 0.001$). Although, there was no difference in overall obesity between cases and controls, there were significantly more overweight individuals in the diabetic group than in the control group. There was however no significant group difference in alcohol and tobacco consumption, in wealth status and education level.

OCP Levels and Diabetes

Lipid-adjusted serum concentrations of OCPs are presented in Table 2. The levels of the four detected OCPs were consistently higher in diabetic than non-diabetic subjects. According to bivariate analyses (Table 3), wealthier people, those with a family history of diabetes, abdominal obesity and high percentage body fat consistently exhibited higher levels of all four OCPs. Overall obesity was associated with high levels of *p,p'*-DDT and trans-nonachlor. People with higher educational level had higher concentrations of *p,p'*-DDE and trans-nonachlor. *p,p'*-DDE and *p,p'*-DDT levels were also significantly higher in office workers and manual workers than in farmers.

According to multivariate logistic regression analyses, the odds ratio (OR) for diabetes increased with abdominal obesity (2.93; 95% confidence interval [CI]: 1.57-5.48), after adjusting for wealth index and a family history of diabetes (data not shown). The OR for diabetes also increased seven-fold with the family history of diabetes (7.58; 95% CI: 3.19-18). The OR for diabetes was 3.29 (95% CI: 1.46-7.4) for highest occupational level (office worker) and remained significant after adjusting for the wealth index (OR = 2.5; 95% CI: 1.6-6.1). The OR for diabetes was 2.17 (95% CI: 1.07-4.4) for higher wealth index, but it was no longer significant after adjusting for occupation (OR = 1.76; 95% CI: 0.79-3.9).

The OR for diabetes was nearly three-fold higher when comparing the third tertile of OCP levels (*p,p'*-DDE, *p,p'*-DDT, and β-HCH) with the first tertile according to logistic regression without adjustment for potential confounders (Table 4). After adjusting for family diabetes, abdominal obesity and wealth index, the association remained significant for *p,p'*-DDE (OR = 2.11; 95% CI: 1.01-4.54), and *p,p'*-DDT (OR = 2.59;

Table 1: Diabetes status (diabetic cases and non-diabetic controls) and risk factors

Variables	Proportion of subjects (%)			*P* value
	All (*N*=212)	Cases (*N*=106)	Controls (*N*=106)	
Education				0.208
No schooling	50.6	45.3	56.6	
Literacy or primary	21.7	22.6	20.8	
College or university	27.4	32.1	22.6	
Occupation				**0.007**
Farmer	42.5	37.7	47.2	
Manual worker	38.7	34.9	42.5	
Office worker	18.9	27.4	10.4	
Wealth index				0.074
Low	27.4	22.6	32.1	
Medium	39.2	36.8	41.5	
High	33.5	40.6	26.4	
Body weight status				**0.004**
Under weight	8.5	5.7	11.3	
Normal weight	49.1	45.5	56.6	
Over weight	27.8	38.7	17.0	
Overall obesity[a]	14.6	14.2	15.1	
High% body fat	28.8	35.8	21.7	**0.023**
Abdominal obesity	48.6	61.3	35.8	**<0.001**
Diabetes in family	20.8	34.9	6.6	**<0.001**
Alcohol consumption	36.8	34.9	38.7	0.569
Tobacco consumption	20.8	16.0	25.5	0.090

Bold values are $P<0.05$ according to Chi-square test. [a]There was no significant difference between cases and controls when obesity was compared with all other body weight categories combined

Table 2: Lipid-adjusted serum concentrations of OCPs in Borgou diabetic cases and non-diabetic controls

OCPs[a]	Adjusted serum concentrations (ng/g total serum lipids)								
	Whole sample (*n*=212)	Cases (*n*=106)	Controls (*n*=106)	Whole sample (*n*=212)					
	Geometric mean (95% CI)			Min	Max	25th	50th	75th	95th
p, p'-DDE	472.8 (385.8-579.5)	599.8 (437.9-821.7)	372.7 (288.9-480.8)	2.2	8360.6	203.2	511.4	1474.3	3798.6
p, p'-DDT	19.2 (14.5-25.4)	32.8 (23.0-46.8)	11.3 (7.4-17.1)	0.3	1074.0	11.1	32.0	74.2	30303
ß-HCH	2.8 (2.3-3.3)	3.8 (3.0-4.9)	2.0 (1.6-2.5)	0.6	351.8	1.1	1.5	6.4	28.1
trans-Nonachlor	1.9 (1.7-2.2)	2.3 (1.9-2.7)	1.6 (1.4-1.9)	0.6	80.5	1.1	1.4	3.1	10.3

[a]For the other OCPs, most concentration values were below the LOD in μg/L and were therefore not reported as ng/g total serum lipids. CI: Confidence interval. Min: Minimum, Max: Maximum, OCPs: Organochlorine pesticides, LOD: Limit of detection

Table 3: OCP concentrations according to personal factors

Variables	Groups	Geometric mean of adjusted serum concentrations (ng/g total serum lipids)			
		p-p'-DDE	*p-p'*-DDT	*β*-HCH	*trans*-Nonachlor
Education	No schooling	374.9	16.5	2.4	1.7
	Literacy or primary	466.9	18.0	3.7	2.3
	College or university	736.1	27.0	3.2	2.4
	P value[a]	**0.007**	0.251	0.096	**0.004**
Occupation	Farmer	375.5	13.6	2.5	1.8
	Manual worker	456.2	21.6	2.8	2.0
	Office worker	855.5	33.4	3.6	2.3
	P value[a]	**0.005**	**0.027**	0.348	0.075
Wealth index	Low	404.3	15.9	2.4	1.8
	Medium	414.1	12.9	2.2	1.8
	High	627.7	35.9	4.3	2.4
	P value[a]	**0.029**	**0.002**	**0.003**	**0.010**
Diabetes in family	Yes	873.9	36.7	5.3	2.7
	No	402.6	16.3	2.4	1.8
	P value[b]	**0.002**	**0.004**	**0.002**	**0.013**
Alcohol	Yes	541.0	16.6	2.8	2.3
	No	437.2	21.0	2.8	1.8
	P value[b]	0.187	0.477	0.794	0.060
Tobacco	Yes	439.4	13.3	2.0	1.7
	No	482.0	21.2	3.1	2.0
	P value[b]	0.230	0.080	0.122	0.158
Body weight status	Under weight	369.2	6.3	1.8	1.4
	Normal weight	417.8	15.6	2.4	1.7
	Over weight	526.4	27.4	3.1	2.5
	Obesity	674.4	37.8	4.4	2.2
	P value[a]	0.181	**0.003**	**0.035**	**0.016**
Overall obesity[c]	Yes	674.4	37.9	4.4	2.2
	No	444.9	17.1	2.6	1.9
	P value[b]	0.223	**0.014**	**0.012**	0.094
High % body fat	Yes	700.9	34.4	3.8	2.4
	No	314.2	10.5	2.0	1.5
	P value[b]	**<0.001**	**<0.001**	**0.003**	**0.001**
Abdominal obesity	Yes	603.5	29.4	3.5	2.4
	No	326.1	10.2	2.0	1.5
	P value[b]	**0.010**	**<0.001**	**<0.001**	**0.001**

Bold values are $P<0.05$. [a]Comparison of concentrations between groups using the Kruskal-Wallis test, [b]Comparison of concentrations between groups using the Mann-Withney test, [c]Comparison of overall obesity with other weight categories combined. OCP: Organochlorine pesticide

95% CI: 1.17-5.42). When education was used as an indicator of socio-economic status in the model instead of wealth index, the OR were similar for *p,p'*-DDE (OR = 2.17; 95% CI: 1.01-4. 65), and *p,p'*-DDT (OR = 2.52; 95% CI: 1.18-5.38). The relationship remained significant only for *p,p'*-DDT, when adjusting for occupation instead of wealth index (OR = 2.52; 95% CI: 1.15-5.39).

OCP Levels and Obesity

Higher *p,p'*-DDT, β-HCH and trans-nonachlor increased the odds of overall obesity and abdominal obesity, according to multivariate logistic regressions, after adjusting for diabetes, gender and wealth index (Table 5). The OR for high percentage body fat was increased only with higher trans-nonachlor levels. The same trends were observed when wealth index was replaced by education level or occupation (data not shown).

DISCUSSION

Our data showed that levels of OCPs were higher in a representative sample of diabetic subjects than in controls, strongly suggesting that increasing environmental exposure to OCPs is associated with diabetes. Our results are consistent with findings of other case-control studies showing that higher serum OCPs may be a major risk factor for diabetes [35,36]. As in our study, other cross-sectional studies have observed this relationship in particular for *p,p'*-DDE [37,38] and *p,p'*-DDT [39-42]. Prospective studies also reported higher risk of diabetes associated with increasing biological levels of some OCPs. [36,43-45]; one tertile-increment in serum *p,p'*-DDE levels more than doubled the risk of diabetes [46]. Our findings also confirmed the suggested dose-response pattern of the association [37,47]. In our study, the relationship remained significant especially for *p,p'*-DDT, after adjusting for a number of confounding variables. This was also the case in several other studies after adjusting for factors such as ethnicity, a family history of diabetes, obesity, economic status, age, gender, and residence area [37,41,48]. The consistency of the relationship for *p,p'*-DDT confirms the higher toxicity of this compound than its *p,p'*-DDE metabolite. In fact, *p,p'*-DDE is less harmful than *p,p'*-DDT, but more persistent and thus more bio-accumulated leading to proportionally higher concentrations in the human body, as observed in the current study. Although associations were also observed in our study between β-HCH and diabetes prior to adjustment for confounders, it did not remain statistically significant after adjustment, as observed in Mexican people [42]. In the current study, trans-nonachlor was not associated with diabetes, which is in contrast with findings of some countries [37,42,49]. β-HCH and trans-nonachlor may have been less widely used in the past, which could explain the lower background level in serum and the poor association with diabetes. Moreover, the elimination of these pesticides in urine is faster than that of *p,p'*-DDT [50].

Table 4: Serum OCP concentrations and diabetes

OCPs	Tertile (T)	OR (CI)[a]	*P* value*	Adjusted OR[b] (CI)	*P* value*	Adjusted OR[c] (CI)	*P* value*	Adjusted OR[d] (CI)	*P* value*
p, p'-DDE	T1	1		1		1		1	
	T2	1.9 (0.9-3.8)	0.051	2.0 (0.9-4.1)	0.058	2.0 (0.9-4.2)	0.058	1.8 (0.8-3.8)	0.100
	T3	**2.93** (1.4-5.8)	**<0.002**	**2.1 (1.03-4.5)**	**0.041**	**2.1** (1.0-4.5)	**0.048**	1.9 (0.8-4.1)	0.093
p, p'-DDT	T1	1		1		1		1	
	T2	1.82 (0.9-3.5)	0.081	1.7 (0.8-3.6)	0.116	1.7 (0.8-3.7)	0.124	1.8 (0.8-3.8)	0.106
	T3	**3.68** (1.8-7.3)	**<0.001**	**2.5 (1.2-5.4)**	**0.016**	**2.5** (1.1-5.4)	**0.018**	**2.5** (1.1-5.3)	**0.020**
β-HCH	T1	1		1		1		1	
	T2	0.81 (0.4-1.6)	0.559	0.6 (0.3-1.4)	0.294	0.6 (0.3-1.3)	0.283	0.6 (0.3-1.4)	0.330
	T3	**2.90** (1.4-5.8)	**0.002**	1.8 (0.8-3.9)	0.099	1.8 (0.8-3.9)	0.112	1.8 (0.8-4.0)	0.102
trans-Nonachlor	T1	1		1		1		1	
	T2	0.6 (0.3-1.2)	0.201	0.5 (0.2-1.1)	0.080	0.5 (0.2-1.07)	0.076	0.4 (0.2-1.01)	0.055
	T3	1.7 (0.9-3.4)	0.093	1.07 (0.5-2.2)	0.843	1.04 (0.4-2.21)	0.902	1.01 (0.4-2.1)	0.987

**P* value according to logistic regression. [a]Unadjusted OR, [b]OR adjusted for diabetes in family and abdominal obesity, [c]OR adjusted for diabetes in family, abdominal obesity and wealth index, Values are similar when wealth index was substituted by education level, [d]OR adjusted for diabetes in family, abdominal obesity and occupation. OCP: Organochlorine pesticides, CI: Confident interval, Bold values are $P<0.05$, OR: Odds ratio, OCP: Organochlorine pesticide

Table 5: Serum OCP concentrations and obesity indicators

OCPs	Tertile (T)	Overall obesity		Abdominal obesity		High % body fat	
		Adjusted OR[a] (CI)	*P* value	Adjusted OR[a] (CI)	*P* value	Adjusted OR[a] (CI)	*P* value
p, p'-DDE	T1	1		1		1	
	T2	1.5 (0.5-4.5)	0.43	1.5 (0.7-3.3)	0.31	1.0 (0.4-2.3)	0.89
	T3	1.9 (0.7-5.2)	0.19	1.8 (0.8-4.1)	0.13	1.1 (0.5-2.5)	0.73
p, p'-DDT	T1	1		1		1	
	T2	1.3 (0.4-4.0)	0.541	1.5 (0.7-3.3)	0.32	1.7 (0.7-4.0)	0.18
	T3	**3.1** (1.1-8.9)	**0.037**	**2.4** (1.1-5.3)	**0.034**	1.9 (0.8-4.4)	0.12
β-HCH	T1	1		1		1	
	T2	2.3 (0.7-7.2)	0.139	1.9 (0.9-4.4)	0.93	1.3 (0.5-2.9)	0.51
	T3	**3.9** (1.2-11.5)	**0.026**	**3.8** (1.5-7.5)	**0.003**	1.6 (0.7-3.6)	0.23
trans-Nonachlor	T1	1		1		1	
	T2	1.4 (0.5-4.4)	0.48	1.3 (0.6-2.9)	0.51	1.3 (0.5-3.0)	0.49
	T3	**3.0** (1.2-8.8)	**0.043**	**4.9** (2.1-11.3)	**<0.001**	**2.3** (1.01-5.1)	**0.042**

[a]OR adjusted for diabetes, gender and wealth index status. Bold values are $P<0.05$ at multivariate logistic regression. OR: Odds ratio, OCP: Organochlorine pesticide

In our work, we did not adjust our regression analyses models for diet and physical activity, but we did for obesity indicators, which could be considered as a reflection of energy balance [51]. High percentage body fat or abdominal obesity appeared more closely associated with OCP levels than overall obesity, as reported in the US [52]. In contrast, overall obesity was only associated with levels of *p,p'*-DDT and β-HCH in our study, suggesting a more recent contamination at least for *p,p'*-DDT. The percentage body fat and abdominal obesity correlated well with serum OCPs in our study as in previous ones [53,54]. Obesity could then partly explain the relationship between diabetes and OCP exposure, even if there is a direct relationship between OCP levels and diabetes. We also found that higher OCP levels were reflected in a three-fold increase in ORs for all three obesity indicators assessed. BMI was strongly associated with internal level of *p,p'*-DDE in Flemish or American people and the association was even stronger among obese subjects [36,47].

Diabetic subjects were those with the most favorable socio-economic status. Indeed, diabetic subjects were more likely than controls to be office workers, with a higher education level and wealth index. The same findings were reported in other African and non-African studies showing that a high socio-economic status was closely related to the development of diabetes or obesity [55-59]. Wealth and higher education level were strongly associated with the development of obesity in Benin as confirmed in a recent study [60]. In contrast, it was observed in a Ghanaian study that a lower socio-economic status was associated with increased risk of diabetes [61]. This can be ascribed to the higher energy density of the cheaper foods purchased by low-income people and the resulting higher rate of obesity observed among them, at least in higher income countries [62]. Obesity indicators (abdominal obesity, high body fat percentage) and a family history of diabetes were also more prevalent in diabetics than in control subjects, which confirm that these are strong determinants of diabetes [61,63,64].

The effects of OCPs on obesity and diabetes could result from different pathways [65-67]. The precise mechanism explaining a potential causal relationship between OCP pesticides and diabetes has not been clearly elucidated yet, but several mechanisms are suggested [13,68]. Pesticides affect glucose homeostasis by altering various pathways including inflammations, mitochondrial dysfunction with endoplasmic reticulum stress, endocrine disruption, genetic damage and epigenetic modifications [69,70]. OCPs are known

to accumulate in adipose tissues, thus possibly disrupting lipid metabolism, and elevating levels of triacylglycerol and fasting glucose. OCPs have also been documented to decrease metabolic activity of adipocytes, which may increase obesity and reduce insulin sensitivity, thus gradually enhancing the risk of diabetes [70]. The physiological or oxidative stress induced by pesticides through the accumulation of fatty acids is a threat for glucose and lipid homeostasis affecting the immune, neuroendocrine, and autonomous nervous systems [12]. It has also been suggested that exposure to some pesticides can potentiate other risk factors of diabetes, such as obesity, by disturbing neural circuits that regulate feeding behavior or by altering differentiation of adipocytes [13,71]. Metabolic dysfunctions, insulin resistance in particular, were strongly associated with OCP exposure in some studies in humans [66,67,72]. Animal studies further support the obesogenic role of pesticides and their involvement in the development of diabetes [73,74].

Our study has limitations related to the fact that a causal relationship between OCP levels and diabetes could not be identified, but the design of the study helped to observe strong associations. Controls were adequately selected to avoid any bias.

CONCLUSION

In the Borgou area of Benin, we found for the first time a strong relationship between serum concentrations of OCPs and diabetes. *p,p'*-DDT was consistently associated with diabetes. Obesity may partly explain the relationship. Except for *p,p'*-DDE, OCP levels were associated with overall obesity, high percent body fat and abdominal obesity. The contribution of pesticide exposure to the development of obesity and diabetes, and the potential modulation of this association by physical inactivity, unhealthy diet and income need to be further explored in order to assess whether lifestyle modification could reduce harmful effects of pesticides. As glucose tolerance and insulin resistance effects were described for organophosphates and pyrethroids [74-77], it would be relevant to extend future investigation to other categories of pesticides, which are now widely used in Africa.

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