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Original Contribution

Association Between Chlorinated Pesticides in the Serum of Prepubertal Russian Boys and Longitudinal Biomarkers of Metabolic Function

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Organochlorine pesticides (OCPs) have been linked to adult metabolic disorders; however, few studies have examined these associations in childhood. We prospectively evaluated the associations of baseline serum OCPs (hexachlorobenzene, β-hexachlorocyclohexane, and *p*,*p*′-dichlorodiphenyldichloroethylene) in Russian boys with subsequent repeated measurements of serum glucose, insulin, lipids, leptin, and calculated homeostatic model assessment of insulin resistance (IR). During 2003–2005, we enrolled 499 boys aged 8–9 years in a prospective cohort; 318 had baseline serum OCPs and serum biomarkers measured at ages 10–13 years. Multivariable generalized estimating equation and mediation regression models were used to examine associations and direct and indirect (via body mass index (BMI) (weight (kg)/height (m)^2)) effects of prepubertal OCP tertiles and quintiles with biomarkers. In multivariable models, higher *p*,*p*′-dichlorodiphenyldichloroethylene (quintile 5 vs. quintile 1) was associated with lower leptin, with relative mean decreases of 61.8% (95% confidence interval: 48.4%, 71.7%) in models unadjusted for BMI and 22.2% (95% confidence interval: 7.1%, 34.9%) in models adjusted for BMI; the direct effect of *p*,*p*′-dichlorodiphenyldichloroethylene on leptin accounted for 27% of the total effect. IR prevalence was 6.6% at ages 12–13 years. Higher hexachlorobenzene (tertile 3 vs. tertile 1) was associated with higher odds of IR in models adjusted for BMI (odds ratio = 4.37, 95% confidence interval: 1.44, 13.28). These results suggest that childhood OCPs may be associated with IR and lower leptin.

children; insulin resistance; leptin; metabolism; pesticides

Abbreviations: β-HCH, β-hexachlorocyclohexane; BMI, body mass index; HCB, hexachlorobenzene; HOMA-IR, homeostatic model assessment for insulin resistance; IR, insulin resistance; LOD, limit of detection; OCP, organochlorine pesticide; *p*,*p*′-DDE, *p*,*p*′-dichlorodiphenyldichloroethylene; T2D, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; TV, testicular volume.

The prevalence of type 2 diabetes mellitus (T2D) and other obesity-related disorders has risen dramatically among children, raising concerns about increased risk for future cardiovascular disease (1). Although diet and declining physical activity are major contributors to obesity and metabolic dysfunction, there is increasing evidence that environmental exposures may play a role (2). Organochlorine pesticides (OCPs) are endocrine disrupting chemicals (3), and experimental evidence suggests they may elicit proinflammatory responses (4) and disrupt energy metabolism by interfering with glucose uptake (5) and adipocyte function (6). A recent National Toxicology Program review found convincing evidence linking dichlorodiphenyltrichloroethane and its metabolite, *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE), with adult-onset T2D (7).

OCP exposure is ubiquitous because of extensive historical agricultural use and environmental persistence, with biomagnification through the food chain (8). Although hexachlorobenzene (HCB) and α- and β-hexachlorocyclohexane are banned, and dichlorodiphenyltrichloroethane is greatly restricted (9), environmental contamination continues from stockpiled chemicals that have long half-lives and from

ongoing generation of α- and β-hexachlorocyclohexane from lindane production (10). The most common route of OCP exposure is diet (11), with children also exposed via transplacental and lactational transfer (12) and their "hand to mouth" behaviors (13).

Rapid growth during adolescence is accompanied by developmental increases in muscle and fat mass and dynamic pubertal changes in metabolic processes. Sex steroids and counterregulatory hormones, such as growth hormone, rise at pubertal onset. During pubertal maturation, there is an initial decline in insulin sensitivity that later improves at sexual maturation (14). Therefore, the pubertal period may be a window of heightened vulnerability to metabolic perturbation by endocrine disrupting chemicals. Although childhood insulin resistance (IR) and dyslipidemia are known risk factors for adult metabolic disorders (e.g., T2D) (15), the role of childhood OCP exposures in later metabolic dysregulation has not been studied. We prospectively examined the relationship of prepubertal serum OCPs with serial serum indicators of metabolic and adipocyte function in a cohort of Russian boys during early adolescence.

METHODS

Study population

The Russian Children's Study is a prospective cohort study of 499 boys in Chapaevsk, Russia, enrolled from 2003 to 2005 at ages 8–9 years, and is described in detail elsewhere (16). The study was approved by the human studies institutional review boards of the participating institutions. Parent or guardians signed an informed consent and the boys signed an assent form. The study initially focused on dioxins; thus, serum OCPs were not measured for the first 144 boys recruited. Of the 355 boys enrolled later with OCP measurements, 5 boys were excluded because of chronic illnesses that affect growth, and an additional 32 boys with OCP measurements were excluded because of unavailable metabolic biomarker data, leaving 318 boys with at least 1 metabolic biomarker measurement during the follow-up period.

At study entry, each boy's parent or guardian completed a nurse-administered health and lifestyle questionnaire (17) that included birth and medical history and demographic and socioeconomic status indicators. A validated Russian Institute of Nutrition semiquantitative food frequency questionnaire was used to ascertain each child's dietary intake (18).

At study entry and annual follow-up visits, a standardized anthropometric examination by a trained research nurse and pubertal staging by a single clinician were performed per written protocol and without knowledge of the boys' pesticide levels (19). Pubertal status was determined by Tanner staging (20) and measurement of testicular volume (TV) using a Prader orchidometer. Age-adjusted z scores were calculated for height and body mass index (weight (kg)/height $(m)^2$) (BMI) using the World Health Organization standards (21).

Blood samples

At study entry and biennially, fasting blood samples were collected. Sera from blood samples taken at enrollment were stored at −35°C until shipment on dry ice to the US Centers for Disease Control and Prevention (Atlanta, Georgia) for organochlorine analysis. The samples, including method blank and quality control samples, were analyzed for OCPs and total lipids as described previously; no lipid or OCP measurements were below the limit of detection (LOD) (11). Blood samples from follow-up visits at ages 10–11 years ($n = 315$) and 12–13 years ($n = 290$) were analyzed at the Endocrinology, Physical Culture, and Sports Laboratory (Moscow, Russia) for glucose, insulin, lipids (total cholesterol (TC) and triglycerides (TG)), and leptin. An enzymatic hexokinase reference method for glucose (interassay coefficients of variation, 8.0%–8.5%) and an enzymatic colorimetric assay for lipids (interassay coefficients of variation for TC and TG were 7.6%–8.0% and 13.4%–20.8%, respectively) were used (COBAS INTEGRA 400 plus, Hoffmann-La Roche, Basel, Switzerland). A chemiluminescent immunometric assay (DPC Immulite 2000, Siemens, Munich, Germany) with a LOD of 2 μIU/mL was used for insulin, with values less than the LOD (6.7%) reanalyzed using a more sensitive electrochemiluminescent assay (Elecsys 2010, Hoffmann-La Roche) with a LOD of 0.2 μIU/mL (interassay coefficients of variation, 5.8%–7.2%). Leptin was measured by enzyme-linked immunoabsorbant assay with interassay coefficients of variation of 13.5%–15.5%; for the 2.5% of values that fell below the LOD of 1.0 ng/mL, the laboratory provided quantitative values less than the LOD, which we used in the analysis (22). One sample batch analyzed for glucose and lipids was removed from the analysis because of implausibly low values.

Homeostatic model assessment for insulin resistance (HOMA-IR), a continuous measure of insulin sensitivity, was calculated using the formula [(fasting insulin in μIU/mL × fasting glucose in mmol/L) / 22.5] (23). IR was defined as HOMA-IR greater than 2.5 for prepubertal children (Tanner stage 1) and greater than 4.0 for pubertal-aged children (Tanner stage >1) (24). Metabolic syndrome was defined according to the International Diabetes Federation criteria, using age, waist circumference, TG, high-density lipoprotein cholesterol, blood pressure, and glucose (25).

Statistical analysis

We evaluated the associations of boys' prepubertal (at ages 8–9 years) serum OCP concentrations at baseline with serum glucose, insulin, TG, TC, and leptin at follow-up (at ages 10–13 years), as well as with IR using both HOMA-IR (continuous outcome) and occurrence of IR (binary outcome) with generalized estimating equations for repeated measures with an exchangeable covariance. Serum wet-weight HCB, β-hexachlorocyclohexane (β-HCH), and p,p'-DDE concentrations were modeled as quintiles, with the lowest quintile as the referent; however, because IR prevalence was low, OCPs were modeled as tertiles for this outcome, with the lowest tertile as the referent. In our statistical models, we adjusted for total serum lipids at the time of OCP measurement as a covariate to minimize potential bias (26). Because of skewed distributions, all continuous outcomes except TC were $\log_e$-transformed for analysis. We evaluated bivariate associations on the basis of prior literature, then used a

Table 1. Descriptive Characteristics of 350 Boys With Serum Organochlorine Pesticide Measurements at Entry Into the Russian Children's Study, 2003–2005

Characteristic	Median	Range	No.	%
Physical characteristics				
Height, *z* score[a]	0.14	−0.58, 0.77[b]		
Body mass index[c], *z* score[a]	−0.31	−1.01, 0.51[b]		
Overweight			36	10
Obese			22	6
Birth and neonatal history[d]				
Birth weight, kg	3.4	3.1, 3.7[b]		
Gestational age, weeks	40	38, 40[b]		
Duration of breastfeeding, weeks	13.0	4.3, 30.3[b]		
Breastfed			297	85
Boys' daily dietary intakes[d]				
Total calories	2,520	2,039, 3,256[b]		
Calories from carbohydrates, %	55	50, 59[b]		
Calories from fat, %	34	30, 38[b]		
Calories from protein, %	11	10, 12[b]		
Boys' daily physical exercise levels[d]				
None			109	31
<2 hours/day			124	36
≥2 hours/day			116	33
Household characteristics[d]				
Maximum parental educational level				
Secondary education or less			29	8
Junior college/technical training			198	57
University graduate			121	35
Household income				
<$175/month			107	31
$175–$250/month			88	25
>$250/month			154	44
Serum organochlorine pesticides				
Lipid-standardized measures, ng/g lipid				
Hexachlorobenzene	162	80, 392[e]		
β-Hexachlorocyclohexane	165	81, 407[e]		
p,p′-Dichlorodiphenyldichloroethylene	288	122, 835[e]		
Wet-weight measures, ng/g				
Hexachlorobenzene	770	383, 1,973[e]		
β-Hexachlorocyclohexane	800	405, 2,078[e]		
p,p′-Dichlorodiphenyldichloroethylene	1,410	596, 4,106[e]		

[a] World Health Organization age-adjusted *z* scores (21).
[b] Values represent the 25th and 75th percentiles.
[c] Weight (kg)/height (m)^2.
[d] Missing information on birth weight ($n = 1$), gestational age ($n = 2$), whether or not breastfed ($n = 5$), dietary intakes ($n = 3$), physical activity ($n = 1$), parental education ($n = 2$), and household income ($n = 1$).
[e] Values represent the 10th and 90th percentiles.

forward stepwise selection process including all covariates with P values of 0.20 or less, and finally reduced to a model including covariates with P values less than 0.10. We considered continuous predictors as both continuous and categorical variables, and we examined point estimates and model goodness-of-fit measures to determine the most appropriate coding. We constructed separate models for each outcome. Age was included as a covariate in all models

Table 2. Fasting Serum Biomarkers by World Health Organization Body Mass Index[a] *z* Score Category and Age in the Russian Children's Study, 2005–2009

Biomarker	Body Mass Index Category				*P* Value[e]
	Normal[b]		Overweight[c,d]		
	Median	25th, 75th Percentiles	Median	25th, 75th Percentiles	
	Boys Aged 10–11 Years[f]				
Glucose, mg/dL	81	74, 89	82	75, 90	0.26
Insulin, μIU/mL	4.84	2.97, 7.00	7.18	5.67, 9.60	<0.001
HOMA-IR	0.98	0.60, 1.45	1.50	1.07, 2.04	<0.001
Triglycerides, mg/dL	64	50, 87	84	63, 103	0.002
Total cholesterol, mg/dL	159	143, 182	175	156, 190	0.001
Leptin, ng/mL	3.12	2.23, 4.92	15.40	11.50, 23.40	<0.001
	Boys Aged 12–13 Years[g]				
Glucose, mg/dL	87	82, 91	89	83, 95	0.09
Insulin, μIU/mL	5.25	3.67, 7.59	9.46	6.62, 12.95	<0.001
HOMA-IR	1.12	0.78, 1.60	2.04	1.37, 2.82	<0.001
Triglycerides, mg/dL	71	54, 96	91	65, 133	0.008
Total cholesterol, mg/dL	165	144, 188	183	155, 200	0.001
Leptin, ng/mL	2.70	1.74, 4.18	16.90	11.40, 25.70	<0.001

Abbreviation: HOMA-IR, homeostatic model assessment of insulin resistance.
[a] Weight (kg)/height $(m)^2$.
[b] *n* = 256 boys aged 10–11 years and 226 boys aged 12–13 years.
[c] Included boys were overweight (>1 standard deviation above the mean) or obese (>2 standard deviations above the mean) according to the World Health Organization criteria (21).
[d] *n* = 59 boys aged 10–11 years and 64 boys aged 12–13 years.
[e] *P* values (2-sided) were based on the Wilcoxon rank-sum test.
[f] Overweight prevalence was 19%.
[g] Overweight prevalence was 22%.

except that for leptin. Models for insulin, HOMA-IR, TC, and leptin included percent calories from fat, and ordinal tertiles of total daily caloric intake were retained in the leptin model. Models for IR and TG retained an indicator of pubertal onset (TV >3 mL), and more advanced pubertal stage (TV ≥15 mL) was retained in the leptin model. Only the TC model retained an indicator of socioeconomic status (maximum parental education, categorized as secondary school or less, junior college, or university degree). All analyses were conducted with SAS, version 9.2, software (SAS Institute, Inc., Cary, North Carolina), and statistical significance was set at $\alpha = 0.05$. Tests for trend over OCP levels were performed by modeling tertiles or quintiles of exposure as a continuous variable. For clarity, continuous outcomes except TC were expressed as relative percent change in means ($[\exp(\beta) - 1] \times 100$), and binary outcomes were expressed as adjusted odds ratios.

Because BMI *z* score may be on the causal pathway between OCPs and biomarkers (27), it was not included in initial OCP models. However, we conducted sensitivity analyses including BMI *z* score at the time of biomarker measurement in the final models. We also used mediation models (28) to assess the total, direct, and indirect (via BMI *z* score as a mediator) effects of OCPs (continuous log-transformed) on biomarkers of metabolic function. To determine whether OCPs' indirect effects depended on the mediator's level, we used a Wald test for interaction between the exposure and mediator (BMI *z* score). When significant interaction was observed ($P \leq 0.05$), direct effects were estimated for both approximately normal-weight (BMI *z* score = 0) and overweight (BMI *z* score = 2) boys. Initially, we assessed mediation separately at ages 10–11 and 12–13 years and then used linear regression models for repeated measures. The indirect effect was estimated as the difference between the total effect (unadjusted for BMI *z* score) and direct effect (adjusted for BMI *z* score). Formal mediation analysis was not performed if the dose-response relationship was nonlinear, or for IR because of low prevalence.

RESULTS

Study population and serum OCP concentrations

Baseline anthropometric measurements and diet, birth, maternal, and household characteristics among the 350 boys with OCP measurements are presented in Table 1. At baseline, 13% of the boys had entered puberty (TV > 3 mL), and 16% were overweight or obese. From ages 10 to 13 years, the prevalence of overweight (based on a repeated measures model accounting for within-boy correlation) increased significantly ($P = 0.009$). The boys demonstrated a wide range of OCP concentrations (Table 1), with few differences in

Table 3. Adjusted Percent Change in Biomarkers by Baseline Quintiles of Serum Wet-Weight Organochlorine Pesticides at 2 Follow-up Visits in the Russian Children's Study, 2003–2009

Metabolic Biomarker, by Quintile of Serum OCP Level	Serum OCPs								
	HCB, pg/g[a]			β-HCH, pg/g[b]			*p,p′*-DDE, pg/g[c]		
	% Change[d]	95% CI	*P* Value[e]	% Change[d]	95% CI	*P* Value[e]	% Change[d]	95% CI	*P* Value[e]
Leptin ($n = 599$)[f,g]									
First quintile		Referent			Referent			Referent	
Second quintile	1.8	−24.7, 37.5	0.91	−2.3	−28.5, 33.6	0.89	−39.2	−55.3, −17.3	0.002
Third quintile	−37.3	−54.1, −14.3	0.003	−44.4	−58.5, −26.6	<0.001	−43.8	−57.7, −25.4	<0.001
Fourth quintile	−43.4	−57.7, −24.3	<0.001	−53.5	−65.6, −37.0	<0.001	−49.7	−62.5, −32.4	<0.001
Fifth quintile	−46.2	−59.9, −27.3	<0.001	−50.6	−63.6, −33.1	<0.001	−61.8	−71.7, −48.4	<0.001
P for trend			<0.001			<0.001			<0.001
Insulin ($n = 602$)[f,h]									
First quintile		Referent			Referent			Referent	
Second quintile	10.1	−7.9, 31.7	0.29	−10.5	−27.0, 9.7	0.29	−10.6	−26.3, 8.5	0.26
Third quintile	10.2	−10.3, 35.5	0.35	−25.5	−38.8, −9.4	0.003	−15.3	−30.7, 3.4	0.10
Fourth quintile	−8.9	−24.1, 9.2	0.31	−33.2	−45.1, −18.6	<0.001	−8.0	−24.9, 12.6	0.42
Fifth quintile	2.8	−16.5, 26.5	0.80	−19.0	−33.9, −0.7	0.04	−23.1	−36.4, −7.0	0.006
P for trend			0.53			0.002			0.02
HOMA-IR ($n = 602$)[f,h]									
First quintile		Referent			Referent			Referent	
Second quintile	9.5	−9.1, 31.8	0.34	−12.3	−29.2, 8.5	0.23	−8.8	−25.5, 11.8	0.38
Third quintile	10.5	−11.0, 37.1	0.37	−26.6	−40.2, −9.8	0.003	−13.4	−29.8, 6.8	0.18
Fourth quintile	−8.4	−24.4, 10.9	0.37	−34.7	−46.8, −19.7	<0.001	−5.9	−24.0, 16.7	0.58
Fifth quintile	1.9	−17.7, 26.0	0.87	−20.8	−36.1, −1.9	0.03	−22.3	−36.2, −5.4	0.01
P for trend			0.53			0.002			0.04

Abbreviations: β-HCH, β-hexachlorocyclohexane; CI, confidence interval; HCB, hexachlorobenzene; HOMA-IR, homeostatic model assessment of insulin resistance; OCP, organochlorine pesticide; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene.

[a] HCB quintiles: first, 169–462 pg/g; second, 463–655 pg/g; third, 656–906 pg/g; fourth 910–1,284 pg/g; fifth, 1,295–15,482 pg/g.

[b] β-HCH quintiles: first, 209–518 pg/g; second, 519–690 pg/g; third, 691–982 pg/g; fourth, 985–1,460 pg/g; fifth, 1,461–13,733 pg/g.

[c] *p,p′*-DDE quintiles: first, 261–818 pg/g; second, 832–1,199 pg/g; third, 1,203–1,716 pg/g; fourth, 1,720–2,659 pg/g; fifth, 2,683–41,302 pg/g.

[d] Adjusted percent change = $[\exp(\beta) - 1] \times 100$, reflecting percent change in biomarker for each quintile versus the lowest quintile.

[e] *P* values (2-sided) were based on the Wald test.

[f] *n* represents number of observations.

[g] Generalized estimating equations for repeated measures regression model adjusted for baseline total serum lipids, testicular volume ≥15 mL, ordinal tertiles of dietary total calories, and percent dietary fat.

[h] Generalized estimating equations for repeated measures regression model adjusted for baseline total serum lipids, age, and percent dietary fat.

baseline characteristics between the 350 boys and the entire cohort of 499 boys (11) and no differences between boys with ($n = 318$) versus those without ($n = 32$) measured biomarkers. The retention rate was 86% after 4 years.

Metabolic function biomarkers

Median concentrations of metabolic function biomarkers were within age-appropriate ranges, with higher values for insulin, HOMA-IR, lipids, and leptin among overweight boys (Table 2) (29–31). Overall, the prevalence rates of IR and metabolic syndrome were 3.8% ($n = 12$) and 0.6% ($n = 2$) at ages 10–11 years and 6.6% ($n = 19$) and 2.1% ($n = 6$) at ages 12–13 years, respectively. Unlike metabolic syndrome, both normal-weight and overweight boys had IR. Covariates retained in multivariable-adjusted models are summarized in Web Table 1, available at http://aje.oxfordjournals.org/. Consistent with prior literature (1, 29), higher BMI *z* score was associated with higher levels of metabolic function biomarkers most closely tied to IR (i.e., glucose, insulin, TG, leptin, and calculated HOMA-IR).

Association of OCPs with metabolic function biomarkers

In models adjusted for covariates (without BMI *z* score), higher prepubertal serum OCPs were associated with lower serum leptin concentrations over 4 years of follow-up (Table 3). Higher quintiles of *p,p′*-DDE compared with the lowest quintile were associated with lower leptin concentrations, with a monotonically decreasing trend. Higher quintiles of both β-HCH and HCB compared with the lowest

Table 4. Adjusted Percent Change in Biomarkers by Baseline Quintiles of Serum Wet-Weight Organochlorine Pesticides at 2 Follow-up Visits in the Russian Children's Study, 2003–2009, With Additional Adjustment for Body Mass Index[a] *z* Score

Metabolic Biomarker, by Quintile of Serum OCP Level	Serum OCPs								
	HCB, pg/g[b]			β-HCH, pg/g[c]			*p,p′*-DDE, pg/g[d]		
	% Change[e]	95% CI	*P* Value[f]	% Change[e]	95% CI	*P* Value[f]	% Change[e]	95% CI	*P* Value[f]
Leptin ($n = 599$)[g,h]									
First quintile		Referent			Referent			Referent	
Second quintile	20.4	2.3, 41.6	0.03	8.4	−8.1, 27.7	0.34	−10.6	−23.7, 4.7	0.16
Third quintile	8.3	−9.9, 30.1	0.39	−3.7	−18.8, 14.3	0.67	−12.4	−25.0, 2.4	0.10
Fourth quintile	−0.3	−15.9, 18.3	0.98	−8.2	−23.2, 9.7	0.35	−16.2	−29.5, −0.4	0.05
Fifth quintile	−1.3	−16.8, 17.1	0.88	−3.3	−18.6, 14.9	0.70	−22.2	−34.9, −7.1	0.006
P for trend			0.27			0.30			0.007
Insulin ($n = 602$)[g,i]									
First quintile		Referent			Referent			Referent	
Second quintile	17.6	1.4, 36.3	0.03	−7.8	−22.8, 10.1	0.37	1.6	−13.7, 19.6	0.85
Third quintile	35.3	12.7, 62.4	0.001	−11.4	−25.7, 5.6	0.18	−1.5	−17.2, 17.2	0.87
Fourth quintile	12.8	−4.1, 32.7	0.15	−16.7	−30.6, −0.1	0.05	−9.0	−9.0, 30.5	0.35
Fifth quintile	29.0	7.4, 54.9	0.006	0.7	−16.3, 21.2	0.94	−2.2	−17.5, 16.0	0.80
P for trend			0.03			0.76			0.89
HOMA-IR ($n = 602$)[g,i]									
First quintile		Referent			Referent			Referent	
Second quintile	17.2	0.04, 36.7	0.04	−9.6	−25.0, 9.0	0.29	4.5	−11.9, 23.9	0.62
Third quintile	37.0	12.9, 66.1	0.001	−11.9	−26.8, 6.0	0.18	1.8	−15.2, 22.1	0.85
Fourth quintile	14.6	−3.6, 36.2	0.12	−17.8	−32.2, 0.3	0.03	12.8	−6.7, 36.3	0.21
Fifth quintile	29.3	7.1, 56.2	0.008	−0.6	−18.2, 20.7	0.95	0.02	−15.9, 19.5	0.98
P for trend			0.03			0.70			0.70

Abbreviations: β-HCH, β-hexachlorocyclohexane; CI, confidence interval; HCB, hexachlorobenzene; HOMA-IR, homeostatic model assessment of insulin resistance; OCP, organochlorine pesticide; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene.

[a] Weight (kg)/height (m)^2.

[b] HCB quintiles: first, 169–462 pg/g; second, 463–655 pg/g; third, 656–906 pg/g; fourth, 910–1,284 pg/g; fifth, 1,295–15,482 pg/g.

[c] β-HCH quintiles: first, 209–518 pg/g; second, 519–690 pg/g; third, 691–982 pg/g; fourth, 985–1,460 pg/g; fifth, 1,461–13,733 pg/g.

[d] *p,p′*-DDE quintiles: first, 261–818 pg/g; second, 832–1,199 pg/g; third, 1,203–1,716 pg/g; fourth, 1,720–2,659 pg/g; fifth 2,683–41,302 pg/g.

[e] Adjusted percent change = $[\exp(\beta) - 1] \times 100$, reflecting percent change in biomarker for each quintile versus the lowest quintile.

[f] *P* values (2-sided) were based on the Wald test.

[g] *n* represents number of observations.

[h] Generalized estimating equations for repeated measures regression model adjusted for baseline total serum lipids, testicular volume ≥15 mL, ordinal tertiles of dietary total calories, percent dietary fat, and World Health Organization body mass index *z* score (21).

[i] Generalized estimating equations for repeated measures regression model adjusted for baseline total serum lipids, age, percent dietary fat, and body mass index *z* score.

quintile were associated with lower leptin, although the dose-response relationship did not exhibit a linear decline (Table 3). In sensitivity analyses including BMI *z* score, the highest 2 quintiles of *p,p′*-DDE were significantly associated with lower leptin (Table 4).

After adjustment, higher β-HCH was associated with lower mean insulin, although the decreasing trend was attenuated at the fifth quintile (Table 3). Higher *p,p′*-DDE was associated with lower insulin; although the test for trend was statistically significant, some departures from a strictly linearly decreasing trend were apparent. In sensitivity analyses further adjusting for BMI *z* score, β-HCH, and *p,p′*-DDE were no longer associated with log insulin (Table 4). In adjusted models not including BMI *z* score, no association between serum HCB and insulin was observed; however, after adjustment for BMI *z* score, higher serum HCB was associated with higher insulin. In all adjusted models, associations of OCPs with HOMA-IR were similar to those with insulin (Tables 3 and 4). We observed no association of OCPs with either glucose or lipids (Web Tables 2 and 3).

In mediation models (Table 5) using continuous log OCPs, with continuous BMI *z* score as a mediator, higher prepubertal serum *p,p′*-DDE concentrations were associated with significant negative direct, indirect, and total effects on log leptin at ages 10–13 years, with no interaction between *p,p′*-DDE and BMI *z* score. However, the direct effect of *p,p′*-DDE comprised only 27% of the total effect on leptin, whereas the indirect effect mediated through BMI *z* score

Table 5. Mediation Analysis Between Baseline Continuous Log Serum Wet-Weight Organochlorine Pesticides and Log Leptin With Body Mass Index *z* Score as the Mediator at 2 Follow-up Visits in the Russian Children's Study, 2003–2009

Mediation Model[a] Effects on Leptin, by Log OCP	Serum OCPs											
	HCB Quintiles, pg/g[b]				β-HCH Quintiles, pg/g[c]				*p,p′*-DDE Quintiles, pg/g[d]			
	β Estimate	95% CI	*P* Value[e]	% Total Effect	β Estimate	95% CI	*P* Value[e]	% Total Effect	β Estimate	95% CI	*P* Value[e]	% Total Effect
Generalized linear model: ages 10–11 years (1 measurement per boy)												
Total effect[f]	−0.82	−1.16, −0.48	<0.001		−0.95	−1.28, −0.63	<0.001		−0.81	−1.08, −0.54	<0.001	
Direct effect[g]	−0.15	−0.38, 0.08	0.19	18	−0.11	−0.33, 0.12	0.35	12	−0.27	−0.45, −0.09	0.004	33
Indirect effect[h]	−0.67	−0.94, −0.40	<0.001	82	−0.85	−1.11, −0.59	<0.001	88	−0.54	−0.76, −0.33	<0.001	67
P for interaction[i]			0.05				0.11				0.14	
Generalized linear model: ages 12–13 years (1 measurement per boy)												
Total effect[f]	−0.97	−1.39, −0.59	<0.001		−1.14	−1.53, −0.75	<0.001		−0.84	−1.17, −0.50	<0.001	
Direct effect[g]	−0.20	−0.46, 0.07	0.14	21	−0.06	−0.32, 0.21	0.69	5	−0.10	−0.31, 0.12	0.38	12
Indirect effect[h]	−0.78	−1.11, −0.44	<0.001	79	−1.08	−1.41, −0.76	<0.001	95	−0.74	−1.01, −0.47	<0.001	88
P for interaction[i]			0.23				0.30				0.97	
Longitudinal generalized estimating equations for repeated measures model: ages 10–13 years (up to 2 measurements per boy)												
Total effect[f]	−0.92	−1.26, −0.59	<0.001		−1.09	−1.40, −0.79	<0.001		−0.88	−1.13, −0.62	<0.001	
Direct effect[g]	−0.17	−0.37, 0.02	0.08	19	−0.14	−0.34, 0.06	0.18	13	−0.24	−0.39, −0.08	0.003	27
Indirect effect[h]	−0.76	−1.05, −0.47	<0.001	81	−0.96	−1.22, −0.69	<0.001	87	−0.64	−0.86, −0.41	<0.001	73
P for interaction[i]			0.07				0.13				0.43	

Abbreviations: β-HCH, β-hexachlorocyclohexane; CI, confidence interval; HCB, hexachlorobenzene; OCPs, organochlorine pesticide; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene.

[a] Models adjusted for baseline total serum lipids, testicular volume ≥15 mL, ordinal tertiles of dietary total calories, and percent dietary fat.

[b] HCB range, 169–15,482 pg/g.

[c] β-HCH range, 209–13,733 pg/g.

[d] *p,p′*-DDE range, 261–41,302 pg/g.

[e] *P* values (2-sided) were based on the Wald test.

[f] Total effect estimated from the model without World Health Organization body mass index (weight (kg)/height $(m)^2$) *z* score adjustment (21).

[g] Direct effect estimated from the model with body mass index *z* score adjustment.

[h] Indirect effect approximates the difference between the total and direct effects.

[i] Testing for interaction between log OCP and body mass index *z* score.

Table 6. Associations Between Baseline Tertiles of Serum Wet-Weight Organochlorine Pesticides and Insulin Resistance at 2 Follow-up Visits in the Russian Children's Study, 2003–2009

IR Model, by Tertile of Serum OCP Level[a]	Serum Organochlorine Pesticides											
	HCB, ng/g[b]				β-HCH, ng/g[c]				*p,p′*-DDE, ng/g[d]			
	Odds Ratio	95% CI	*P* Value[e]	No. of Boys With IR	Odds Ratio	95% CI	*P* Value[e]	No. of Boys With IR	Odds Ratio	95% CI	*P* Value[e]	No. of Boys With IR
Unadjusted for BMI[f] *z* score[g]												
First tertile		Referent		7		Referent		17		Referent		13
Second tertile	1.63	0.61, 4.35	0.33	11	0.37	0.14, 0.94	0.04	7	0.81	0.32, 2.03	0.65	11
Third tertile	1.96	0.74, 5.21	0.18	13	0.35	0.13, 0.82	0.03	7	0.51	0.19, 1.35	0.18	7
P for trend			0.18				0.03				0.17	
Adjusted for BMI *z* score[h]												
First tertile		Referent		7		Referent		17		Referent		13
Second tertile	2.83	1.72, 0.98	0.06	11	0.59	0.24, 1.48	0.26	7	1.55	0.52, 4.63	0.43	11
Third tertile	4.37	1.44, 13.28	0.009	13	0.78	0.29, 2.11	0.63	7	1.18	0.41, 3.42	0.76	7
P for trend			0.007				0.54				0.69	

Abbreviations: β-HCH, β-hexachlorocyclohexane; BMI, body mass index; CI, confidence interval; HCB, hexachlorobenzene; IR, insulin resistance; OCP, organochlorine pesticide; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene.

[a] IR was defined on the basis of homeostatic model assessment of IR greater than 2.5 for prepubertal children (Tanner stage 1) and greater than 4.0 for pubertal-aged children (Tanner stage >1).

[b] HCB tertiles: first, 169–602 pg/g; second, 603–987 pg/g; third, 988–15,482 pg/g.

[c] β-HCH tertiles: first, 209–636 pg/g; second, 637–1,091 pg/g; third, 1,092–13,733 pg/g.

[d] *p,p′*-DDE tertiles: first, 261–1,070 pg/g; second, 1,071–2,007 pg/g; third, 2,008–41,302 pg/g.

[e] *P* values (2-sided) were based on the Wald test.

[f] Weight (kg)/height (m)^2.

[g] Generalized estimating equations for repeated measures regression model adjusted for baseline serum total lipids, age, and testicular volume >3 mL.

[h] Generalized estimating equations for repeated measures regression model adjusted for baseline serum total lipids, age, testicular volume >3 mL, and World Health Organization BMI *z* score (21).

accounted for 73% of the total effect. The direct effect of *p,p′*-DDE was 33% of the total effect at the first follow-up visit (at ages 10–11 years) but only 12% of the total effect at ages 12–13 years. Both higher β-HCH and HCB concentrations measured at ages 8–9 years were associated with significant total and indirect effects on leptin at ages 10–13 years, but direct effects were not significant. There was no interaction between β-HCH and BMI *z* score, but a marginal interaction ($P = 0.07$) between HCB and BMI *z* score. At ages 10–11 years, there was a significant interaction ($P = 0.05$) between HCB and BMI *z* score (Table 5); the direct effect of HCB for overweight boys (BMI *z* score = 2.0) was significant ($P = 0.02$) and accounted for 33% of the total effect, whereas the direct effect for boys with normal weight (BMI *z* score = 0) accounted for only 8% of the total ($P = 0.30$) (data not shown). At ages 12–13 years, there was no significant interaction between HCB and BMI *z* score.

In multivariable models examining associations of OCPs with IR (Table 6), the highest HCB tertile compared with the lowest was associated with nonsignificant 2-fold higher odds of IR; after further adjustment by BMI *z* score, there was a significant 4-fold higher odds of IR. Higher β-HCH concentrations were associated with decreased odds of IR; after adjustment for BMI *z* score, the association was attenuated. *p,p′*-DDE was not associated with IR either with or without adjustment for BMI *z* score.

DISCUSSION

A review of the epidemiologic literature on OCPs and adult T2D concluded that most cross-sectional studies and all but 1 prospective study have found associations of higher serum concentrations of *p,p′*-DDE and HCB with higher risk of T2D (7). Fewer studies have evaluated associations of β-HCH with T2D; the cross-sectional studies reported both positive and null associations, and the results of a prospective study were null (7). Although epidemiologic data support a likely association of OCPs with adult T2D, the underlying biological mechanisms are poorly understood. Furthermore, we are unaware of any epidemiologic studies that examine the prospective association between childhood OCP exposures and metabolic dysregulation during later childhood or adulthood.

We found that higher prepubertal HCB concentrations were associated with greater odds of IR among Russian boys and that, after adjustment for BMI *z* score, this association was strengthened, and higher HCB was associated with higher serum insulin and HOMA-IR levels. These results are consistent with a meta-analysis of data from prospective

epidemiologic studies (32) and a prospective cohort study of older Swedish women (33); both reported that higher HCB was associated with a doubling of risk for T2D. Similar to dioxins, HCB binds the aryl hydrocarbon receptor, although with lower affinity (34); aryl hydrocarbon receptor activation has been associated with interference with cellular glucose uptake and lipid metabolism and release of inflammatory cytokines (35, 36). Our results suggesting that higher serum HCB may be associated with higher risk of IR may provide initial evidence for a link between higher childhood serum HCB concentrations and adult T2D. However, given the low prevalence of IR in our study and the uncertainty about the underlying mechanism, our findings should be interpreted with caution.

In contrast to those of HCB, higher concentrations of another organochlorine pesticide, β-HCH, were associated with lower insulin concentrations and HOMA-IR, and therefore lower odds of IR. However, after adjustment for BMI *z* score, these associations were greatly attenuated and no longer statistically significant. We reported previously that higher prepubertal serum OCP concentrations, including β-HCH, were associated with subsequent lower BMI *z* score (27). We speculate that the associations of BMI-unadjusted higher serum β-HCH with lower insulin and IR were caused primarily by the association of higher serum β-HCH with lower BMI *z* score, which, in turn, typically reduces the risk of IR. Adjustment for BMI *z* score therefore diminished the associations. We were, however, unable to formally assess these associations using mediation analysis because of the nonlinear associations between β-HCH and serum insulin and HOMA-IR and the low prevalence of IR.

Higher prepubertal serum concentrations of all OCPs were associated with lower subsequent leptin measures; however, only the association between serum *p*,*p*′-DDE and leptin remained significant after adjustment for BMI *z* score. Serum leptin is positively correlated with body fat (37); therefore, the inverse association between OCPs and serum leptin is in accord with our previous finding that higher prepubertal OCPs were associated with lower subsequent BMI *z* score (27). Consistent with this, our mediation analysis found that all OCPs had indirect effects on serum leptin via BMI *z* score, with only *p*,*p*′-DDE exerting an independent, direct effect on serum leptin. Our baseline OCP concentrations were measured at ages 8–9 years, when the majority of the boys were prepubertal. Serial samples for biochemical analysis were collected through ages 10–13 years, when the majority of the boys had entered puberty. Our results suggest that higher prepubertal OCPs may be associated with an alteration of the normal adipose tissue expansion that occurs during pubertal growth, and that *p*,*p*′-DDE is also directly associated with lower secretion of leptin by adipocytes. Leptin regulates appetite and cellular energy (38), stimulates skeletal muscle glucose uptake (39), and increases fatty acid oxidation and lipolysis (40); therefore, diminished leptin secretion may affect insulin sensitivity. In experimental data, *p*,*p*′-DDE perturbs adipocyte function by increasing fatty acid uptake (6) and stimulating release of adipokines (6, 41). *p*,*p*′-DDE and other OCPs have long biological half-lives and concentrate in adipocytes. This internal reservoir of OCPs results in ongoing OCP exposures from adipocyte stores (42). We speculate that adipocyte function may be affected by chronic exposure, and there could be proinflammatory changes similar to those observed with obesity, which has implications for future metabolic homeostasis (42).

We found complex interrelationships among serum OCPs, growth, body fat, and metabolic function biomarkers that we attempted to account for in our statistical approaches. However, there may be model misspecification that affects the interpretability of our findings. Also, we used BMI *z* score for estimating body fat, which is an indirect measure. We hypothesized that the prepubertal period was a vulnerable developmental window for metabolic effects; however, our follow-up was relatively short, and our last study visit with metabolic measurements occurred at ages 12–13 years, before completion of growth and pubertal development.

The generalizability of our results may be limited for several reasons. First, the community has experienced long-term environmental contamination with multiple chemicals and metals, leading to exposure to complex mixtures. Also, the upper ranges of serum HCB and β-HCH concentrations in this population were much higher than in most other communities (11). Second, the community has limited economic resources, which may affect diet and lifestyle factors related to metabolic health. Finally, although we adjusted for many potential confounders, there may be residual confounding or unrecognized confounders that affect our results.

Strengths of our study include a high retention rate and collection of detailed data on growth, puberty, and important covariates, including dietary intake and socioeconomic status indicators. We used mediation analysis, an innovative approach to assess OCPs' direct and indirect effects on metabolic function biomarkers.

Our findings suggest that higher prepubertal serum HCB and *p*,*p*′-DDE may impair insulin sensitivity and disrupt adipocyte function, and that the prepubertal period is a vulnerable exposure window. This may provide insight into the biological pathways by which HCB and *p*,*p*′-DDE are associated with adult T2D. Our analysis is based on a limited follow-up period and ended before adolescent growth and pubertal development were complete; thus, we are unable to say whether these associations are indicators of transient metabolic dysregulation or are precursors of later metabolic outcomes (e.g., T2D and dyslipidemia). Further research is needed to establish whether these initial associations will persist and to determine whether they are on the pathway to adult metabolic disease.

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