SERUM CHLORINATED PESTICIDES AND LONGITUDINAL SERUM BIOMARKERS OF ENERGY HOMEOSTASIS AMONG RUSSIAN BOYS

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Introduction

Chapaevsk (population 72,000) is a small city located in central Russia (950 km south-east of Moscow) with an area of 187 km^2, half of which is occupied by chemical industries. The Khimprom Chemical Plant in Chapaevsk produced chlorine-containing industrial and agricultural chemicals, such as the pesticides hexachlorobenzene (HCB) and beta-hexachlorocyclohexane (βHCH), and as a by-product polychlorinated dibenzodioxins/dibenzofurans (PCDDs/PCDFs). Release of these compounds led to substantial contamination of the city's air, soil, water and food supply, including locally raised animals and vegetables, with on-going human exposure.[1] The town is also environmentally contaminated with polychlorinated biphenyls (PCBs) and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE).[2] The primary aim of the Russian Children's Study is to examine the association of these organochlorine compounds (OCs) with longitudinal measures of the boys' physical growth and sexual maturation. In the present report, we examined whether serum concentrations of OCPs were associated with serum biomarkers of energy homeostasis among Chapaevsk boys over four years of follow-up.

Materials and Methods

Study Population: The Russian Children's Study is an ongoing prospective cohort study of 499 peri-pubertal boys, enrolled at ages 8 and 9 years from 2003 to 2005. The boys' initial study visit included physical examinations, fasting blood samples for organochlorine compounds (OC) and metabolic analyses, and completion of health, dietary, lifestyle and SES questionnaires. 350 boys had complete data on serum concentrations of the OC pesticides (OCPs) HCB, βHCH, and p,p'-DDE, and 318 of these had at least one measure of glucose, insulin, lipids, or leptin during follow-up.

Sample Collection and Analysis: Biennial fasting blood samples were collected at follow-up visits, and kept at -35° C. The baseline samples were shipped on dry ice to the National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), Atlanta, GA for chemical analyses. The OCP samples were spiked with $^{13}C_{12}$-labeled pesticides, extracted by solid phase extraction (SPE)[3] using Oasis HLB, followed by automated acid and neutral silica gel and analyzed using high resolution mass spectrometry in selective ion monitoring.[4] Blood samples from follow-up visits 2 and 4 were analyzed at the EFIS Laboratory in Moscow, Russia for glucose, insulin, and leptin. Glucose was analyzed using the COBAS INTEGRA 400 plus, specifically the enzymatic reference method with hexokinase for glucose. Insulin was analyzed using a chemiluminescent immunometric assay (DPC Immulite 2000) with a limit of detection (LOD) of 2 uIU/ml, with values below the LOD (6.7%) re-analyzed using a more sensitive electrochemiluminescent assay (Elecsys 2010) with a LOD of 0.2uIU/ml. Leptin was measured using an ELISA based on the sandwich principle, with an LOD of 1.0 ng/ml and 2.5% of values below the LOD. The homeostatic model assessment (HOMA-IR), a continuous measure of insulin sensitivity, was calculated from fasting glucose and insulin.[5]

Statistical Methods: Summary statistics for glucose, insulin, HOMA-IR, and leptin were calculated at each follow-up exam. HOMA-IR was used to define insulin resistance (IR), a dichotomous measure, for boys (pre-pubertal ([G=1] - HOMA >2.5; pubertal boys ([G≥2] - HOMA > 4.0) and evaluated for increasing trends over time. Generalized estimating equations (GEEs) for repeated measures with an exchangeable covariance were used to examine the associations between baseline serum OCPs with longitudinal fasting serum glucose, insulin, and leptin as well as HOMA-IR, using data at baseline (N=318), exam 2 (N=312) and exam 4 (N=290). Due to their highly skewed distributions, glucose, insulin, and leptin were log-transformed for analysis. Serum OCPs were divided into tertiles, with the lowest tertile serving as the reference. Covariates included in the model were boy's age, an indicator for low birthweight (<2.5 kg), indicators for pubertal stage (pre-pubertal -Tanner genitalia (G) stage 1, early puberty - Tanner G stages 2,3, late puberty -Tanner G stage 4,5) with pre-puberty used as the reference group, total daily caloric intake, and percent calories from protein, fat and carbohydrate.

Results and Discussion

Demographics

Of the 318 boys included in our analysis, 84% were 8 years old at enrollment, 9% were born prematurely (<37 weeks), 5 % had low birth weight, 88% were breast fed, and baseline nutrition was within age and gender appropriate guidelines.[6] Most of the parents (92%) had higher than secondary education, and 44% of households had incomes in the highest category (> 250 US dollars/month).

Serum Organochlorine Pesticides

There was a wide range of serum HCB, βHCH, and p,p'-DDE concentrations among the 318 boys (Table 1); median serum HCB and βHCH concentrations were more than 10-fold higher, and median serum p,p'-DDE more than 3-fold higher, than median serum levels among 12 to 19 year old children in the U.S.[7]

Table 1. Distribution of serum pesticides (ng/g lipid) among 8-9 year old Russian boys (N=318)

Percentiles	10th	25th	Median	75th	90th
Hexachlorobenzene (HCB)	80	106	159	244	359
β-hexachlorocyclohexane (βHCH)	85	117	171	272	436
p,p'-dichlorodiphenyldichloroethylene (p,p′-DDE)	122	189	296	494	912

Physical Characteristics of the Boys during the Follow-up Period

At study entry, the boys' mean (SD) age-adjusted BMI and height z-scores were -0.21 (1.28) and 0.11 (1.01), respectively. The BMI and height z-scores did not change significantly over four years of follow-up. At enrollment, 9% of the boys were overweight (defined as > 1 SD above and < 2 SD below the mean BMI z-score), and 6% were obese (defined as ≥ 2 SD above the mean BMI z-score).[8] At exam year 4, 14% of the boys were overweight, and 8% were obese

Serum Glucose, Insulin, HOMA-IR, and Leptin

Table 2. Serum Biomarkers Among Russian Boys (median: 25th, 75th percentiles)†

Biomarkers	Visit 2 (Age 10-11) (n=312)	Visit 4 (Age 12-13) (n=290)	p-value for age
Glucose (mg/dl)	81 (75, 89)	87 (82, 92)	<0.001
Insulin (uIU/ml)	5.4 (3.3, 7.6)	5.9 (4.0, 8.8)	0.04
HOMA-IR	1.1 (0.7, 1.6)	1.3 (0.9, 1.9)	0.001
Leptin (ng/ml)	3.7 (2.4, 7.9)	3.4 (2.0, 7.6)	0.91

†Serum measurements restricted to boys with follow-up data.

Median serum glucose, insulin, HOMA-IR, and leptin were within normal ranges at exam 2 (median age 10.1 years) and exam 4 (median age 12.0) (Table 2). However, glucose, insulin, and HOMA-IR were significantly higher as the boys aged (Table 2). Serum leptin did not differ with increasing age. The prevalence of insulin resistance (IR) was

3% at exam 2 and 4% at exam 4. Median serum values for insulin were twice as high for obese boys compared to boys of normal weight (10.7 vs. 5.1uIU/ml) while serum leptin was more than 7-fold higher (21.2 vs. 3.0 ng/ml). The prevalence of IR was seven time higher for obese boys as compared to boys of normal weight (28% vs. 4%).

Predictors of Serum Glucose, Insulin, HOMA-IR, and Leptin

Increased age was associated with higher glucose, insulin, and HOMA-IR, although we only measured these biomarkers twice, at follow-up visits 2 and 4. Age was not associated with serum leptin or the odds of IR. BMI z-score was positively associated with all serum biomarkers and with odds of IR. Early pubertal stage was protective against the odds of IR, but was not associated with any serum biomarkers. As compared to pre-puberty, pubertal onset and development was associated with higher serum insulin and HOMA, but not glucose or the odds of IR, while puberty was associated with a significant decrease in serum leptin. Low birth weight was not associated with any serum biomarkers, but was marginally associated (p=0.06) with a threefold increased odds of IR. Higher total caloric intake was associated with lower leptin, but was not associated with any other biomarker or the odds of IR. Higher percent caloric intake from fat was associated with higher serum insulin, leptin, and HOMA-IR, but was not associated with serum glucose or the odds of IR. Dietary protein was not associated with any serum biomarkers or with the odds of IR.

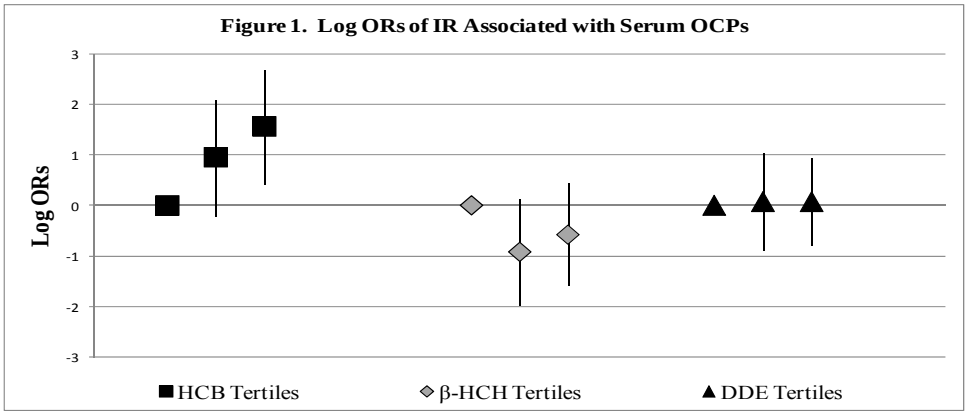
Table 3. Associations of log-transformed Serum OCPs with log-transformed Glucose, Insulin, HOMA-IR and Leptin

	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Log transformed Glucose						
	HCB		βHCH		p,p'-DDE	
Tertile 3 (highest)	0.01 (-0.02, 0.03)	0.45	-0.01 (-0.04, 0.02)	0.44	0.02 (-0.006, 0.04)	0.13
Tertile 2	0.0001 (-0.02, 0.02)	0.94	0.004 (-0.02, 0.03)	0.75	0.03 (0.003, 0.05)	0.03
Tertile 1 (lowest)	Reference		Reference		Reference	
Log transformed Insulin						
	HCB		βHCH		p,p'-DDE	
Tertile 3 (highest)	0.15 (0.004, 0.29)	0.04	-0.06 (-0.21, 0.09)	0.43	-0.01 (-0.15, 0.13)	0.85
Tertile 2	0.07 (-0.07, 0.21)	0.35	-0.13 (-0.27, 0.007)	0.06	-0.02 (-0.16, 0.12)	0.78
Tertile 1 (lowest)	Reference		Reference		Reference	
Log transformed HOMA-IR						
	HCB		βHCH		p,p'-DDE	
Tertile 3 (highest)	0.16 (0.007, 0.31)	0.04	-0.07 (-0.22, 0.09)	0.39	0.006 (-0.14, 0.15)	0.93
Tertile 2	0.07 (-0.08, 0.21)	0.37	-0.13 (-0.27, 0.02)	0.08	0.006 (-0.14, 0.15)	0.93
Tertile (lowest)	Reference		Reference		Reference	
Log transformed Leptin						
	HCB		βHCH		p,p'-DDE	
Tertile 3 (highest)	-0.15 (-0.33, 0.03)	0.11	-0.19 (-0.43, 0.04)	0.10	-0.28 (-0.46, -0.09)	0.004
Tertile 2	-0.05 (-0.22, 0.12)	0.55	-0.22 (-0.42, - 0.01)	0.04	-0.15 (-0.33, 0.02)	0.09
Tertile 1 (lowest)	Reference		Reference		Reference	

[a]HCB tertiles: Q1 31 – 124; Q2 125 - 206; Q3 207 – 2660; [b]βHCH tertiles: Q1 39 – 130; Q2 131 – 224; Q3 225 – 2860; [c]ppDDE tertiles: Q1 48 – 222; Q2 223 – 417; Q3 418 – 9370[1]GEE repeated measures regression model adjusted for age, WHO BMI z-score, pubertal stage (prepubertal, early puberty, late puberty, low birth weight, total calories consumed, percent calories from carbohydrate, protein, and fat.

Serum OCPs Associations with Serum Glucose, Insulin, HOMA-IR, and Leptin

In multivariate models, boys with the highest tertile of HCB compared with the lowest had significantly higher serum insulin and higher HOMA-IR (Table 3) over four years of follow-up. Although serum HCB was not associated with serum glucose, boys with the highest tertile of HCB also had significantly increased odds of IR (OR=4.74: 95% CI 1.52, 14.77) (Figure 1). In multivariate models, higher tertiles of serum p,p'-DDE were associated with lower serum leptin over four years of follow-up (Table 3). Higher tertiles of βHCH were associated with lower serum leptin, although only the middle tertile was statistically significant.



Conclusions

In a longitudinal study of Russian boys, higher serum HCB measured at age 8 to 9 years old was associated with higher serum insulin, HOMA-IR, and the odds of IR over 4 years of follow-up. Higher baseline serum p,p'-DDE and βHCH were associated with lower serum leptin over 4 years of follow-up although the βHCH association was nonlinear. The biological mechanisms for these OCPs on glucose metabolism and serum leptin concentrations are not well characterized. Although our preliminary results suggest that OCPs may have an effect on glucose metabolism and serum leptin concentrations, more research is needed to investigate these relationships and possible mechanisms.

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