Effects of perinatal and current dioxin exposure on energy metabolism in humans

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Introduction

Various studies have shown an association between persistent organic pollutants, especially organochlorine compounds, and insulin resistance and diabetes [(1) (2)]. However, none of the studies were prospective, but rather cross-sectional. Furthermore, an influence of the diabetes itself cannot be excluded: the metabolic changes caused by the disease might result in higher levels of xenobiotics.
A prospective cohort study dating from 1987 and 1990/1991, allowed us to study effects of dioxins in relation to energy metabolism in the developing child.
In the neonatal period we already saw a significant decrease in retinol binding protein (RBP) in association with a higher lactational exposure in our cohort, suggesting an influence on fat metabolism [(3)]. This protein is an essential part of fat metabolism. We therefore assessed the energy metabolism during follow-up in adolescence.

Methods and materials

Study population:

This study is part of a longitudinal cohort study of currently 14-19 year old children, studied during their neonatal (n=60) [(4)], toddler (n=60) [(5)] and pre-pubertal period (n=41) [(6)]. All 33 children (18 girls and 15 boys) participating in the current follow-up were born in the Amsterdam/Zaandam region. Twenty-five of the children are still inhabitants of the region. Dioxin exposure was determined in the perinatal period in breast milk. Of the total cohort of 41 subjects who participated in the pre-pubertal study, one subject was excluded from the current follow-up because of an Ewing sarcoma and one was partly excluded because of an extra Y-chromosome. Five subjects declined to participate in the new follow-up, three could not be traced. Of the 33 examined adolescents 2 refused to undergo vena puncture and 2 refused a second vena puncture after blood clotting in the first needle. The study was approved by the institutional medical ethics committee. All participants of the study and their parents signed an informed consent.

Laboratory analyses

For measurements of fasting glucose, insuline, total cholesterol and lipid spectrum (HDL, LDL, triglycerides), leptin and current PCDD/F, dl-PCB and PBDE serum concentrations, 30 subjects underwent vena puncture, following a starvation period of at least four hours. Serum was obtained from 200 ml of blood and stored at -20° C until analysis. Perinatal PCDD/F levels and current serum levels of PCDD/Fs, dl-PCBs and PBDEs were determined in an uncontaminated laboratory at the Department of Environmental Chemistry of the University of Amsterdam. Concentrations of the 19 most toxic dioxin congeners (seven PCDDs and twelve PCDFs) and the concentration of 3 dioxin-like PCBs (77, 126, 169) and 8 PBDEs (28, 47, 99, 100, 153, 154 and 183) were determined. The concentration of dioxin and dioxin-like PCB (dl-PCB) congeners are expressed in toxic equivalents (TEQ) ng/kg fat.
An activated carbon column (Carbosphere) was used for separation of the chemicals. The dioxin fraction was isolated and a clean-up was performed using a column of $AgNO_3$ on silica gel and a column of activated Al_2O_3 on silica gel. After concentrating the sample, quantification was done using hr-GC/hr-MS. As an internal standard, a mixture of ^{13}C-labelled PCDD/Fs, dl-PCBs and PBDEs were used. More detailed information about the analysis will be published elsewhere [(7)] .

Dioxin concentrations were measured in the mothers' milk 3-4 weeks after birth, which is indicative of the prenatal exposure. The cumulative total postnatal/lactational exposure was calculated: prenatal exposure multiplied by the total breast milk intake [(8)] .

Statistical analyses
For statistical analyses linear regression was performed and the non-parametric Spearman's correlation coefficient was calculated using SPSS® software package.

Results/discussion

Characteristics of the cohort (age, BMI and perinatal PCDD/F exposure) and the current serum PCDD/Fs and dl-PCBs are shown in table 1.

Glucose levels
Fasting glucose and insulin levels were determined in the serum of the adolescents. Preliminary results show a positive trend between current serum dioxin and fasting glucose levels (P=0.087).
No relation between dl-PCBs, PBDEs or perinatal dioxin exposure and fasting glucose levels were seen.

Insulin and glucose/insulin ratio
A negative correlation was found between current insulin levels and prenatal dioxin exposure (p=0.014) (figure 1), as well as with lactational exposure (p=0.022). For the current serum dioxin and current serum dl-PCBs, no correlation was seen with insulin levels.
For the ln glucose/insulin ratio a positive correlation was seen with the prenatal (p=0.018) (figure 2) and lactational (p=0.051) dioxin exposure using Spearman's correlation coefficient. No correlation was seen with the current levels of dioxins and dl-PCBs.

A toxic effect on the β-cell of the pancreas is the most likely explanation for our findings. Signs of insulin resistance, as demonstrated in this study, can predict the development of diabetes type 2. A study on US Vietnam War veterans found a correlation between diabetes and dioxin exposure [9]. Higher incidence of diabetes is also found after background dioxin exposure levels [10].

Body Mass Index (BMI)
No correlation was seen between the current body mass index (BMI) and the prenatal or lactational dioxin exposures, nor with the current dioxin, dl-PCB and PBDE levels.

Leptin levels
Levels of leptin were measured in the serum of the adolescents. As expected, a clear correlation between body mass index (BMI) and leptin was seen (p=0.006) using Spearman's correlation coefficient.
We found a negative correlation between leptin and current serum dioxin concentrations using Spearman's correlation coefficient (p=0.006) . We also found a negative correlation between prenatal dioxin exposure and leptin levels (p=0.03) (figure 3) using Spearman's correlation. PBDE and dl-PCB levels showed no correlation with leptin levels.

As expected, a correlation between BMI and leptin was seen. However, possibly due to the limited number of subjects, we found no correlations with the measured chemical compounds and current BMI. A decreased leptin level causes an increased appetite. Direct satiation after a meal is correlated with levels of free fatty acids, insulin and gastric factors.
We hypothesise that around birth the set point for energy metabolism is established in the hypothalamus. Leptin plays an important role in this mechanism. Taylor's mice studies support this [11] .

Lipid profile
In the subjects, levels of cholesterol, triglycerides, HDL and LDL were determined. No significant correlations were seen for any of the measured endpoints.
No relation between dl-PCBs, PBDEs or perinatal dioxin exposure and fasting glucose levels was seen.

Conclusion
This study indicates that leptin and insulin levels are influenced by (perinatal) dioxin exposure. Our results may be ominous for an increased risk of obesity with higher perinatal dioxin exposure. It might be possible that the dioxin exposure perinatally has modulated the setpoint for energy metabolism, when shortly after birth a metabolic revolution takes place, from a continuous supply of glucose to an intermittent supply of lactose and fat.

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Figure 1: Insulin levels and prenatal dioxin exposure (p=0.014).

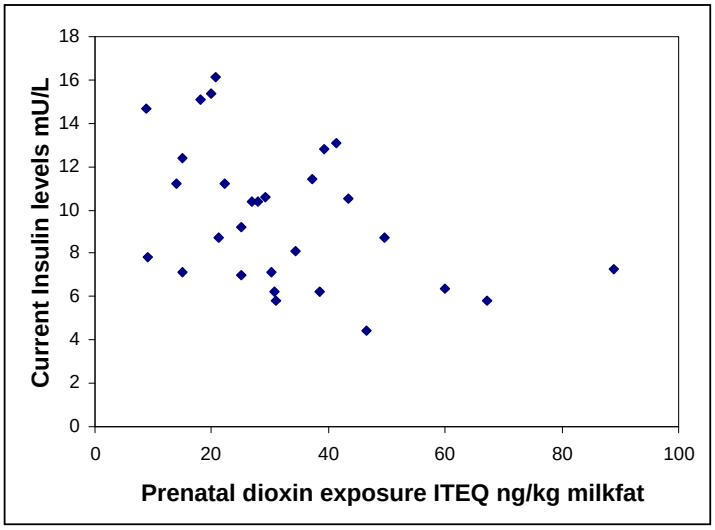

Figure 2: Glucose/insulin levels and prenatal dioxin exposure (p=0.006).

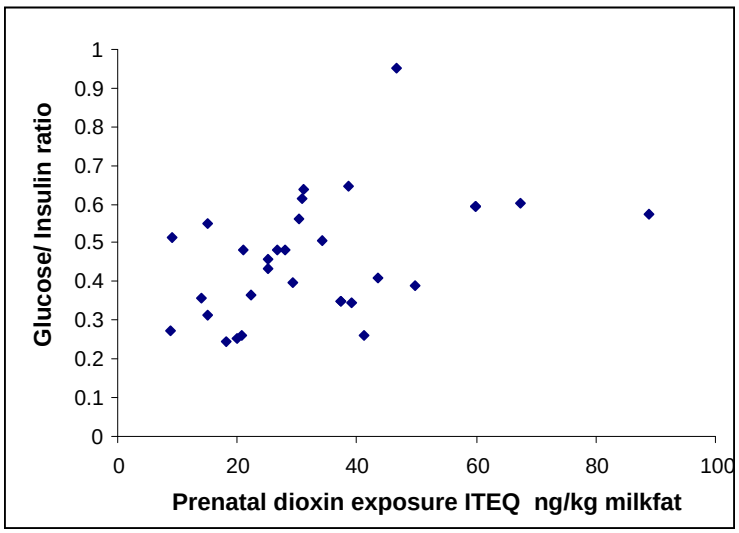

Figure 3: Leptin levels and prenatal dioxin exposure (p=0.03).

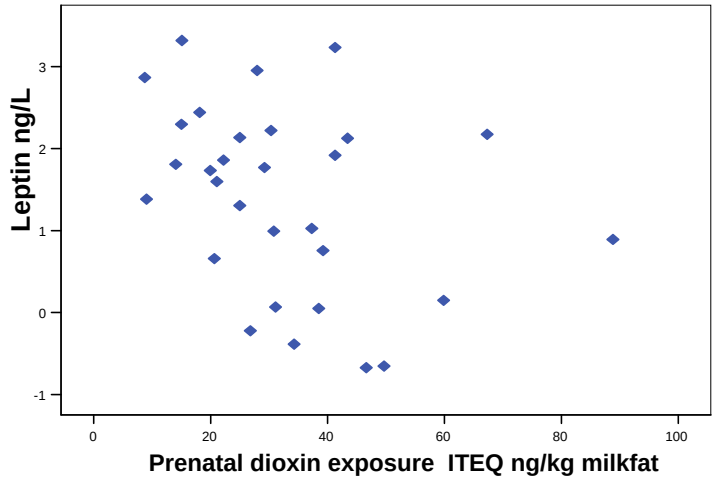

Table 1: Mean age, BMI, PCDD/F and dl- PCB exposure

	Median	Mean	Median (girls: n=19)	Median (boys: n=14)	Range	95th - percentile
Age (years)	14.3	15.0	14.3	14.3	14.0-18.7	18.5
Prenatal PCDD/F exposure TEQ (pg/g lipid)	29.8	32.6	29.2	28.6	9.05-88.8	74.8
Lactational PCDD/F exposure TEQ (ng)	45.9	66.9	45.9	42.0	4.34-279	239.1
BMI (kg/m^2)	19.7	21.0	19.7	19.5	17.4-30.9	29.9
Current serum PCDD/F TEQ (pg/g lipid)	1.6	2.2	1.1	2.3	0.4-6.1	6.1
Current serum dl-PCBs TEQ (pg/g lipid)	1.8	2.2	1.7	1.5	0.04-7.8	7.3