A Long term follow-up study; Metabolic and other effects of dioxins, PCBs and PBDEs in adolescent

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Introduction:
In 1987 the Amsterdam/Zaandam study cohort was initiated in order to study the possible effects of perinatal dioxin exposure on health parameters, including growth and development (1,2). Dioxins (PCDD/Fs) were measured in the mothers' milk to evaluate the children's dioxin exposure. Since 1987, 4 follow-up studies of the cohort have shown effects on the thyroid hormone, immunological- and haematological systems, behaviour, neuromotor development, lung function, pubertal development and energy metabolism (3,4,5,6).
Results of the most recent study round will be given in this abstract with an emphasis on those concerning the metabolic problems.

Dioxins, PCBs and PBDEs are toxic environmental pollutants which accumulate in humans. Exposure is mainly through ingestion (food), however exposure through inhalation is a major exposure route for PBDEs especially in children (7,8).
Diabetes and obesity are currently epidemic in many countries. Although changes in diet and less physical activity are the main factors of the increased prevalence in obesity, many other cofactors like sleep deprivation, increased maternal age at delivery, maternal smoking and more stable home temperatures (9) may play a role. Endocrine disruptors might also contribute to the increasing incidence of obesity.
Due to their lipophilic properties, dioxins, PCBs and PBDEs are mainly stored in adipose tissue. The mechanism of possible contribution of these chemicals to obesity remains unclear. Various studies, however, have already shown an association between persistent organic pollutants, especially organochlorine compounds, and insulin resistance and diabetes (10) (11).
In our prospective study a significant decrease in retinol-binding protein was found in an earlier follow-up in the neonatal period in correlation with a higher lactational exposure to dioxin, suggesting an effect on fat metabolism (12). As a result of this finding we analysed the cohort's leptin levels during puberty. Leptin, a hormone produced by white adipose tissue, has an important function in the regulation of appetite in the hypothalamic appetite centre, giving a feeling of satiation in order to limit caloric surplus. It also plays a role in the energy production from fatty acids in the skeletal muscle cell (13).

Methods:
Two decades ago this study was initiated with 120 mother-baby pairs, all Caucasian women aged between 23 and 38 years of age. Sixty-one of these pairs who were breast fed for at least 2 months, following an optimal pregnancy and with normal birth weight, were included in the cohort. The children were evaluated during their neonatal (61), toddler and pre-pubertal period (41). In the current follow-up, 33 of the traceable children of the prepubertal period were studied, now aged between 14-19 years. The following objectives were achieved:

- Current levels of PCDD/Fs (19 most toxic congeners), dl-PCBs (77, 126, 169) and PBDEs (28, 49, 85, 99, 100, 153, 154, 183) were measured. Determinations were performed using high resolution GC/MS.

- Growth and pubertal development was assessed (Tanner stages, testicle volume, height, weight and Body Mass Index (BMI)).
- Lung function was determined (spirometry, body box and diffusion measurements).
- Immunological and haematological parameters were assessed (leukocyte count and differentiation, haemoglobin, thrombocyte count and thrombopoietin).
- Thyroid hormone homeostasis was evaluated (TSH, T_3, T_4, FT4, TBG and anti TPO).
- Metabolic function was documented (fasting glucose, insulin, leptin and lipid spectrum).
- Behavioural problems were assessed.

Statistical analyses

For statistical analyses linear regression was used and with multiple variables, multiple regression was used using SPSS®. For the metabolic parameters dependent values we used serum glucose, serum insulin, the glucose:insulin ratio, leptin, BMI and BMI:leptin ratio. The prenatal, lactational and current serum PCDD/Fs and the current serum dl-PCB and PBDEs levels were the independent values using the Spearman's correlation coefficient. To calculate the relationship between the variables insulin-leptin and leptin-BMI, the Pearson's correlation coefficient was used. To adjust for other variables the partial correlation coefficient was used. Evaluation of variables or confounding factors (age, sex and BMI) was performed.

Results and discussion

The mean levels of PCDD/Fs measured in the serum of the adolescents amounted to 2.2 pg TEQ/g lipid (0.36-6.1) and those of dl-PCBs amounted to 2.2 pg TEQ/g lipid (0.04-7.8). This is much lower than the prenatal exposure level of 33 pg/g lipid milk fat and mean cumulative lactational exposure of 68 ng. There was no relation between the perinatal PCDD/F levels and the current PCDD/F or dl-PCB levels. The mean concentration of the sum of PBDEs in serum was 14 ng/g lipid (4.9-74) including one extremely high value, excluding this one, the mean was 8.7 ng/g lipid (14).

Energy Metabolism

The energy metabolism was evaluated by measuring fasting serum levels of glucose, insulin, leptin and lipid spectrum. The current glucose:insulin and the BMI:leptin ratios were calculated.

The current serum PCDD/Fs and total TEQ (dl-PCBs+PCDD/Fs) were positively related to current serum glucose levels ($p=0.015$ and $p= 0.037$). The prenatal dioxin (PCDD/F) exposure was positively related to the glucose:insulin ratio ($p=0.024$) and negatively to the current insulin levels ($p=0.017$). Lactational PCDD/F exposure was also negatively related to insulin levels ($p=0.028$). These findings partly confirm findings in other human and animal studies (15,16).

The glucose metabolism could be influenced directly by a dioxin effect on the beta-cells of the pancreas. Influencing (hypothalamic) metabolic set-points in the prenatal and lactational period via the genomic pathway of the Ah receptor could also be an explanation for the finding of lower current insulin levels. The crucial role of the hypothalamus in the control of food intake and regulation of energy balance has been described (17).

Epigenetic changes are also an important way how dioxins might influence metabolism.

No correlation was seen between the current BMI and the prenatal ($p=0.920$) or lactational ($p=0.177$) dioxin exposures, nor with the current dioxin ($p=0.951$), dl-PCB ($p=0.788$) and PBDE ($p=0.735$) levels. Separate analysis for boys and girls also showed no significant relationships. No relationship was found with age ($p=0.772$). However we have to keep in mind that the children were all in puberty, a period with changing BMIs. A Belgian study found a positive association between intrauterine PCB exposure and BMI in 3 year old children; however the measured PCBs were different congeners than the ones measured in our study (18). Higher BMI is also seen in one other study in relation with OCDD (19).

Levels of leptin were measured in the serum of the adolescents. A clear relationship between BMI and leptin was found ($p<0.001$) using Pearsons correlation coefficient. As expected, a relationship between leptin and insulin was also seen ($p=0.034$). Using the partial correlation coefficient, gender and age did not influence these relationships. The statistical analysis on leptin levels in relation to the environmental pollutants was strongly influenced by gender, therefore a confounding factor. Analysis stratified by gender showed no significant results

(p=0.979 for boys and p=0.230 for girls). Similarly no significant results were found for the BMI:leptin ratio with gender stratified analysis.
One animal study on rat cells showed no relation between leptin and TCDD levels (16). Other effects on fat metabolism are however seen in animal studies. Body weight gain and disturbances in adipocyte differentiation are seen in relation to dioxin exposure (20).
Possible limitations of our study are the small number of participants and the fact that only breast fed participants were included. We could not assess a positive effect modulation of breast feeding. Effects of compounds not measured, like DDT (dichlorodiphenyl dichloroethene), hexachlorobenzene, non-dioxin-like PCBs and PBBs (polybrominated biphenyls) cannot be excluded.

Puberty

A relationship between prenatal and lactational dioxin exposure and the initiation of breast development (p=resp. 0.023 and 0.048) was found. In the boys we saw indications of a delay in the age of first ejaculation with higher current dl-PCBs. No relationships were seen between current Tanner stages, testicular volume and BMI with the determined compounds (21).

Thyroid function

One of the hormones important for growth and development is the thyroid hormone. Previous human and animal studies have shown that the thyroid hormone status is influenced by the chemicals we studied. In the current study a positive association was found between dl-PCBs and T_3, and with T_3 and BDE 99. No correlations were found with (perinatal) dioxin exposure.
T_3 and T_4 are responsible for a normal (energy) metabolism through up-regulation of carbohydrate catabolism, lipid catabolism and protein synthesis.

Immunology and haematology

In an earlier study of the cohort, during the neonatal period, an effect on the polymorphic neutrophil granulocytes was seen with higher perinatal dioxin exposure.
In the current study, a lower number of polymorphic neutrophils with increasing dl-PCB exposure (p=0.021) was evident. PBDEs had a negative effect on the number of lymphocytes (p=0.001). A positive relationship between serum haemoglobin (Hb) levels and serum ∑PBDEs (p=0.003) was found (22).

Lung Function

After evaluation of the children's long function, the decrease in lung function present in the pre-pubertal period in relation to increasing prenatal and lactational dioxin exposure is no longer visible. However, a decrease in lung function is now seen in relation to increasing BDE exposure in the teenagers. A significant negative relation was found with sum BDE and FEF 50 (p=0.016). For BDE 100 and BDE-99 a significant negative relation was found with FEV1/VCMAX (p=0.031 and p=0.049). A positive relationship was seen between current dioxin body burdens and FEV_1 (p=0.038) and TLC (p=0.026) in the girls.

Behavioural Problems

Dioxin-like compounds are also well-known neurotoxicants. During earlier study of the same cohort in the pre-pubertal period an increase in social problems, thought problems, anxious/depressed feelings, social problems and aggressive behaviour was seen. In this re-evaluation of behavioural problems, using CBCL and TRF lists, we could not confirm these findings.

Final thoughts and future study perspectives

It is alarming that not only the rather high dioxin levels in the neonatal period, but also the lower current levels of PCDD/Fs, dl-PCBs and PBDEs have (persistent) effects on the evaluated health parameters. To our knowledge, this is the first study showing effects in puberty following perinatal exposure. Furthermore, this is also the first study showing effects of PBDEs on the human immune system, hematologic parameters and lung function. The results concerning the metabolic effects and the lung function are novel findings and have not been published yet.
Our cohort is limited and we would like to encourage larger studies to confirm or correct our findings.
We have to be alert for long term effects of these relatively small developmental disturbances, which could be a co-factor for illness in later life. It is quite possible that exposure to POPs perinatally may be a co-factor in the rising incidence of breast cancer and diabetes, seen over the last decade.

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