CLINICAL STUDY

Spermaturia and serum hormone concentrations at the age of puberty in boys prenatally exposed to polychlorinated biphenyls

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Abstract

Objective: To determine whether prenatal exposure to polychlorinated biphenyls (PCBs) with possible hormone-disrupting effects is capable of affecting sexual differentiation in boys at the age of puberty. **Design:** Following analysis for PCB in their umbilical cords, 196 boys from a Faroe birth cohort were examined for the development of puberty at 14 years of age.

Methods: Physical examination included determination of Tanner stages and testicular size. A morning urine sample was centrifuged and examined for the presence of sperm. Serum was analyzed for sex hormones.

Results Twenty boys (10.2%) had abnormalities in testicular development, mainly cryptorchidism. Only three of them had a positive spermaturia test, but the level of exposure to PCBs in this group had not been increased. Occurrence of spermaturia in 58 of the remaining 176 boys was also not associated with PCB exposure but showed highly significant associations with Tanner stages and testicular size. Serum concentrations of testosterone, FSH and LH were higher in boys with spermaturia, while sex hormone-binding globulin was lower and no difference occurred in inhibin B. Serum hormone parameters showed only weak associations with the level of prenatal PCB exposure.

Conclusions: These findings support the validity of spermaturia as a useful indicator of puberty, although a substantial rate of false negatives must be taken into account. Despite the wide range of exposure to PCB, the findings did not reveal any definite associations with the development of puberty. However, because of the limited size of the cohort, small effects cannot be excluded.

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Introduction

Widely occurring food pollutants, such as polychlorinated biphenyls (PCBs), are suspected of causing endocrine disruption (1). Increased exposure to persistent PCB congeners occurs in fishing populations whose diet includes marine species high in the food chains. Substantially increased body burdens have thus been documented in the Faroe Islands, where the pilot whale is part of the traditional diet (2).

Exposure to endocrine disruptors is of particular importance during development, where the fetal testicles are regarded as highly sensitive to changes in hormone concentrations, especially the balance between oestrogen and androgen (1, 3). The effects of an imbalance may include developmental abnormalities, such as hypospadias and cryptorchidism, decreased sperm count

and increased risk of testicular cancer (1, 3, 4). Experimental animal studies also suggest effects on pubertal development (5–8).

We have followed a birth cohort of children born in 1986–1987 in the Faroe Islands, where increased PCB exposure has been documented (2, 9). This population includes subjects who only rarely eat contaminated food items, thereby providing a built-in control group. The cohort members have now been examined at puberty. In boys, gonadal maturation is not characterized by any visible event, and assessment of the development of puberty by questionnaire will probably not be reliable. In an effort to determine the possible effects of increased prenatal PCB exposure on sexual differentiation in boys, we carried out detailed clinical examinations of the cohort at the age of puberty and included analysis of urine samples for the

presence of spermatozoa (spermaturia) and determination of serum hormone concentrations (10, 11).

Subjects and methods

Cohort subjects

The birth cohort was generated from consecutive births at the three Faroese hospitals in Tórshavn, Klaksvík and Suduroy during a 21-month period starting 1 March 1986 (12). A high consent rate, successful co-operation within the health care system and minimal practical problems ensured that about 75% of all births were included in the cohort. The study protocol was prepared in accordance with the most recent version of the Helsinki Declaration and was approved by the Faroese ethical review committee.

An umbilical cord specimen was collected by the midwife from almost all cohort members. For boys examined at 7 years of age in 1993, the concentrations of major PCB congeners were determined by capillary gas chromatography with electron-capture detector, and the sum of major congeners 138, 153 and 180 multiplied by 2.0 was used as a measure of the total PCB concentration (9). Because of the long biological half-life of these congeners, the cord concentration is likely to reflect the level of exposure throughout gestation. To assess the validity of this long-term exposure biomarker, PCBs were also measured in 50 matching samples of cord blood; the two parameters were found to correlate very well ($r = 0.90$) (9). *p,p'*-dichlorodiphenyldichloroethane (*p,p'*-DDE) was also detected in the samples, but was not considered in the statistical analysis because of its close association with PCB.

The clinical examination of the oldest part of the cohort was scheduled during the spring of 2000 at approximate mid-puberty, i.e. at an average of about 13 years and 9 months. A total of 196 cohort boys completed the examinations and provided a urine sample for spermaturia assessment. A PCB result was available for 175 of the boys examined.

Clinical examination

All boys underwent clinical examination by a paediatrician. Pubic hair and external genital development was graded in Tanner stages (13). Testicular volume was determined by a Prader orchidometer and, if possible, by ultrasound using a sector scanner with an Aloka 7.5 MHz transducer with a water-containing front manufactured for near-field scanning (Aloka, Zug, Switzerland). The transducer was placed directly on the skin of the exposed scrotum. Each testicle was measured using electronic calipers along the longest axis (A) and on a perpendicular section in two mutual dimensions (B and C). The volume was calculated using the formula of an ellipsoid: $\text{volume} = 4/3 \times 22/7 \times A \times B \times C \times 1/8$ (12). In 54 boys examined by

both methods, the ultrasound reading averaged 75% of the orchidometer result. This ratio is very similar to the one observed in adult men (14, 15). In children who had been examined by ultrasound only, the result was therefore divided by this conversion factor.

Spermaturia assessment

Every boy was asked to bring a 50 ml sample of the first morning urine for the spermaturia assay, which was carried out by a slight modification of a previously used method (10). The sample was centrifuged at 3600 rounds per minute (r.p.m.) for 30 min. The supernatant was decanted and the sediment was resuspended in approximately 3–5 ml of the remaining supernatant. The resuspended sediment was transferred to a 15 ml Falcon tube and centrifuged at 3600 r.p.m. for 30 min. The supernatant was decanted again and the sediment resuspended in approximately 0.5–1.5 ml of the remnant supernatant. Then two-drop samples (10 µl each) on Superfrost Plus glass and one smear sample (10 µl) were made, and the slides were subjected to fixation in 96% alcohol for 5 min followed by Papanicolaou staining (10). After Pertex mounting of the stained slides, microscopic examination was performed at a 400-fold magnification. If less than four spermatozoa were present in the first drop sample the second drop sample was examined and, if the second drop sample was negative, then the smear. If no spermatozoa were present in any of these then an additional drop sample was prepared and only if this was also without spermatozoa was the spermaturia assessment considered to be negative.

Serum hormone analyses

After completion of clinical examination, a blood sample was obtained from almost all subjects either at 1200 h or at 1700 h. Serum testosterone was measured by radioimmunoassay (Coat-a-Count; DPC, Los Angeles, CA, USA) with a detection limit of 0.23 nmol/l and intra- and interassay coefficients of variations were both below 10%. The concentrations of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and sex hormone-binding globulin (SHBG) were determined by time-resolved immunofluorometric assays (DELFI; Wallac, Turku, Finland). The sensitivities in these assays were 0.06 IU/l, 0.05 IU/l and 0.23 nmol/l respectively and both the intra- and interassay variation values were below 8% in all three assays. Serum inhibin B was measured in an enzyme immunometric assay (Oxford Bio-Innovation Ltd, Oxford, Oxon, UK). This assay is specific for the bioactive inhibin B dimer (α-βB). The sensitivity of the inhibin B assay was 18 pg/ml and the intra- and interassay coefficients of variation were <12% and <17% respectively.

Table 1 Means $\pm$ S.D. for the main characteristics of 176 boys exposed to PCB at the approximate age of puberty according to the presence of spermatozoa in a morning urine sample. The 95% confidence interval (CI) for the difference between the two groups is indicated.

	Positive	Negative	P value	95% CI
Number	61	135	—	—
Birth weight (g)	3789 $\pm$ 564	3751 $\pm$ 476	0.64	–120; 196
Maternal age at parturition (years)	27.1 $\pm$ 5.4	27.9 $\pm$ 5.3	0.33	–2.5; 0.9
Cord tissue PCB (ng/g)*	1.96 (1.14–3.38)	1.82 (1.09–3.69)	0.59	
Age at examination (years)	13.76 $\pm$ 0.23	13.75 $\pm$ 0.24	0.93	–0.07; 0.07
Average Tanner stage				
Public hair	2.5 $\pm$ 0.8	1.9 $\pm$ 0.9	<0.001	0.4; 1.0
External genitals	2.7 $\pm$ 0.7	2.0 $\pm$ 0.8	<0.001	0.4; 1.0
Testicular size (ml)				
Right	10.0 $\pm$ 3.6	6.8 $\pm$ 3.9	<0.001	1.8; 4.6
Left	9.8 $\pm$ 3.1	6.4 $\pm$ 3.6	<0.001	2.2; 4.7

* Geometric mean with interquartile range in parenthesis (95% CI for difference not calculated).

Statistical analysis

PCB concentrations showed a highly skewed distribution; a logarithmic transformation approached Gaussian distribution and provided a better fit of the regression models. On the basis of the PCB concentration, the boys were split into approximate tertile exposure groups with low, intermediate and high PCB levels. Parametric methods were used throughout, and two-sided *P* values were calculated. For the ordered categorical Tanner stage results, the significance of the calculations was confirmed using standard non-parametric tests.

Results

Sixty-one (31.1%) of the 196 boys examined had spermatozoa in the urine. The age of the boys varied from 13.3 to 14.2 years, with no difference between

boys with spermaturia and those without. Descriptive data are shown in Table 1.

Data from clinical examinations at 7 and 14 years of age and past medical history revealed 19 cases of cryptorchidism, eight of which were bilateral, and one case of torsio testis treated with ablatio. Only three of these 20 boys had spermaturia (*P* = 0.13 by Fisher's exact test, as compared with 58 of 176 boys without abnormalities). The cord PCB concentration did not differ between boys with these abnormalities (geometric mean, 1.99 ng/g) and those without (1.85 ng/g) (*P* = 0.73). Statistical calculations were therefore carried out for the whole group, with supplementary calculations after exclusion of the 20 boys with abnormalities.

Clinical examination data showed considerable variability in secondary sex characteristics in the boys, although only two had reached Tanner stage 4. Both testicular size and Tanner stage were significantly (*P* < 0.001) associated with the presence of

Figure 1 Presence (open bars) and absence (hatched bars) of spermaturia in relation to average testicular size (ml) in 145 Faroese boys at the age of puberty.

Figure 2 Presence (open bars) and absence (shaded bars) of spermaturia in relation to Tanner stages of (A) pubic hair and (B) external genitalia in 170 Faroese boys at the age of puberty.

spermaturia (Figs 1 and 2). The main serum sex hormone concentrations were higher in boys with spermaturia, but the reverse was true for SHBG, and no appreciable tendency was apparent for inhibin B (Table 2). Testosterone was slightly lower ($P = 0.16$) and LH higher ($P = 0.03$) in the subjects from whom a blood sample was taken late in the day. The time of venipuncture was not associated with other outcome variables or the PCB exposure level.

Table 2 Average $\pm$ s.d. for serum concentrations of hormonal parameters in 158 boys at 14 years of age in relation to positive or negative examination for spermatozoa in the urine.

	Positive	Negative	P value
Testosterone (nmol/l)	8.45 $\pm$ 5.25	4.56 $\pm$ 4.88	<0.001
Inhibin B (ng/l)	200 $\pm$ 46	193 $\pm$ 71	0.49
SHBG (nmol/l)	51.4 $\pm$ 27.8	66.8 $\pm$ 34.8	0.005
FSH (IU/l)	2.78 $\pm$ 1.72	2.32 $\pm$ 1.32	0.064
LH (IU/l)	1.96 $\pm$ 1.31	1.47 $\pm$ 1.09	0.013

The range of exposures to PCB covered a span of 100-fold, but the PCB concentration in cord tissue showed no difference in regard to the spermaturia result (Table 1). Likewise, tertile PCB groups showed no difference in hormone concentrations, Tanner stage or testicular size (Table 3), and correlation coefficients were low and far from significant. However, visual inspection of the association between PCB and serum testosterone (Fig. 3) suggests that boys with high hormone concentrations were exposed to excess amounts of PCB prenatally. The time of venipuncture did not affect this association.

Logistic regression analyses were then carried out with spermaturia as the dependent variable. As expected, the logarithmic transformation of the PCB concentration showed a better fit than the linear variable. After adjustment for age, testicular size and serum testosterone concentration, the PCB concentration showed a regression coefficient of 0.18 ± 0.66 ($P = 0.79$). This result is in accordance with the unadjusted comparison (Table 1). Likewise, linear regression

Table 3 Average $\pm$ S.D. for serum concentrations of hormonal parameters, testicular size and Tanner stages in 156 boys at 14 years of age in tertile groups of PCB concentrations (ng/g wet weight) in umbilical cord sampled at birth.

	Low (<1.5) (n = 57)	Intermediate (1.5–3.0) (n = 61)	High (>3.0) (n = 57)	P for trend
Testicular size (ml)	6.83 $\pm$ 3.19	8.89 $\pm$ 4.18	7.50 $\pm$ 3.66	0.30
Tanner stage				
Public hair	1.94 $\pm$ 0.90	2.38 $\pm$ 0.82	1.88 $\pm$ 0.88	0.63
External genitals	2.06 $\pm$ 0.88	2.45 $\pm$ 0.78	2.12 $\pm$ 0.82	0.25
Testosterone (nmol/l)	4.87 $\pm$ 4.55	7.44 $\pm$ 5.71	5.46 $\pm$ 5.74	0.26
Inhibin B (ng/l)	189 $\pm$ 51	191 $\pm$ 73	195 $\pm$ 69	0.37
SHBG (nmol/l)	61.8 $\pm$ 35.6	55.8 $\pm$ 28.4	65.2 $\pm$ 34.8	0.95
FSH (IU/l)	2.31 $\pm$ 1.16	2.84 $\pm$ 1.75	2.44 $\pm$ 1.78	0.37
LH (IU/l)	1.52 $\pm$ 1.05	1.95 $\pm$ 1.33	1.47 $\pm$ 1.15	0.58

Figure 3 Serum testosterone concentrations (nmol/l) in 156 boys in relation to their prenatal PCB exposure ($r = 0.09$; $P = 0.26$). Boys with testicular abnormalities are indicated by solid circles, boys with a positive spermaturia test with open circles, and those with a negative test with crosses.

analyses using testicular size and serum hormone concentrations as the dependent variables failed to show any clear PCB effect. These findings were replicated after exclusion of boys with testicular abnormalities.

Discussion

The onset of puberty is determined by a complex interaction between a multitude of hormones (11, 16). An imbalance caused by external exposure to xeno-oestrogens, especially during sensitive developmental stages, could potentially lead to changes in pubertal development. Previous experimental research in this field is somewhat equivocal about whether precocious or delayed sexual maturation might be expected in males prenatally exposed to endocrine disruptors (5–8). Thus, an increased occurrence of genital abnormalities in male mice and earlier sexual maturation in females were reported after exposure to high doses of oestrogenic chemicals (5). In female

guinea pigs, exogenous oestrogen exposure during the prenatal period delayed the onset of puberty in a dose-dependent fashion, perhaps due to an inhibitory or androgenizing effect on the hypothalamo–hypophysial axis (8). However, patients with congenital adrenal hyperplasia tend to show central precocious puberty, and girls are more likely to develop precociously, while there is a predominance of delayed puberty in males (17, 18).

The examination for spermaturia is known to exhibit a high rate of false negative results (10, 11, 19, 20). Spermaturia has an intermittent appearance and emission of spermatozoa does not occur daily in subjects with confirmed spermatogenesis. Also, the occurrence of few spermatozoa may be missed by the examiner, or the cells may be lost in the sample preparation before examination. However, the prevalence of negative urine samples does not seem to change after the first observed spermaturia (19).

Approximately half of the boys in Tanner stage 3 tested positive for spermaturia, and the same was true

for the boys with a testicular volume above 9 ml (Figs 1 and 2). This rate of positive results is similar to the one obtained after documented spermarche in a longitudinal study where 24-h urine samples were used (19). Thus, the examination of a 50 ml morning urine sample seems to be appropriate and does not appear to cause a rate of false negative results above expectation (20). Likewise, the association of serum hormones with other markers of puberty development are in accordance with expectation, and the hormone concentrations are similar to previous findings in boys of this age group (21).

Determination of the age of spermarche has been the object of considerable interest. Although an early study suggested a mean age around the 13th birthday (22), most authors describe the mean age of spermaturia around the 14th birthday (11, 16). Because of the false negative samples, spermarche may precede the age at which spermaturia is first observed considerably; in a prospective study with collection of 24-h urine samples every 3 months, the underestimation was estimated to be 0.41 years (19). Thus, the boys might be further in their pubertal development and maturity than assumed from their spermaturia status. If the age at which a 50% rate of spermaturia is reached is considered an indication of puberty, then the Faroese results suggest that puberty is reached by about or slightly above the age of 14 years. Taking an underestimation of about 0.4 years into account, the true age at which spermarche is reached may more likely average about 13.6 years. This estimate is in good agreement with previous observations from populations without known exposures to endocrine disruptors (11, 16, 19).

Overall, no association was detected between the degree of prenatal PCB exposure level and spermaturia or other markers of puberty development. This conclusion must be interpreted in the light of the limited sensitivity of the method for detection of spermaturia and the temporal changes occurring in other indicators of the development of puberty. Also, boys with the highest serum testosterone concentrations tended to belong to the group with the highest prenatal PCB exposure levels. This possible association may be reason for further exploration of these issues.

The validity of the PCB exposure biomarker must also be considered in regard to the general issue of developmental exposure to endocrine-disrupting chemicals. On the one hand, the cord PCB concentration is likely to be a good indicator of the exposure level throughout gestation (9). However, while some PCB congeners may have oestrogenic effects, others may be anti-oestrogenic, and some associated contaminants, such as *p,p'*-DDE, may have anti-androgenic effects (1, 23). Assessment of the three major PCB congeners may therefore be an imprecise indicator of the total xeno-oestrogenic exposure level. In addition, the exposure assessment did not include postnatal

intake levels from human milk (2) or diets including contaminated seafood.

In conclusion, increased prenatal PCB exposure did not affect, to any significant degree, the prevalence of spermaturia at 13.75 years of age and was only weakly associated with other markers of the development of puberty. However, presence of spermaturia was closely associated with other puberty indicators. The failure to detect a clear PCB effect on these parameters should not be interpreted as evidence that these exposures are harmless. Although the methods applied and the size of the exposed population did not allow any detection of a significant association, other effects of endocrine disruption may be more likely, perhaps including increased incidence of hormone-dependent cancers (1). Also, PCBs are thought to cause neurotoxic and other adverse health effects (24) that were not considered in the present study. Nonetheless, because of the evidence on decreasing sperm quality and other adverse effects on male reproductive function, further studies in this field are highly warranted.

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