High Growth Rate of Girls with Precocious Puberty Exposed to Estrogenic Mycotoxins

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Objective To test the hypothesis that human puberty timing can be advanced by environmental estrogen exposure.

Study design We analyzed serum mycoestrogen contamination via high-performance liquid chromatography (HPLC) in 32 girls affected by central precocious puberty (CPP) and in 31 healthy female control subjects. All 32 patients received triptorelin (TR) for more than 12 months after diagnosis.

Results Increased serum levels of zearalenone (ZEA; 933.7 ± 200.3 pg/mL; 95% CI, 723.5-1143.9) and of its congener α-zearalenol (106.5 ± 1.9 pg/mL; 95% CI, 104.5-108.5) contaminated 6 girls with CPP, who were from a bounded Tuscany area. At diagnosis, ZEA levels correlated with patient height ($r = 0.906$, $P < .05$) and weight ($r = 0.887$, $P < .05$), but not with bone age. In patients who were mycotoxin-positive, height ($F = 4.192$; $P < .01$), weight ($F = 3.915$; $P < .01$), and height velocity ($F = 2.777$, $P < .05$) were higher than patients who were mycotoxin-negative during 12-months TR treatment. Height correlated with weight both in patients who were mycotoxin-positive ($r = 0.986$, $P < .001$) and in patients who were mycotoxin-negative ($r = 0.994$, $P < .001$). Body mass index, bone age, and gonadal secretion was not different in patient groups before and during TR treatment ($P > .05$).

Conclusions Mycoestrogenic zearalenone is suspected to be a triggering factor for CPP development in girls. Because of its chemical resemblance to some anabolic agents used in animal breeding, ZEA may also represent a growth promoter in exposed patients. *(J Pediatr 2008;152:690-5)*

Since the 1970s, there has been a worldwide scientific discussion on potential health consequences of human exposure to estrogen disruptors. Many environmentally persistent compounds are toxic estrogen agonists, androgen antagonists, or both. Thus, they can disregulate the hypothalamic-pituitary-gonadal (HPG) axis, potentially inducing central precocious puberty (CPP).[1]

In humans, little is known about pollutant influence on premature sexual development. In a study of patients with precocious puberty, serum dichloro-diphenyl-dichloro ethylene (DDE) levels were 10-fold higher in foreign girls than in native Belgian girls.[2] The authors hypothesized that migration interrupted exposure to estrogen disruptors, inducing accelerated maturation of HPG axis.[2] Because native girls without precocious puberty served as control subjects, the association remains speculative. Since it was banned in the late 1960s, DDT and its metabolites are not proposed as major endocrine pollutants in Italy, though DDT/DDE may be detected in biological samples.[3]

In 1979, Fara et al[4] described a school epidemic of premature thelarche in Northern Italy. Italians frequently consumed meat of young animals such as poultry, pig, calf, and lamb, which can be treated with anabolic steroids to increase growth rate. After this episode, the European Union banned the application/use of anabolic growth promoters in agriculture since 1985.[5,6]

From 1978 to 1984, an epidemic of premature thelarche and precocious puberty occurred in Puerto Rico.[7-10] It was suggested that dairy and meat products could be contaminated with anabolic estrogens such as zeranol (α-zearalanol; α-ZAL) or diethylstilberstrol, which were used for increasing muscle mass in cattle and poultry[7,8]; Schoental suggested the possibility of Fusarium toxin contamination of grain products as

α-ZAL	α-zearalanol	E_2	17β-estradiol
α-ZOL	α-zearalenol	GnRH	Gonadatropin-releasing hormone
β-ZAL	β-zearalanol	HPG	Hypothalamic-pituitary-gonadal
β-ZOL	β-zearalenol	HPLC	High-performance liquid chromatography
BA	Bone age	Ht	Height
BMI	Body mass index	HV	Height velocity
CA	Chronological age	TR	Triptorelin
CPP	Central precocious puberty	Wt	Weight
DDE	Dichloro-diphenyl-dichloro ethylene	ZEA	Zearalenone

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the causative agent.[11] An increased incidence of early thelarche/mastopathy patients in the southeastern Hungary since 1989 was also observed.[12] In that study, estrogenic mycotoxins were detected in 5 of 36 early thelarche patients with serum zearalenone (ZEA) level of 18.9 to 103 μg/L as well.[12] ZEA, α-ZAL, and their metabolites are able to adopt a conformation that sufficiently resembles 17β-estradiol (E_2) to allow it to bind to estrogen receptors in target cells exerting estrogenic (agonist) action.[13-15]

A high incidence of CPP has been reported in a small region of North-West Tuscany: the incidence of CPP in the pediatric population of Viareggio countryside was 22 to 29 times higher compared with that in neighboring areas.[16] Although the cause for this difference remains unexplained, the geographic distribution of CPP strongly suggests the involvement of an environmental estrogen exposure in the onset of CPP.[16]

The aim of this study was to perform serum measurements of mycotoxin ZEA and its metabolites (ie, α-ZAL, β-zearalanol [β-ZAL], α-zearalenol [α-ZOL], and β-zearalenol [β-ZOL]) in North-West Tuscany patients affected by idiopathic CPP and to evaluate the mycoestrogen exposure as triggering factor for premature sexual development.

METHODS

Subjects

To assess serum mycotoxin contamination, we selected 32 girls with idiopathic CPP who came as outpatients to the Pediatric Endocrine Center of Pisa between 2001 and 2005; 17 girls with CPP were from the Viareggio countryside (group A), and 15 patients were from Pisa (group B).[16] In addition, 31 healthy age- and sex-matched control subjects were selected from Viareggio (n = 15; group C) and Pisa (n = 16; group D). After approval from the local institutional review board, informed consent was obtained from all the parents of patients before the study.

Diagnostic criteria for CPP were consistent with a recent study.[17] At CPP diagnosis, all 32 patients (group A and B) had a history of increased growth velocity, Tanner breast stage of at least 2, and bone age (BA) was advanced >1 year; luteinizing horomone, and follicle-stimulating hormone responses to the gonadatropin-releasing hormone (GnRH) stimulation test (100 mg/m^2, intravenous bolus dose) were in the pubertal range.[18] Clinical diagnosis of CPP was also confirmed with E_2 levels ≥25 pg/mL and by age ≤8 years.

After diagnosis, 32 girls with CPP were treated with triptorelin (TR) depot intramuscularly (Decapeptyl, Ipsen Pharma Biotech SA, Toulon Cedex, France) at 0.1 mg/ kg body weight every 28 days for >12 months. TR doses were adjusted during treatment according to patient weight. Data on the auxology and the pubertal development were obtained at 3- to 6-month intervals, with BA yearly determined with the Greulich and Pyle method. According to Italian standards,[19] height (Ht), weight (Wt), height velocity (HV), and body mass index (BMI; weight in kilograms/height in m^2) are expressed as SD score either for chronological age (SDS_{CA}) and for BA (SDS_{BA}). TR-induced suppression of gonadotropin secretion was checked every 3 or 6 months with radioimmunoassays.

Mycotoxin Measurement

Mycotoxin standards of ZEA, α-ZOL, β-ZOL, α-ZAL, and β-ZAL (Sigma-Aldrich, Milan, Italy), and immunoaffinity columns ZearaStar (Romer Labs, Herzogenburg, Austria) were purchased. Acetonitrile and methanol were of high-performance liquid chromatography (HPLC) grade, and other reagents were of analytical grade (Baker Analyzed Reagent, J.T. Baker, Deventer, The Netherlands).

For mycotoxin analysis, serum samples were collected during the GnRH-stimulating test[17] and at 12 months of GnRHa treatment for CPP, as during both routine evaluations (at baseline and after 12 months) for control subjects.

Serum concentrations of mycotoxins were HPLC-assayed. 5 mL plasma was mixed with 5 mL of 0.2 M sodium acetate buffer with a pH level of 5.5. This solution was incubated for 16 hours at 37°C with 50 μL of glucuronidase solution before it was mixed with 15 mL of phosphate buffer saline and adjusting to a pH level of 7.4 with 1 M NaOH. After centrifugation at 3000 rpm, the clear part was passed through ZearaStar column at flow-rate of 1 to 2 drops/s. The column was washed with 20 mL water (1-2 drops/s). Elution was performed with 1.5 mL of methanol. The eluate was evaporated to dryness under a stream of nitrogen. The residue was re-dissolved in 100 μL of HPLC mobile phase and injected into the HPLC system. The chromatographic system consisted of a Jasco 880 pump and Jasco 821 fluorescence detector (Jasco, Tokyo, Japan). Jasco Borwin software was used for data processing. Excitation wave-length (λex) and emission wave-length (λem) were set at 274 and 440 nm, respectively. The reversed-phase column was a Spherisorb 3 μm C_{18} column (150 × 4,60 mm) from Waters (Milford, Mass). The HPLC was operated with mobile phase system consisting of H_2O/ACN (50/50, v/v) at a flow rate of 1 mL/min. For both ZEA and α-ZOL, the limit of detection and limit of quantification were 0.025 ng/mL and 0.05 ng/mL, respectively. For β-ZOL, α-ZAL, and β-ZAL, the limit of detection and limit of quantification were 0.25 ng/mL and 0.5 ng/mL, respectively. The recoveries of ZEA, α-ZOL, β-ZOL, α-ZAL, and β-ZAL were 87.1% ± 0.3%, 84.2% ± 0.2%, and 80.1% ± 0.3%, 79.5% ± 0.5%, and 82.1% ± 1%, respectively. The intra-assay and interassay coefficients of variation were <10% for each compounds.

Statistical Analysis

Values are expressed as mean ± SD, unless otherwise stated. Statistical analysis was performed by using 1-way analysis of variance and the Fisher exact test. Bonferroni's adjustment to a *P* value was applied when appropriate. Correlations in 2 variables were determined with Pearson's cor-