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Gender differences in cadmium and cotinine levels in prepubertal children

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ABSTRACT

Susceptibility to environmental stressors has been described for fetal and early childhood development. However, the possible susceptibility of the prepubertal period, characterized by the orchestration of the organism towards sexual maturation and adulthood has been poorly investigated and exposure data are scarce. In the current study levels of cadmium (Cd), cotinine and creatinine in urine were analyzed in a subsample 216 children from 12 European countries within the DEMOCOPHES project. The children were divided into six age–sex groups: boys (6–8 years, 9–10 years and 11 years old), and girls (6–7 years, 8–9 years, 10–11 years). The number of subjects per group was between 23 and 53. The cut off values were set at 0.1 μg/L for Cd, and 0.8 μg/L for cotinine defined according to the highest limit of quantification. The levels of Cd and cotinine were adjusted for creatinine level. In the total subsample group, the median level of Cd was 0.180 μg/L (range 0.10–0.69 μg/L), and for cotinine the median wet weight value was 1.50 μg/L (range 0.80–39.91 μg/L). There was no significant difference in creatinine and cotinine levels between genders and age groups. There was a significant correlation between levels of cadmium and

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creatinine in all children of both genders. This shows that even at such low levels the possible effect of cadmium on kidney function was present and measurable. An increase in Cd levels was evident with age. Cadmium levels were significantly different between 6–7 year old girls, 11 year old boys and 10–11 year old girls. As there was a balanced distribution in the number of subjects from countries included in the study, bias due to data clustering was not probable. The impact of low Cd levels on kidney function and gender differences in Cd levels needs further investigation.

1. Introduction

Prepuberty and puberty are critical periods of maturation during which hormones orchestrate the development of secondary sexual characteristics, skeleton and behavior. Estrogen and testosterone levels during these periods may be disturbed by environmental stressors from air, water, and food. A large number of xenobiotics/xenoestrogens couple estrogen receptors at different efficiency, thus redirecting biological pathways of maturation. The fact that some agents such as cotinine and fungicides act as aromatase inhibitors by lowering levels of estrogen and increasing levels of testosterone (Barbieri et al., 1986; Trosken et al., 2006) has not been investigated to the same extent. The currently reported increased incidence of early maturation or delay in onset of puberty in industrialized countries may have been related to environmental settings contaminated with hormonally active agents (Jacobson-Dicman and Lee, 2009). Additionally, exposure to endocrine disruptors during prepuberty may have significant implications on health risks during adulthood such as an increase in sterility of both genders, as well as increased incidence of testicular cancer and higher incidence of breast cancer in younger age groups (Znaor et al., 2013; Giannandrea et al., 2013; Toppari and Juul, 2010; Fucic et al., 2012).

Estrogen is defined as an endocrine and paracrine agent and neuromodulator that acts via three receptors so far known. By interacting with receptors xenoestrogens may have broad biological effects on the human organism. The impact of endocrine active agents on carcinogenesis is of great significance as according to current knowledge all cancers are accompanied by disturbances in estrogen receptor distribution and/or estrogen and aromatase levels (Fucic et al., 2012; Fucic et al., 2010; Yakimchuk et al., 2012; Okitsu et al., 2010; Kim et al., 2013). However, xenoestrogenic effects are not limited to carcinogenesis since other systems like the cardiovascular system and the urinary system are also modulated by estrogen (Hagenfeldt and Eriksson, 1988; Robinson et al., 2013). Also, disturbances of androgen receptors and testosterone levels are related with for example breast, lung and prostate cancer (Thike et al., 2014; Chang et al., 2013).

Cadmium is one of the most common endocrine disruptors in a child's living environment which may be present in food and air (due to environmental tobacco smoke, ETS) (Satarug and Moore, 2004, Watanabe et al., 2013). Rice is the most frequent dietary source of Cd (Kipppler et al., 2012). Furthermore, the dietary intake of toxic metals including Cd may be higher in children than in adults (Liu et al., 2010).

Cadmium is a well-known toxic metal that binds to estrogen receptors (metalloestrogen) (Crespo-Lopez et al., 2009; Buzard and Kasprzak, 2000; Byrne et al., 2013; Darbre, 2006; Nasiadek et al., 2011). This toxic metal may cause different neurotoxic effects, but also inflammation which is associated with Cd-related cardiovascular diseases and oxidative stress (Colacino et al., 2014).

The benchmark dose for Cd in adults for renal effects has been calculated to be 0.6–1.1 μg/g creatinine (Suwazono et al., 2006). A recent study in children showed that exposures even below the reference level for Cd based on renal effects, set by the European Food Safety Authority to 1 μg/g creatinine and to 5.24 μg/g creatinine by to the World Health Organization, still cause learning disabilities (Ciesielski et al. 2012). In an adult population of Swedish women tubular and glomerular effects in the kidney were already significantly increased at mean levels of 0.8 μg/g creatinine (Akesson, et al., 2005). A long term study conducted in Japan suggested irreversible and slowly progressive renal tubular dysfunction after exsposure to environmental Cd even after reduction of exposure (Iwata et al., 1993). Additionally, Cd exposure has been associated with a decrease of estradiol levels (Zhang et al., 2008). The specific susceptibility of the prepubertal period for toxic metals has been shown in an animal model, where exposure to lead until puberty caused higher renal toxicity than exposure during adulthood (Berrahal et al., 2011).

Metallothioneines (MT) are metal binding proteins present in humans in four isoforms and regulated by hormones. The complex of Cd and MT is nephrotoxic (Klaassen et al., 2009) and its bioaccumulation during prepuberty and puberty, as well as its specific gender related susceptibility, has not yet been investigated.

Apart from Cd, nicotine is another common pollutant present in the life environment of children, and potentially has an impact on the developing hormone system. Environmental tobacco smoke has mainly been known to be associated with an increased incidence of asthma, wheezing illnesses, bronchiolitis and cognitive abilities even at very low levels (Hwang et al., 2012; Tung et al., 2013; Yolton et al., 2005). Despite global efforts for reducing the number of smokers, children are not protected as they are predominantly exposed at home. Cigarette smoke is a source of both chemical agents and radiation (polonium). Cotinine, a metabolite of nicotine, is a commonly used biomarker of exposure to tobacco smoke. However, cotinine is also an active molecule that can have impact on human health via several mechanisms. Additionally to aromatase inhibition (an anti-estrogen activity) (Barbieri et al., 1986; Kadohama et al., 1993; Myong et al., 2013; Soldin et al., 2011; Zeller and Berger, 1989), cotinine is mitogenic for smooth muscle cells at concentrations seen among passive smokers. This represents a potential risk for blood vessel intimal hyperplasia (Calphor et al., 1997). Cotinine has also been shown to be an immunosuppressor in animal models, but whether this effect is a consequence of cotinine or testosterone, which is also known to be immunosuppressive (Furman et al., 2013), is unknown. An increase of testostosterone by aromatase inhibition has not been proven (Kalra et al., 2004). Exposure to ETS decreases levels of estradiol in women (Soldin et al., 2011).

The prepubertal period may be a vestibule for puberty and thus crucial in the final physiological preparation for puberty, which therefore makes it specifically vulnerable to the environmental contaminants (Mantovani and Fucic, 2014). The possible impact of hormonally active agents from the living environment on the prepubertal period has still not been investigated.

The COPHES and DEMOCOPHES twin projects, which represent the first European harmonized biomonitoring study on children and their mothers in 17 countries, included measurements of urinary cadmium (Cd) and cotinine. Children were the primary target group of the study because the EU Environment and Health

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Action Strategy (SCALE) was focused on children for their particular and universally recognized vulnerability (Becker et al., 2014; Den Hond et al., 2014; Casteleyn et al., this issue).

The aim of the current study was to investigate possible age and gender differences in levels of Cd and cotinine between the prepubertal period defined as age 6–7 and 6–8 years for girls and boys, respectively (Berberoglu, 2009; Golub et al., 2008) and the early pubertal period. In the study a subample of COPHES/DEMOCOHES cohort was analyzed. As this is the first study to compare the possible biological effects of Cd and cotinine during the prepubertal and early pubertal period, it was not possible to refer to any already agreed criterion in regard to the selection of age groups and the investigated biomarkers, thus the age groups were selected arbitrarily taking into account that girls enter puberty before boys.

Although most studies investigate ETS in children using questionnaires it has been shown that the method is unreliable as smokers may not report their smoking habits acurately. Therefore, cotinine levels in urine are a much more objective measurement (Butz et al., 2011). In the current study Cd was measured in urine as a reliable biomarker of exposure (Trzcinka-Ochocka et al., 2004). As increased creatinine clearance has recently been shown to be a good indicator of tubular damage in populations environmentally exposed to Cd (Kobayashi et al., 2008) a correlation analysis of Cd and creatinine was performed. Additionally, impact of Cd on levels of creatinine was investigated and compared between selected gender and age groups.

2. Subjects and methods

2.1. Population

The DEMOCOPHES study protocol of the EU Life+ project was approved by the ethical committee of each European country included. Participants or their respective mothers (depending on age) gave their informed consent. In the current study results of the analysis of chidren from Slovenia, Spain, Belgium, Denmark, Luxemburg, Czech Republic, Cyprus, Sweden, Romania, Poland, Slovakia and Hungary were included. In these countries the levels of Cd and cotinine were higher than the mean values of Cd and cotinine in all countries included in the DEMOCOPHES project. The selection of subsample of the analyzed children population based on Cd and cotinine levels as described in Methods consisted of 216 children (117 boys and 99 girls). All of the subjects in the listed countries within the age range of 6–11 years were analyzed. Approximately 3 subjects per age/sex group for each country were analyzed.

Children were recruited in schools between 2011 and 2012 and had to be living at the same location for at least the five previous years. Children living in hospitals or institutions, being homeless, or presenting metabolic disturbances or abnormal urine excretion were excluded.

For the purpose of the study children were divided into 6 "age–sex" groups: boys 6–8 years ($n=53$), girls 6–7 years ($n=30$), boys 9–10 years ($n=41$), girls 8–9 years ($n=32$), boys 11 years ($n=23$), and girls 10–11 years ($n=37$).

Mothers were provided with materials for urine collection. The first morning urine was collected in a 250 mL polypropylene vessel previously rinsed with 10% HNO_3 to avoid contamination (from the material). Urine samples, initially kept at 4 °C, were aliquoted and stored at −20 °C until analysis.

3. Methods

Cadmium, cotinine and creatinine were analyzed in European laboratories that successfully passed the Quality Assurance program established by COPHES. For each parameter, the laboratories used different laboratory procedures but all followed the same procedures for quality assurance and quality control requested by the COPHES Quality Assurance Unit. Each laboratory had standard operating procedures (SOP) (for details, see Schindler et al., 2013). Additionally, two External Quality Assessment Schemes and two Interlaboratory Comparison Investigations were provided by the COPHES Quality Assurance Unit to ensure quality and comparability of results. Each laboratory had standard operating procedures(SOP). To guarantee comparability and quality of chemical analysis, quality assurance program was designed by COPHES/DEMOCOPHES. The laboratories could use which analytical method they choose but only laboratories that successfully completed the COPHES/DEMOCOPHES quality assurance program were qualified to analyze the samples from the study. Detailes of quality assessment are avaliable in Schindler et al. (2013) Children with levels lower than 0.1 μg/L for Cd, and 0.8 μg/L for cotinine were excluded (levels falling below the highest limits of quantification).

4. Statistical analysis

Statistical analysis was performed using StatSoft, Inc. (2011). STATISTICA, version 10 (StatSoft, Inc. Tulsa, OK, USA). Basic descriptive summaries of data were obtained using mean, standard deviation (SD), median, interquartile range (IQR) and range. Differences between groups were calculated using Student's *t*-test or Mann–Whitney *U*-test for gender differences and analysis of variance (ANOVA) or Kruskal–Wallis ANOVA for gender–age groups (for respectively normal and non-normal distributed results). Associations between variables were tested using Spearman's rank order correlations. $P<0.05$ was considered statistically significant for all analyzes.

5. Results

Results for Cd and cotinine levels and creatinine were available for 216 children (117 boys and 99 girls aged 6–11 years). Levels of Cd and cotinine were also adjusted for creatinine level. Data showing levels of Cd, cotinine, creatinine and adjusted levels of Cd and cotinine for the levels of creatinine are presented in Table 1.

Levels of Cd were in the range of 0.10–0.69 μg/L of urine with a mean (SD) of 0.20 μg/L (0.104). No significant difference was observed between boys and girls for levels of Cd ($t=0.585$, $P=0.559$, Student's *t*-test) or for Cd/creatinine ($Z=0.096$; $P=0.923$, Mann–Whitney *U* test). A significant difference in the levels of Cd through "age–sex" groups was found ($H=16.824$, $P=0.005$, Kruskal–Wallis ANOVA) with the lowest median values in youngest groups and highest values in the oldest groups (significantly different between girls 6–7 years old and 11 years old boys, $P=0.038$; and between girls 6–7 years old and 10–11 years old girls, $P=0.024$; Graph 1), but no significant difference was found for Cd/creatinine (μg/g) values between "age–sex" groups ($F=0.869$, $P=0.503$, ANOVA). This is most probably due to the significant association between Cd and creatinine levels ($R=0.420$, $P<0.001$, Spearman's rank order correlation, (Graph 2). The same level of association could be followed through all "age–sex" groups ($R=0.335$–0.617) and was comparable in boys ($R=0.387$, $P<0.001$) and girls ($R=0.453$, $P<0.001$). Also a significant difference was found between Cd levels between subgroups based on the creatinine level (≤ 1400 vs. >1400 – high creatinine level). A subgroup with high creatinine level had a significantly higher level of Cd (mean ± SD; 0.233 ± 0.106 vs. 0.179 ± 0.096; $t=3.913$, $P=0.0001$).

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Table 1
Values of Cd, cotinine and creatinine in each age group according to gender.

Parameter μg/L	Gender–age group	*N*	Mean	SD	Median	Range		IQR		Statistics[a]
Cd	All	216	0.204	0.104	0.180	0.100	0.690	0.130	0.240	n/a
	Boys	117	0.208	0.107	0.180	0.100	0.640	0.130	0.240	$t=0.585$; $P=0.559$
	Girls	99	0.199	0.101	0.180	0.100	0.690	0.130	0.230	
	B6-8	53	0.190	0.112	0.160	0.100	0.640	0.120	0.220	$H=16.824$; $P=0.005$
	G6-7	30	0.157	0.055	0.150	0.100	0.360	0.120	0.200	
	B9-10	41	0.209	0.081	0.190	0.100	0.390	0.140	0.260	
	G8-9	32	0.209	0.125	0.185	0.100	0.690	0.135	0.230	
	B11	23	0.246	0.128	0.200	0.100	0.590	0.150	0.360	
	G10-11	37	0.225	0.097	0.210	0.100	0.550	0.150	0.290	
Cotinine	All	216	3.154	4.738	1.505	0.800	39.910	1.000	2.576	n/a
	Boys	117	3.037	4.264	1.500	0.800	26.400	1.000	2.500	$Z=-0.155$; $P=0.877$
	Girls	99	3.294	5.263	1.600	0.800	39.910	1.000	3.310	
	B6-8	53	2.360	2.857	1.300	0.800	15.570	0.994	2.500	$H=6.552$; $P=0.256$
	G6-7	30	2.164	1.639	1.734	0.800	7.200	1.100	2.000	
	B9-10	41	2.895	4.676	1.510	0.800	26.400	1.100	1.875	
	G8-9	32	3.366	7.047	1.214	0.800	39.910	0.900	2.429	
	B11	23	4.849	5.678	1.900	0.800	16.770	1.100	5.679	
	G10-11	37	4.148	5.353	1.760	0.800	20.097	1.000	4.940	
Creatinine	All	216	1395.309	530.229	1351.000	341.380	3061.027	970.000	1683.500	n/a
	Boys	117	1402.069	497.635	1362.000	460.000	2653.795	1036.000	1724.742	$t=0.203$; $P=0.839$
	Girls	99	1387.321	568.812	1335.000	341.380	3061.027	950.000	1671.914	
	B6-8	53	1268.831	412.745	1183.000	585.803	2290.000	969.000	1551.000	$F=1.808$; $P=0.113$
	G6-7	30	1262.767	465.978	1290.000	341.380	2142.000	886.514	1508.000	
	B9-10	41	1488.980	534.277	1485.643	610.000	2653.795	1080.000	1976.206	
	G8-9	32	1464.635	571.336	1436.500	576.000	3001.000	1025.000	1754.706	
	B11	23	1554.166	550.494	1615.158	460.000	2530.000	1191.736	1956.000	
	G10-11	37	1421.444	636.653	1394.770	540.000	3061.027	950.000	1696.000	
Cd/creatinine	All	216	0.160	0.097	0.136	0.040	0.959	0.106	0.188	n/a
	Boys	117	0.163	0.111	0.137	0.040	0.959	0.108	0.180	$Z=0.096$; $P=0.923$
	Girls	99	0.156	0.077	0.135	0.066	0.527	0.105	0.196	
	B6-8	53	0.158	0.095	0.134	0.065	0.560	0.110	0.179	$F=0.869$; $P=0.503$
	G6-7	30	0.145	0.095	0.111	0.066	0.527	0.093	0.151	
	B9-10	41	0.152	0.065	0.141	0.040	0.312	0.111	0.175	
	G8-9	32	0.149	0.067	0.130	0.071	0.361	0.099	0.200	
	B11	23	0.191	0.187	0.133	0.077	0.959	0.099	0.205	
	G10-11	37	0.172	0.066	0.170	0.072	0.347	0.119	0.214	
Cotinine/creatinine	All	216	2.688	4.824	1.156	0.316	43.713	0.734	2.526	n/a
	Boys	117	2.539	4.297	1.138	0.316	33.000	0.755	2.168	$Z=-0.305$; $P=0.761$
	Girls	99	2.865	5.399	1.254	0.359	43.713	0.680	2.833	
	B6-8	53	2.102	2.578	1.154	0.489	12.144	0.725	2.168	$H=5.282$; $P=0.383$
	G6-7	30	1.924	1.350	1.469	0.556	5.547	0.749	2.535	
	B9-10	41	2.249	4.026	1.025	0.378	24.513	0.754	1.547	
	G8-9	32	2.910	7.618	0.963	0.473	43.713	0.609	2.083	
	B11	23	4.061	7.008	1.526	0.316	33.000	0.886	4.329	
	G10-11	37	3.589	5.161	1.364	0.359	25.272	0.743	3.407	

SD – standard deviation, IQR – inter-quartile range; B6-8 – boys 6–8 years; G6-7 – girls 6–7 years; B9-10 – boys 9–10 years; G8-9 – girls 8–9 years; B11 – boys 11 years; G10-11 – girls 10–11 years of age.

[a] Statistical analyses were based on Student's *t*-test (*t*), Mann–Whitney *U* test (*Z*), analysis of variance (ANOVA-F), and Kruskal–Wallis ANOVA by ranks (*H*).

Levels of cotinine were in the range of 0.80–39.91 with a median (IQR) of 1.505 (1.000–2.576). No significant difference was found between boys and girls for levels of cotinine ($Z=-0.155$, $P=0.877$, Mann–Whitney *U* test) or for cotinine/creatinine ($Z=-0.305$; $P=0.761$, Mann–Whitney *U* test). No significant difference in the levels of cotinine through "age–sex" groups was found ($F=1.808$, $P=0.113$, ANOVA) although a trend for higher values was found towards the oldest groups. Furthermore, no significant difference was found for cotinine/creatine values between "age–sex" groups ($H=5.282$, $P=0.383$, Kruskal–Wallis ANOVA) but with lowest median values among groups according to age. Contrary to a significant association seen between Cd and creatinine, overall no significant association was found between cotinine and creatinine levels ($R=-0.002$, $P=0.973$, Spearman's rank order correlation, Graph 3).The same level of association could be followed through all "age–sex" groups ($r=0.05$–0.15) and was comparable in boys ($r=0.04$, $P=0.656$) and girls ($r=-0.03$, $P=0.765$).

No significant association was found between levels of Cd and cotinine ($R=0.013$, $P=0.844$) but a mild significant association was found for values of Cd and cotinine when adjusted for creatinine ($R=0.244$, $P<0.001$).

6. Discussion

During the last decade, a significant progress in the elucidation of the complexity of effects of hormonally active substances from

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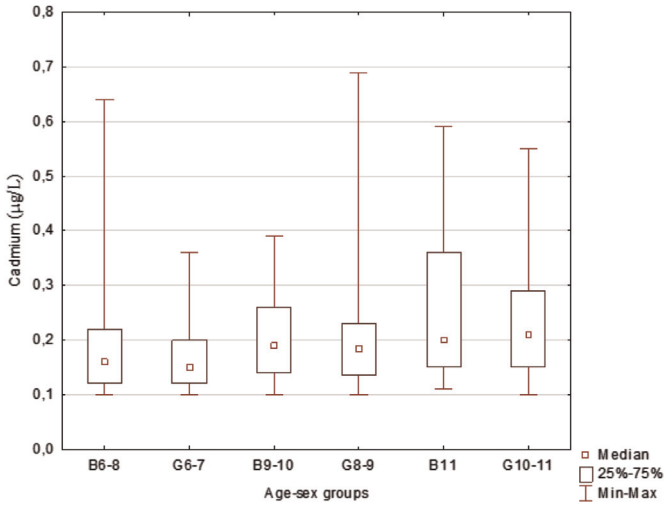
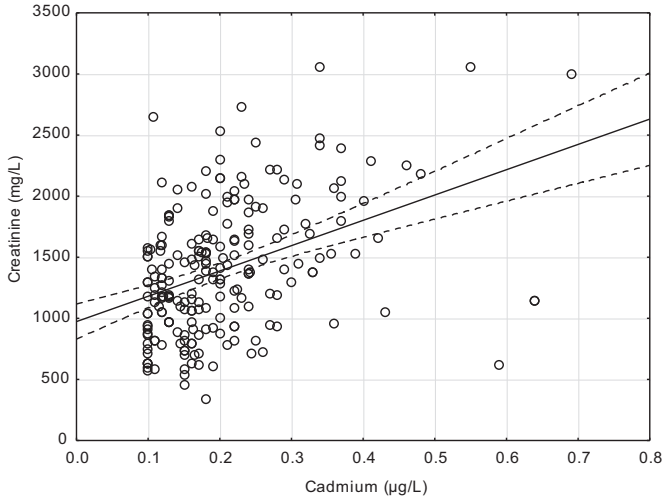
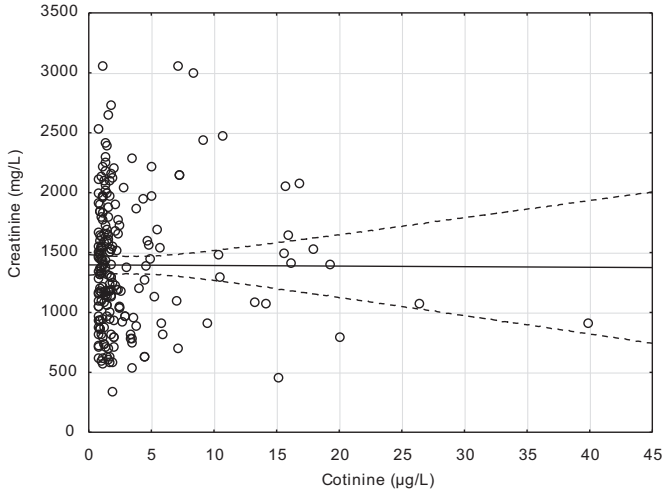


Graph 1. Categorized box and whisker plot of Cd levels (μg/L) in age–sex groups ($N=216$); B6-8 – boys 6–8 years; G6-7 – girls 6–7 years; B9-10 – boys 9–10 years; G8-9 – girls 8–9 years; B11 – boys 11 years; and G10-11 – girls 10–11 years of age.



Graph 2. Scatter plot of the association of Cd and creatinine levels ($N=216$); $r=0.42$, and $P<0.001$. Full line represents the linear correlation equation and dotted lines 95% confidence intervals.



Graph 3. Scatter plot of the association of cotinine and creatinine levels ($N=216$); $r=-0.002$, and $P=0.973$. Full line represents the linear correlation equation and dotted lines 95% confidence intervals.

the environment has been made. Not only reproductive organs, but also other organ systems, such as cardiovascular and nervous systems are affected and concentrations in humans exceeding effect levels have been measured (Montano et al., 2013). Similarly, in addition to investigations of the transplacental action of environmental agents on fetal development, specific attention is currently focused on puberty as a developmental period in which the balance of estrogen, testosterone and its receptors is of particular importance as a prerequisite of normal maturation. Contrary to recognized gender differences in response to hormonally active agents from the environment during adulthood, a knowledge gap in gender differences of endocrine disruptor impact on development during prepuberty and early puberty exists.

In the current study, data of subsample from the first harmonized European biomonitoring study within the twin projects COPHES and DEMOCOPHES were used in order to investigate possible gender and age differences in levels of Cd and cotinine during the prepubertal and early pubertal period and their correlation with creatinine levels.

Results showed that overall Cd and cotinine levels in children aged beteween 6 and 11 year old were similar to those described in previous studies (Cerna et al., 2012; Horton et al., 2013). Levels of Cd and cotinine increased with age but there was no significant difference within the prepubertal and early pubertal age groups.

The results of the current study showed significantly lower levels of urine-Cd in 6–7 years old girls compared to 10–11 years old girls and 11 years old boys. Such transient lower levels of urinary Cd may have been correlated with higher testosterone levels in prepubertal girls than in boys and girls during early puberty (Courant et al., 2010; Veldhuis et al., 2001; Belgorosky and Rivarola, 1988) which is in concordance with the earlier onset of puberty in girls. In older age groups, the Cd level in girls increased to that of boys (Graph 1). It could be hypothesized that relatively higher levels of testosterone in girls during this narrow developmental window of prepuberty stimulate metallothionein (MT) synthesis, which causes a more efficient coupling of Cd as urine levels of Cd are described to be correlated with MT levels (Kido et al., 1991). This more efficient coupling of Cd in prepubertal girls may furthermore result in higher risks of kidney damage in girls than in boys. Similar mechanisms are present in women who smoke and second hand smokers, where cotinine, as an aromatase inhibitor, causes increased testosterone levels and consequently significantly higher levels of MT (Ronco et al., 2005; Milnerowicz, 1997).

A significant correlation between Cd and creatinine in our study in all age groups for both genders suggests that even very low levels of Cd may cause disturbances in kidney functions. Significant increase of Cd levels in children with creatinine levels higher than reference values suggests health risks already at low doses. Cadmium persists in the kidneys of humans for decades and its nephrotoxicity has been reported at low level of exposure (Satarug and Moore, 2004). Thus urinary Cd with levels above 1 μg/L in adults showed increased health risks for albuminuria and chronic kidney disease (Ferrara et al., 2010). Cadmium at low levels exposures can also cause the early onset of diabetic renal complications, osteoporosis, blood pressure problems and increased cancer risk (Nakagawa and NIshio, 1996; Brzoska, 2012; Zhao et al., 2014).

All of the children in this study had levels of cotinine less than 60 μg/L which corresponds to the reported range for non-smoking persons and is similar to levels measured in the US (Butz et al., 2011). There was no difference in cotinine levels between the oldest groups of boys and girls, despite the fact that in this period of life an increase of estrogen and a consequentional acceleration of nicotine metabolism could be expected (Benowitz et al., 2006). There was also no correlation of cotinine with creatinine levels.

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During prepuberty children have the same metabolism and elimination of nicotine as adults (Hukkanen et al., 2005; Leong et al., 1998) but we are yet to investigate whether the same levels of cotinine have different effects on organ systems during puberty maturation. Furthermore the effects of cotinine as an aromatase inhibitor on prepubertal and pubertal development have also not yet been investigated.

Exposure to ETS in children is associated with respiratory diseases such as asthma, respiratory infections and bronchitis (Tung et al., 2013; Hwang et al., 2012). Asthma caused by ETS during puberty is more pronounced in girls (Strong and Chang, 2013). Girls exposed to ETS and allergic sensitization during prepuberty are at greater risk of decreased lung function later in adulthood compared to non-sensitized girls and boys (Brunst et al., 2012). As no gender differences in cotinine levels were detected in our study, it could be hypothesized that cotinine, as an aromatase inhibitor, may have gender related differences in its effects on the respiratory system. The gender differences of incidence and survival in patients with lung cancer is based on differences in estrogen receptor distributions and disturbances and underlines the importance of estrogen in lung tissue (Fucic et al., 2010).

In conclusion, the analysis of Cd urine levels in 6–11 year old children showed gender differences between 6–7 year old girls, 11 year old boys and 10–11 year old girls which could have been a consequence of increased MT levels stimulated by increased testosterone levels in prepubertal girls. An observed correlation between Cd levels and creatinine levels suggested an impact on the kidney function even at very low doses. There was no gender or age difference in cotinine levels and correlation with creatinine. All current recommendations of Cd levels are based on adult populations. There are no data on its specific effects during developmental stages especially during prepuberty and puberty when its xenoestrogenic mechanisms could be expected to have the most impact due to the interaction with increased estrogen and testosterone levels and the specific distribution of ER important for the maturation of all organic systems (Rogol et al., 2002). The possible bias of the current study is its age group selection, as there are no agreed criteria for the definition of age groups with regard to the investigated biomarkers. Thus, the results of this study may serve as initial insight for further studies on susceptibility during prepubertal and early pubertal period. Future research should focus on Cd specific damage of kidney function in prepubertal girls, impact of ETS on osteoporosis in prepubertal and pubertal period and investigation of polycystic ovaria incidence in ETS exposed girls.

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