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Metals, hormones and sexual maturation in Flemish adolescents in three cross-sectional studies (2002–2015)

Sam De Craemer [a,*], Kim Croes [a], Nicolas van Larebeke [a,b], Stefaan De Henauw [c], Greet Schoeters [d], Eva Govarts [d], Ilse Loots [e], Tim Nawrot [f,g], Vera Nelen [h], Elly Den Hond [h], Liesbeth Bruckers [i], Yue Gao [a], Willy Baeyens [a]

[a] Department of Analytical, Environmental and Geochemistry (AMGC), Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Elsene, Belgium
[b] Study Centre for Carcinogenesis and Primary Prevention of Cancer, Department of Radiotherapy and Nuclear Medicine, Ghent University, Belgium
[c] Department of Public Health, Ghent University, Ghent, Belgium
[d] Environmental Risk and Health, Flemish Institute for Technological Research (VITO), Mol, Belgium
[e] Faculty of Political and Social Sciences, University of Antwerp, Antwerp, Belgium
[f] Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium
[g] Department of Public Health & Primary Care, Occupational & Environmental Medicine, Leuven University (KU Leuven), Leuven, Belgium
[h] Provincial Institute for Hygiene, Antwerp, Belgium
[i] Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Diepenbeek, Belgium

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ABSTRACT

Sex hormone levels and timing of sexual maturation are considered important markers for health status of adolescents in puberty, and previous research suggests they might be influenced by metal exposure. In three campaigns of the Flemish Environment and Health Study (FLEHS I 2002–2006; FLEHS II 2007–2011 and FLEHS III 2012–2015), data were collected on internal exposure to metals (Cd, Cu, Pb, Cr, Mn, Tl, Ni, Sb, Hg, As and As species) and sexual maturation in 2671 14–15 years old adolescents. All metals were measured in blood and/or urine, except total- and methylmercury which were measured in hair samples. Sex hormone levels were measured in blood serum of adolescent males of the cohorts of FLEHS I and FLESH II. The use of a uniform methodology in successive campaigns allows to confirm associations between exposure and health in different cohorts and over time. Furthermore, mathematical and statistical density correction methods using creatinine or specific gravity were tested for urinary markers.

Significant associations between sex hormones and maturity markers were observed in the FLEHS I and II campaigns, when both were assessed together. Regardless of the applied correction method, creatinine correction systematically introduced bias due to associations of creatinine with sex hormones and maturation markers, especially in adolescent males, while this is not the case for specific gravity. A series of exposure-response associations were found, but several involving Cd, Pb, As, Tl and Cu persisted in different FLEHS campaigns. The effects of Pb and Cu on luteinizing hormone, (free) testosterone, (free) oestradiol and maturation support a xenoestrogenic agonistic action on the feedback of oestradiol to the hypothalamus-pituitary-gonadal axis.

Our results suggest that specific care should be taken when selecting urine density correction for investigating associations with hormonal and maturation markers in adolescent males. Furthermore, the possibility of xenoestrogenic effects of certain metals in environmentally exposed adolescents warrants further investigation.

1. Introduction

Puberty is suspected to be a period where an individual is especially vulnerable to environmental exposures to pollutants. Since the body and especially the brain are under development, external substances that influence this maturation could have long lasting effects on an individual (Sato et al., 2008). An important step in the onset of puberty is the activation of the hypothalamus-pituitary-gonadal (HPG) axis, which leads to increased formation of gonadal hormones involved in sexual maturation (Foster et al., 2006; Sisk and Foster, 2004). Epidemiological research has also reported correlations between sex hormone levels and the progression of puberty, reflecting the link between these processes (Den Hond et al., 2011; Kletter et al., 1993). It follows that external disturbances on the normal functioning of the HPG axis could affect puberty (including sexual and brain development) through influencing concentrations of hormones (Sato et al., 2008).

* Corresponding author.
E-mail address: sam.de.craemer@vub.be (S. De Craemer).

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Exposure to certain metals has been observed to influence concentrations of sex hormones and/or maturation (positively or negatively) in tests on animals (Al-Hamood et al., 1998; Cheng et al., 2003; Dearth et al., 2002; Liu et al., 2013; Srivastava et al., 2004), clinical tests on humans (Ayala et al., 2008) or in epidemiological research (Interdonato et al., 2015; Meeker et al., 2010; Schell and Gallo, 2010; Zawatski and Lee, 2013). Some of the effects of metals have been linked to xenoestrogenic actions in vitro (Darbre, 2006). Adolescents have potential exposure pathways to several of these metals, so from a health perspective it would be interesting to check associations in this age group between exposure to metals on one hand, and maturity markers and levels of HPG related hormones on the other hand. However, metals other than Cd and Pb were considered in very few studies.

In three successive Flemish Environment and Health Studies (FLEHS I, FLEHS II and FLEHS III) data were collected on exposure and effect markers through human biomonitoring and questionnaires (Schoeters et al., 2012). Adolescents aged 14–15 years were one of the focus groups during all three campaigns of FLEHS (2002–2006; 2007–2011; 2012–2015). The gathered information includes concentrations of several metals and maturity markers in all adolescents for all three campaigns, and information on sex hormone concentrations in adolescent males for the first and second campaign (FLEHS I and II). As a first step, the datasets were used to explore significant associations between exposure and effect markers. Subsequently, those associations that were supported by the literature or several FLEHS campaigns were further investigated and discussed. Where possible, suggestions are made for the mechanism by which the exposure markers affect sex hormones and/or maturity.

Another goal of this study was to determine a reliable urine density correction factor for urinary concentrations. Therefore, we compared the use of creatinine and specific gravity, as evidence is emerging that creatinine correction is less reliable in adolescents since it is significantly affected by skeletal muscle mass and diet, and has been shown to increase during maturation (Martin et al., 2008; Weaver et al., 2015).

2. Methods

2.1. Recruitment, sampling and questionnaires

Recruitment, sampling and use of questionnaires have been described in the literature for FLEHS I (Den Hond et al., 2011), FLEHS II (Croes et al., 2014b) and FLESH III (De Craemer et al., 2016). In studies were sex hormones were measured in adolescent males (FLEHS I and II), samples were taken around the same time in the morning (8:00 to 12:00) to limit the influence of diurnal variability of hormones. The number of participants in each study for whom metal exposure were measured was 1659, 606 and 406 respectively, although not all exposure and effect markers were measured in each individual. The total number of observations for each association investigated can be found in the relevant tables in Sections 6, 7 and 8 of the Supplementary material. An informed consent was signed by both the participants and their parents, and the biomonitoring studies were approved by the Ethical Committee of the University of Antwerp, Belgium (FLEHS I and II) and of the University hospital of Antwerp (FLEHS III).

The biomarkers of effect considered in this study are presented in Table 4. The questionnaires served to get personal information on lifestyle, education, living environment and other factors that could have an influence on metal concentrations or effect markers in the individual. Assessment of sexual development has been previously described for FLEHS I and II (Croes et al., 2014b; Den Hond et al., 2011). Briefly, development of genitals in adolescent males, breasts in adolescent females and pubic hair in both sexes was scored using the international scoring criteria of Marshall and Tanner, where stage 1 corresponds to the start of puberty and stage 5 to the adult stage. Information on menarche was obtained through self-assessed questionnaires.

2.2. Description and measurement of exposure and effect markers

2.2.1. Exposure markers

Table 1 indicates which markers were measured in which study, and whether they were measured in blood (B), urine (U) and/or hair (H). Trace element analysis in blood and urine in FLEHS I (Schroijen et al., 2008) and II (Baeyens et al., 2014; Vrijens et al., 2014) has been previously described, induced coupled plasma mass spectrometry (ICP-MS) was used for most markers, except As-metabolites, toxicologically relevant arsenic (TRA), total mercury (THg) and methylmercury (MeHg). Trace element analysis in FLEHS III was identical to the analysis in FLEHS II for the reported elements, except for arsenic species in urine. In FLEHS II, TRA was measured directly using flow injection –hydride generation atomic absorption spectrometry (Baeyens et al., 2014). In FLEHS III, trivalent As(III), pentavalent As(V), monomethyl arsenic acid (MMA), dimethyl arsenic acid (DMA) were measured using High Performance Liquid Chromatography-ICP-MS, with a Dynamic Reaction Cell, and TRA was calculated as the sum of these markers. The measurement of total and methylmercury in hair samples in FLEHS II was described by Croes et al. (2014a).

In urine specific gravity and creatinine were determined respectively by densitometer and the Jaffe method, and used to correct for urine density.

In general, urinary metal concentrations were corrected for urine density by specific gravity using Eq. (1), with SG as specific gravity and C and C_{SG}.as uncorrected and corrected biomarker concentrations respectively.

$$C_{SG} = C\frac{1.024-1}{SG-1} \quad (1)$$

Creatinine correction was done by dividing the metal concentrations by the concentration of creatinine of that sample.

2.2.2. Effect markers

Tables 2 and 3 describe the effect markers in our populations. Sex hormones investigated in this study were oestradiol (E2), testosterone (T), free oestradiol and testosterone (fE2 and fT), sex hormone binding globulin (SHBG), luteinizing hormone (LH) and follicle stimulating hormone (FSH). Hormone levels in adolescent males were measured in blood serum using commercial immunoassays as previously described for FLEHS I and II (Croes et al., 2014b; Den Hond et al., 2011), FSH as described in Dhooge et al. (2006). Aromatase was calculated as the ratio of testosterone to oestradiol (T/E2).

The effect markers for sexual development were binary markers based on the Tanner stage reached. For genital and pubic hair development in adolescent males, the value 1 was assigned if they were at stage 4 or 5. For breast and pubic hair development in adolescent females, the value 1 was assigned if they were at stage 5. Otherwise, the value 0 was assigned to these markers. These cut-offs were chosen in order to have reasonably large subgroups below and above the cut-off in each campaign of FLEHS. The percentage at or above these cut-offs were around 70% in adolescent males, and around 60% in adolescent females.

2.3. Statistical analysis

Since not all exposure and effect markers were measured in all campaigns of FLEHS, and study designs differed slightly between the campaigns, it was decided not to work on a pooled dataset. Instead, we used identical statistical methods for the datasets of each campaign separately. Continuous effect or exposure markers below the limit of detection (LOD) were replaced by LOD/2.

To assess exposure-response associations, multiple regression was performed by linear regression models for continuous effect markers (hormones in adolescent males and age at menarche), and logistic