Clinical Endocrinology (2015) **83**, 357–362 doi: 10.1111/cen.12704

ORIGINAL ARTICLE

Cadmium delays puberty onset and testis growth in adolescents

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Summary

Objective Cadmium (Cd) has been shown to impair pubertal development in experimental animals. However, no data are available for male adolescents with increased urinary cadmium levels.

Design The aim of this cross-sectional study was to evaluate pubertal onset and pituitary–gonadal axis hormones in male adolescents with increased urinary levels of Cd.

Subjects We studied 111 males, aged 12–14 years living in the Milazzo-Valle del Mela area. A control age-matched population ($n = 60$) living 28–45 km far from the industrial site was also enrolled.

Measurements Pubertal stages were assessed by clinical examination according to Tanner's score. Mean testicular volume was also investigated by ultrasound examination. Urinary Cd concentration and blood levels of FSH, LH, testosterone and inhibin B were also investigated.

Results Cd levels were significantly higher in adolescents living in the Milazzo-Valle del Mela area, compared to both age-matched subjects living far from the industrial plants and the reference values. Our population showed also a delayed onset of puberty, a smaller testicular volume and lower testosterone levels. An inverse correlation was found between urinary Cd and testicular volume ($r = -0{\cdot}25$; $P = 0{\cdot}0008$), testosterone levels (Spearman's $r = -0{\cdot}0{\cdot}37$; two-tailed $P < 0{\cdot}0001$) and LH levels (Spearman's $r = 0{\cdot}048$; $P < 0{\cdot}05$). Testosterone levels were positively correlated with testicular volume (Spearman's $r = 0{\cdot}48$; $P < 0{\cdot}0001$).

Conclusions This study, for the first time, suggests that increased Cd burden is associated with delayed onset of puberty in male adolescents and impaired testicular growth.

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(Received 23 July 2014; returned for revision 19 November 2014; finally revised 3 December 2014; accepted 15 December 2014)

Introduction

Heavy metals are endocrine disruptors that cause oestrogenic or anti-androgenic effects and interfere with the reproductive system and the normal development.[1–3] These metals, produced by the emissions of the industrial plants, are taken up by the ecosystem components and enter the food chain. For this reason, besides the industrial workers, the risk of human exposure increases also for people living near polluted areas.[4,5] Among the several heavy metals, cadmium (Cd) is an occupational and environmental pollutant that represents one of the major hazards to the human health. It has a long half-life (20–30 years in humans) and a low excretion rate (<1–2 μg/day), and, as a consequence, it is accumulated in tissues of the male reproductive system.[6] Furthermore, it has been shown that cadmium negatively influences male reproductive health acting at each level of the hypothalamic–pituitary–gonadal axis and impairing the control of several developmental processes[7] dependent on follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, as reported in rats[8] and in adult males.[9]

To the best of our knowledge, the effects of cadmium on the onset of puberty and reproductive hormones in male adolescents have not been investigated, so far. Our research group is investigating the presence of some heavy metals (arsenic, cadmium, chromium, lead, mercury, nickel and vanadium) in biological matrices obtained from adolescents living in a polluted area of Sicily, named Milazzo-Valle del Mela.[10,11] Among the several heavy metals, adolescents living in the polluted area had only increased levels of cadmium, likely due to an enhanced burden of exposure to this heavy metal.[10] In fact, the residential area surrounding the industrial plants of Milazzo-Valle del Mela (i.e. refinery, batteries recycling implant, power plant) is considered to be at high risk of environmental crisis by local authorities.[10] However, the area is also of volcanic origin, and this may concur to the enhanced cadmium burden.

Taking into account that increased cadmium might impair the hypothalamic–pituitary–gonadal axis in males, we evaluated

pubertal status (according to Tanner stages),[12] testicular volume and blood concentration of reproductive hormones in male adolescents of the Milazzo-Valle del Mela area.

Materials and methods

Study design and population

This study was a part of a biomonitoring investigation aimed at evaluating the levels of several heavy metals in adolescents.[10,11] This cross-sectional study was carried out in 111 male children, aged 12–14 years and living in the seven municipalities of the Milazzo-Valle del Mela area (namely, Condrò, Gualtieri Sicaminò, Milazzo, Pace del Mela, San Filippo del Mela, Santa Lucia del Mela and San Pier Niceto). A control age-matched population ($n = 60$) living 28–45 km far from the industrial site and resident in the municipalities of Montalbano Elicona ($n = 16$) and Messina ($n = 44$) was also enrolled. These two sites were chosen to rule out any possible role of other environmental (as volcanic activity from Mount Etna) or anthropogenic factors (as vehicular traffic): in fact, Montalbano Elicona is a rural area, while Messina is a city with a congested urban traffic. Inclusion in the study was on a volunteer base. We met the parents in the different schools, and, after an exhaustive explanation of the project, those concerned for the possible exposure to heavy metals signed the informed consent. We enrolled only healthy, no smoker subjects of Sicilian origin that have been living in the selected area from at least 10 years. Medical visits were performed on Monday, Tuesday and Wednesday afternoon in the outpatient clinic of Milazzo Hospital. During the medical visit, all the children were evaluated by specifically trained personnel that measured height and weight, calculated body mass index (BMI) with the following formula: weight (kg)/height (m^2) and scored pubertal development, according to Tanner.[12] Clinical onset of puberty (stage G2) was considered when testicular volume was >3 ml.

All the subjects underwent an ultrasound evaluation to measure testis volume in upright position with a My Gold Lab 25 (Esaote SpA, Genoa, Italy) equipped with linear, high-resolution and high-frequency (7·5–14 MHz) probe dedicated to the study of soft parts and with colour Doppler for detecting slow flows. Volume was calculated automatically by the software by applying the ellipsoid formula (length × width × thickness × 0·52). Parenchyma echo-structure was considered normal in the presence of thin, densely packed and homogeneously deployed echoes.

Urine levels of cadmium

All children were provided with urine collection containers for 24-h specimens, and their parents were instructed for appropriate procedure and storage. Urine collection was performed 1 or 2 days before the medical visit and stored at 2–6 °C to avoid any loss of sample or contamination. Cadmium urine samples were analysed by blinded technicians on coded samples using an atomic absorption spectrometer procedure.[10,11] The results in the form of descriptive statistics were expressed in μg/l.

Hormone levels

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and inhibin B were evaluated in duplicate by ELISA (Enzyme Linked Immune Absorbent Assay), in agreement with the manufacturers' protocol (DRG International, Inc. Hamburg, Germany). The mean absorbances were calculated and interpolated with those from standard curves, and results were expressed in mIU/ml for FSH and LH, in pg/ml for inhibin B and in nmol/l for testosterone.

Statistical analysis

Data were processed using the statistical software package Stata/MP version 13.0 for Windows. Results were expressed as median values and 95% confidence interval (C.I.), if not stated differently. Data normality was tested with D'Agostino-Pearson normality test, and the differences between Milazzo-Valle del Mela and control population were evaluated with *t*-test for parametric data and Mann–Whitney *U*-test for nonparametric ones. As cadmium levels were detectable in both adolescent groups, all the correlation and linear regressions were calculated in the whole population, using the Spearman's method for nonparametric data or Pearson's test for the parametric ones. To estimate the influence of confounding factors (i.e. Tanner stage and BMI), a multiple linear regression was performed.

Results

Anthropometric measurements

Table 1 shows median and 95% C.I. of age (years), testicular volume (mean and 95% C.I.), urinary cadmium (mean and SD), height (mean and SD) and weight (mean and SD) of the male adolescents living in Milazzo-Valle del Mela area, stratified by Tanner stage. No difference was observed in the main anthropometric measurements (results not shown), of children living in Milazzo-Valle del Mela or in control areas, at least for the Tanner stages G3 and G4 (the only two stages present in the control population). When comparing BMI values of all enrolled adolescents with the chart of body mass index-for-age percentiles developed by the National Center for Chronic Disease Prevention and Health Promotion,[13] no significant difference in centile distribution for Tanner stage 4 was observed (results not shown).

Urinary levels of cadmium

Urinary cadmium levels detected in our population were compared with the values of control group (Table 2) and were also stratified by age. Statistical analysis revealed that cadmium was higher in the Milazzo-Valle del Mela adolescents than in the age-matched controls. Furthermore, cadmium levels (geometric mean 0·58 μg/l) were higher in the studied population as

Table 1. Median and 95% C.I. of age (years), testicular volume (mean and 95% C.I.), urinary cadmium (mean and SD), height (mean and SD) and weight (mean and SD) at each stage of puberty in children from Milazzo-Valle del Mela

Tanner stage (*n*)	Age (12–14 years)	Testicular volume (ml)	Cadmium (μg/l)	Height (cm)	Weight (kg)
G1 (18)	12·72 (12·46–12·98)	1·88 (1·55–2·21)	0·84 ± 0·53	151·4 ± 9·1	49·9 ± 15·1
G2 (34)	13·04 (12·80–13·28)	4·65 (4·32–4·97)	0·68 ± 0·73	156·5 ± 7·4	51·3 ± 14·1
G3 (51)	13·40 (13·24–13·57)	8·37 (7·94–8·80)	0·76 ± 0·56	162 ± 6·7	57·3 ± 12·7
G4 (8)	13·63 (13·36–13·91)	13·93 (12·92–14·93)	0·86 ± 0·19	164·8 ± 8·6	60·7 ± 18·7

Table 2. Mean urinary cadmium (μg/l) and Tanner stages in children from Milazzo-Valle del Mela compared to controls

Age	Milazzo-Valle del Mela	Mean urinary cadmium (95% C.I.)	Tanner stage (n; %)	Controls	Mean urinary cadmium (95% C.I.)	Tanner stage (n; %)
12 years	28	0·59* (0·45–0·73)	G1* (7; 25%) G2* (12; 42·8%) G3* (9; 32·1%) G4 (0)	21	0·11 (0·06–0·16)	G1 (0) G2 (0) G3 (20; 95·2%) G4 (1; 4·7%)
13 years	53	0·84* (0·63–1·05)	G1* (7; 13·2%) G2* (16; 30·1%) G3 (24; 45·2%) G4** (6; 11·3%)	26	0·13 (0·08–0·18)	G1 (0) G2 (0) G3 (11; 42·3%) G4 (15; 57·6%)
14 years	30	0·74* (0·60–0·87)	G1** (4; 13·3%) G2* (6; 20%) G3*** (18; 60%) G4** (2; 6·6%)	13	0·12 (0·05–0·19)	G1 (0) G2 (0) G3 (5; 38·4%) G4 (8; 61·5%)

*$P < 0·0001$ *vs* age-matched controls.
**$P < 0·01$ *vs* age-matched controls.
***$P = 0·008$ *vs* age-matched controls.

compared to age-matched nonexposed adolescents living in Germany[14] (0·08 μg/l) or Canada[15] (0·27 μg/l). In addition, mean cadmium levels were higher than the reference value of 0·50 μg/l, above which an increased risk for adverse health effects has been reported.[16] This clearly indicates that adolescents have an increased cadmium burden.

Sexual maturation: age in a stage

Participants were stratified on the basis of the age at which the various stages of puberty were reached (Table 2). Male adolescents from Milazzo-Valle del Mela showed a delayed sexual maturation compared with age-matched controls. In fact, of the 111 examined adolescents, 18 were still in G1 at a median age of 12·72 years, while only 51 were in stage G3 and 8 in stage G4, indicating that only the 53·15% was in the proper Tanner stage for chronological age. On the other hand, none of the 60 age-matched control adolescents was in stages G1 or G2. In addition, children from Milazzo-Valle del Mela had a significant lower testicular volume in stages G3 and G4 compared with control adolescents ($P = 0·001$ and $P = 0·03$, respectively; Fig. 1a).

Hormonal status and correlation with pubertal stages and cadmium levels

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and inhibin B levels were measured in Milazzo-Valle del Mela adolescents and compared with control population, according to pubertal stages (Table 3). Testosterone levels were significantly lower for stage G3 and G4 ($P = 0·0022$ and $P = 0·0052$, respectively; Table 3, Fig. 1b). LH levels were significantly higher in the Milazzo-Valle del Mela children than in controls at stages G3 and G4 ($P < 0·0001$; Table 3, Fig. 1c). FSH levels were not significantly different between groups (Table 3, Fig. 1d). At stage G3, inhibin B was significantly higher ($P = 0·03$) in adolescents from Milazzo-Valle del Mela compared with controls (Table 3, Fig. 1e). Furthermore, we subdivided adolescents from Milazzo-Valle del Mela into two groups according to being in a proper Tanner stage for chronological age or in an impaired Tanner stage. As shown in Fig. 1f, cadmium levels in children with impaired Tanner stage were significantly higher ($P = 0·0047$).

Correlations and linear regressions

We also performed a correlation analysis between the several parameters, evaluating the influence of confounding factor such as BMI and Tanner stage. Testis volume was significantly related with testosterone levels (Spearman's $r = 0·48$; two-tailed $P < 0·0001$), as shown in Fig. 2a; the linear regression analysis was also statistically significant ($r^2 = 0·56$; P value for slope nonzero: <0·0001). Moreover, testis volume was inversely related with urinary Cd concentration (Spearman's $r = -0·046$; two-tailed $P < 0·001$), and the linear regression was statistically

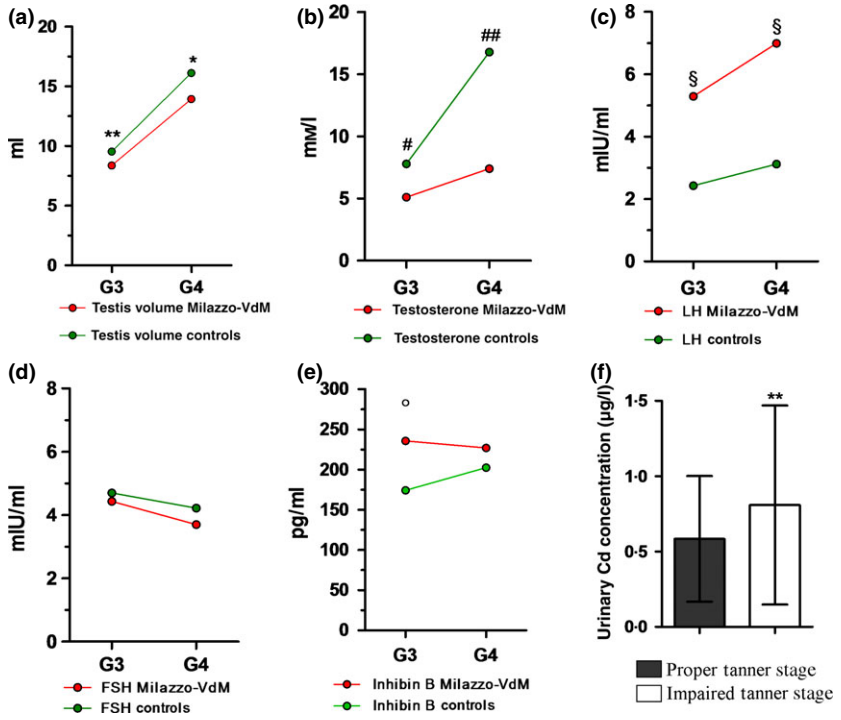

Fig. 1 Mean values reported for adolescents living in Milazzo-Valle del Mela compared with age-matched controls. (a) Mean testicular volume $^{*}P = 0{\cdot}03$; $^{**}P = 0{\cdot}001$. (b) Mean testosterone levels $^{\#}P = 0{\cdot}0022$; $^{\#\#}P = 0{\cdot}0052$. (c) Mean LH levels $^{\S}P < 0{\cdot}0001$. (d) Mean FSH levels. (e) Mean inhibin B levels $P = 0{\cdot}0329$. (f) Urinary cadmium (Cd) levels in male adolescents with a proper or delayed Tanner stage for chronological age. Data are plotted as mean ± SEM. $P = 0{\cdot}0047$ *vs* proper Tanner.

Table 3. Mean ± SD of FSH, LH, testosterone and inhibin B values at each stage of puberty in the Valle del Mela male adolescents compared with values reported for our age-matched control population

	Milazzo-Valle del Mela				Controls			
Tanner (n)	G1 (18)	G2 (34)	G3 (51)	G4 (8)	G1 (0)	G2 (0)	G3 (36)	G4 (24)
FSH (mIU/ml)	1·89 ± 1·29	3·75 ± 2·76	4·43 ± 3·27	3·70 ± 2·40	–	–	4·64 ± 1·99	4·3 ± 2·04
Min–Max	0·28–4·32	0·78–13·35	1·06–18·04	0·5–7·43			1·21–9·36	1·12–9·65
LH (mIU/ml)	3·18 ± 2·20	4·10 ± 2·43	5·29 ± 2·37*	6·99 ± 2·75*	–	–	2·43 ± 1·05	3·12 ± 0·88
Min–Max	1·31–8·76	0·83–10·99	2–10·99	3·31–10·51			1·11–5·89	2·14–5·4
Testosterone (nmol/l)	0·5 ± 0·2	2·6 ± 2·9	5·1 ± 3·4**	7·4 ± 5·3***	–	–	7·77 ± 4·52	16·78 ± 8·15
Min–Max	0·1–0·8	0·1–9·9	0·7–18·7	1·7–13·7			4·01–24·64	7·82–35·9
Inhibin B (pg/ml)	214 ± 183·5	269·2 ± 177·3	182·8 ± 104·2****	258·1 ± 173	–	–	174·3 ± 40·18	202·5 ± 49·20
Min–Max	65·11–704·4	72·09–704·4	57·44–705·7	114·1–539·3			108·2–265·2	112·2–298·0

*$P < 0{\cdot}0001$ *vs* Controls at same stage.
**$P = 0{\cdot}0022$ *vs* Controls at same stage.
***$P = 0{\cdot}0052$ *vs* Controls at same stage.
****$P = 0{\cdot}03$ *vs* Controls at same stage.

significant ($r^2 = 0{\cdot}11$; P value for slope nonzero: <0·0001; Fig. 2b). Testosterone and Cd levels were inversely related (Spearman's $r = -0{\cdot}037$; two-tailed $P < 0{\cdot}0001$), with a significant linear regression ($r = 0{\cdot}2$; P value for slope nonzero: <0·0001; Fig. 2c). Furthermore, we performed the same analysis considering the LH serum levels as dependent variable and urinary Cd concentration as independent variable: the correlation was statistically significant (Spearman's $r = 0{\cdot}048$; two-tailed $P < 0{\cdot}05$), and the linear regression analysis showed an $r^2 = 0{\cdot}09$ (P value for slope nonzero: <0·05; Fig. 2d). Finally, we did not find any significant correlation between urinary cadmium levels and inhibin B or FSH nor between inhibin B and the several hormones.

Discussion

Cadmium has several adverse effects on human health[17]; occupational investigations in adult men and experimental studies,[8,9] with high exposure levels, support an adverse and harmful role for cadmium; however, there is a paucity of data in childhood. Children may be more susceptible to toxic exposure than adults because they eat more food contaminants and they are subjected to susceptible socio-behavioural activities, multiple exposure pathways and active developmental processes.[18]

Our population had urinary Cd levels higher than those reported by the human biomonitoring (HBM) programmes in adolescents.[14–16] The proposed HBM level I represents the

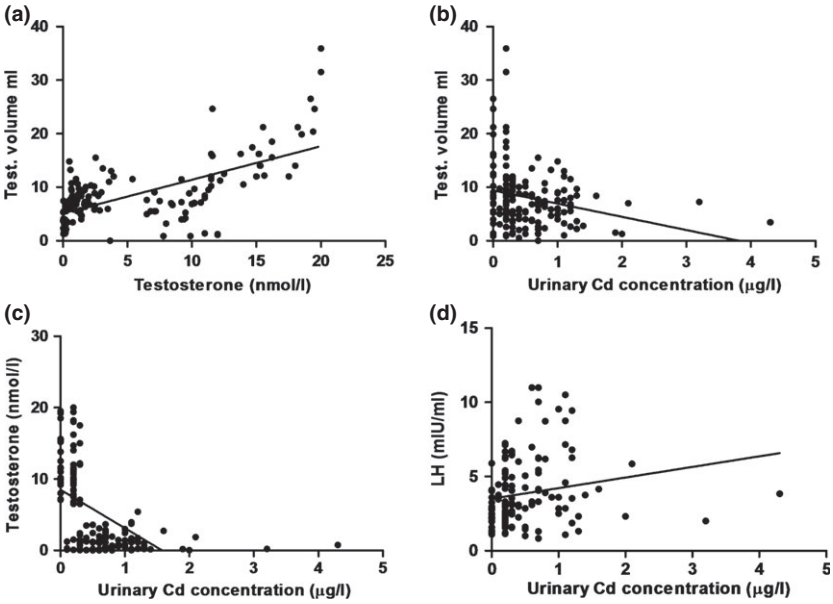

Fig. 2 (a) Linear regression analysis between testicular volume and testosterone levels in male adolescents. $r^2 = 0{\cdot}4$; $P < 0{\cdot}0001$. (b) Linear regression analysis between testicular volume and urinary cadmium (Cd) in male adolescents. $r^2 = 0{\cdot}06$; $P = 0{\cdot}0012$. (c) Linear regression analysis between urinary cadmium (Cd) and testosterone levels in male adolescents. $r^2 = 0{\cdot}28$; $P < 0{\cdot}0001$. (d) Linear regression analysis between urinary cadmium (Cd) and LH levels in male adolescents. $r^2 = 0{\cdot}09$; $P < 0{\cdot}05$.

concentration of a substance, in human biological material, below which there is no risk for adverse health effects and therefore no need for action, while the HBM level II is the concentration of a substance above which there is an increased risk for adverse health effects and, consequently, an urgent need to reduce exposure and to provide individual biomedical care. The observed values fell in the range between HBM I and HBM II, suggesting a chronic exposure and possible negative health effects on adolescents living in the Milazzo-Valle del Mela area, an industrial site in northern Sicily (south of Italy). However, we do not know whether this may be influenced by the geographical position: in fact, we lack data about urinary cadmium levels in adolescents resident in the industrialized sites of northern Italy. Indeed, we showed for the first time that the increased cadmium burden delays the onset of puberty in male adolescents, resident in a high risk area of Sicily. The increased levels of Cd caused remarkable changes in the mean ages of Tanner stages for chronological age and reduced testicular volume and testosterone levels. Thus, it can be speculated that testicular development is negatively influenced by an enhanced burden of Cd exposure in male adolescents, as previously suggested in experimental studies.[19–23] Cd is characterized by long-lasting biological half-life likely due to its low excretion rate from the kidney. As a consequence of this kinetic profile, Cd prolonged exposure exerts toxic effects in several tissues, including the testis.[19–24] Furthermore, it may increase the blood–brain barrier permeability and may cause neurotoxic effects likely due to biochemical cell alterations and functional changes in the central nervous system.[25] *In vivo* experimental studies have also shown that Cd affects the HPG axis function by acting at each of the three levels, and the net result of this interaction depends on the age and the timing of exposure.[26] Furthermore, this xenotoxic may also play a role in the aetiology of gonadal dysfunction.[23,27] Accordingly, testosterone levels were markedly lower in our adolescents when compared with controls. This led us to hypothesize that the delay in the onset of puberty with the concomitant impaired testis growth may be due to a direct toxic effect of cadmium on the Leydig cells. For this reason, we performed a linear regression analysis to correlate urinary Cd levels with testosterone in all included children and we found a significant correlation, suggesting that cadmium exposure disrupts testosterone production at testis level, likely interfering with secretion and/or production of the hormone by the Leydig cells, as previously reported in experimental animal models.[21,22] However, we did not find any significant change in the anthropometric parameters in our studied populations: this discrepancy might be due to the complex interplay of several hormones and genetic factors influencing growth.

We therefore tried to identify other possible site(s) of the endocrine-disrupting effects of cadmium by measuring the levels of FSH, LH and inhibin B. FSH levels were not significantly changed in our children when compared to controls. Moreover, LH levels were significantly higher in our adolescents compared with the children living far from the industrial plants. Furthermore LH levels showed a positive correlation with urinary cadmium levels, taking in consideration the Tanner stage and the BMI as confounding factors. This latter result may be due to the feedback loop triggered by the reduced testosterone levels that lead to an exaggerated release of LH, as a compensatory mechanism to counteract the reduced testosterone production. In G3 stage, inhibin B levels were significantly higher in the studied children compared with controls: this may happen when testis growth and pubic hair appearance are poor, as previously reported.[28] Furthermore, no correlation was found between urinary cadmium and inhibin B levels.

The present study has some limitations as we did not measure sex hormone-binding protein (SHBG) because of the insufficient amount of sera, nor spermatogenesis (that would be useful to understand the relationship between cadmium and sexual maturation) because of bioethical issues. Furthermore SHBG is

included into the diagnostic regimens for suspected hypogonadism only when total testosterone results are equivocal.[29] Nevertheless, this study is of particular importance pointing out the need of a close health risk assessment to favour the implementation of preventive measures in male adolescents exposed to Cd and to avoid late complication on fertility and gonad function.

Acknowledgements

Nothing to declare. The authors are grateful to Dr. Roberto Vita for his support. The investigation was granted by a Sicilian Government funding.

References

1 Ademuyiwa, O., Agarwal, R., Chandra, R. *et al.* (2010) Effects of sub-chronic low-level lead exposure on the homeostasis of copper and zinc in rat tissues. *Journal of Trace Elements in Medicine and Biology*, **24**, 207–211.
2 Kakkar, P. & Jaffery, F.N. (2005) Biological markers for metal toxicity. *Environmental Toxicology and Pharmacology*, **19**, 335–349.
3 Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C. *et al.* (2009) Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine Review*, **30**, 293–342.
4 Mazej, Z., Al Sayegh-Petkovsek, S. & Pokorny, B. (2010) Heavy metal concentrations in food chain of Lake Velenjsko jezero, Slovenia: an artificial lake from mining. *Archives of Environmental Contamination and Toxicology*, **58**, 998–1007.
5 Qiao, M., Cai, C., Huang, Y. *et al.* (2011) Characterization of soil heavy metal contamination and potential health risk in metropolitan region of northern China. *Environmental Monitoring and Assessment*, **172**, 353–365.
6 Aitken, R.J. & Baker, M.A. (2013) Causes and consequences of apoptosis in spermatozoa; contributions to infertility and impacts on development. *International Journal of Developmental Biology*, **57**, 265–272.
7 Lafuente, A. (2013) The hypothalamic-pituitary-gonadal axis is target of cadmium toxicity. An update of recent studies and potential therapeutic approaches. *Food and Chemical Toxicology*, **59**, 395–404.
8 Lafuente, A., Márquez, N., Pérez-Lorenzo, M. *et al.* (2000) Pubertal and postpubertal cadmium exposure differentially affects the hypothalamic-pituitary-testicular axis function in the rat. *Food and Chemical Toxicology*, **38**, 913–923.
9 Ciarrocca, M., Capozzella, A., Tomei, F. *et al.* (2013) Exposure to cadmium in male urban and rural workers and effects on FSH, LH and testosterone. *Chemosphere*, **90**, 2077–2084.
10 Interdonato, M., Bitto, A., Pizzino, G. *et al.* (2014) Levels of heavy metals in adolescents living in the industrialised area of milazzo-valle del mela (northern sicily). *Journal of Environmental and Public Health*, **2014**, 326845.
11 Pizzino, G., Bitto, A., Interdonato, M. *et al.* (2014) Oxidative stress and DNA repair and detoxification gene expression in adolescents exposed to heavy metals living in the Milazzo-Valle del Mela area (Sicily, Italy). *Redox Biology*, **2**, 686–693.
12 Tanner, J.M. & Whitehouse, R.H. (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of Disease in Childhood*, **51**, 170–179.
13 Kuczmarski, R.J., Ogden, C.L., Guo, S.S. *et al.* (2002) 2000 CDC growth charts for the United States: Methods and development. National Center for Health Statistics, Vital and Health Statistics Series.
14 Umweltbundesamt (UBA). Report 2010 Health and Environmental Hygiene: German Environmental Survey (GerES). Umweltbundesamt, Berlin. http://www.uba.de
15 Health Canada. (2010) Report of human biomonitoring of environmental chemicals. Results of the Canadian Health Measures Survey Cycle 1 2007–2009, Ottawa.
16 Schulz, C., Wilhelm, M., Heudorf, U. *et al.* (2012) Update of the reference and HBM values derived by the German Human Biomonitoring Commission. *International Journal of Hygiene and Environmental Health*, **215**, 149.
17 Bernhoft, R.A. (2013) Cadmium toxicity and treatment. *Scientific World Journal* **2013**, 394652. Review.
18 Au, W.W.. (2002) Susceptibility of children to environmental toxic substances. *International Journal of Hygiene and Environmental Health* **205**, 501–503. Review.
19 Ji, Y.L., Wang, H., Liu, P. *et al.* (2010) Pubertal cadmium exposure impairs testicular development and spermatogenesis via disrupting testicular testosterone synthesis in adult mice. *Reproductive Toxicology*, **29**, 176–183.
20 Liu, Q., Gu, J.H., Yuan, Y. *et al.* (2013) Effect of cadmium on rat Leydig cell testosterone production and DNA integrity in vitro. *Biomedical and Environmental Sciences*, **26**, 769–773.
21 Luca, G., Lilli, C., Bellocci, C. *et al.* (2013) Toxicity of cadmium on Sertoli cell functional competence: an in vitro study. *Journal of Biological Regulators & Homeostatic Agents*, **27**, 805–816.
22 Ohtani, K., Yanagiba, Y., Ashimori, A. *et al.* (2013) Influence of injection timing on severity of cadmium-induced testicular toxicity in mice. *Journal of Toxicological Sciences*, **38**, 145–150.
23 Wang, B. & Du, Y. (2013) Cadmium and its neurotoxic effects. *Oxidative Medicine and Cellular Longevity* **2013**, 898034. Review.
24 Foote, R.H. (1999) Fertility of rabbit sperm exposed in vitro to cadmium and lead. *Reproductive Toxicology*, **13**, 443–449.
25 Lafuente, A., Márquez, N., Pérez-Lorenzo, M. *et al.* (2001) Cadmium effects on hypothalamic-pituitary-testicular axis in male rats. *Experimental Biology and Medicine*, **226**, 605–611.
26 Lafuente, A., González-Carracedo, A., Romero, A. *et al.* (2004) Cadmium exposure differentially modifies the circadian patterns of norepinephrine at the median eminence and plasma LH, FSH and testosterone levels. *Toxicology Letters*, **146**, 175–182.
27 Lafuente, A. & Esquifino, A.I. (1999) Cadmium effects on hypothalamic activity and pituitary hormone secretion in the male. *Toxicology Letters*, **110**, 209–218.
28 Andersson, A.M., Juul, A., Petersen, J.H. *et al.* (1997) Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *Journal of Clinical Endocrinology and Metabolism.*, **82**, 3976–3981.
29 Ho, C.K., Stoddart, M., Walton, M. *et al.* (2006) Calculated free testosterone in men: comparison of four equations and with free androgen index. *Annals of clinical biochemistry*, **43**, 389–397.