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Semen quality and reproductive endocrine function with regard to blood cadmium in Croatian male subjects

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Abstract

In 123 Croatian men with no occupational exposure to metals, the influence of cadmium on reproductive parameters was examined after adjusting for age, smoking, alcohol, and biomarkers of lead, copper, zinc, and selenium. The following variables were measured: blood cadmium (BCd), blood lead (BPb), activity of δ-aminolevulinic acid dehydratase (ALAD), erythrocyte protoporphyrin, serum copper (SCu), serum zinc (SZn), serum selenium (SSe), activity of glutathione peroxidase (GPx) in blood, testis size, semen quality (including sperm concentration, motility, viability, and morphology), indicators in seminal fluid (the lactate dehydrogenase isoenzyme LDH-C_4, fructose, zinc, acid phosphatase, and citric acid), and hormones in serum (follicle-stimulating hormone – FSH, luteinizing hormone, prolactin, testosterone, and estradiol). The median and range BCd values were 2.94 (0.49-11.93) μg/L in 61 smokers and 0.59 (0.20-3.71) μg/L in 62 nonsmokers ($p < 0.0001$). Smoking habits (cigarettes/day) highly significantly correlated with BCd ($p < 0.0001$). After adjusting for potential confounding variables by multiple regression, BCd was significantly associated with a decrease in testis size ($p < 0.03$) and an increase in serum estradiol ($p < 0.005$), FSH ($p < 0.03$), and testosterone ($p < 0.04$). Smoking was significantly associated with a decrease in serum prolactin ($p < 0.006$) and LDH-C_4 in seminal fluid ($p < 0.03$). Several reproductive parameters were significantly associated with BPb and ALAD, biomarkers of lead, and/or with SCu, SZn, SSe, and GPx. The necessity of controlling for various metals, and other potential confounders when assessing the influence of a particular metal on reproductive function in men, is emphasized.

Introduction

Cadmium (Cd) and lead (Pb) are toxic metals, pervasive in the human environment and are known to accumulate in the human body over a lifetime, including prenatal life (especially Pb). Many experimental animal studies have shown that both Cd and Pb can adversely affect the mammalian male reproductive system. On the other hand, epidemiologic studies are equivocal about the effects of Cd and/or Pb on human sperm parameters, hormone concentrations, and male fertility. For example, decreased sperm concentration and sperm count values in apparently healthy men were associated with Cd but not Pb (Xu *et al.* 2003), whereas the opposite was observed in another study of subjects with relatively higher Pb exposure (Telišman *et al.* 2000). It was also noticed that selenium (Se) showed the reverse reproductive effects to those of Cd (Xu *et al.* 2003), whereas zinc (Zn) showed the reverse reproductive effects to those of Pb, and Cd (Telišman *et al.* 2000) in the same subjects. This is in accordance with the results of experimental animal studies, indicating that Se and Zn can reduce the toxicity of both Cd and Pb (Nordberg 1978, Telišman 1995). Furthermore, recent evidence suggests that oxidative damage to sperm DNA plays a critical role in the etiology

Table 1. Median and range values of relevant parameters in 123 Croatian male subjects with no occupational exposure to metals

Parameter	Median (range)
Age (years)	31 (19-48)
Smoking (cigarettes/day)	0 (0-40)
Alcohol (drinks*/week)	3 (0-56)
BCd (μg/L)	0.85 (0.20-11.93)
BPb (μg/L)	57 (25-149)
ALAD (European units)	52.4 (22.8-96.4)
EP (μmol/L erythrocytes)	0.68 (0.38-1.68)
SCu (μg/L)	1112 (763-1662)
SZn (μg/L)	961 (734-1213)
SSe (μg/L)	72.7 (51.9-106.9)
GPx (U/g Hb)	132.5 (91.0-164.5)

*One drink = 3 dL beer, 1 dL wine, or 0.3 dL brandy

of poor semen quality and male infertility (Kodama *et al.* 1997, Shen *et al.* 1999), implicating a possible role of Cd (Xu *et al.* 2003) and Pb (Quintanilla-Vega *et al.* 2000) in this respect. Namely, Cd and Pb can interfere with the absorption, retention, distribution, and biologic availability of Se, Zn, and copper (Cu) in the body, resulting in relative deficiency of these essential elements (Nordberg 1978, Thijs *et al.* 1992, Telišman 1995, Jurasović *et al.* 2000, Pizent *et al.* 2003), which are required for optimum activity of the important antioxidant enzymes, superoxide dismutase (Cu,Zn-SOD) and glutathione peroxidase (Se-GPx), involved in the protection of cells and DNA against oxidative damage.

Although it is known that various toxic and essential metals are interactive, very little information is available on their possible *combined* effect on human male reproductive function. For example, human occupational and environmental exposure to Cd and Pb is often accompanied by considerable exposure to Zn, and *vice versa*, which may act as an atagonist and thus mask the Cd- and/or Pb-related effects; both Cd and Pb can adversely affect Zn metabolism, and possibly Cu metabolism; and Cu and Zn can antagonistically affect each other's absorption rate and metabolism (Telišman 1995). The present study considers the relationship between simultaneously measured parameters of semen quality and of reproductive endocrine function with respect to blood Cd (BCd) in men with no occupational exposure to metals, after adjusting for possible confounding variables such as age, smoking habits, alcohol consumption, and biomarkers of Pb, Cu, Zn, and Se by multiple regression. Activity of δ-aminolevulinic acid dehydratase (ALAD) in blood and erythrocyte protoporphyrin (EP) concentration, biomarkers of Pb exposure and/or effect, were used in addition to blood Pb (BPb) for better evaluation of long-term cumulative exposure to Pb (Alessio *et al.* 1981, Telišman *et al.* 1982). Serum concentrations of Cu (SCu), Zn (SZn), and Se (SSe) were used as common biomarkers of the status of these essential elements in the body. Activity of glutathione peroxidase (GPx) in blood was used in addition to SSe for better evaluation of Se status in the body. Possible influence factors of age, smoking habits, and alcohol consumption on reproductive parameters were evaluated, bearing in mind that they commonly correlate with biomarkers of Cd, Pb, Cu, Zn, or Se in humans. With regard to the hypothesis that Cd induces reproductive effects in men, these variables may be both potential confounders and risk factors.

Materials and methods

Study population

The study was cross-sectional in design. It was carried out in 123 Croatian male subjects, 19–48 years of age, who had never been occupationally exposed to metals. The subjects were randomly selected among those reporting for examination in the andrology unit of the Vuk Vrhovac Clinic in Zagreb. The group contained subjects from couples suspected of infertility of unknown etiology (who were not necessarily abnormal themselves) and voluntary semen donors for artificial insemination, examined in random order under identical conditions. None of the selected subjects had been occupationally exposed to pesticides, herbicides, ionizing or microwave irradiation, organic solvents, anesthetics and vinyl chloride, or had urinary tract infection, varicocele, cryptorchidism, hypogonadism, digitorectal indication of prostatitis, indication of chronic orchoepididymitis and history of genital region trauma, or used dietary supplements containing Cu, Zn, Se, Mg and Fe, which could influence male reproductive parameters or metal metabolism. The participation rate was 91% of eligible subjects based on the above-mentioned selection criteria. Namely, 12 individuals out of the 135 originally subscribed subjects refused to participate in the study. All subjects gave informed consent before inclusion in the study. The study was performed in accordance with

the ethical principles for medical research involving human subjects (the Helsinki Declaration, as revised in October 2000) and was approved by the Institutional Review Board.

A questionnaire including data on age, dietary habits, smoking, alcohol consumption, and professional and medical history was completed by a physician for each of the 123 subjects. Most of the subjects lived in the area of Zagreb, whereas the remaining lived in other parts of Croatia (86 and 37 subjects, respectively). All of the subjects consumed a mixed diet (none were vegetarian). There were 61 smokers and 62 nonsmokers, and 95 alcohol consumers and 28 nonconsumers of alcohol.

Sampling and analyses of biologic specimens

The ejaculate (by masturbation) and venous blood were sampled between 0800 and 1000 h for each subject. All subjects were required to fast in the preceding 10 h, abstain from alcohol in the preceding 24 h, and avoid any sexual activity in the preceding 4 days. The specimens of each subject were sampled in the Vuk Vrhovac Clinic. Special care was taken to avoid any contamination with metals during the ejaculate and blood sampling, storage, and analyses. The chemicals used for metal analyses were of analytical grade for spectroscopy (Merck, Darmstadt, Germany).

The BCd and BPb measurements were performed by the electrothermal atomic absorption spectrometry (AAS) method, essentially the same as the one described for seminal fluid Pb and Cd determination (Jurasović & Telišman 1993), and controlled each day for accuracy by analyzing three reference blood samples with certified BCd and BPb values: BCR No. 194-196 (Community Bureau of Reference, European Commission, Brussels, Belgium). The accuracy of both BCd and BPb measurements was also controlled by the laboratory's regular participation in the National External Quality Assessment Scheme (NEQAS, Birmingham, UK) and our mean running variance index score (MRVIS) was consistently lower than the average MRVIS for all participants. ALAD activity was measured < 5 h after blood sampling (blood was stored at 4 °C) using the European standardized method (Berlin & Schaller 1974). EP was measured by spectrofluorometric method (Chisolm & Brown 1975) and the accuracy was controlled by the laboratory's regular participation in the Erythrocyte Protoporphyrin Proficiency Testing Program (EPPTP, Madison, WI, USA). Blood hemoglobin (Hb) and hematocrit were measured by standard hematologic method. The SCu and SZn measurements were performed by flame AAS method (Pizent & Telišman 1996) and controlled each day for accuracy by analyzing two reference serum samples with certified SCu and SZn values: Cation-Cal (American Dade, Miami, FL, USA) and Seronorm (Nycomed Pharma, Oslo, Norway). SSe was measured by electrothermal AAS method (Gammelgaard & Jøns 1997) and the accuracy was controlled each day by analyzing two reference serum samples with certified SSe values: Seronorm (Nycomed Pharma, Oslo, Norway) and Second Generation (J. Versieck, Gent, Belgium). The accuracy of SCu, SZn, and SSe measurements was also controlled by the laboratory's regular participation in the Trace Elements External Quality Assessment Scheme (TEQAS, Guildford, UK) and our results were categorized as being Acceptable (as opposed to Borderline or Unacceptable as the remaining two options). GPx activity in blood was measured by the European standardized method (Belsten & Wright 1995) and the results are expressed per gram Hb (U/g Hb).

Measurements of reproductive parameters included: testis size; semen volume, pH, and liquefaction time; percentages of leucocytes, erythrocytes, and immature sperm cells; sperm concentration and count, sperm motility, viability, morphology, and hypoosmotic swelling (HOS) test; indicators in seminal fluid: the lactate dehydrogenase isoenzymes fraction C_4 (LDH-C_4), fructose, Zn (SfZn), acid phosphatase, and citric acid; and hormones in serum: follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, and estradiol.

Macroscopic and microscopic examination of semen was performed according to World Health Organization (WHO) guidelines (WHO 1999). Sperm concentration and motility were determined at 37 °C within 1 h after the sampling of ejaculate, by using computer-aided sperm analysis (CASA) technique on a Hamilton-Thorne Research IVOS, version 10, semen analyzer. CASA outcomes included indicators of sperm progression, and vigor. Sperm morphology was evaluated on air-dried Papanicolau-stained samples with regard to sperm shape (thin, tapered, round, and amorph), sperm size (too long, too short, too wide, and too narrow), acrosome presence, and midpiece and tail abnormalities.

The LDH-C_4 (which is often called LDH-X) in seminal fluid was measured by electrophoresis (Gavella *et al.* 1982), whereas fructose (Gavella 1981), SfZn and acid phosphatase (Gavella 1988), and citric

Table 2. Spearman correlation coefficient and the level of significance (r, p) for relationships between relevant parameters in 123 male subjects

	Smoking	Alcohol	BCd	BPb	ALAD	EP	SCu	SZn	SSe	GPx
Age	0.043	0.020	0.046	0.201*	0.004	0.051	0.197*	−0.022	0.121	0.016
Smoking	–	0.208*	0.808****	0.042	−0.248**	0.055	0.227*	−0.055	−0.261**	−0.302***
Alcohol		–	0.099	0.293***	−0.362****	−0.036	0.243**	−0.068	−0.140	0.041
BCd			–	0.083	−0.137	0.166	0.160	−0.145	−0.196*	−0.189*
BPb				–	−0.267**	−0.028	0.112	−0.113	0.031	−0.012
ALAD					–	0.065	0.004	0.116	−0.011	0.003
EP						–	−0.020	−0.069	0.084	0.143
SCu							–	−0.042	−0.070	−0.018
SZn								–	0.198*	−0.022
SSe									–	0.308***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

acid (Gavella 1983) in seminal fluid were measured by automated methods using an Abbot bichromatic analyzer.

Serum concentrations of FSH, LH, prolactin, testosterone, and estradiol were measured by fluoroimmunoassay using commercial kits (DELFIA, Pharmacia, Uppsala, Sweden).

Statistical methods

Because of the skewed distribution of most of the measured parameters, the results are presented as median and range, and the significance of the difference between subgroups was calculated by using the Mann-Whitney U-test (z, p). Spearman correlation (r, p) was calculated for the association between different parameters. Forward stepwise multiple regression was used to calculate the inter-relationship of all the parameters considered possible explanatory variables (which were simultaneously introduced in the model) with respect to each of the measured reproductive parameters.

Results

Table 1 shows data for relevant parameters in the study population, whereas Table 2 shows the correlation results between these parameters. Increased exposure to Cd was mostly through smoking and thus the difference in BCd was highly significant ($z = 8.544$, $p < 0.0001$) between the 61 smokers and 62 nonsmokers; the median and range BCd values were 2.94 (0.49-11.93) μg/L and 0.59 (0.20-3.71) μg/L, respectively. A highly significant correlation was found between smoking habits (cigarettes/day) and BCd in all 123 subjects ($p < 0.0001$) (Table 2). In addition, smoking significantly correlated with an increase in SCu ($p < 0.05$) and alcohol consumption (drinks/week) ($p < 0.05$), and with a decrease in ALAD ($p < 0.01$), SSe ($p < 0.01$) and GPx ($p < 0.001$), whereas BCd significantly correlated with a decrease in SSe ($p < 0.05$) and GPx ($p < 0.05$). These and other significant correlations (Table 2) indicate a complex association among most of the variables, suggesting the possibility that certain variables may act as confounding factors and thus mask a causal relationship between BCd and reproductive parameters.

The study results showed no significant correlation between BCd and parameters of semen quality, whereas a significant correlation was found between BCd or smoking and certain parameters of male reproductive endocrine function. For example, serum prolactin significantly inversely correlated with BCd ($r = -0.271$, $p < 0.01$) and age ($r = -0.245$, $p < 0.01$) and positively with ALAD ($r = 0.191$, $p < 0.05$) and SZn ($r = 0.193$, $p < 0.05$); serum testosterone inversely correlated with age ($r = -0.269$, $p < 0.01$) and smoking ($r = -0.257$, $p < 0.01$); and serum estradiol positively correlated with BCd ($r = 0.257$, $p < 0.01$) and smoking ($r = 0.263$, $p < 0.01$). Table 3 shows data on the serum concentrations of reproduction hormones in the study population, which ranged from normal to pathologic as compared to the reference range of these parameters.

Because a combined influence of certain variables may have masked possible Cd-related reproductive effects, multiple regression was used to evaluate the association of reproductive parameters with BCd after adjusting for potential confounding variables. In sep-

Table 3. Median and range values of serum hormones in 123 male subjects, compared to the reference range

Parameter	Median (range)	Reference range
FSH (U/L)	3.8 (0.6–40.5)	1.0–10.5
LH (U/L)	3.5 (1.0–23.1)	1.0–5.8
Prolactin (μg/L)	5.4 (1.9–16.6)	2.3–11.5
Testosterone (nmol/L)	21.1 (9.3–39.7)	8.7–33.0
Estradiol (nmol/L)	0.10 (0.02–0.20)	0–0.13

arate regression models, the inter-relationship of BCd and all of the remaining potential explanatory variables (BPb, ALAD, and EP, respectively, and SCu, SZn, SSe, GPx, age, smoking, and alcohol) with respect to each of the measured reproductive parameters was calculated by forward stepwise multiple regression. Different models included either nontransformed or log-transformed values of BCd and BPb. Table 4 shows a summary of multiple regression results concerning significant ($p < 0.05$) associations between certain reproductive parameters and potential explanatory variables in the study population. Beta is the standardized regression coefficient, whereas the coefficient B value relates to the units of measurement actually used for the dependent variable and independent (explanatory) variables in the equation. The results showed that an increase in BCd was significantly associated with a decrease in testis size ($p < 0.03$) and an increase in serum estradiol ($p < 0.005$), FSH ($p < 0.03$), and testosterone ($p < 0.04$), but not with any parameter of semen quality. In addition, significant ($p < 0.05$) associations were found between several reproductive parameters and biomarkers of Pb (BPb and ALAD) as well as SCu, SZn, SSe, GPx, age, smoking, or alcohol (Table 4).

Discussion

The BCd level in the study population (Table 1) is slightly higher as compared to general population groups from other countries. This can mainly be ascribed to smoking habits and the relatively high Cd content in Croatian cigarettes; in heavy smokers, BCd levels of up to 13 μg/L are commonly found (Vahter 1982, Telišman *et al.* 1986, 1997, 2000). The reported range of tobacco Cd content in several brands of domestic cigarettes purchased in Zagreb, Croatia, was 1.08–4.33 μg/cigarette (Ivičić *et al.* 1985), with an average Cd level of approximately 2.6 μg/g which is very similar to the reported average Cd level of 2.7 μg/g in Mexican cigarettes (Watanabe *et al.* 1987). The published range of Cd content in cigarettes produced in different countries was 0.19–3.04 μg/g (Elinder *et al.* 1983) and 0.29–3.38 μg/g (Watanabe *et al.* 1987); both studies found the lowest Cd level in cigarettes from India and the highest Cd level in cigarettes from Mexico. It is interesting to note that Cd content in cigarettes was low in India (Elinder *et al.* 1983, Watanabe *et al.* 1987) where the cigarette smoking-induced increment in BCd was very small (Vahter 1982), whereas it was high in Mexico (Elinder *et al.* 1983, Watanabe *et al.* 1987) and in Croatia (Ivičić *et al.* 1985) where the increment in BCd was large (Vahter 1982). The geometric mean BCd values reported for 10 countries, including Belgium, China, India, Israel, Japan, Mexico, Peru, Sweden, USA and Croatia (in former Yugoslavia), ranged from 0.2 μg/L (Sweden) to 1.5 μg/L (Belgium) for nonsmokers, and from 0.6 μg/L (India) to 2.8 μg/L (Croatia) for smokers (Vahter 1982), whereas those of a meta analysis in Italian subjects were 0.52 μg/L for nonsmokers and 1.47 μg/L for smokers (Alessio *et al.* 1993). The BCd levels for nonsmokers in the present study (median BCd 0.59 μg/L), as well as in our previous studies (Vahter 1982, Telišman *et al.* 1986, 1997, 2000), are comparable to those in many other countries.

The BPb level in the study population (Table 1) is considerably lower as compared to the median BPb value of 135 μg/L in 60 Croatian male teachers examined in 1981 (Vahter 1982), which is mainly because of a less prevalent use of gasoline containing Pb. However, the BPb level is still relatively high as compared to developed countries where efforts for abatement of Pb exposure started earlier and have been more efficient. For example, the reported geometric mean BPb values for the general population groups from the USA (Brody *et al.* 1994) and Japan (Watanabe *et al.* 1996) were 28 μg/L and 23.2 μg/L, respectively.

The levels of SCu, SZn, and SSe in the study population (Table 1) are comparable to those of healthy adult general population groups in many other countries, although the range of published values is relatively wide. This may partly be attributed to concomitant exposure to other metals and their interaction (especially in industrial areas) as well as different dietary habits and contents of Cu, Zn, Se, and other metals in food. For example, the reported range for

Table 4. Summary of multiple regression results concerning significant ($p < 0.05$) associations between reproductive parameters and potential explanatory variables (BCd, BPb, ALAD, EP, SCu, SZn, SSe, GPx, age, smoking, and alcohol) in 123 male subjects

Variable	Beta	Coefficient (B)	Standard error of B	p_B
Testis size				
ALAD	0.213	0.357	0.148	0.018
BCd	−0.284	−2.249	0.965	0.021
Slow sperm				
Log BPb	0.222	8.423	3.682	0.024
Pathologic sperm				
SCu	−0.236	−0.014	0.006	0.021
Normal sperm				
SCu	0.204	0.016	0.008	0.045
BPb	−0.197	−0.084	0.042	0.050
SSe	0.213	0.191	0.096	0.048
Subnormal sperm				
BPb	−0.290	−0.035	0.012	0.0041
SSe	0.274	0.069	0.025	0.0061
Too short sperm				
SCu	−0.206	−0.007	0.004	0.046
Too wide sperm				
BPb	0.350	0.142	0.038	0.00029
Thin sperm				
SSe	−0.215	−0.141	0.067	0.038
Tapered sperm				
Alcohol	0.243	0.204	0.083	0.017
GPx	−0.213	−0.101	0.050	0.049
Amorph sperm				
SCu	−0.237	−0.015	0.006	0.019
SSe	−0.245	−0.180	0.077	0.022
LDH-C_4				
ALAD	0.254	0.251	0.089	0.0056
Smoking	−0.216	−0.209	0.089	0.021
SfZn				
SZn	0.197	0.150	0.070	0.036
Acid phosphatase				
SZn	0.183	0.533	0.269	0.050
FSH				
Log BCd	0.194	2.458	1.099	0.027
LH				
SCu	0.216	0.004	0.001	0.016
SZn	−0.180	−0.004	0.002	0.050
Prolactin				
Smoking	−0.263	−0.062	0.022	0.0058
Age	−0.222	−0.106	0.045	0.019
Testosterone				
Age	−0.266	−0.256	0.084	0.0027
Log BCd	0.188	2.411	1.126	0.034
Estradiol				
Log BCd	0.271	0.020	0.007	0.0042

SCu was 600–1760 μg/L in Italian subjects (Minoia *et al.* 1990) and 585–2027 μg/L in German subjects (Rükgauer *et al.* 1997), and that for SZn was 540–1510 μg/L in Italian subjects (Minoia *et al.* 1990) and 608–1510 μg/L in German subjects (Rükgauer *et al.* 1997). Variations in SSe levels are particularly pronounced, both between different countries and within some countries such as the USA and China, which is mainly because of the large differences in Se concentration in soil (Alfthan & Neve 1996); among 20 different countries considered, the lowest average SSe values (< 80 μg/L) are found in New Zealand and generally in the eastern European countries, whereas the highest average SSe values have been reported from the seleniferous regions in the USA (198 μg/L) and China (490 μg/L).

After adjusting for potential confounding variables by multiple regression, the results (Table 4) show a significant association between an increase in BCd and decreasing size of testes ($p < 0.03$) and increasing levels of serum estradiol ($p < 0.005$), FSH ($p < 0.03$), and testosterone ($p < 0.04$). Many experimental studies in rodents have shown that Cd reduces the size of testes and other androgen dependent organs as well as serum testosterone levels (Nordberg 1975, Zylber-Haran *et al.* 1982, Laskey *et al.* 1984, Clark *et al.* 1994, Waalkes *et al.* 1997); the reduction in testosterone after injection of a single dose of Cd was always followed by serious damage to the testes. It has also been shown that rodent testes are extremely susceptible to Cd-induced damage (Nordberg 1972, Waalkes *et al.* 1988) and that even low doses of Cd produce hemorrhagic necrosis despite the fact that relatively little Cd distributes to the testes (Nordberg 1972). There are no known published data concerning the effect of Cd on testis size in humans. As opposed to the majority of published experimental animal studies that applied a single Cd dose by $s.c.$ or $i.p.$ route, a recent study of chronic oral Cd exposure in rats (Zeng *et al.* 2003) showed a significant Cd-related increase, rather than decrease, in serum testosterone. This finding is in accordance with the results of the present study (Table 4), and our previous study of subjects with no occupational Cd exposure (Telišman *et al.* 2000) as well as with the results of a recent study of occupationally Cd-exposed male workers (Zeng *et al.* 2002). In addition, the latter study showed a significant positive association between urinary Cd and serum LH levels, but no significant association with serum FSH levels, after adjusting for confounding factors such as age, smoking habits, and alcohol consumption (Zeng *et al.* 2002). Other studies of occupationally Cd-exposed male workers showed no significant Cd-related influence on reproductive endocrine function (Mason 1990, McGregor & Mason 1991). There are no known published data concerning the effect of Cd on serum estradiol levels in human males. In general, there are very few literature data related to Cd effects on the human male reproductive system, as indicated in recent reviews on the subject (Tas *et al.* 1996, Benoff *et al.* 2000).

The results (Table 4) show no significant Cd-related influence on parameters of semen quality in the study population. Published data concerning groups of male subjects suspected of infertility (including nonsmokers and smokers) showed a significant positive correlation between abnormal sperm morphology and BCd levels (Chia *et al.* 1992, 1994, Telišman *et al.* 2000) and an inverse correlation between sperm motility and BCd (Telišman *et al.* 2000), between semen volume and either BCd (Chia *et al.* 1992) or seminal fluid Cd (Xu *et al.* 1993, Chia *et al.* 1994), and between sperm concentration and either BCd (Xu *et al.* 1993, Chia *et al.* 1994) or seminal fluid Cd levels (Xu *et al.* 2003). On the other hand, significant positive correlation was found between sperm motility and linear and curvilinear velocity with respect to semen Cd (Noack-Füller *et al.* 1993), or no significant correlation was found between conventional parameters of semen quality and either semen Cd (Oldereid *et al.* 1994) or seminal fluid Cd levels (Saaranen *et al.* 1989, Noack-Füller *et al.* 1993, Keck *et al.* 1995) in the same kind of subjects. No significant influence of occupational Cd exposure on fertility of male workers was found (Gennart *et al.* 1992).

Contrary to the observed reproductive effects of Cd, the study results show a significant Pb-related influence on certain parameters of semen quality but no significant relationship with serum levels of reproduction hormones (Table 4). After adjusting for potential confounding variables by multiple regression, an increase in BPb is significantly associated with increasing percentages of slow sperm ($p < 0.03$) and too wide sperm ($p < 0.003$) and with decreasing percentages of morphologically normal ($p = 0.05$) and subnormal ($p < 0.005$) sperm, whereas a decrease in ALAD is significantly associated with a decreasing size of testes ($p < 0.02$) and seminal fluid LDH-C_4 level ($p < 0.01$). The results (Table 4) also show a significant association between a decrease in SCu and an increasing percentage of pathologic sperm ($p < 0.03$), particularly amorph sperm ($p < 0.02$) and too short

sperm ($p < 0.05$), and decreasing serum LH level ($p < 0.02$); between a decrease in SZn and increasing serum LH ($p = 0.05$) and impaired prostate secretory function, that is, a decrease in SfZn ($p < 0.04$) and acid phosphatase in seminal fluid ($p = 0.05$); between a decrease in SSe and decreasing percentages of morphologically normal ($p < 0.05$) and subnormal ($p < 0.01$) sperm and increasing percentages of amorph sperm ($p < 0.03$) and thin sperm ($p < 0.04$); and between a decrease in GPx and increasing percentage of tapered sperm ($p < 0.05$). Increase in age was found to be significantly associated with a decrease in serum testosterone ($p < 0.003$), and prolactin ($p < 0.02$) levels. Smoking significantly contributed to a decrease in seminal flud LDH-C_4 level ($p < 0.03$) and serum prolactin ($p < 0.006$). Alcohol consumption was found to significantly contribute to increasing percentage of tapered sperm ($p < 0.02$).

In conclusion, the results indicate the necessity of controlling for various toxic and essential metals as well as other potential confounders (including age, smoking, and alcohol) when evaluating the influence of a particular metal on reproductive function in men. Within the Cd exposure range in the study population (BCd 0.20–11.93 μg/L), a significant Cd-related decrease in testis size and an increase in serum estradiol, FSH, and testosterone levels was observed after adjusting for age, smoking, alcohol, and biomarkers of Pb, Cu, Zn, and Se by multiple regression.

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