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Review

Adverse Effects of Low Level Heavy Metal Exposure on Male Reproductive Function

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Abbreviations: TE: testosterone; hCG: human chorionic gonadotrophin; ROS: reactive oxygen species; OS: oxidative stress; ATSDR: Agency for Toxic Substances and Disease Registry; SFH: follicle stimulating hormone; B: beta; MT: metallothionein; L-VDCC: L-type voltage-dependent ion channels; CFTR: cystic fibrosis transmembrane conductance regulator gene; CDC: Center for Disease Prevention and Control; ALAD: δ-aminolevulinic acid dehydratase; VDR: vitamin D receptor; OR: odds ratios; ppm: per million; GSH: glutathione; GCL: glutamyl-cysteine ligase; GCLC: glutamyl-cysteine ligase catalytic subunit; GCLM: glutamyl-cysteine ligase modifier subunit; GST: glutathione-s-transferase; GSTP1: GST pi 1; MMA: monomethylated arsenic; DMA: dimethylated arsenic; AS3MT: As^{+3} methyltransferase.

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Lead, cadmium, mercury, and arsenic, often referred to as "heavy metals", are toxic for wildlife, experimental animals, and humans. While experimental animal and human occupational studies with high exposure levels generally support an adverse role for these metals in human reproductive outcomes, information on the effects of low, environmentally-realistic exposure levels of these metals on male reproductive outcomes is limited. We review the literature on effects of exposure to low levels of these metals on measures of male fertility (semen quality and reproductive hormone levels) and provide supporting evidence from experimental and occupational studies. Potentially modifying effects of genetic polymorphisms on these associations are discussed. A brief review of the literature on the effects of three trace metals, copper, manganese, and molybdenum, that are required for human health, yet may also cause adverse reproductive effects, follows. Overall, there were few studies examining the effects of exposure to low levels of these metals on male reproductive health. For all metals, there were several well-designed studies with sufficient populations appropriately adjusted for potential confounders and many of these reported harmful effects. However, many studies lacked sufficient numbers of participants to be able to detect differences in outcomes between exposed and non-exposed individuals, did not clearly identify the source and characteristics of the participants, and did not control for other exposures that could alter or contribute to the outcomes. The evidence for the effects of low exposure was strongest for cadmium, lead, and mercury and less certain for arsenic. The potential modifying effects of genetic polymorphisms has not been fully explored. Additional studies on the reproductive effects of these toxic ubiquitous metals on male reproduction are required to expand the knowledge base and to resolve inconsistencies.

KEYWORDS arsenic, cadmium, human semen quality, lead, mercury, reproductive hormones

INTRODUCTION

Lead, cadmium, mercury, and arsenic are often referred to as "heavy metals" and their defining chemical characteristic is high density ($>5 g/cm^3$). These metals are found naturally in geological formations in the earth's crust and are released by natural forces and human activities. Exposure to all four metals usually occurs accidently, in certain occupations, through consumption of contaminated food and water, or inhalation of contaminated air. While they are not required for human health and are not intentionally part of the normal diet, food consumption is a major source of worldwide exposure. Human exposure to these metals has become widespread as documented in several population-based surveys [CDC 2003, 2005] and is even increasing in some parts of the world. At high doses, all four metals are toxic to the reproductive systems of wildlife, animals, and humans [ATSDR 1999, 2007a, 2007b, 2008a], and consequently are targets of public health monitoring and interventions [Jarup 2003].

Most reports on the male reproductive effects of heavy metals are from experimental animal, epidemiological, and occupational studies usually involving high doses not commonly encountered by the general population. Due to the widespread exposure of humans and known toxicity of these metals, concern is growing that low level exposure may also adversely affect male reproductive outcomes. We thus focused this review on the human male reproductive consequences of exposure to the low levels of these metals routinely found in the environment. For each metal, we present information on exposure sources, relevant animal work, a brief summary of occupational studies, human environmental exposure reference levels, and then the literature on the human male reproductive effects including potential effect modification by genetic polymorphisms.

CADMIUM

Cadmium occurs in nature at low concentrations, mainly associated with ores of zinc, lead, and copper. Human activities and industrial uses release cadmium to the environment [ATSDR 2008a; Jarup et al. 1998]. Consumption of contaminated shellfish and rice and cigarette smoking are the main sources of human exposure [ATSDR 2008a; Cheng et al. 2006]. The biological half life of cadmium in humans is long ranging from 7 to 26 years in kidney and 3 to 4 months in blood. Due to its low rate of excretion, cadmium accumulates over time in the kidneys, liver, semen, ovaries, and placenta [Akinloye et al. 2006; Ronco et al. 2005; Varga et al. 1993] with a preference for male reproductive organs [Danielsson et al. 1984]. The cadmium level to which the general population is exposed has been estimated in a representative, population-based survey of the general USA population [CDC 2005]. The estimated blood geometric mean for cadmium, unadjusted for smoking, for individuals 20 y and older was 0.412 μg/L, and for all males 1 y and older was 0.403 μg/L.

Animal and In Vitro Studies

Of the heavy metals, cadmium has been the most widely studied and is considered a reproductive toxicant in experimental animals when administered as a single high dose [Benoff et al. 2000; Thompson and Bannigan 2008]. Several other studies, however, have also reported adverse effects at lower environmental doses. A single low dose of cadmium (0.05 or 1.0 mg/kg body weight), administered to adult rats resulted in failure of spermiation, the final phase of sperm differentiation [Hew et al. 1993], and in reduced sperm concentration and motility [Xu et al. 2001]. Chronic exposure of adult rats to low doses of cadmium (1.6 or 7.4 μg/kg for 14 days) significantly depressed sperm concentration, and human chorionic gonadotrophin (hCG)-stimulated serum testosterone (TE) concentration [Laskey et al. 1984]. Exposure of rats to cadmium in drinking water (5 mg/L or 50 mg/L for 4 weeks) produced a dose-dependent decrease in sperm motility [Benoff et al. 2008]. At doses that did not affect most organs, cadmium caused damage to the testes within 24–48 h. Cadmium also has the ability to disrupt the blood-testis barrier (reviewed in [Siu et al. 2009a]). *In vitro* addition of cadmium to Sertoli cell cultures (reviewed in [Siu et al. 2009b]) or of Sertoli cells and spermatocytes [Chung and Cheng 2001], disrupts the tight (occludins) junctions between cells, thus, providing a mean of entry into cells. Cadmium also significantly decreased hCG-stimulated TE production by Leydig cells *in vitro* or *in vivo* at doses that did not affect viability [Laskey and Phelps 1991; Phelps and Laskey 1989]. Thus, both Sertoli

and Leydig cells appear to be targets for cadmium's adverse effects. Cadmium has also been shown to accumulate in the hypothalamus and pituitary and to decrease the level of prolactin [Lafuente et al. 2001].

Collectively, these results suggest both direct (via testicular and hypothalamus-pituitary toxicity) and indirect (via altered hormone secretion) effects may be involved in cadmium's reproductive effects. Consistent with these results is the hypothesized role of cadmium as a metallohormone [Byrne et al. 2009]. Once cadmium enters cells the damage it induces has been attributed primarily to its interference with zinc-mediated metabolic processes, possibly by molecular mimicry of zinc [Bridges and Zalups 2005]. Although cadmium is not a redox-active metal and does not generate reactive oxygen species (ROS) via the Fenton-like reactions, cadmium can bind to sulfhydryl groups of the ROS regulators, such as glutathione. By decreasing the ability of sulfhydryl molecules to scavenge ROS, cadmium can indirectly increase levels of ROS and induce oxidative stress (OS) [Stohs and Bagchi 1995; Valko et al. 2005] leading to lipid peroxidation [El-Demerdash et al. 2004], DNA damage, and cell death (reviewed in [Siu et al. 2009a]). Cadmium has also been shown to increase the level of apoptotic biomarkers in several species [Migliarini et al. 2005; reviewed in Thompson and Bannigan 2008].

Human Studies

Few occupational studies on cadmium exposure and reproductive outcomes have been conducted [Gennart et al. 1992; Saaranen et al. 1989]. Accordingly, the Agency for Toxic Substances and Disease Registry (ATSDR), found there was insufficient evidence to determine the effects of inhaled cadmium on human reproductive outcomes [ATSDR 2008a]. Nevertheless, several studies have examined the male reproductive effects of exposure to low levels of cadmium, and have provided some evidence in support of decreased semen quality and/or altered level of reproductive hormones. Studies of Asian men attending infertility clinics with blood cadmium levels between 0.78 μg/L and 1.31 μg/L reported negative correlations between blood cadmium level and sperm density ($r = -0.24$, $p < 0.05$), number of sperm per ejaculate ($r = -0.27$, $p < 0.05$) and semen volume ($r = -0.29$, $p < 0.05$) [Xu et al. 1993]. The blood cadmium level (mean 1.35 μg/L) was also negatively correlated with semen volume ($r = -0.37$, $p < 0.05$) and positively correlated with sperm midpiece defects ($r = 0.42$, $p < 0.05$) and immature forms ($r = 0.45$, $p < 0.05$) [Chia et al. 1992]. Men with low sperm motility had significantly higher blood cadmium levels than did men with normal sperm motility ($p < 0$ .025). Potential confounding variables, including cigarette smoking, were not controlled in these studies.

A Nigerian study recruited 60 male partners of couples attending an infertility clinic, excluding men who smoked, consumed alcohol, used steroids or fertility drugs, or had medical conditions that might impair spermatogenesis [Akinloye et al. 2006]. The mean serum cadmium level was higher in the men with low or no sperm compared to men with a normal number of sperm (230 μg/L (low sperm), 460 μg/L (no sperm) vs. 210 μg/L (normal sperm)), while mean seminal cadmium levels were higher only in men with no sperm (1570 μg/L no sperm; 1100 μg/L normal sperm). Serum levels of luteinizing hormone and follicle stimulating hormone (FSH), TE, and prolactin were significantly higher in the men with low or no sperm. While these men did not have occupational exposures, the authors noted that Nigeria is reported to be highly polluted by toxic metals, especially by cadmium and lead, which may account for their unusually high cadmium levels. A similar study of Indian infertility clinic attendees observed that the median seminal fluid cadmium level in fertile men was 0.5 μg/L while that of infertile men was 10.4 μg/L [Pant et al. 2003a]. Cadmium was significantly negatively correlated with sperm motility and sperm concentration in infertile men with few motile sperm ($r = -0.50$ and $r = -0.63$, $p < 0.05$ for both). However, in models adjusted for smoking, cadmium was not associated with sperm concentration or motility.

A study of 123 Croatian men attending an infertility clinic with median blood cadmium levels of 0.85 μg/L found significant negative associations between blood cadmium level and testis size (regression coefficient beta (B) = -0.284, $p = 0.021$; the change in the dependent variable that results from a change of one standard deviation in the dependent variable), but not with the sperm parameters in models adjusted for potential confounders [Jurasovic et al. 2004]. Blood cadmium was positively associated

with FSH ($B = 0.194$, $p = 0.0027$), TE ($B = 0.188$, $p = 0.034$), and estradiol ($B = 0.271$, $p = 0.0042$) levels. Blood cadmium was also positively associated with seminal fluid acid phosphatase ($p = 0.027$), an indicator of prostate function.

In contrast to the above study, no effects of blood cadmium levels were found on the sperm parameters of 219 male partners of couples attending infertility clinics in Michigan, USA [Meeker et al. 2008a]. The median blood cadmium level, 0.20 μg/L, was much lower than those reported in the above [Jurasovic et al. 2004] and other studies, and may explain the lack of association with the sperm parameters. The same group of investigators, however, found that cadmium was positively associated with serum inhibin B levels in a model adjusted for age, BMI, and current smoking (p for trend = 0.03), and this association persisted even when the effects of the other metals were controlled (p for trend = 0.005) [Meeker et al. 2008b]. No effects were seen on FSH or TE. While inhibin B is considered the best available endocrine marker of spermatogenesis in subfertile men [Pierik et al. 1998], another study also found it did not correlate with sperm parameters [Kumanov et al. 2006].

A study of 98 industrial workers (median blood cadmium level 3.40 μg/L) and 51 subjects not occupationally exposed (median cadmium level 1.83 μg/L) found significant positive correlations between blood cadmium levels and pathologic sperm ($r = 0.158$, $p < 0.05$), and LH ($r = 0.158$, $p < 0.05$) and TE ($r = 0.1295$, $p < 0.01$) levels and a negative correlation with prolactin level ($r = -0.168$, $p < 0.05$) in the total study population [Telisman et al. 2000].

Two other small studies did not find significant effects of cadmium exposure on sperm parameters. A small Finnish study compared sperm and seminal fluid concentrations of cadmium in 27 industrial or refinery workers and 45 consecutive sperm donor candidates [Hovatta et al. 1998]. The level of cadmium in sperm of the industrial workers (mean 0.04 mg/kg) was significantly higher than that of the sperm bank donors (0.005 mg/kg). Sperm cadmium, however, was not a significant contributor to abnormal sperm motility, concentration, or morphology in regression models. The models were not adjusted for smoking although the authors state that smoking and alcohol habits were similar in the two groups. A small German study found similar semen levels of cadmium in men with proven fertility, infertility patients with normal sperm parameters, and patients with abnormal semen parameters (0.43 μg/L for fertile patients and 0.43–0.44 μg/L for the infertile groups) [Keck et al. 1995]. No correlations between the semen cadmium level and the sperm parameters were reported. The lack of differences in cadmium levels between the fertility groups may have been due to their inability to control for smoking, as smoking information was not collected on all participants. This may have masked any differences associated with fertility status. Additionally, several studies have not found significant correlations between blood and semen or sperm cadmium levels [Kasperczyk et al. 2004; Kiziler et al. 2007], making it especially difficult to interpret these results. Similar difficulties comparing levels of lead in blood and semen have been reported [Alexander et al. 1998].

Taken together, these studies suggest that moderate to high levels of environmental cadmium exposure adversely affect sperm parameters and alter hormone levels. However, too few studies controlled for potential cofounders such a cigarette smoking or had large enough study populations to be able to detect differences if present. While animal experiments support an adverse effect of low cadmium exposure on semen parameters, more research is needed to clarify this relationship in human males.

Gene Polymorphisms: Potential Gene-Environment Interactions

Some of the conflicting results of the human studies may conceivably be due to variations in genes involved in cadmium (as well as other metal) metabolism and in genes involved in protecting cells from the harmful effects of metals. Polymorphisms have been identified in genes coding for proteins involved in metal transport, in structures that allow the metals to gain access to the cell, and in enzymes that protect against OS. Much of what is known about these polymorphisms has come from experiments in mice in which cadmium is an established testicular toxicant. Several metal binding proteins have been reported to protect cells from cadmium-induced toxicity. Once cadmium enters the body, it is bound by thiol containing proteins, such metallothionein (MT) and reduced glutathione and cysteine, which, along with red blood cells, have

been regarded as the principal carriers of cadmium in the blood. MT is involved in metal transport and is a main carrier for zinc [Coyle et al. 2002]. MT can also bind cadmium thus sequestering it and reducing its toxicity. Four major isoforms of MT (1–4) have been identified in humans, although MT-1 and MT-2 are the main isoforms and are expressed in all tissues of as to whether humans and rodents.

There appears to be a question, however, as to whether MT expressed constitutively in rodent testes or is it induced in response to cadmium treatment (see [Ren et al. 2003a] for discussion). Two recent papers shed some light on the controversy. In rats treated with a single low dose of cadmium (4 μmol/kg 0.45 mg/kg), liver cadmium levels increased rapidly over 6 h, but remained undetectable in testicular interstitial cells (18% Leydig cells) [Ren et al. 2003b]. The levels of MT-1 and MT-2 mRNA were relatively high in interstitial cells from untreated rats and increased after cadmium treatment peaking at levels approximating those measured in the liver at 6 h. While high basal levels of MT protein were detected in both interstitial cells and liver, the addition of cadmium increased protein levels in the liver, but slightly decreased levels in the interstitial cells. Similar results were found when MT-1 and MT-2 mRNAs were measured in rat testicular Sertoli cells and spermatogenic cells, although spermatogenic cell MT-2 mRNA levels decreased slightly in response to cadmium treatment [Ren et al. 2003a]. As with the interstitial cells, no increase in MT protein was observed in Sertoli or spermatogenic cells as compared to liver cells in response to cadmium.

These results suggest that within the same rodent strain the transcriptional response of MT to cadmium is organ specific, cell type specific, and time-dependent. More importantly they suggest that MT translation in response to cadmium is minimal. This finding is in line with the previous suggestion that the vulnerability of testicular tissue to heavy metal toxicity may be due to its inability to produce MT. They also suggest that at least in certain rodent strains, MT may not be the principle cadmium binding protein in the testes and may not play a major role protecting testicular cells from cadmium-induced toxicity. Several other cadmium transporters present in the testes have recently been identified including ZIP8 and tesmin, which contains an MT-like motif [Dalton et al. 2005; He et al. 2009; Martin et al. 2007; Sugihara et al. 1999]. These transporters may play more prominent roles in testicular metal binding. Polymorphisms in these genes have not yet been identified.

While the role of MT in binding and sequestering cadmium in the testes is not clear, it has been proposed that one way cadmium exerts its toxic effects is by displacing zinc from MT thereby reducing zinc bioavailability. Zinc is an essential trace element required for the maintenance of germ cells, spermatogenesis, and regulation of sperm motility [Yamaguchi et al. 2009] and in spermiogenesis [Merker and Gunther 1997]. Zinc is also a co-factor for enzymes involved in preventing OS and has been shown to modulate the testicular toxicity of cadmium [Parizek 1957]. MT also functions as an antioxidant by scavenging ROS [Coyle et al. 2002]. Thus MT may still play an important although less direct role in reducing cadmium-induced testicular toxicity.

At least 10 isoforms of human MT have been identified resulting from postranslational modifications and/or the metal that is bound (reviewed in [Coyle et al. 2002]). The selective expression of these isoforms may alter cadmium sensitivity. Polymorphisms have also been found in MT-1 and MT-2 [Giacconi et al. 2008, 2005; Yang et al. 2008]. A polymorphism in MT-1A for example, was associated with type 2 diabetes in patients with cardiovascular complications [Giacconi et al. 2008]. Less zinc was released from stimulated blood leucocytes in these patients, suggesting loss of zinc bioavailability. The ability of the variant MT-1A to function as an antioxidant may also be compromised. The role of these variants in the effects of human cadmium toxicity have not been investigated.

Variation in genes coding for proteins that comprise plasma membrane ion channels and transporters could affect sensitivity to cadmium. While these proteins normally regulate calcium flux, other cations, including cadmium and lead have been shown to utilize these channels as well [Kiss and Osipenko 1994]. L-type voltage-dependent ion channels (L-VDCC) usually provide cellular access for calcium, with the ion selectivity determined by binding sites in the pore area of the channel [Heinemann et al. 1992; Leinders et al. 1992]. Several L-VDCC isoforms exist with variation in their pore-forming units, one of which, α1C, is testes specific [Goodwin et al. 2000]. A splice variant of α1C is

altered in the domain responsible for ion channel activation. Two thirds of men with varicocele had the L-VDCC α1C splice variant [Benoff et al. 2005]. Men with the variant had significantly higher levels of testicular cadmium than men without. After varicoelectomy, the sperm counts of patients expressing the normal L-VDCC α1C transcripts increased significantly while the sperm counts of men expressing the altered transcripts did not. While the results of this study will require confirmation, they suggest that men with calcium channel variants may be at increased risk for the severity of varicocele-associated infertility and for higher levels of testicular cadmium.

Other data suggest that variation in the function of ion channels or voltage gated channels can impact health, including male reproductive health. Deletions in the L-VDCC α1C subunit have been shown to have a functional role in calcium flow in rat hepatocytes [Brereton et al. 1997], and deletions in VDCC have been associated with familial hemiplegic migraine and episodic ataxia type-2 disorder [Ophoff et al. 1996]. Polymorphisms in ion channels in neural cells have been associated with neuro-psychiatric disorders [Kleiderlein et al. 1998]. Alterations in channel function and in ion transport have been found in reconstituted human sperm plasma membranes from infertile but not fertile men [Ma and Shi 1999]. Consistent with this observation, almost 100% of cystic fibrosis patients who have mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR), a cAMP-activated chloride channel structurally similar to L-VDCC [Sheppard et al. 1994], are infertile due to obstructive azoospermia [Chillón et al. 1995]. Overall, these results are intriguing, but further animal and human studies will be required to elucidate the role of variability in ion transporter function on cadmium-mediated male reproductive toxicity.

LEAD

Lead is a naturally-occurring element found in mineral deposits usually combined with other elements. Natural levels of lead in soil are usually low, but human activities have resulted in substantial increases in lead levels in the environment, especially near mining and smelting sites, near some types of industrial and municipal facilities, and adjacent to highways [Juberg et al. 1997; Pirkle et al. 1998]. For adults the major pathway for occupational lead exposure is inhalation of lead-containing dusts and fumes [Gittleman et al. 1994]. In non-occupational settings, consuming food, and drinking water contaminated with lead leached from lead-containing pipes or from natural geological formations are the primary routes of exposure. Once absorbed, lead accumulates in the blood, soft tissues, and bone with a half life of 35 days in blood to 20–30 y in bone. Lead has also been reported to accumulate in male reproductive tissues [Danielsson et al. 1984; Oldereid et al. 1993] and is considered a male reproductive toxicant [ATSDR 2007b]. A study of a representative sample of the USA population reported that the average adult blood lead level was 19 μg/L [Jones et al. 2009], well below the Center for Disease Prevention and Control (CDC)'s action level of 10 μg/dL or 100 μg/L [Brody et al. 1994].

Animal and In-Vitro Studies

Experimental animal studies have shown that lead exposure can lead to testicular atrophy, changes in the weights of accessory glands, alterations in semen quality, and disruption of the hypothalamic-testicular pituitary axis [Sokol et al. 1985; Thoreux-Manlay et al. 1995]. Leydig cells appear to be a target as lead exposure results in suppression of TE synthesis [Thoreux-Manlay et al. 1995], while Sertoli cell function does not appear to be affected [Nathan et al. 1992]. Lead accumulates preferentially in the epididymus and other accessory glands [Marchlewicz et al. 1993; Oldereid et al. 1993]. The findings of the large majority of these studies, however, are based on a single, large dose of lead (and/or cadmium) administered by injection, which substantially differs from human occupational and environmental exposure conditions (including that through smoking).

Mechanisms involved in lead-induced toxicity include its ability to displace zinc in MT resulting in alterations in zinc bioavailability and its interference with calcium-mediated processes including disruption of the blood-testis barrier by replacing calcium in zona adherens junctions. Lead is considered a calcium mimic and may affect a variety of systems [Bridges and Zalups 2005]. Lead is a redox metal and generates ROS and OS in a variety of systems (reviewed in [Nemsadze et al. 2009; Patrick 2006]). As mentioned for cadmium, ROS can inhibit the

production of sulfhydryl antioxidants, damage nucleic acids and inhibit DNA repair, and initiate membrane lipid peroxidation. Specific targets of lead include inhibition of enzymes involved in heme production, possibly due to its accumulation in erythrocytes, and induction of inflammation in vascular endothelial cells. Lead may also be involved in apoptosis (reviewed in [Pulido and Parrish 2003; Rana 2008]), although its role has not been well studied.

Human Studies

In occupational studies, blood lead level has consistently been associated with reduced sperm count, poor sperm motility, and abnormal sperm morphology [Alexander et al. 1998, 1996; Assennato et al. 1987; Eibensteiner et al. 2005; Gennart et al. 1992; Hu et al. 1992; Lancranjan et al. 1975; Lerda 1992; Plechaty et al. 1977; Robins et al. 1997] . Effects on reproductive hormones have been less consistent [Assennato et al. 1987; Rodamilans et al. 1988].

Low lead exposure levels have been associated with adverse human male reproductive effects in several studies. A group of Croatian men with low to moderate lead levels (median blood lead 367 μg/L) were compared to men not occupationally exposed to lead (103 μg/L) [Telisman et al. 2000]. Their median sperm density, sperm count, and number of motile sperm were significantly lower in the low to moderately exposed men. Blood lead was negatively correlated with sperm count ($r = -0.177$, $p < 0.05$) and progressively motile sperm ($r = -0.179$, $p < 0.05$), and positively correlated with abnormal sperm head morphology ($r = 0.209$, $p < 0.01$) and with TE ($r = 0.188$, $p < 0.05$) and estradiol ($r = 0.201$, $p < 0.01$) levels. Smoking was significantly positively correlated with the levels of both TE and estradiol ($r = 0.193$ and $r = 0.180$, respectively, $p < 0.05$), suggesting it may have confounded those relationships. A decrease in markers of prostate secretory function (seminal zinc level, acid phosphatase activity, and citric acid level) were observed with higher blood lead level.

In 123 Croatian men with no occupational exposure to metals attending an andrology clinic, the median blood lead level was 57 μg/L [Jurasovic et al. 2004]. In regression models adjusted for confounders (age, smoking, alcohol, blood cadmium, and serum copper, zinc, and selenium), blood lead level was positively associated with the percentage of slow sperm ($B = 0.222$, $p = 0.024$) and too wide sperm ($B = 0.350$, $p = 0.0003$), and negatively associated with normal sperm ($B = -0.197$, $p = 0.050$) [Jurasovic et al. 2004; Telisman et al. 2007]. In a subsequent report with a larger study population (240 men), median blood lead level was 49.2 μg/L [Telisman et al. 2007]. Blood lead level was positively associated with pathologic sperm ($B = 0.31$, $p < 0.0002$), wide sperm (Beta = 0.32, $p < 0.0001$), round sperm ($B = 0.16$, $p < 0.03$), serum TE ($B = 0.21$, $p < 0.003$) and estradiol ($B = 0.22$, $p < 0.0008$), and negatively associated with serum prolactin ($B = -0.18$, $p < 0.007$). A decrease in δ-aminolevulinic acid dehydratase (ALAD), an indicator of long term lead exposure, was associated with decreased seminal plasma zinc levels ($p < 0.04$) indicating the adverse effects of lead on prostate function.

A study on the effects of environmental levels of metal exposure on measures of male reproduction recruited subjects from two infertility clinics in Michigan, USA. The median blood lead level of 219 participants was 15 μg/L [Meeker et al. 2008a]. No associations were observed between blood lead and sperm concentration, sperm motility, or sperm morphology in models adjusted for age and current smoking. In a model for sperm concentration adjusted for molybdenum, manganese, cadmium, and mercury, however, the middle lead quantile was a significant predictor (OR = 3.94, 95% CI 1.15–13.6), although the test for trend was marginally non-significant ($p = 0.07$). In another report by the same group, blood lead level was significantly inversely associated with prolactin and thyroid stimulating hormone levels in models adjusted for age, BMI, and current smoking (p for trend = 0.002 and 0.03, respectively) [Meeker et al. 2009]. In models adjusted for other metals, lead remained a significant negative predictor for levels of both hormones (p for trend for lead = 0.0002 and 0.02, respectively).

In a small study of men with proven fertility and men with fertility problems, infertile men with low or no sperm or with sperm with low motility had significantly higher mean seminal fluid lead levels (mean lead levels 104 – 150 μg/L) than did men of proven fertility (60 μg/L) [Pant et al. 2003b]. The lead level was significantly negatively correlated with sperm motility ($r = -0.5$, $p < 0.05$) and concentration ($r = -0.63$, $p < 0.05$). However in regression models

adjusted for smoking, lead was not associated with low sperm concentration or motility.

A small study (55 men) compared blood and semen lead levels in fertile Egyptian men without occupational exposure, infertile men without occupational exposure, and infertile men with occupational exposure [El Zohairy et al. 1996]. The highest blood lead level was found in infertile men with occupational exposure (370.2 μg/L) while the lowest was in fertile men without occupational exposure (169 μg/L) ($p < 0.01$). TE level was not different between the groups, but LH and FSH levels were significantly higher in the infertile men with occupational exposure compared to fertile men without occupational exposure (9.86 mIU/ml vs. 6.90 mIU/mL and 13.15 mIU/mL vs. 5.48 mIU/mL, respectively). Occupations in this study varied widely from painters to storage battery workers and most likely included exposures to other chemicals.

Urban Mexican men with a mean blood lead level of 93 μg/L had mean sperm lead levels of 0.047 ng/10^6 cells and seminal fluid lead levels of 2.02 μg/L [Hernandez-Ochoa et al. 2005], suggesting that the male reproductive tract accumulated lead, although these levels were not correlated. The lead level of sperm was significantly negatively associated with sperm motility ($B = -2.12$), normal morphology ($B = -1.42$), viability ($B = -1.30$) and concentration (adjusted for smoking) ($B = -17.17$) (all $p < 0.05$). In a study of 74 IVF patients, seminal plasma lead levels were inversely correlated with fertilization rate ($r = -0.447$, $p < 0.0001$) and with two sperm function biomarkers (mannose receptor expression, mannose-induced acrosome reaction) [Benoff et al. 2003]. Paternal lead exposure was also associated with an increased risk of infertility, defined as the non-occurrence of a marital pregnancy, in men monitored for exposure to inorganic lead [Sallmen et al. 2000]. In this group the risk of infertility increased with paternal lead exposures, with odds ratios (OR) and 95% confidence intervals ranging from 1.27 (1.08–1.51) for men exposed to 0.5 μmol/L (104 μg/L) blood lead to 1.90 (1.30–2.59) for exposure to 2.5 μmol/L (520 μg/L).

Other studies have not found associations between lead exposure and male reproductive outcomes. A small German study observed that sperm lead levels in sperm bank donors were lower than those in industrial workers (mean 0.03 mg/kg vs. 0.10 mg/kg) and did not find associations between seminal plasma lead level and sperm concentration, motility, or morphology [Hovatta et al. 1998] possibly due to the similar lead levels. A study of Singapore men did not find correlations between blood or seminal plasma lead levels and sperm concentration, motility, or morphology [Xu et al. 1993], but did not adjust for potential confounders, including cigarette smoking, a potential source of lead exposure. Another small study of 22 German volunteers with mean semen lead level of 98 μg/L did not find significant correlations between lead level and sperm parameters [Noack-Fuller et al. 1993]. The population was not described and there was no adjustment for potential confounders. One other small study with an undefined population and lack of adjustment for possible confounders also did not find significant relationships between lead exposure and semen parameters [Butrimovitz et al. 1983]. Overall, many of these studies were well-designed, were performed in different countries, and found significant negative effects of low to moderate lead exposure levels on human sperm parameters, and alterations in hormone levels. Large studies adjusted for appropriate confounders evaluating low lead exposure levels are needed to confirm or refute these findings.

Gene Polymorphisms: Potential Gene-Environment Interactions

Polymorphisms in several genes implicated in physiological processes involving metals have been associated with lead level. The vitamin D receptor (VDR) is involved in intestinal calcium absorption and storage in bone. Both calcium and lead are stored in bone, and lead is considered a molecular mimic of calcium (reviewed in [Bridges and Zalups 2005]). Interactions between lead and calcium have been reported in numerous studies and it has been suggested that lead may gain entry into cells through one or more of the different types of Ca^{2+} channels. A polymorphism in the VDR gene was shown to affect bone mineral density and lead accumulation in bone. Individuals with the variant allele (VDR B) had higher blood and bone lead levels than did individuals with the wild-type allele [Schwartz et al. 2000]. The enzyme δ-aminolevulinic acid dehydratase (ALAD) is required for heme synthesis and is considered the major intracellular

ligand for lead in erythrocytes. The ALAD G177C polymorphism results in two codominant alleles, ALAD-1 and ALAD-2. ALAD-2 carriers compared to ALAD-1 homozygotes had a higher lead erythrocyte binding capacity resulting in higher blood lead levels. It has been suggested that ALAD might sequester lead thus protecting target organs from lead-induced toxicity [Gundacker et al. 2009].

An Austrian study of 324 medical students with low lead levels (urine median 1.90 μg/g creatinine, blood median 19.8 μg/L, and hair median 665 ng/g) found that the VDR B genotype was an independent predictor for an increased lead level in urine ($\beta = 0.179$, $p = 0.019$) [Gundacker et al. 2009] in models adjusted for consumption of red wine and poultry, family history of cancer, and BMI. The combination of the VDR B and ALAD-2 polymorphisms was also associated with an increase in urine lead levels ($\beta = 0.222$, $p < 0.001$) in a similar model. This study also examined the effect of metallothionein polymorphisms and found the MT-2a variant was negatively associated with blood lead level ($\beta = -0.184$, $p = 0.011$). Other studies, however, did not find associations between the ALAD polymorphism and risk of lead toxicity (reviewed in [Kelada et al. 2001]). Variation in study populations, numbers of participants, lead level, and health outcomes may explain the discrepant results. Additionally, the effect of the polymorphisms may be context dependent, with effects differing in individuals according to genetic background (e.g. VDR genotype) or level of exposure. The potential effect of these polymorphisms on the relationship between lead exposure and animal or human male reproductive outcomes has not been assessed.

MERCURY

Mercury and mercury-containing compounds have been used for thousands of years in activities as diverse a vaccine preservation, treatment for syphilis, skin creams, dental amalgams, extraction of gold, and haberdashery [Clarkson and Magos 2006]. Elemental (monatomic) or metallic mercury occurs in vapor and liquid forms and is very stable. Evaporation of mercury vapor from land and sea surfaces, from volcanic activity, or through burning fossil fuel has resulted in its worldwide distribution. In the upper atmosphere mercury vapor is oxidized to water-soluble ionic inorganic mercury and returns to earth in rain. The major routes for non-occupational inorganic mercury exposure include dental amalgams, pharmaceutical applications, cosmetic preparations, and residential and school exposures to mercury vapors off-gassing from gymnasium and indoor track flooring [ATSDR 1999; Beaulieu et al. 2008]. Organic or methyl mercury is found in water sediments, where microorganisms methylate inorganic mercury converting it to methyl mercury. Methyl mercury is persistent and bioaccumulates in the food chain with predator species, such as fish and raptors, having the highest levels. The major route for methyl mercury exposure for the USA population is consumption of fish, both ocean and fresh water [Mahaffey et al. 2004]. The largest survey of mercury levels in the general male USA population (1,127 men, mean age 52.8 y) occurred in the mid to late 1990s and reported a mean total blood mercury of 2.55 μg/L [Kingman et al. 1998]. Total blood mercury includes inorganic and organic forms, while urinary or plasma levels reflect inorganic mercury exposure. Other more current surveys conducted in Europe that included men found lower levels (0.58 μg/L and 0.78 μg/L) [Becker et al. 2002; Benes et al. 2000].

Animal and In Vitro Studies

Low dose mercury exposure has been reported to negatively affect measures of male reproductive health (reviewed in [Clarkson and Magos 2006; Tan et al. 2009]). It is reported to cross the blood-testes barrier and accumulate in Sertoli and Leydig cells [Ernst et al. 1991], in the testes of experimental animals [Lee and Dixon 1975; Schuurs 1999]. Treatment of rats with inorganic (50 or 100 μg/kg) or organic (5 or 10 μg/g) mercury for 90 days induced Leydig cell disintegration, inhibited the activity of 3β-hydroxysteroid dehydrogenase (3-β-HSD), an enzyme critical for TE production, and decreased TE levels [Chowdhury et al. 1985; Vachhrajani and Chowdhury 1990]. Rat Sertoli cells incubated *in vitro* with 31 μM (6.22 mg/L) of inorganic mercury produced lower levels of inhibin b [Monsees et al. 2000]. The number of rat epididymal sperm declined after incubation with inorganic mercury and a dose-dependent decrease in motility was also noted [Rao and Gangadharan 2008]. Monkeys administered methyl mercury orally at 50 or 70 μg/kg/day for

20 weeks had non-significantly elevated blood mercury levels (approximately 2 ppm) [Mohamed et al. 1987]. The percent motile sperm decreased in a dose-dependent fashion and was significantly different than the controls (approximately 58% and 52% vs. approximately 66% motile, respectively). The percent total tail defects increased significantly in the methyl mercury treated groups compared to controls (approximately 16% vs. approximately 33% for both treated groups). In an earlier report the same investigators noted that incubation of sperm with similar concentrations of methyl mercury also decreased motility via interference with the Dynein/microtubule sliding assembly [Mohamed et al. 1986]. *In vitro* treatment of human sperm with mercury concentrations from 50 to 800 μmol/L (10.0 to 160.4 μg/L) induced membrane lipid peroxidation and DNA breaks, lowered sperm viability, and decreased the rate of the acrosome reaction leading to sperm dysfunction [Arabi and Heydarnejad 2007].

Human Studies

In humans methyl mercury has been detected in semen [Rignell-Hydbom et al. 2007] and these levels have correlated with poor male reproductive outcomes. Male partners of infertile couples with abnormal sperm parameters (below World Health Organization cut-off levels for normal) [WHO 2003] had significantly higher blood mercury levels than did men of proven fertility (44.2 mmol/L vs. 31.2 mmol/L, $p = 0.03$; or 8.9 μg/L vs. 6.3 μg/L) [Choy et al. 2002a]. Individuals with elevated blood mercury levels also consumed more seafood than did individuals with lower mercury levels ($p = 0.02$). In a study of male partners of infertile couples from Hong Kong, the seminal fluid mercury level (mean overall 41.4 nmol/L or 8.3 ng/L) was significantly positively correlated with abnormal sperm morphology ($r = 0.26$, $p < 0.02$) and negatively correlated with normal sperm motion characteristics ($r = -0.20$ to -0.21, $p < 0.03$ to 0.4) [Choy et al. 2002b]. Subfertile men had approximately 40% more methyl mercury in hair than did fertile age-matched men (4.5 parts per million (ppm) vs. 3.9 ppm) [Dickman et al. 1998] (n = 159). Men in the highest mercury tertile (3.70–25.28 ppm) had almost double the risk of being subfertile than did men in the lowest tertile (OR = 1.95, 95% CI, 1.61–2.37).

A small study of infertility patients in Singapore (21 men) found that men with high total blood mercury concentrations (71.2 mmol/L or 14.4 ng/L) as compared to those with lower levels (31.5 mmol/L or 6.3 ng/L) had lower sperm concentration (41.6×10^6/mL vs. 65.7×10^6/mL), percentage of morphologically normal sperm (40.0% vs. 50.0%), and percentage of motile sperm (45.4% vs. 49.0 %). The differences were not statistically significant possibly due to the small number of participants [Leung et al. 2001].

Swedish fishermen who consumed high levels of methyl mercury-contaminated fish from the Baltic Sea had twice the blood methyl mercury level compared to men with low fish consumption [Svensson et al. 1995]. A recent study of this population found no associations between blood or semen methyl mercury levels and sperm motility, sperm concentration, total sperm count, sperm chromatin integrity, or on the proportion of Y-chromosome bearing sperm and blood methyl mercury levels (median 2.25 μg/L) [Rignell-Hydbom et al. 2007]. The study had a low rate of participation (38% and 44% for two combined cohorts of fishermen) and low numbers in certain groups after categorization for some outcomes. This may have prevented them from finding significant associations if present.

In a small study of infertility clinic attendees in Singapore, no difference in blood methyl mercury levels was found between men with less than 40% motile sperm and men with greater than 40% motile sperm (mean blood methyl mercury level 180 μg/L and 191 μg/L, respectively) [Chia et al. 1992]. A study of infertility patients in Michigan, USA with a median total blood mercury level of 1.10 μg/L did not find adverse effects on sperm parameters [Meeker et al. 2008a; Ramamoorthi et al. 2008] or significant alterations in reproductive hormone levels [Meeker et al. 2008b]. The latter studies included men with low blood mercury levels, possibly too low to observe adverse effects.

Taken together, there is a suggestion that men with somewhat higher blood mercury concentrations (above 8 μg/L) were more likely to have lowered sperm parameters than men with a somewhat lower concentration of mercury. Caution must be used when interpreting these results especially since the studies showing significant negative effects were also populations consuming higher levels of fish and

seafood that may contain other contaminants with known adverse reproductive effects, such as organochlorine compounds. Additionally the number of participants was often low and possible confounding factors were not addressed.

Gene Polymorphisms: Potential Gene-Environment Interactions

There is great variation in the elimination half-life of methyl mercury with a range of 45 to 190 days in bile [Clarkson and Magos 2006]. Several polymorphisms have been identified that affect mercury retention and thus may affect health outcomes. As mentioned above, MT expression is induced by exposure to several metals including mercury. Cadmium is regarded as the most potent inducer followed by zinc, mercury, and silver. Exposure of rats to 50 μg/kg/day to 100 μg/kg/day mercury for 14 days significantly increased the levels of MT-1 and MT-2 RNA in the epididymus, although protein levels were not measured [Dufresne and Cyr 1999]. Polymorphisms in MTs would also be expected to alter the bioavailability of mercury and thus their toxicity. Methyl mercury is eliminated as a complex bound to glutathione (GSH) in bile and polymorphisms in GSH-synthesizing or GSH-conjugating genes may alter the rate of elimination. The rate-limiting enzyme for GSH synthesis is glutamylcysteine ligase (GCL), having a catalytic subunit (GCLC) and a modifier subunit (GCLM). Polymorphisms in both subunits have been identified. Substitution of C to T at position −129 in the GCLC gene (GCLC-129) [Koide et al. 2003] and a C to T substitution at position −588 in the GCLM gene (GCLM-588) [Nakamura et al. 2002] both affect promoter activity and thus GSH production. The glutathione-s-transferase (GST) enzymes are also involved in conjugating a variety of electrophilic compounds as part of its phase II enzyme detoxification function. GST pi 1 (GSTP1) has two polymorphisms resulting in changes in its amino acid sequence (Ile05Val and Ala114Val) that are associated with differences in enzyme activity and substrate preference [Sundberg et al. 1998; Zimniak et al. 1994].

Polymorphisms in GCLM, GCLC, or GST genes altered the association between the level of methyl mercury, measured as erythrocyte total mercury, and the plasma level of polyunsaturated fatty acids. This was demonstrated in a cardiovascular disease study where one variant allele for either GCLC-129 or GSTP1-114 was found to have significantly higher levels of erythrocyte total mercury [Custodio et al. 2004]. In a subsequent study on the modifying effects of genetic polymorphisms on retention of inorganic mercury in 309 men exposed to mercury vapors, men with a GCLM-588 allele had increased blood, plasma, and urinary methyl mercury levels compared to men without the variant allele [Custodio et al. 2005]. A study of 324 medical students with low mercury exposure (urine median 1.24 μg/g, blood median 1.34 μg/g and erythrocyte median 202 ng/g) in Austria observed that polymorphisms in GSTP1 (GSTP1-114) and in MT-4 significantly increased erythrocyte mercury levels ($p = 0.04$ and $p = 0.0003$, respectively) [Gundacker et al. 2009]. The gene combinations of GSTP1-114/GSTT1 and GSTP1-105/GCLC also significantly increased erythrocyte mercury levels ($p = 0.003$ and $p = 0.0003$, respectively). Taken together, these results suggest that the polymorphisms in the GCL subunits may increase both inorganic and organic mercury retention while GSTP1 and GSTT1 polymorphisms may play roles in conjugating methyl mercury and/or in the defense against oxidative stress. Studies remain to be carried out that evaluate the effects of these polymorphisms on the relationship between mercury exposure and male reproductive outcomes.

ARSENIC

Arsenic is a metalloid widely distributed in the earth's crust. It can exist in several chemical states of which the most common forms are arsenate (As^{+5}) and arsenite (As^{+3}) (reviewed in [ATSDR 2007a]). Arsenic and its compounds usually occur in trace quantities in rock, soil, water, and air. Concentrations may be higher in certain areas as a result of weathering and anthropogenic activities including metal mining and smelting and during fossil fuel combustion. Other sources of contamination are the manufacture and use of wood preservatives. The most common exposure sources for arsenic are inhalation of air containing arsenic, air from contaminated workplaces, sawdust or smoke from wood treated with arsenic, living near uncontrolled

hazardous waste sites containing arsenic, and living in areas with unusually high natural levels of arsenic in rock. A representative sample of USA population found that the 50th percentile of combined urinary arsenic species was approximately 6 μg/L [Caldwell et al. 2009], while earlier studies from European countries and the USA found levels of ≤ 10 μg/L [Cleland et al. 2009].

Animal and In-Vitro Studies

The few experimental animal studies available consistently found harmful effects of arsenic on measures of male reproductive health. Mice were treated with drinking water containing arsenite (533.9 μmol/L (69.4 mg/L) for 35 days), the form of arsenic most frequently found in drinking water [Pant et al. 2001]. Arsenic levels increased in the testes (0.52 to 5.26 mg/kg, $p < 0.05$), epididymis (2.70 to 4.70 mg/kg, $p < 0.05$), and in the seminal vesicles ($p < 0.05$). No signs of toxicity were detected. Changes were observed in sperm motility (72% to 60%, $p < 0.05$), total epididymal sperm count (5.62×10^6 to 3.84×10^6, $p < 0.05$), and the percent of sperm with morphological abnormalities (5.6% to 8.8%, $p < 0.05$). The activity of 17β-HSD, an enzyme involved in TE metabolism, was decreased from 3.28 units to 1.50 units ($p < 0.05$). Mice injected subcutaneously with 3 mg/kg arsenic trioxide accumulated arsenic in the testes and plasma and exhibited inhibition of spermatogenesis. The total number of sperm (53.3% of control), sperm motility (48.7% of control), and sperm viability (53.3% of control) were significantly decreased [Chiou et al. 2008]. Plasma and testicular TE levels decreased by 38.2% and 59.4%, respectively, and plasma LH level decreased by 51.6%. Both the expression and activities of 3β-SD and 17β-HSD were decreased in rodents administered low levels of arsenic (20–40 mg/L drinking water, 5 mg/k or 3 mg/k) [Chang et al. 2007; Chinoy et al. 2004; Chiou et al. 2008]. Several studies suggest that the hypothalamus and brain may be the major targets of arsenic's effects, leading to hormone dysregulation and decreased sperm concentrations [Biswas et al. 1999; Jana et al. 2006; Sarkar et al. 2003]. However, the substantial amounts of arsenic detected in testes, epididymus, seminal vesicle, and ventral prostate suggest a possible direct effect on testicular tissue. Arsenic is electrophilic and can bind to the electron-rich sulfhydryl groups in proteins and may thus directly modulate the activities of key enzymes involved in TE production. Arsenic has been shown to bind to glutathione and several antioxidant enzymes thus decreasing the reducing capacity of cells and inducing oxidative stress [Valko et al. 2005]. Blood levels of arsenic were associated with increased level of ROS and increased level of lipid peroxides in exposed individuals in two Asian studies [Pi et al. 2002; Wu et al. 2001].

Human Study

We found only one study investigating the effects of low level arsenic exposure on human male reproductive outcomes. A cross-sectional study of men attending infertility clinics in Michigan, USA found a significantly increased risk for low sperm motility with exposure to environmental levels of arsenic, after adjusting for smoking and age [Meeker 2008a]. The odds ratio for low sperm motility with the highest arsenic quartile was 3.80 (1.38–10.4). Arsenic was also a significant risk factor for low semen volume in a multi-metal model ($p = 0.05$). In another report by the same authors, increasing arsenic level was associated with increasing odds for low LH levels (p for trend $= 0.04$), after adjusting for age, BMI, and current smoking [Meeker 2008b]. Although the data is suggestive, further research on the effects of exposure to environmental levels of arsenic on human semen quality is needed before any conclusions can be drawn.

Gene Polymorphisms: Potential Gene-Environment Interactions

Susceptibility to the adverse effects of arsenic exposure is in part due to large individual variability in arsenic metabolism affecting both retention and distribution of arsenic metabolites. Recent studies suggest a role for polymorphisms in genes involved the biotransformation of As^{+5} and As^{+3} to the methylated forms excreted in urine [Hayakawa et al. 2005]. GSTs are involved in catalyzing the formation of arsenic-glutathione conjugates which are required for urinary arsenic excretion. Polymorphisms in GSTM1, GSTT1, and GST omega-1-(GSTO1) have been associated with variation in

urinary arsenic metabolite levels [Chiou et al. 1997; Kile et al. 2005; Marnell et al. 2003]. The most toxic arsenic metabolite is monomethylated arsenic (MMA), while the metabolite with the shortest retention time is dimethylated arsenic (DMA). Both are measured in urine. The fraction of MMA in urine, which reflects MMA in tissues, has been linked to risk of a variety of health conditions. Polymorphisms in As^{+3} methyltransferase (AS3MT), the main gene involved in catalyzing arsenic methylation, has been shown to alter the relative urinary percentages of arsenic excreted as MMA and DMA [Meza et al. 2007]. The potential effects of these polymorphisms on reproductive outcomes have not been investigated.

ESSENTIAL METALS

Reports in the last several years suggest that certain essential or trace metals, including copper, manganese, and molybdenum, can also have adverse effects on male reproduction. Copper is a trace metal important for the function of many enzymes including those involved in redox cycling, mitochondrial respiration, iron absorption, and free radical scavenging (reviewed in [Tapiero et al. 2003]). Several studies have examined the relationship between exposure to environmental levels of copper and male reproductive outcomes. In a study on the effects of trace metal exposure on semen quality and endocrine function in 123 Croatian men, median serum copper level was 1112 µg/L [Jurasovic et al. 2004]. Multiple regression models indicated serum copper level was positively associated with normal sperm ($B = 0.204$, $p = 0.045$) and LH ($B = 0.216$, $p = 0.016$) and negatively associated with pathologic sperm ($B = -0.236$, $p = 0.021$), too short sperm ($B = -0.206$, $p = 0.046$), and amorphous sperm ($B = -0.237$, $p = 0.019$). A second report from the same researchers investigated the effect of copper exposure on male reproductive endpoints among a group of men with low lead exposure. Serum copper (median copper level = 1066 µg/L) was negatively associated with thin sperm ($B = -0.20$, $p \leq 0.01$) [Telisman et al. 2007]. In a third report by the same group, copper level did not differ significantly between men without occupational lead exposure and those exposed to slight to moderate lead levels (median copper level 1175 µg/L vs. 1132 µg/L, respectively) [Telisman et al. 2000]. Although copper was negatively correlated with motile sperm count ($r = -0.168$, $p \leq 0.05$) and viable sperm count ($r = -0.170$, $p \leq 0.05$) in the lead exposed workers, it was not a predictor of any of the sperm parameters in the final models. It was, however, a positive predictor of acid phosphatase (an indicator of prostate secretory function) in a model containing blood lead, serum zinc, and alcohol ($r = 0.437$, $p = 0.0000$).

Several studies have focused on the effects of copper exposure on men with different fertility status. An early British study compared fertile with infertile men, defined as male partners of couples failing to achieve a pregnancy for at least 1 y and having either sperm counts less than 20×10^6 sperm/mL or as greater than 20×10^6 sperm/mL [Stanwell-Smith et al. 1983]. Both groups of infertile men had plasma copper levels significantly greater than the fertile men (15.6 µmol/L or 991.4 µg/L and 16.3 µmol/L or 1035.9 µg/L vs. 14.5 µmol/L or 921.5 µg/L, $p = 0.007$). Subanalyses found no differences between fertile and infertile men with respect to age, height, weight, smoking, or alcohol consumption. A study from Turkey collected blood and semen samples from 60 suspected subfertile men attending an infertility clinic and 40 volunteers with normal semen parameters, none of whom had common infertility disorders, drank alcohol, or smoked [Aydemir et al. 2006]. Both serum copper (1115 µg/L vs. 941 µg/L) and seminal plasma copper (37 µg/L vs. 34 µg/L) levels were significantly higher in the subfertile group. Seminal copper level was positively correlated with sperm reactive oxygen species ($r = 0.299$, $p < 0.05$), but not with the sperm parameters or measures of lipid peroxidation. A German study of 172 infertile men attending an infertility clinic and 18 men of proven fertility did not find differences in seminal copper level between the two groups (1950 µg/L vs. 1834 µg/L) [Jockenhovel et al. 1990]. They did, however, find significant correlations between copper level and sperm concentration ($r = 0.32$, $p < 0.001$), progressively sperm motility ($r = 0.23$, $p < 0.005$), and normal sperm morphology ($r = 0.22$, $p < 0.005$) in the combined groups. No information was provided on how infertility was defined, on demographics, or on possible confounders. Seventy semen samples were collected from healthy college students and from adult men undergoing infertility evaluation in Taiwan [Huang et al. 2000]. The men were divided

into normospermic, oligospermic, asthenospermic, and oligoasthenospermic. Only men with asthenospermia had significantly higher copper seminal plasma concentrations compared to normospermic men (0.23 ppm or 230 μg/L vs. 0.17 ppm or 170 μg/L, $p < 0.05$). In the abnormal sperm groups, copper level was positively correlated with sperm count ($r = 0.23$, $p < 0.05$), but not with motility. A study from Taiwan compared men with proven fertility to subfertile male partners of couples who failed to conceive after one y of unprotected intercourse. No significant differences in blood or seminal plasma copper levels were found between the two groups [Wong et al. 2001]. Although these studies varied in their definition of infertile and in adjustment for confounders, several found that men with fertility problems had higher copper levels than those of men with proven fertility [Aydemir et al. 2006; Huang et al. 2000; Stanwell-Smith et al. 1983].

In the study of Michigan USA infertility clinic patients, increasing copper level was significantly associated with decreasing sperm morphology (OR for highest copper tertile = 2.41, 95% CI 1.10–5.84) (p for trend = 0.05) after adjusting for age and current smoking [Meeker et al. 2008a]. In the model for sperm morphology that included molybdenum, copper was a negative predictor ($p = 0.05$). In an analysis to assess metal-metal interactions using U ranks, a method in which multivariate data are grouped, [Wittkowski et al. 2004] by the same investigators, copper and manganese individually were significantly negatively correlated with total motile sperm [Ramamoorthi et al. 2008]. Copper paired with cadmium, manganese, or molybdenum was significantly negatively correlated with total motile sperm, and the correlations for the pairs were stronger than those for the individual metals. Additionally, copper paired with lead or molybdenum was significantly correlated with sperm morphology, while all three individually were not. When the effect of copper on reproductive hormone levels was investigated by the same group, increasing copper levels were associated with increasing levels of testosterone (p for trend = 0.03) after adjustment for age, BMI, and current smoking [Meeker et al. 2008b], but not in the testosterone model that included molybdenum. Copper was significantly negatively associated with decreasing levels of LH in a model that also contained selenium (p for trend = 0.02). Copper was also negatively associated with prolactin in a model adjusted for age, BMI, and current smoking (B for the third quartile = −0.25, −0.041, −009) (p for trend = 0.04) and was also a negative predictor for prolactin in a model adjusted for chromium, lead, and molybdenum (p for trend = 0.01) [Meeker et al. 2009]. Although these results need to be replicated in larger studies, they suggest that copper can have both negative and positive effects on male reproductive outcomes. Studies also suggest potential interactions between copper and other metals, at both high and low environmentally relevant levels.

It is interesting to note that in the studies evaluating the relationship between copper levels and sperm parameters, despite several similar well designed studies that defined the study populations and controlled for potential confounders, the findings were inconsistent. Since the levels of copper were approximately the same in these populations, other characteristics of the study populations such as exposure to metals affecting copper adsorption and bioavailability (molybdenum, cadmium, and zinc) [Linder and Hazegh-Azam 1996; Telisman 1995; Vyskocil and Viau 1999], and/or other environmental contaminants, may have contributed to these differing results. Studies with other populations with well-defined exposures are needed to understand the effects of copper on semen quality.

Manganese at trace levels is required for normal sperm function [Aschner and Aschner 2005], and has been detected in human seminal fluid [Abou-Shakra et al. 1989; Dawson et al. 2000; Lafond et al. 1988]. However, men exposed to high occupational levels were impotent [ATSDR 2008b; Penalver 1955], had decreased birth rates [Lauwerys et al. 1985], and decreased semen parameters [ATSDR 2008b]. Animal and *in vitro* experiments indicate that high manganese exposure decreased sperm motility and concentration [Huang et al. 2001; Ponnapakkam et al. 2003]. In reports from a Michigan USA study on environmental exposures and male reproductive outcomes in infertility clients, high environmentally relevant manganese level (greater than the 75th percentile) was associated with increased risk of low sperm motility (OR = 5.4, 95% CI 1.6–17.6) and low sperm concentration (OR = 2.4; 95% CI 1.2–4.9) [Wirth et al. 2007]. In the same models, high zinc level reduced the risk of the low sperm parameters, suggesting a beneficial effect. Of interest, U-shaped dose-response

relationships between quartiles of manganese exposure and all three sperm parameters were observed. In a separate analysis assessing the effects of metal interactions using U-ranks, manganese paired with cadmium, copper, or molybdenum was significantly negatively correlated with total motile sperm [Ramamoorthi et al. 2008]. Although manganese and copper were significant when unpaired, the correlation was stronger when paired. When sperm morphology was added to the outcome, all three pairs as well as the pair of manganese and lead were significantly negatively correlated with the combined outcomes. None of the metals alone affected sperm morphology. Manganese levels were also positively associated with inhibin B level (p for trend = 0.04) and negatively associated with prolactin level (p for trend = 0.03) in models adjusted for age, BMI, and smoking, but not in models adjusted for other metals [Meeker et al. 2008a, 2008b]. Thus at environmentally relevant levels, manganese decreased sperm parameters and altered hormone levels, suggesting an endocrine disrupting effect. Confirmation or refutation of these findings awaits other studies of populations exposed to environmentally relevant manganese levels.

Molybdenum, another trace metal, is a cofactor for enzymes involved in the carbon, nitrogen, and sulfur cycles [Chan et al. 1998] and is, thus, essential for human health. Although the effects of molybdenum on reproduction have not been well studied, its reproductive toxicity was described in several animal studies. Male rats given rather high doses of molybdenum (20, 80, or 140 mg/kg) and low doses of copper (5 mg/kg) for 13 weeks after weaning had reduced fertility (75% infertile) and showed histological degeneration of seminiferous tubules [Jeter and Davis 1954]. Female mice mated with molybdenum-treated males had fewer litters, more stillbirths, and more offspring dying before 21 days old. No effects on females were observed, suggesting that males were more susceptible to the effects of molybdenum than females. In male rats fed 30 or 50 mg sodium molybdate/kg 5 days per week for 60 days compared to untreated rats, epididymal and accessory organ weights were significantly decreased, as were the percent motile sperm (86.0% vs. 49.1%) and the percent of sperm with normal morphology (89.7% vs. 76.9%) [Pandey and Singh 2002]. Molybdenum accumulated in the epididymus, seminal vesicle, and in the prostate, but not in the testis. The authors also reported evidence of damage to seminiferous tubules and male-mediated embryotoxicity (e.g. reduced implantation, increased pre/post implantation losses, and reduced fetal growth) associated with sodium molybdate exposure.

Only one group of investigators has studied the effects of exposure to environmentally relevant levels molybdenum on human reproductive outcomes. In the study of infertility clinics in Michigan USA, a dose-dependent decline in sperm concentration with increasing levels of molybdenum was found (OR for highest molybdenum tertile = 3.5, 95% CI 1.1, 10.8; p for trend = 0.04) in a model adjusted for age and current smoking [Meeker et al. 2008a]. In a multiple regression model that adjusted for other metals, the negative association between molybdenum and sperm concentration persisted, although it was marginally non-significant (p for trend = 0.07). In a model for sperm morphology adjusted for copper and BMI, the highest molybdenum level was negatively associated with sperm morphology (OR = −1.59; 95% CI −3.03 to −0.15) (p for trend = 0.03). In a stratified analysis used to assess metal-metal interactions, the adjusted OR for men with high molybdenum and low copper was elevated for both low sperm concentration and morphology (OR = 14.4, 95% CI 1.6 – 21 and OR = 13.7, 95% CI 1.6–144, respectively). These results suggest that low copper may be a risk factor for low sperm parameters in men with elevated molybdenum levels, as suggested by the experiments of Jeter and Davis [1954]. In an analysis using U ranks, the same group found molybdenum paired with cadmium, copper, or manganese was significantly negatively correlated with total motile sperm, while the pair of copper and molybdenum was significantly negatively correlated with sperm morphology. The effects of the pairs were stronger than that of the metals alone [Ramamoorthi et al. 2008]. In an analysis of effects of molybdenum on reproductive hormones, molybdenum was negatively associated with testosterone level (adjusted B for the highest molybdenum tertile = −60 (−97.2 to −22.8) (p for trend = 0.001) [Meeker et al. 2008b]. Molybdenum was also a significant predictor in models adjusted for other metals for testosterone level and for the free androgen index. Interestingly, copper was positively associated with testosterone in the adjusted model, and in the multiple metal model

for free androgen index. An exploratory analysis to assess metal-metal interactions indicated that testosterone levels were considerably lower among men with high molybdenum and lower copper or zinc levels. Additionally, in a model adjusted for copper, lead, age, race, and smoking status, molybedenum level was a predictor of prolactin level (p for trend = 0.03) [Meeker et al. 2009]. While these results need to be repeated in a larger population, they suggest that molybdenum, a common contaminant of food and drinking water, may adversely affect measures of male reproduction.

CONCLUSIONS

This survey of the effects of low-level heavy and trace metal exposure on human reproduction demonstrates that some concern about exposure is warranted. Several carefully conducted studies suggest that certain changes in semen quality and reproductive steroidogenesis occur at low to moderate levels of exposure. In spite of these intriguing findings, for many metal-outcome relationships there are inconsistencies and gaps in our knowledge that prevent full interpretation of studies. Furthermore, it is difficult to assess risk associated with exposure at these levels as data showing dose-response relationships between exposure and effect are limited. Additionally, few studies reporting null findings, perhaps representing exposures with little or no effect, are available.

Using a combination of approaches these gaps in our knowledge can be filled and uncertainty minimized. First, *in vitro* and animal studies could make better use of exposure levels found to cause adverse reproductive effects in epidemiological studies of non-occupationally exposed human populations. There are, however, several caveats. It is difficult to extrapolate doses used *in vivo* or from human studies to the appropriate *in vitro* dose. The pharmacokinetics of the metals in animals can result in differences between the dose administered and the biologically effective dose. There are also considerable interspecies differences in susceptibility to male reproductive toxicity of metals between humans and other mammals (particularly rodents). Additionally there is a deficiency of relevant information for establishing quantitative dose-response relationships and no-adverse-effect exposure thresholds for metal-induced reproductive effects in men.

Second, studies using human subjects need to have a sufficient number of participants to justify their conclusions. They also need to provide a description of the basis for participant selection and of the population characteristics that might influence the outcome or interpretation of results. Where possible, adjustment for exposures (e.g. smoking) or individual characteristics (e.g. age) that may confound metal-outcome relationships should be considered. Other challenges include assessing the effects of genetic variation in modifying the relationships between metal exposure and male reproductive outcomes. While critically important, such studies usually require large populations and are expensive to conduct.

Although not extensively addressed in this review, humans are exposed to many metals simultaneously and these metals can interact additively, synergistically, or antagonistically [ATSDR 2004]. The presence of trace metals may also alter the effects of heavy metal exposure. Furthermore, diet, particularly intake of calcium, iron, and antioxidants, can modify the effect of a given metal exposure on health endpoints including reproductive ones [Dhooge et al. 2007; Kefer et al. 2009; Kordas et al. 2007; Telisman et al. 2007]. It is thus difficult to assign specific effects to a metal if it is the only one evaluated and results may be inconsistent if levels of other metals or dietary constituents that can modify effects are not considered. Despite the above, progress has been made in these areas as demonstrated by many of the studies reviewed. Additional well designed *in vitro*, animal, and human studies should continue to shed light on the complex and fascinating interplay between metals and male reproduction.

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