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Urinary excretion of mercury, copper and zinc in subjects exposed to mercury vapour

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The excretion of mercury, copper and zinc in urine, and mercury in whole blood and plasma, was determined in 40 chloralkali workers exposed to mercury vapour and 40 age-matched referents. The Hg concentrations in whole blood, plasma and urine were higher in the exposed group (35 nmol l^{-1}, 30 nmol l^{-1}, and 11.5 nmol $mmol^{-1}$ creatinine, respectively) in comparison with the reference group (15 nmol l^{-1}, 6.3 nmol l^{-1}, and 1.8 nmol $mmol^{-1}$ creatinine, respectively). The urinary copper excretion was similar in the two groups, while U-Zn excretion was significantly higher ($P = 0.04$) in the exposed group, median 0.83 μmol $mmol^{-1}$ creatinine versus 0.76 μnmol $mmol^{-1}$ creatinine in the reference group. In a subgroup of exposed workers with current U-Hg above 11.5 nmol l^{-1} $mmol^{-1}$creatinine (20 μg g^{-1} creatinine) the median U-Zn was 1.1 μmol $mmol^{-1}$ creatinine. In both groups smokers had high U-Zn levels than non smokers. When both U-Hg and smoking were taken into account in a linear regression model, there was a significant association between U-Hg and U-Zn in the combined group of exposed and referents ($P = 0.002$). This study indicates that mercury exposure in humans, as in animals, causes increased urinary excretion of zinc. The mechanisms may be induced synthesis of metallothionein in the kidneys, displacement of Zn from preexisting metallothionein by Hg, or a decreased reabsorption of zinc in the kidneys owing to a slight tubular dysfunction.

Keywords: copper, exposure, mercury, smoking, zinc

Introduction

Exposure to high levels of mercury (Hg) vapour may be found in mercury mines and certain industries, e.g. plants for production of chlorine, fluorescent tubes or thermometers. Low-level exposure is common in dentistry and is also seen in the general population as a result of release from dental amalgam fillings. Mercury vapour is readily absorbed through the lungs and distributed to various organs before elimination by urinary and faecal excretion. The central nervous system and the kidney are the target organs for inorganic mercury exposure (WHO 1991)

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Metallothioneins (MT) are low-molecular weight proteins with high cysteine content, and thus a high affinity for certain metal cations such as mercury, cadmium, zinc (Zn), silver and copper (Cu). In short-term animal studies mercuric chloride has been shown to induce MT in the kidney (Piotrowski *et al*. 1974, Chmielnicka *et al*. 1986, Chan *et al*. 1992, Liu *et al*. 1992), to increase Cu and Zn levels in kidney (Bogden *et al.* 1980, Lee *et al.* 1983, Chmielnicka *et al*. 1986, Liu *et al*. 1992), and to increase the excretion in urine of copper and zinc (Chmielnicka *et al*. 1986, Liu *et al*. 1992). Similar findings were reported for mercury vapour (Cherian & Clarkson 1976, Yoshida *et al*. 1991). Little, however, is known about the possible influence of Hg exposure on Cu and Zn metabolism in humans.

The aim of this study was to investigate whether occupational exposure to inorganic mercury vapour affects urinary Cu and Zn excretion.

Subjects and methods

Subjects

We examined mercury, zinc and copper in urine in 40 male chloralkali workers exposed to mercury vapour at a chloralkali plant and 40 age-matched, unexposed referents at the same company. Table 1 shows their ages and duration of Hg exposure. During the last years of exposure, typical air-Hg concentrations at ordinary work in the plant were 20–50 $\mu g\ m^{-3}$, but during maintenance the levels were higher (Sällsten *et al*. 1992). Alcohol consumption was moderate, and there were no differences between the exposed and referents. The median fish consumption was one meal per week in both groups. There were 17 smokers in the exposed group and 19 among the referents.

Sampling

Blood and morning urine samples were obtained in metal-free tubes and bottles, respectively, during the same period for exposed and control subjects. After separation of plasma, the samples were stored at –25°C. In order to correct for differences in urinary flow rate, the results were related to the creatinine concentration (Barregård 1993), analysed using a modified Jaffé method with an unprecision below 2%. The bottles for urine sampling contained a small amount of sulfamic acid as a preservative and the average pH of the final urine samples was 5 (range 3–7).

Mercury, zinc and copper determinations

Mercury in whole blood (B-Hg), plasma (P-Hg) and urine (U-Hg) was analysed by cold vapour atomic absorption spectrometry (Skare 1972, Einarsson *et al*. 1984). The detection limit was 1 nmol l^{-1}. Precision and accuracy were acceptable, as presented earlier (Barregård *et al.* 1994). For the exposed group, we also calculated a cumulative exposure index for each subject by adding their yearly mean B-Hg values.

Zinc in urine (U-Zn) was analysed using the flame atomic absorption technique. One ml 0.3 M nitric acid was added to one ml urine, and absorbance was measured at 215 nm. Copper in urine (U-Cu) was analysed using graphite furnace atomic absorption at 324.8 nm. Urine samples were diluted (1:5) with Triton solution including saturated ammonium oxalate solution as a modifer. Urine, spiked with Zn or Cu, was used for calibration. Each sample was analysed in duplicate. The detection limit for Cu was 0.1 $\mu mol\ l^{-1}$, and for Zn 1.0 $\mu mol\ l^{-1}$. The unprecision was below 5% for U-Zn concentrations in the range 3–30 $\mu mol\ l^{-1}$ and about 5% for U-Cu in the range 0.1–5 $\mu mol\ l^{-1}$. Quality control with a reference sample of lyophilized urine (Seronorm™ batch 108, Nycomed, Oslo, Norway), measured together with the samples, showed acceptable accuracy. The results, U-Zn 10.8 $\mu mol\ l^{-1}$ (SD = 0.4, $N = 18$) and U-Cu 718 nmol l^{-1} (SD = 7, $N = 38$), accorded with the certified values of 10 $\mu mol\ l^{-1}$ and 709 nmol l^{-1}.

Statistical analyses

Group comparisons were performed using Student's *t*-test for paired observations or Wilcoxon's rank sum test. Spearman's rank correlation coefficient (r_s) was used to express correlations between single variables. Associations between more than two variables were analysed using the multiple linear regression technique. Statistically significant refers to $P < 0.05$ in two-tailed tests.

Results

As expected, the Hg concentrations in whole blood, plasma and urine were higher in the exposed group in comparison with the reference group (Table 1). The Hg concentrations in these media were also highly intercorrelated in both groups. The urinary

Table 1. Background factors, mercury concentrations in blood, plasma and urine, and concentrations of zinc and copper in urine among chloralkali workers and referents

	Referents ($n = 40$)			Exposed ($n = 40$)		
Variables	Mean	Median	Range	Mean	Median	Range
Age (*y*)	36	38	18–61	36	35	19–65
Exposure time (*y*)	–	–	–	9.6	10	0–31
B-Hg nmol l^{-1}	17	15	8–60	47	35	8–160
P-Hg nmol l^{-1}	6.9	6.3	2–12	37	30	14–119
U-Hg nmol $mmol^{-1}$ creatinine	2.0	1.9	0.5–5	15	12	4.5–53
Cumulative exposure index[a]	–	–	–	810	530	15–4300
U-Zn $\mu mol\ mmol^{-1}$ creatinine	0.78	0.76	0.25–2.2	0.88	0.83	0.39–1.8
U-Cu nmol $mmol^{-1}$ creatinine	15	15	4–44	15	14	3–32

[a]Sum of yearly mean B-Hg

Cu excretions were similar in the two groups, while the median urinary zinc excretion was higher in the exposed group, although the difference was not statistically significant (Table 1).

The ranges for U-Zn were similar in the two groups but, as shown in the distribution of U-Zn concentrations (Figure 1), there was one outlier among the referents, having an over-diluted urine sample (3.6 mmol creatinine l^{-1}). In the further analysis two subjects, one exposed and one referent, with creatinine concentrations less than 4.4 mmol l^{-1} (0.5 g l^{-1}) were excluded since such overdiluted samples are considered inadequate for measuring excretion rates (Alessio *et al.* 1985). The median U-Zn was still higher in the exposed group (0.83 μmol $mmol^{-1}$ creatinine) compared with the reference group (0.76 μmol $mmol^{-1}$ creatinine) and a significant difference in U-Zn between the groups ($n = 38$ pairs) was then found ($P = 0.04$). Moreover, the U-Zn concentrations were even higher for the subgroup of exposed workers with current U-Hg above the median 11.5 nmol $mmol^{-1}$ creatinine (20 μg g^{-1} creatinine), Table 2.

Urinary Zn excretion was, however, also affected by smoking. Among the referents ($n = 39$) the median U-Zn was 0.82 μmol $mmol^{-1}$ creatinine in smokers and 0.61 in nonsmokers ($P = 0.04$). Among the Hg-exposed workers ($n = 39$), it was 1.07 μmol $mmol^{-1}$ creatinine in smokers and 0.75 in non-smokers. In the subgroup with high current U-Hg, the median U-Zn was 1.09 μmol $mmol^{-1}$ creatinine in smokers and 0.86 in nonsmokers, both higher than in smoking and non-smoking referents ($P =$ 0.05 and 0.03, respectively)

When both U-Hg and smoking were taken into account in a linear regression mode, there was a significant and positive association between U-Hg and U-Zn in the combined group of exposed and referents ($P = 0.002$). When the exposed group was analysed separately, the relation between U-Hg and U-Zn was similar to that for the combined group, but

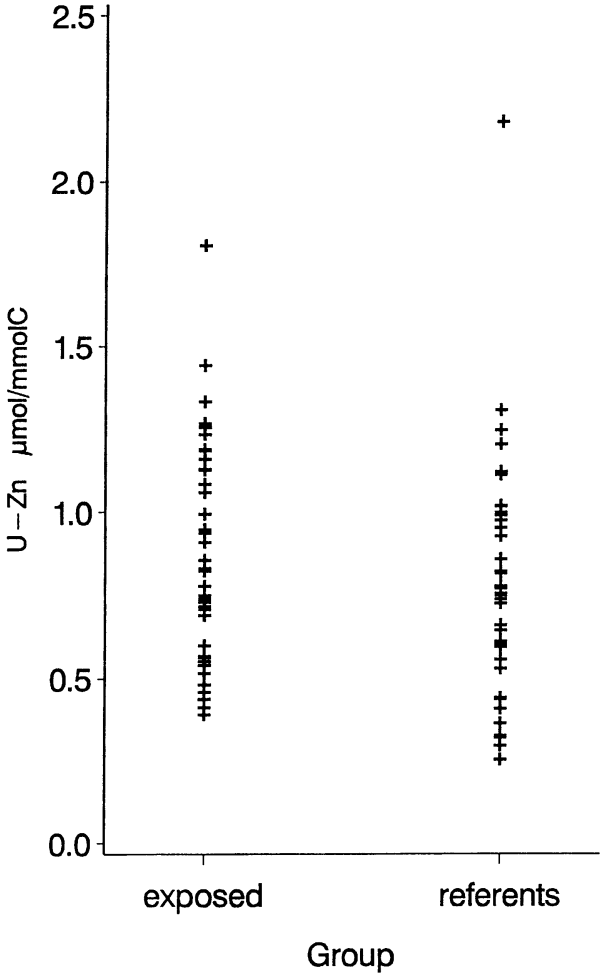

Figure 1. Zinc concentration in urine (U-Zn, μmol $mmol^{-1}$ creatinine) among 40 chloralkali workers exposed to mercury vapour and 40 referents.

Table 2. Median mercury concentrations in blood, plasma and urine, and median concentrations of zinc and copper among referents and chloralkali workers. The exposed workers have been divided into two subgroups according to the median U-Hg for the whole group (High if U-Hg > 11.5 nmol $mmol^{-1}$ creatinine). Two subjects with creatinine values below 4.4 mmol l^{-1} have been excluded

	Referents ($n = 39$)		Exposed, High U-Hg ($n = 19$)		Exposed, Low U-Hg ($n = 20$)	
Variables	Median	Range	Median	Range	Median	Range
B-Hg nmol l^{-1}	15	8–60	55**	15–160	30**	8–55
P-Hg nmol l^{-1}	6.3	2–12	46**	21–119	24**	14–42
U-Hg nmol $mmol^{-1}$ creatinine	1.8	0.5–5	21**	12–53	8.4**	4.5–11
U-Zn μmol $mmol^{-1}$ creatinine	0.76	0.25–1.3	1.1*	0.54–1.8	0.73	0.39–1.3
U-Cu nmol $mmol^{-1}$ creatinine	14	4–27	14	3–32	13	5–27

[a] $P < 0.005$ (pair differences).
** $P < 0.0005$ (pair differences).

the regression coefficient did not significantly differ from zero ($p = 0.07$). Age had no impact on U-Zn.

No correlations were found between U-Zn and mercury concentrations in blood (B-Hg or P-Hg). For copper, no significant correlations were found between U-Cu, on the one hand, and mercury in any media, or U-Zn on the other.

Discussion

The present study indicates that mercury exposure in humans, as in animals, causes increased urinary excretion of zinc. For copper, no such effect was seen.

The impact of Hg exposure on Zn metabolism may be related to metallothionein induction. In mammals, the synthesis of MT can be induced in various organs after exposure to these metals (Cherian & Goyer 1978). The biological role for MTs is unclear, though several functions such as detoxification and storage of heavy metals, and regulation of cellular Cu and Zn metabolism have been suggested (Bremner 1987).

A possible mechanism explaining why Hg exposure increases urinary Zn excretion could be that Hg, having a higher affinity for metallothionein than Zn, displaces zinc from preexisting MT (Day *et al.* 1984, Funk *et al.* 1987). Displacement of Zn by Hg has been shown to occur *in vitro* and *in vivo* in liver MT (Funk *et al.* 1987). Such an effect would result in at least a temporary increase of urinary Zn excretion.

An alternative explanation could be that Hg exposure induces increased MT synthesis in the kidneys. It remains elevated as long as kidney Hg is high, resulting in an increased number of binding sites not only for Hg but also for Zn. The result would be increased kidney Zn concentrations. Animal studies (Chmielnicka *et al.* 1986, Yoshida *et al.* 1991) have shown increased kidney Zn and U-Zn at Hg exposure. For cadmium, which also induces MT, a clear positive correlation between kidney cadmium levels and kidney Zn levels has been shown in humans (Elinder *et al.* 1977, Bem *et al.* 1993).

None of the subjects in this study had an increased albuminuria but a slight effect on renal tubules, as shown by an increased excretion of NAG (*N*-acetyl-beta-glucosaminidase), was seen in the highest exposed group (Barregård *et al.* 1988). After glomerular filtration the majority of Zn is reabsorbed in the tubules (Alfrey 1985) and therefore the increased Zn excretion in the subgroup with high exposure may be caused by a slight tubular dysfunction.

Normally, about 25% of Zn elimination in humans takes place by urinary excretion and about 75% by the faecal route (Elinder 1986). Increased urinary Zn loss is probably balanced by a decrease in faecal Zn, since the homeostasis of Zn seems to be maintained by intestinal Zn secretion (Elinder 1986). Therefore, it seems unlikely that the moderate increase in urinary Zn excretion found in the Hg-exposed workers could contribute to Zn deficiency. Body pools of Zn were, however, not investigated in the present study.

Smokers had higher U-Zn levels than nonsmokers. It has been shown that Zn in kidney cortex is positively correlated to cadmium in kidney cortex (Elinder *et al.* 1977, Bem *et al.* 1993). Since kidney cadmium levels are higher in smokers (Elinder *et al.* 1976, Bem *et al.* 1993), smokers could be expected to have higher kidney Zn levels as well. No such effect was found for the kidney cortex in the study by Bem *et al.* (1993). In another study, however, the kidney Zn levels were higher among smokers than nonsmokers, especially in the medulla (Hardell *et al.* 1994). There are few previous reports on the impact of smoking on urinary Zn excretion. Elinder *et al.* (1978) and Schuhmacher *et al.* (1994) found that smokers had about 30–50% higher U-Zn than nonsmokers, but the differences were not statistically significant.

We found no effect of Hg exposure on U-Cu. Urinary copper excretion only accounts for 1% or less of total copper elimination in humans (Aaseth & Norseth 1986). Kidney-Cu and U-Cu are much lower than kidney-Zn and U-Zn. Interestingly, Funk *et al.* (1987) found that Hg displaces preexisting Zn but not Cu from liver MT. The reason, according to Funk, is that Hg displaces Zn from cluster B, one of the two metal clusters in metallothionein, while Cu remains or even increases in this cluster, since cluster B binds copper more avidly than Zn. Langworth *et al.* (1992) found no effect of Hg exposure on urinary copper excretion in humans, and the average U-Cu levels were the same as in the present study. In summary, moderate Hg exposure seems to have no effect on copper metabolism.

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