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Renal function of chloralkali workers after the cessation of exposure to mercury vapor

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Objectives The aims of the study were to assess renal function in chloralkali workers previously exposed to mercury vapor and to assess the impact of selenium status on the biomarkers of kidney function.

Methods Forty-nine chloralkali workers previously exposed to mercury vapor were compared with 49 age-matched referents in a cross-sectional study. Selected biomarkers of kidney function and biomarkers of selenium status were measured. The index group had been exposed for 13.1 (range 2.8–34.5) years on the average at a mean urinary mercury excretion of 9.3 (range 4.0–25.4) nmol/mmol creatinine a year. The exposure had ceased on an average of 4.8 (range 4.2–10.0) years prior to the examinations.

Results No statistically significant differences were found between the groups for the measured biomarkers of kidney function. The serum selenium concentration and serum glutathione peroxidase activity were associated with the activity of N-acetyl- β -D-glucosaminidase in urine (U-NAG). The results indicate that having higher glutathione peroxidase activity or a higher serum selenium concentration results in a lower excretion of U-NAG. This effect was the most pronounced in the oldest third of the participants. Apparently the well-known association between U-NAG and age could only be found for the participants with a lower selenium status.

Conclusions Increased activities of U-NAG during ongoing exposure to mercury vapor appear to be reversible upon cessation of exposure. Selenium status has a substantial impact on U-NAG activity and should be considered in studies of U-NAG excretion.

Key terms albumin; glutathione peroxidase; kidney; N-acetyl- β -D-glucosaminidase; past exposure; selenium.

The kidney is recognized as a target organ for the toxicity of mercury vapor (1). Exposure to mercury vapor is still common in parts of the chloralkali industry, where metallic mercury is used as a cathode in the production of chlorine and caustic soda by the electrolysis of brine. The uptake of mercury by these workers is mainly through the inhalation of mercury vapor.

Most epidemiologic studies on the urinary excretion of albumin (U-Alb) in people currently exposed to mercury vapor are nonpositive (2–10), although increased concentrations have been reported (11). Animal studies suggest that pathological changes at the glomerular basement membrane are attributed to immunologic

mechanisms (12). An increased prevalence of autoantibodies to the glomerular basement membrane (anti-GBM) may be found, but substantial variability in the strain and species in the susceptibility of this response suggests a genetic control (12). Generally, transient immunologic changes may occur after exposure to mercuric chloride. Only one epidemiologic study has reported an elevation of anti-GBM however (3), and elevation is contradicted by several nonpositive investigations (4, 8–10, 13–15).

Cross-sectional studies of workers during ongoing exposure to mercury vapor have shown an elevated activity of the kidney proximal tubular cell enzyme

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N-acetyl- β -D-glucosaminidase in urine (U-NAG) or associations between U-NAG and the mercury concentration in urine (U-Hg) (4, 5, 7–10). Increased U-NAG activity in chloralkali workers has been associated with exposure levels corresponding to U-Hg of about 5 nmol/mmol creatinine (10).

Chronic adverse effects of past exposure are much less well documented. Former chloralkali workers had no different level of serum creatinine, creatinine clearance, U-Alb, or U-NAG 5.7 years, on the average, after the cessation of exposure than their referents (16). Likewise, no differences for U-Alb, anti-GBM, or U-NAG were found about 12 years after exposure cessation between formerly exposed persons and referents (17). None of these studies presented data on kidney function during the time when the participants were still exposed. Mortality studies have not convincingly shown increased mortality in relation to kidney disease (18–20).

Selenium may reduce the kidney toxicity of inorganic mercury in animals (21), in particular in the proximal tubule cells (22). Selenium may also protect the proximal tubule cells in patients receiving *cis*-platinum (23). A previous epidemiologic study of chloralkali workers suggested that selenium status may modify the effect of exposure to mercury vapor on U-NAG activity (10). The human kidney is rich in selenium, and selenium-dependent glutathione peroxidase (GSHpx) in plasma is, to a large extent, synthesized in the proximal tubule cells (24–26).

This cross-sectional study investigated kidney function in chloralkali workers about an average of 5 years after the cessation of occupational exposure to mercury vapor in a plant that converted to a new technology not involving the use of mercury. Most of the participants had also been examined before the exposure ceased, as their concentration of U-NAG was increased (10). Thus the primary aim of this study was to assess the kidney function and to study a potential reversibility of selected biomarkers of renal toxicity. A further aim was to address the potential impact of selenium status on the applied biomarkers, in particular on the activity of U-NAG.

Study population and methods

Study design and study population

All of the men who had been exposed to mercury vapor for at least 1 year were eligible for inclusion in this cross-sectional study. Furthermore, exposure was required sometime during the last 10 years prior to the examinations. Administrative staff members were not eligible for inclusion. The study was based on individ-

ual matches of pairs of one exposed person with one referent for age (± 3 years). Details have been published elsewhere (27).

In short, 63 previously exposed persons fulfilled the inclusion criteria, of whom five were excluded due to medical conditions recorded by the occupational health service. Of the 58 persons invited to the examinations, 9 declined to participate. Thus 84.5% of the eligible, previously exposed persons participated in the study. Forty-one had participated in a similar investigation while still exposed, shortly before the plant was converted to new technology (10), and thus the study also included longitudinal data.

For comparison, 57 potential referents were invited. Among them, were also referents who had participated previously (10). The referents were recruited from men employed in the lignine drying and production plant and in the cellulose bleaching, drying, and packing facilities located in the same industrial complex as the chloralkali plant. As none of the participants were excluded but four people declined to participate, 93.0% of the eligible referents participated in the study. Four referents could not be included because they did not fulfill the criterion for age-matching, mainly because the previously exposed person for whom they had served as a referent previously (10) did not participate in this study. Thus, 40 of the 49 referents investigated in this study had also attended the examinations prior to the conversion of the technology.

The exclusion criteria were identical for both groups. Alcohol abuse, metabolic disorders, and major psychiatric or neurological diseases causing severe disability were important criteria for exclusion. No persons were excluded due to kidney disease.

The examinations and a structured interview, including self-reported intake of alcohol during the last year (28), were carried out at the occupational health service by its personnel. The background characteristics are shown in table 1.

The study protocol was approved by the Regional Ethics Committee for Medical Research, and informed written consent was obtained from all of the participants.

Exposure assessment

The exposed participants were regularly monitored by measurements of the urinary mercury content, mainly in samples collected after a shift, from the start of production in 1949 until the conversion in 1997. U-Hg was determined by cold vapor atomic absorption spectrometry (CVAAS) during the time the participants in this study were employed (since the mid-1970s). With few exceptions, the U-Hg measurements used for the calculation of the historical exposure estimates were based on this method.

Table 1. Background characteristics of 49 chloralkali workers previously exposed to mercury vapor and 49 referents.

Group	Age (years)		Education (years)		Shift worker (%)		Alcohol consumption (l/year)		Smokers and snuffers (%)		Smokers only (%)		Tobacco consumption (g/week)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Exposed	46.4	28.9–66.9	10.5	7–14	47	–	3.2	0–17.3	55	–	45	–	42	0–150
Referents	46.4	28.2–68.8	11.0	8–17	61	–	3.2	0–13.5	45	–	33	–	37	0–200

The method of adjusting the U-Hg concentrations according to the individual urinary creatinine concentration was introduced in 1982. In order to adjust the concentrations measured prior to 1982, the mean of the individual urinary creatinine concentrations measured in 1982 and 1983 was used. We are not aware of other changes in the monitoring strategy during the period relevant for this study.

Individual cumulative exposure indices (Cum U-Hg) were calculated on the basis of the historical U-Hg measurements. An individual mean U-Hg was calculated for each quarter of the years of exposure. Quarters without U-Hg measurements were given the mean of the U-Hg before and after the missing quarter. The quarterly means were used to calculate a mean U-Hg for each year of exposure. These individual means were added to yield the Cum U-Hg. The mean Cum U-Hg was 125.2 (range 14.5–490.6) nmol/mmol creatinine. The mean exposure intensity (Cum U-Hg/year) was calculated by dividing the Cum U-Hg by the years of exposure. The mean Cum U-Hg/year was 9.3 nmol/mmol (range 4.0–25.4) creatinine/year during an average exposure duration of 13.1 (range 2.8–34.5) years. On the average 50.1 (range 9–560) U-Hg measurements were identified for each participant. The exposure to mercury vapor ceased on an average of 4.8 (range 4.2–10.0) years before the examinations.

Laboratory measurements

The participants were instructed to bring first-voided morning urine samples from the day of and the day before the examination (collected in 30-ml Sarstedt® tubes, Sarstedt, Nümbrecht, Germany) with them to the examination and to store them in a refrigerator before bringing them to the examinations. Heparinized whole-blood samples were obtained from the cubital vein on the day of the examination (10-ml Venoject® tubes, Terumo Corporation, Belgium). Blood specimens for the determination of creatinine were collected in 5-ml Vacuette® tubes (Greiner Labortechnik, Austria), and in 10-ml Vacuette® tubes for immunologic assays. After centrifugation (1500 revolutions/minute for 10 minutes), serum was stored in NUNC® (Nalge Nunc International, Denmark) 1.8-ml cryotubes. The urinary biomarkers of kidney function were measured in both urine samples

collected from each person, whereas trace elements in urine were measured in the sample collected on the examination day. All biological samples were stored at –20°C until analysis.

Renal markers

Renal markers were determined in urine samples stored without additives. All of the measurements were carried out at day 60 after sampling with a Cobas Mira analyzer (Cobas Instruments, Roche Diagnostic Systems, Switzerland) at 37°C (29).

Creatinine was measured photometrically as an endpoint measurement after 125 seconds at 500 nm, using alkaline picrate (ABX Diagnostics, Montpellier, France). U-NAG (detection limit 0.02 U/l) was measured colorimetrically on the basis of the release of 3-cresolsulfonphthalein from 3-cresol-sulfonphthaleinyl-N-acetyl-β-D-glucosaminide at 580 nm, as an endpoint measurement (Boehringer, Mannheim, Germany). The within and between assay coefficient of variation (CV) was 2.8% and 3.0%, respectively. Alkaline phosphatase in urine (U-ALP) was measured colorimetrically at 405 nm and pH 9.8 by a kinetic method as p-nitrophenol liberated from p-nitrophenyl phosphate (Roche Diagnostics GMBH, Germany). The within and between assay CV was 1.2% and 2.0%, respectively. U-Alb (detection limit 0.02 mg/l) was determined by immunoturbidimetry with the use of a commercial kit as an endpoint measurement at 340 nm (ABX Diagnostics, Montpellier, France). The within and between assay CV was 4.0% and 5.8%, respectively. Autoantibodies to the glomerular basement membrane (anti-GBM) were determined with the use of DIASTAT™ ELISA (enzyme-linked immunosorbent assay) kits from Axis-Shield (Dundee, United Kingdom), following the assay protocol provided by the manufacturer.

Glutathione peroxidase in serum

Glutathione peroxidase was measured by an automated assay as previously described (30) on an Axon autoanalyzer (Technicon Instruments, Bayer Corporation, New York, NY, USA). Enzyme activity was determined in a two-step reaction in which glutathione peroxidase catalyzed the reduction of hydroperoxide by GSH, followed

by the NADPH-dependent reduction of glutathione disulfide catalyzed by glutathione reductase. The disappearance of NADP (nicotinamide adenine dinucleotide) phosphate was measured spectrophotometrically by reduced absorbance at 340 nm with the use of a millimolar extinction coefficient of 6.22 mM/cm for NADPH. The standard curve was linear up to a concentration of 500 U/l. The samples were analyzed in one batch with a within-assay CV of 3.5 %.

Measurements of trace elements

U-Hg was measured by direct CVAAS as previously described (10). The accuracy and reproducibility of the measurements were assessed using lyophilized Seronorm™ trace element quality control materials (STE FE 1114) (Sero Ltd, Asker, Norway). The day-to-day variation was 4% for mercury at an observed average U-Hg concentration of 241 nmol/l, corresponding to the recommended value of 239 nmol/l by the producer. The detection limit of the method was 2 nmol Hg/l ($3 \times \text{SD}$ of the individually prepared blank solutions). Total mercury in whole blood (B-Hg) was measured by inductively coupled plasma high-resolution mass spectrometry. The details have been given elsewhere (27).

Cadmium in urine (U-Cd) was measured using an electrothermal atomic-absorption spectrometric (ETAAS) system (Perkin-Elmer SIMAA 6000, Überlingen, Germany). The accuracy and reproducibility were assessed using Seronorm™ human urines STE FE 1114 and STE 101021. The day-to-day variations were 6% and 11% at observed average U-Cd concentrations of 42 and 2.9 nmol/l, respectively. The recommended concentrations of U-Cd supported by the producer were 44 and 3.1 nmol/l. The detection limit was 0.4 nmol/l of cadmium.

Serum was prepared for the measurement of selenium (S-Se) using the same procedure as for B-Hg (27) and analyzed for selenium by ETAAS calibrated with serum-matched standard solutions. Reduced palladium was used as a chemical modifier. The detection limit was 0.05 mmol Se/l. The accuracy and reproducibility of the measurements were demonstrated using Seronorm™ human-serum STE 605113. The reproducibility of the measurement was $\leq 5\%$, and the measured average selenium concentration was within $\pm 3\%$ when compared with the value recommended by the producer (0.99 mmol/l).

Statistics

The mean concentrations of the biomarkers of renal function measured in the urine samples collected on two consecutive days are presented. Several of the measured variables had skewed distributions. If the skewness ex-

ceeded 2.0 or the distributions to be compared had different variances (Levene's test), the variables were log-transformed, and the geometric means (GM) were presented. An analysis of variance was used for group comparisons. If more than two groups were compared, the least square difference was calculated post hoc to separate the groups that differed. Students t-test for paired samples was applied for assessing differences between the two examinations of the same person. The chi-square test was used for dichotomous variables.

Associations between the effect variables and the independent variables were assessed with multiple linear regression analysis (backward procedure). Independent variables [age, being a smoker or not (1/0), alcohol consumption (liter of pure ethanol/year), U-Cd (log), U-Hg, medication (1/0), and diastolic blood pressure] were assessed together with the exposure variable (1/0). The calculations were performed with the inclusion of S-Se or S-GSHpx separately. The level of statistical significance was set at $P < 0.05$ (two-tailed). The statistical package SPSS®, version 11.5 (SPSS Inc, Chicago, IL, USA) was used.

Results

The mean concentrations of B-Hg [23.1 (range 4.9–76.8) nmol/l versus 17.5 (range 3.1–67.8) nmol/l, $P = 0.049$] and U-Hg [1.7 (range 0.2–5.2) nmol/mmol creatinine versus 1.2 (range 0.3–3.2) nmol/mmol creatinine, $P = 0.003$] were slightly higher in the previously exposed participants than in their referents, whereas the mean concentrations of U-Cd for the exposed [0.4 (range 0.1–1.3) nmol/mmol creatinine] versus the referents [0.3 (range 0.1–3.4) nmol/mmol creatinine] ($P = 0.91$) were similar.

No statistically significant group differences were found between the exposed participants and the referents for the kidney function biomarkers serum creatinine, U-Alb, U-ALP, U-NAG, and anti-GBM (table 2). Nor did the mean concentrations of S-GSHpx and S-Se differ significantly. The Pearson's correlation coefficients calculated between the concentrations (nontransformed) of U-Alb, U-NAG, and U-ALP measured on two successive days were 0.76, 0.66, and 0.95 ($P < 0.001$ for all of the associations), respectively.

The previous exposure to mercury vapor, the current U-Hg and the Cum U-Hg did not contribute to the statistical models for any of the kidney function parameters in the regression analysis (table 3). Age was positively associated with U-NAG and U-Alb. The statistical models also suggested that an increasing concentration of S-Se and an increasing activity of S-GSHpx were associated with lower levels of U-NAG and U-Alb. One

Table 2. Biomarkers of kidney function and selenium status in 49 chloralkali workers previously exposed to mercury vapor and 49 age-matched referents. The difference for the log-transformed variables is expressed as a ratio. (U-NAG = N-acetyl-β-D-glucosaminidase in urine, U-Albumin = urinary excretion of albumin, U-ALP = alkaline phosphatase in urine, S-creatinine = serum creatinine, S-anti-GBM = serum glomerular basement membrane, S-GSHpx = glutathione peroxidase activity in serum, S-Se = selenium concentration in serum, GM = geometric mean, AM = Arithmetic mean, ND = not detected)

	U-NAG (U/mmol creatinine)		U-Albumin (μg/μmol creatinine)		U-ALP (U/mmol creatinine)		S-Creatinine (μmol/l)		S-anti-GBM (U/ml)		S-GSHpx (U/l)		S-Se ^a (μmol/l)	
	GM	Range	GM	Range	GM	Range	AM	Range	AM	Range	AM	Range	AM	Range
Exposed	0.19	0.09–0.77	0.38	0.01–6.79	0.17	0.05–0.79	87	44–112	0.35	ND–1.46	138	103–203	1.4	0.7–2.1
Referents	0.19	0.06–1.88	0.34	0.01–7.99	0.19	0.05–3.31	86	58–105	0.34	ND–1.36	145	105–201	1.5	0.9–2.7
Difference (95% CI)	1.00	(0.81–1.23)	1.11	(0.69–1.81)	0.90	(0.69–1.16)	0.3	(-4.1–4.7)	0.01	(-0.08–0.09)	-7	(-16–2)	-0.06	(-0.21–0.08)

^a Measured in 48 exposed and 48 referents.

Table 3. Results from the multiple linear regression analysis (model; see statistics). (U-NAG = N-acetyl-β-D-glucosaminidase in urine, U-Albumin = urinary excretion of albumin, ns = not significant)

	α	β	Multiple r
U-NAG (log)	-0.62 ^a + 0.006 ^b	Age + 0.15 ^c U-Cd (log) - 0.002 ^c S-GSHpx	0.43 ^a
U-NAG (log)	-0.84 ^a + 0.007 ^a	Age + 0.01 ^c Alcohol - 0.18 ^b S-Se	0.45 ^a
U-Albumin (log)	-0.10 ^{ns} + 0.01 ^c	Age - 0.006 ^c S-GSHpx	0.30 ^c
U-Albumin (log)	-0.26 ^{ns} + 0.01 ^c	Age - 0.44 ^b S-Se	0.35 ^b

^a P<0.001.

^b P<0.01.

^c P<0.05.

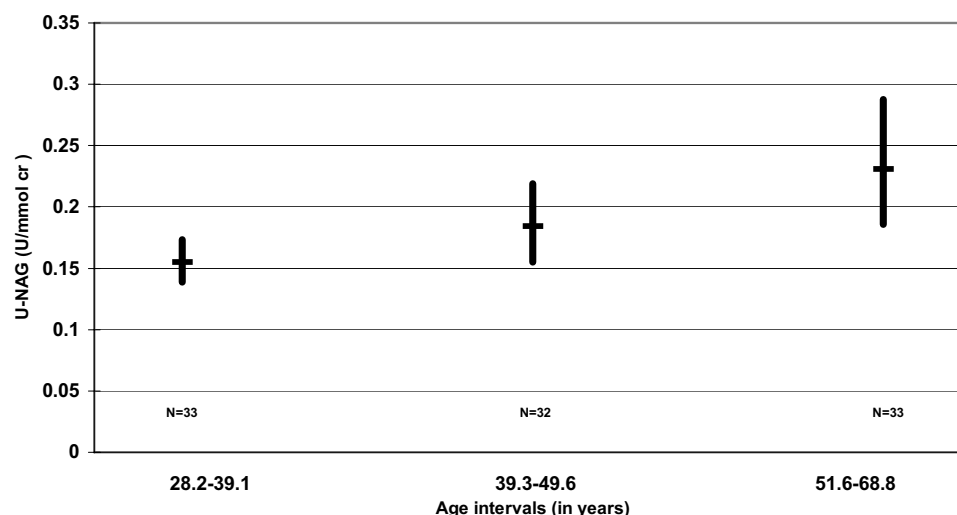


Figure 1. Activities of N-acetyl-β-D-glucosaminidase in urine (U-NAG) (geometric mean and 95% confidence interval) according to age. (cr = creatinine)

of the regression models indicated an association between U-NAG and U-Cd. When the participant with the highest U-Cd (3.4 nmol/mmol creatinine) was removed from the analysis, the association with U-Cd disappeared, but S-GSHpx was still included in the statistical model, and the regression coefficients changed only slightly (revised model: U-NAG (log) = -0.78^{P<0.001} + 0.006^{P=0.001} age + 0.01^{P=0.03} alcohol - 0.002^{P=0.02} S-GSHpx). No association was found between the markers of selenium status and the brush border enzyme alkaline phosphatase.

According to the results from the regression analysis, all of the participants were stratified by age into three equally large groups. The increasing trend in the geometric mean U-NAG activities by age is obvious (figure 1). When it was substratified by S-GSHpx according to the median concentration and the result was combined with the age stratification, the level of S-GSHpx had no influence on the measured U-NAG activities in the younger participants (figure 2). In the oldest age group, the U-NAG activities differed statistically significantly (P=0.02) between the participants with

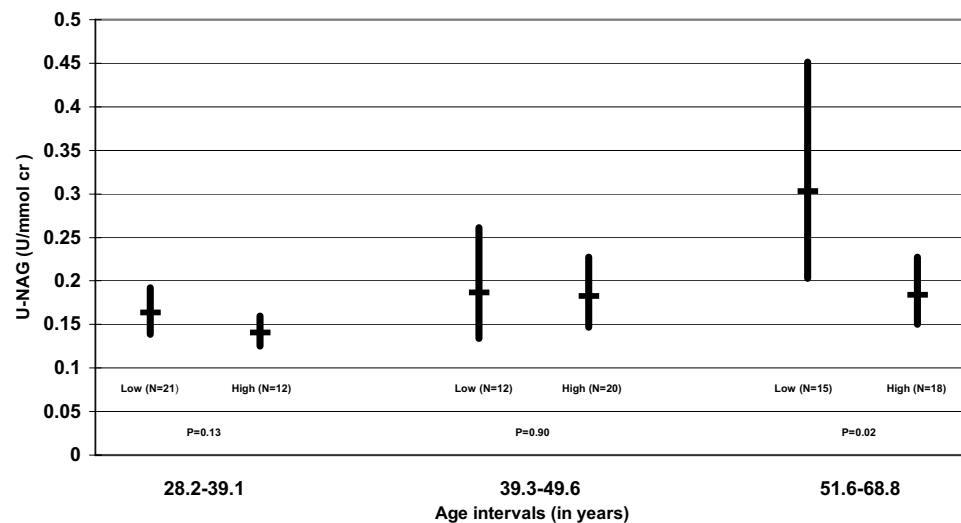


Figure 2. Activities of N-acetyl- β -D-glucosaminidase in urine (U-NAG) (geometric mean and 95% confidence interval) according to age and the S-GSHpx activity. The low selenium-dependent glutathione peroxidase (S-GSHpx) activities ranged from 103 U/l to 140 U/l, and the high activities ranged from 141 U/l to 203 U/l. (cr = creatinine)

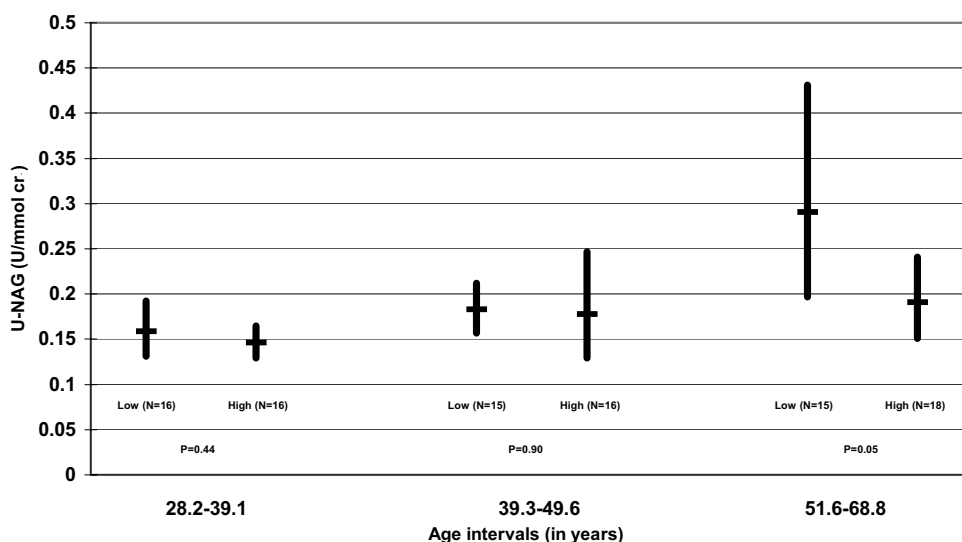


Figure 3. Activities of N-acetyl- β -D-glucosaminidase in urine (U-NAG) (geometric mean and 95% confidence interval) according to age and the serum concentration of selenium. The low serum concentrations of selenium ranged from 0.7 μ mol/l to 1.3 μ mol/l, and the high concentrations ranged from 1.4 μ mol/l to 2.7 μ mol/l. (cr = creatinine)

high and low S-GSHpx. A similar pattern was observed when we stratified according to the median concentration of S-Se (figure 3). Only insignificant changes in the U-NAG activities were calculated when the activities were adjusted as shown in figure 2 and figure 3 according to alcohol consumption and age. No statistically significant associations were found between U-NAG and age for the participants with "high" levels of S-GSHpx or S-Se, respectively, in contrast to the participants with low levels of S-GSHpx ($\text{U-NAG}(\log) = -1.11^{P<0.001} + 0.009^{P=0.005} \text{ age}$) or S-Se ($\text{U-NAG}(\log) = -1.14^{P<0.001} + 0.01^{P=0.001} \text{ age}$).

The biomarkers measured in 41 previously exposed persons and 40 referents that had been studied when they were still exposed more than 4 years earlier were compared with the measurements from this study. The increase in U-NAG between the two occasions was somewhat larger for the referents than for the previously exposed participants (table 4). The increases were of statistical significance for both groups. The concentra-

tions of U-Alb decreased significantly in both groups. No statistically significant group differences were found between the 41 previously exposed persons and the 40 referents at the second examination. However, a P-value of 0.05 was found for the difference in U-NAG between these workers at the first examination.

Discussion

The aim of this study was to assess the long-term consequences of exposure to mercury vapor on the kidney in chloralkali workers after exposure cessation. An additional aim was to examine any influence of selenium status upon the kidney biomarkers, in particular U-NAG. The participation was high in both groups, the finding indicating that selection bias is of little importance. The two groups under study were similar with respect to important background characteristics such as age, duration of formal education, alcohol consumption, and

smoking habits. These background characteristics and the histories as factory workers suggest that the two groups under study had a similar sociodemographic background.

No group differences could be detected for any of the measured kidney parameters. Hence the higher activity of U-NAG measured during ongoing exposure (10) had disappeared. This finding is noteworthy and should indicate that increased U-NAG during ongoing exposure is reversible upon exposure cessation. However, a slightly higher U-Hg in the previously exposed participants could suggest that slight exposure may still occur among some persons. The selenium status, as measured by the concentration of S-Se and the activity of S-GSHpx, appears to have a suppressive effect on U-NAG activity. This finding is consistent with our *a priori* hypothesis. We are not aware of other studies showing this effect of selenium on U-NAG concentration in the absence of occupational nephrotoxic exposures. An association, although weaker, between selenium status and U-Alb was also found. This possibility was not hypothesized *a priori* and should thus be considered an interesting, possibly random finding that deserves further exploration. Age was, as anticipated, related to the activity of U-NAG. However, the association between U-NAG and age was found only for the participants with a low selenium status. Such an association has, to our knowledge, not previously been reported.

Only two studies have addressed the question of the potential long-term consequences of previous exposure to mercury vapor on kidney biomarkers. Our results of the U-NAG activities and U-Alb concentrations, being similar for the workers previously exposed and for the referents, support the results from previous studies (16, 17). Our observation that the increase in U-NAG among the exposed persons between the two examinations was lower than for the referents may suggest that at least the U-NAG activities normalize after the cessation of exposure. While these exposed persons were still under exposure, they had significantly higher U-NAG than their referents (10). Hence one could speculate mechanistically that, after exposure cessation, the U-NAG activity would decrease. This decrease might be related to the decrease in U-Hg, which occurs with a half-time of about 60–80 days (31). After the normalization of U-NAG, the normal process of ageing might occur in the previously exposed persons. For the referents, the U-NAG level increases with age, which is well known. A general problem of studies using longitudinal laboratory data is that slight modifications in the methods related to laboratory analysis may be introduced. This could be one reason for the observation of lower U-Alb concentrations in examination 2 as compared with that in examination 1 (table 4).

Table 4. Concentrations of biomarkers of the kidney function in urine and serum of 41 chloralkali workers previously exposed to mercury vapor and 40 referents examined on two occasions. (U-NAG = N-acetyl- β -D-glucosaminidase in urine, U-Albumin = urinary excretion of albumin, U-ALP = alkaline phosphatase in urine, S-creatinine = serum creatinine, U-Hg = mercury concentration in urine)

	Examination 1		Examination 2		P-value ^a	P-value ^b
	Mean	Range	Mean	Range		
U-NAG (U/mmol creatinine) ^c						
Exposed	0.15	0.05–0.44	0.18	0.09–0.77	0.006	.
Referents	0.12	0.05–0.69	0.19	0.06–1.88	<0.001	0.79
U-Albumin (μg/μmol creatinine) ^c						
Exposed	0.51	0.17–5.2	0.33	0.01–6.8	0.02	.
Referents	0.46	0.19–3.8	0.32	0.01–8.0	0.04	0.89
U-ALP (U/mmol creatinine) ^c						
Exposed	0.18	0.05–0.53	0.17	0.05–0.79	0.38	.
Referents	0.19	0.06–1.12	0.18	0.05–3.31	0.47	0.81
S-Creatinine (μmol/l)						
Exposed ^d	83.6	66–132	86.9	44–112	0.08	.
Referents	84.9	68–106	85.7	58–105	0.56	0.56
U-Hg (nmol/mmol creatinine)						
Exposed	5.9	1.6–16.8	1.7	0.6–5.2	<0.001	.
Referents	1.1	0.2–3.4	1.2	0.3–3.2	0.71	0.01

^a P-value for differences between examination 1 and examination 2 for the exposed group and reference group.

^b P-values between groups at examination 2.

^c Geometric mean.

^d For serum creatinine, 40 previously exposed participants.

We are not familiar with other studies of kidney biomarkers in which a target group has been followed for such a long time after the cessation of exposure to mercury vapor. Thus, within this time-span, there was no evidence for inorganic mercury acting as a so-called “slow toxin”, where the ageing process unmasks a permanently reduced functional capacity. However, the follow-up time was limited to between 4 and 5 years, but cohort studies do not indicate a significantly increased mortality from kidney diseases for people exposed to mercury vapor (18–20).

It has been shown that patients administered the selenium antagonist *cis*-platinum, and who also received selenium in high doses, had a significantly lower increase in U-NAG than patients who did not receive selenium (23). It has also been suggested that patients should be monitored with respect to S-GSHpx before the administration of nephrotoxic drugs so that those with a particular risk associated with certain medications can be identified (26). Our results could have the implication that exposure to other metals (eg, mercury) that are toxic to the proximal tubule cells may have their effects modified by the differences in the selenium status. However, no intervention studies with respect to exposure to mercury vapor have, to our knowledge, been carried out so far with humans.

The negative associations that were found between U-NAG and the markers of selenium status suggest that selenium has an inhibitory effect on the excretion of U-NAG. Selenium has protective properties against the untoward effects of inorganic mercury exposure among animals (21, 22), but little is known for humans. In our previous study, we found that selenium modified the effect of exposure to mercury vapor on the excretion of U-NAG (10). Our present study suggests that selenium may even modify the U-NAG excretion in the absence of known occupational nephrotoxic exposures. We have not found other studies confirming this view. This finding is methodologically important for epidemiologic studies of persons exposed to nephrotoxic compounds because a difference in selenium status between groups under study may operate as an important confounder. It should be noted that the excretion of the brush border enzyme alkaline phosphatase was not associated with selenium status, in contrast to the association with NAG, which is a lysosomal enzyme.

U-NAG is considered to be a marker of toxicity for the kidney proximal tubular cells in the absence of gross glomerular basement membrane defects. These cells are also the major source of extracellular GSHpx (25, 26). However, there were only small differences between S-GSHpx and S-Se in the impact on the U-NAG activities. The low Pearson's correlation coefficient of 0.33 ($P=0.001$) between the two variables does not suggest serious collinearity. Therefore, both markers may be used when the impact of selenium status on the U-NAG activity is assessed.

In conclusion, no effect of previous exposure to mercury vapor was observed for the measured kidney biomarkers. Our study shows that the increased U-NAG activities previously found (10) may be reversible upon the cessation of exposure. Hence the relationship between U-NAG and U-Hg should be investigated further to address the possibility that the reversibility may be related to the reduction in U-Hg when exposure ceases. Selenium status appears to have an important effect on the excretion of U-NAG, and this possibility implies that selenium status markers should be measured in occupational epidemiologic studies of U-NAG so that potential confounding from the difference in selenium status between groups can be ruled out. Another implication of our results could be that the magnitude of the effects of exposure to nephrotoxic substances on the proximal tubule cells may depend on the selenium status of the populations under study.

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