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An epidemiological study of reproductive function biomarkers in male welders

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Abstract

In a cross-sectional study, the serum concentrations of inhibin B and prolactin of 96 male current welders were compared with the concentrations measured in 96 age-matched referents. Also, 23 patients who were all former welders diagnosed as having welding-related manganism were studied. The current welders' geometric mean (GM) airborne exposure to manganese (Mn) was 121 μg m^{-3} (range 7–2320). The serum concentrations of prolactin adjusted for age and smoking habits (GM 193 mIU l^{-1} vs. 166 mIU l^{-1}; $p = 0.047$) and inhibin B adjusted for alcohol consumption (arithmetic mean (AM) 151 ng l^{-1} vs. 123 ng l^{-1}; $p = 0.001$) were higher in the welders compared with the referents. The whole blood Mn concentration was associated with the serum prolactin concentrations. Tobacco smoking resulted in lower serum prolactin concentrations. The GM serum prolactin concentrations of the patients did not significantly differ from that of the referents, but their AM serum inhibin B concentration was statistically significantly lower. The results may suggest an effect of Mn on the pituitary that is reversible upon cessation of exposure. Lower inhibin B concentrations in the patients could point to a functional impairment of the testicular Sertoli cells, that may be caused by a welding fume component or other factors in their work environment.

Keywords: *Manganese, welders, inhibin B, prolactin, epidemiology*

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Introduction

Manganese (Mn) is toxic to the central nervous system (CNS) by causing a disease of movements (manganism) clinically resembling idiopathic Parkinson's disease (Calne et al. 1994). Early case reports suggested that reduced libido or impotency may occur as part of manganism (Baader 1932). Later, disturbances in sexual functions were reported in larger studies of Mn-intoxicated miners (Rodier 1955, Schuler et al. 1957). Studies of Mn-exposed animals have presented evidence of various alterations

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related to male reproductive functions, such as lower testicular weight, the degeneration of seminiferous tubules, lower sperm production and reduced fertility (Chandra et al. 1973, Singh et al. 1974, Chandra et al. 1975, Murthy et al. 1980, Laskey et al. 1982, Elbetieha et al. 2001).

Most of the world production of Mn is used in the production of steel. Manganese is present in welding electrodes, and there are probably several million welders worldwide exposed to Mn-containing welding fumes. Epidemiological studies that have addressed the issue of fertility in male welders reported an odds ratio (OR) of 2 for having poor sperm quality or a crude OR of about 2 for subfertility that was not of statistical significance after adjusting for confounders (Mortensen 1988, Bonde 1990a). Reduced sperm quality reported in one cross-sectional study was not confirmed in other studies (Bonde 1990b, Bonde & Ernst 1992, Hjollund et al. 1998). Thus the available literature shows ambiguous evidence that exposure to welding fumes may cause male sexual functions to deteriorate.

Increased concentrations of prolactin (PRL) in serum have been observed in Mn alloy production workers (Alessio et al. 1989, Mutti et al. 1996, Ellingsen et al. 2003). These observations extend the observation in rats of intraventricularly injected Mn being related to the serum PRL concentrations (Barbeau et al. 1976). A very high concentration of serum PRL is associated with oligospermia and impotency in men (Melmed & Kleinberg 2003). The secretion of PRL from specific anterior pituitary cells (lactotrophs) into the systemic circulation in a circadian and pulsatile rhythm is under tonic hypothalamic inhibition by the neurotransmitter dopamine. Dopamine acts by binding to D2-receptors on the lactotrophs and inhibits both the release and the synthesis of PRL (Thorner et al. 1992). The rate-limiting step of the dopamine synthesis is dependent on the iron (Fe)-containing enzyme, tyrosine hydroxylase (TH), converting tyrosine to L-Dopa. Altered TH activity in several rat brain areas, including the hypothalamus, has been reported after administration of $MnCl_2$ (Bonilla 1980, Deskin et al. 1980, Daniels & Abarca 1991).

Little is known about the testicular uptake of Mn. Iron is utilized by differentiating germinal cells, and it has been suggested that transferrin-bound Fe is internalized into the Sertoli cells. These cells synthesize transferrin in order to shuttle Fe through the cell into the germ cells (Petrie & Morales 1992). Also, a role for the divalent metal transporter 1 for testicular Fe transport has been identified in rats (Griffin et al. 2005). Whether Mn is transported by these Fe transporters in testicular tissues, as in several other tissues, is not known.

Inhibin B is a glycoprotein predominantly produced by the testicular Sertoli cells in man (Kumanov et al. 2005). It is generally believed that the production of inhibin B is stimulated by the follicle stimulating hormone (FSH), and that inhibin B exerts a negative feedback effect on FSH secretion in man (Anderson et al. 1997, Kumanov et al. 2005). Men with normal fertility have higher serum levels of inhibin B than men with impaired fertility (Eckardstein et al. 1999, Ballescá et al. 2000, Brugo-Olmedo et al. 2001). There are also indications of a positive correlation between the concentration of sperm and serum inhibin B (Mahmoud et al. 1998). Inhibin B is currently viewed as a biomarker of Sertoli cell function and a measure of spermatogenesis (Mahmoud et al. 1998, Kumanov et al. 2005).

Sperm measurements are difficult to perform in epidemiological studies due to the often low participation, and there is a need for another functional biomarker of sperm production. In this investigation the concentrations of the reproductive function

biomarkers PRL and inhibin B in the serum of male welders currently exposed to welding fumes were studied. While PRL has been studied in other Mn-exposed populations, it has not been studied in welders. To our knowledge, inhibin B has not been studied in any Mn-exposed populations. Manganism in welders is regarded as a serious occupational health problem in Russia, and data on former welders who have received that diagnosis and who have also been financially compensated for their disease are presented as well. These biomarkers have never been studied in such patients. This work is part of a larger epidemiological study emphasizing nervous system functions of welders. Data on the exposure to welding fumes have been presented (Ellingsen et al. 2006).

Material and methods

Subjects

Details on the study design have been presented (Ellingsen et al. 2006). The welders were recruited from one shipyard and one plant producing heavy machinery in the vicinity of St Petersburg in Russia. One welding department was selected at random from each plant, and one shift of welders from each of the selected departments was eligible for inclusion. The main criteria for inclusion in this cross-sectional pair-matched study were at least 1 year of employment as a welder (men only) and being employed at the time of the examinations. Referents were selected among turners/fitters (men only) working at the same plants. They were pair-matched 1:1 with an exposed subject with a maximum age difference of ± 2 years within a pair.

Subjects who had been on sick leave for more than 14 days on the examination day were not considered for inclusion. Exclusion criteria included known current or previous diseases of the CNS that are probably unrelated to Mn exposure (e.g. brain tumours or transitory ischaemic attacks), and known alcohol (or drug) abuse. Diabetes mellitus, and serious kidney or liver diseases were other criteria for exclusion. Occupational exposure to organic solvents for more than 3 years as painters or spray painters, or ever having been employed at plants producing solvents led to exclusion. Subjects with previous occupational exposure to lead or mercury for more than 1 year were not considered for inclusion.

Five out of a total of 132 welders who were identified were not available for inclusion (sick leave or vacation). Eleven welders were excluded based on information in the records of the occupational health services (OHS), mainly owing to high alcohol intake. Thus, 116 welders were eligible for inclusion, of whom 20 declined to participate, yielding a participation rate of 82.8% among the eligible welders. Fourteen potential referents of those 156 turners/fitters who were identified as working in the departments selected for recruiting the referents were not available for the study due to sick leave or vacation. Twenty-two subjects were excluded due to the violation of the exclusion criteria (mainly because of high alcohol intake). Thus the reference base consisted of 120 eligible potential referents, of whom 24 refused to participate (participation rate 80.0%). Background data of the participants are shown in Table I.

The Northwest Public Health Research Centre (NWPHRC) in St Petersburg (Russia) had recorded 74 subjects with the diagnosis of manganism in their medical files at the time of this investigation. All patients had had their condition officially recognized as an occupational disease in the Russian insurance system. The aim was

Table I. Background characteristics and exposure data in 96 currently exposed welders and 96 age-matched referents.

	Exposed		Referents	
	Mean	Range	Mean	Range
Age (years)	36.3	20–65	36.1	18–66
Smokers (%)	60.4	–	62.5	–
No. of cigarettes/day	9.5	0–40	10.6	0–30
Alcohol consumption (g per year)	7700	0–47300	5300	0–72800
Duration of welding (years)	13.5	1–40	–	–
Air-Mn[a] ($\mu g\ m^{-3}$)	121	7–2320	–	–
B-Mn ($nmol\ l^{-1}$)	154	67–422	124	45–258
B-Pb[a] ($\mu mol\ l^{-1}$)	0.24	0.09–0.81	0.18	0.06–1.04
U-Mn[a] ($nmol\ mmol\ cr^{-1}$)	0.35	0.05–11.4	0.26	0.04–21.0

[a]Geometric mean, otherwise arithmetic mean; cr, creatinine.

to examine half of these patients. Every second individual from an alphabetical list was considered. Two subjects were not available. Four subjects had to be excluded. Because four patients refused to participate, 27 patients (participation rate 87.1%) could be included, of whom all were former welders. Four patients were female, and were thus not further considered in this study of male fertility. Because the youngest of the patients was 41 years of age, all referents and all current welders above this age were used as comparison for the patients. Their background data are shown in Table II.

The examinations, that included a structured interview and the sampling of blood and urine, were carried out at the respective local occupational health clinics for the welders and the referents, and at the NWPHRC for the patients. Participation in the study was voluntary, and all participants signed an informed written consent. The study was approved by the Ethics Committee of St Petersburg Pasteur Institute.

The collection of biological samples

Briefly, the subjects were instructed to bring with them to the examinations a first-voided morning urine sample voided directly into a clean plastic cup before being transferred to a 25 ml Universal container (Nalge Nunc Int. Corp., Rochester, NY, USA). Blood samples were collected between 08.30 and 09.30 the same morning. Heparinized whole blood for the determination of trace elements was collected by using 10 ml Venoject tubes (Terumo Corp., Belgium), while blood specimens for the determination of PRL and inhibin B in serum were collected with Greiner 9 ml tubes (Greiner Labortechnik, Austria). After centrifugation at 1500 rpm (600 g) for 10 min, the serum was stored in Sarstedt 2 ml Cryo tubes (Sarstedt, Nümbrecht, Germany). All biological samples were frozen and stored at the NWPHRC at $-20^{\circ}C$ before shipment to the laboratories.

Analysis of PRL and inhibin B in serum

The serum concentration of PRL was measured by an immunofluorometric assay using kits supplied by Wallac Oy (Turku, Finland). The intra-assay and interassay coefficients of variation (CV) were 3–6% and 8–9%, respectively. The laboratory reference range in men is 50–700 $mIU\ l^{-1}$. The serum concentrations of inhibin B

Table II. Background characteristics, exposure and the serum concentrations of prolactin and inhibin B in 23 male patients (all former welders) who have received the diagnosis of manganism, 42 current welders (exposed) and 42 referents.

	Patients		Exposed		Referents		
	Mean	Range	Mean	Range	Mean	Range	*p* ANOVA
Age* (years)	50.2	41–58	46.8	41–65	48.4	41–66	0.10
Smokers (%)	47.8	–	59.5	–	52.4	–	–
No. of cigarettes per day	6.6	0–20	9.9	0–40	8.6	0–30	0.39
Alcohol consumption* (g per year)	1950	0–8112	10258	0–47320	6349	0–72800	0.01
Duration of welding (years)	22.7	15–30	21.4	1–40	–	–	–
Time since welding cessation (years)	5.7	4–7	–	–	–	–	–
B-Mn*** (μmol l^{-1})	152[d]	94–345	152	84–391	127	68–258	0.07
B-Pb[a],*,*** (μmol l^{-1})	0.17[d]	0.10–0.41	0.24	0.09–0.71	0.18	0.08–1.04	0.01
U-Mn[a],*,** (nmol mmol cr^{-1})	0.13[d]	0.06–0.26	0.25[d]	0.05–2.2	0.38	0.04–21.0	0.001
Prolactin[a] (mIU l^{-1})	151	62–379	173	58–708	162	74–1082	0.59
Adjusted[a],[b] (mIU l^{-1})	150	–	174	–	162	–	0.56
Inhibin B *,**,*** (ng l^{-1})	83	8–200	157	22–348	127	8–242	<0.001
Adjusted[c],*,**,*** (ng l^{-1})	87	–	154	–	128	–	<0.001

*p <0.05 between patients and exposed; **p <0.05 between patients and referents; ***p <0.05 between exposed and referents; [a]geometric mean, otherwise arithmetic mean; [b]adjusted for age and smoking status; [c]adjusted for alcohol consumption; [d]one subject missing; cr, creatinine.

were measured by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using kits supplied by Oxford Bio-Innovation (Oxfordshire, UK). The intra-assay and interassay CV were <7%. The manufacturer's reference range in men is <400 ng l^{-1}.

Trace elements in blood and urine

For the measurement of Mn (B-Mn) and lead (B-Pb) in whole blood at the National Institute of Occupational Health, Norway (NIOH), 1.5 ml 65% ultrapure nitric acid was added to 1 ml of whole blood in a polypropylene digestion tube. After heating to 95°C for 1 h, 200 µl of an internal standard solution was added to the sample. For the measurement of Mn in urine (U-Mn), all samples were heated for 1 h at 95°C prior to analysis. One millilitre of urine was added to 200 µl of an internal standard solution and diluted to volume. The digested blood and diluted urine samples were analysed by inductively coupled plasma sector field mass spectrometry (ICP-SF-MS) using an Element 2 mass spectrometer (Thermo Electron, Bremen, Germany) calibrated with whole blood and urine matrix-matched standard solutions. Seronorm TM Trace Elements human whole blood and urine quality control materials were used for quality assurance. The concentration of creatinine in urine was measured according to Jaffe reaction using a SFA-200 flow injection analyser (Burkard Scientific Ltd., Uxbridge, UK). Details have been presented (Ellingsen et al. 2006).

Air sampling and analysis

Details on air sampling and analysis have been presented (Ellingsen et al. 2006). The exposure was characterized by personal full shift air sampling on the 2 days directly preceding the collection of the biological samples. Millipore plastic filter cassettes (25 mm) equipped with 0.8 μm pore-size cellulose-ester membrane filters (AAWPO2500; Millipore, Bedford, MA, USA,) were placed in the breathing zone underneath the welding helmet. SKC Sidekick personal pumps operated at a constant flow of 2.0 l min^{-1} were used (SKC Ltd., Dorset, UK).

After digestion of the air filters in 2 ml of aqua regia and 0.2 ml of hydrofluoric acid, the samples were analysed at NIOH by inductively coupled plasma optical emission spectrometry (ICP-OES). Reference workroom air filters prepared at NIOH which were traceable to international certified reference materials were used for the quality assurance of the measurements. The long-term daily use at NIOH has permitted the achievement of a 2% or better accuracy and reproducibility (day-to-day variation) for the measured elements.

Estimates of exposure to manganese

The estimates of exposure to Mn were the current individual concentrations of U-Mn and B-Mn. Individual airborne exposure to Mn in the workroom air was measured on the 2 days preceding the collection of the biological samples. The mean concentration of the 2 days of sampling was used as an air exposure estimate (air-Mn) in this study. Details on sampling, analytical methods and quality assurance have been published (Ellingsen et al. 2006). Exposure data are presented in Table I.

Statistics

Distributions of measures with skewness exceeding 2.0 were log-transformed to achieve normalization. Thus, the concentrations of PRL in serum, U-Mn and air-Mn were log-transformed. For log-transformed variables the geometric means (GM) are presented, while the arithmetic means (AM) are otherwise used. The analysis of variance (ANOVA) was used for group comparisons. When more than two groups were compared, the least square difference was calculated. Stepwise multiple linear regression analysis (backward procedure) was performed for all the participants in the cross-sectional part of the investigation in order to assess associations between the dependent variables and the following independent variables; exposed/non-exposed (1/0), age (in years), alcohol consumption (g per year), B-Pb (log), smoking status (1/0) and the use of medication (1/0).

When the dependent variable was associated with the exposure variable, dose–response associations were assessed where B-Mn, U-Mn and air-Mn were included separately into the statistical models instead of exposed/non-exposed (1/0), in addition to the relevant potential confounders. All subjects were included for the assessment of B-Mn and U-Mn. However, only the exposed subjects were assessed for air-Mn because the variable was log-transformed and the referents had the value 0 for exposure, which it is meaningless to transform. The level of significance was set at 0.05 (two-tailed). The statistics were calculated with the data package SPSS 11.5 on a personal computer.

Results

The GM serum PRL concentration of all current welders was nearly significantly higher ($p = 0.051$) when compared to the age-matched referents, while the AM concentration of inhibin B in serum was significantly ($p = 0.001$) higher (Table III). Age ($p = 0.002$) and being exposed as a welder ($p = 0.047$) were significantly positively associated, while being a smoker ($p = 0.006$) was negatively associated with the serum PRL concentrations in the multiple linear regression analysis (not shown). When assessing the concentrations of inhibin B in serum as a dependent variable in a multiple linear regression model, being exposed as a welder or not ($p < 0.001$) was significantly included in the model while alcohol consumption was nearly significantly included ($p = 0.06$) (not shown). The estimates of the concentrations of PRL (adjusted for age and smoking) and inhibin B (adjusted for alcohol consumption) in serum in the two groups and the corresponding group differences after adjusting for these factors are shown in Table III.

Based on the results from the regression analysis of the serum PRL concentrations, the material was stratified according to exposure status and smoking habits (Figure 1). Current smokers had lower serum PRL concentrations than current non-smokers, the difference being of statistical significance ($p = 0.02$) in the referents (adjusted for age). Although the point estimate of the difference was of the same magnitude in the welders, the age-adjusted difference did not attain statistical significance ($p = 0.12$).

Dose–response patterns between serum PRL and B-Mn, U-Mn and air-Mn, were assessed by multiple linear regression analysis. When considering all subjects and taking into account age and smoking habits, B-Mn was of borderline significance ($p = 0.054$) positively associated with the serum PRL concentrations. No association was found between the concentrations of U-Mn and serum PRL.

The welders were subsequently stratified according to their concentration of B-Mn into three equally large groups and compared to their age-matched referents. The differences in the serum PRL concentrations between the welders and the age-matched referents, expressed as a ratio because the variable serum PRL was log-transformed, are presented in Figure 2. The welders with the lowest B-Mn had nearly identical serum PRL concentrations when compared with their age-matched referents, while the third of the welders with the highest B-Mn had a significantly higher GM serum PRL concentration ($p = 0.05$). Air-Mn was negatively associated with serum PRL. However, using the same approach as above of stratifying the welders according to the level of air-Mn, a statistically significant difference was found between the

Table III. The serum concentrations and the mean group differences (and 95% confidence interval (CI)) of prolactin and inhibin B in 96 welders and 96 referents matched for age. The group differences for the prolactin concentrations in serum are expressed as ratios, because the variable was log-transformed.

	Exposed		Referents		Group difference		
	Mean	Range	Mean	Range	Mean	95% CI	*p*
Prolactin[a] (mIU l^{-1})	193	58–1320	166	51–1082	1.16	1.00–1.35	0.051
Adjusted[a,b] (mIU l^{-1})	193	–	166	–	1.16	1.00–1.34	0.047
Inhibin B (ng l^{-1})	152	22–348	122	8–310	30.0	13.5–46.5	0.001
Adjusted[c] (ng l^{-1})	151	–	123	–	28.0	11.4–44.5	0.001

[a]Geometric mean, otherwise arithmetic mean; [b]adjusted for age and smoking status; [c]adjusted for alcohol consumption.

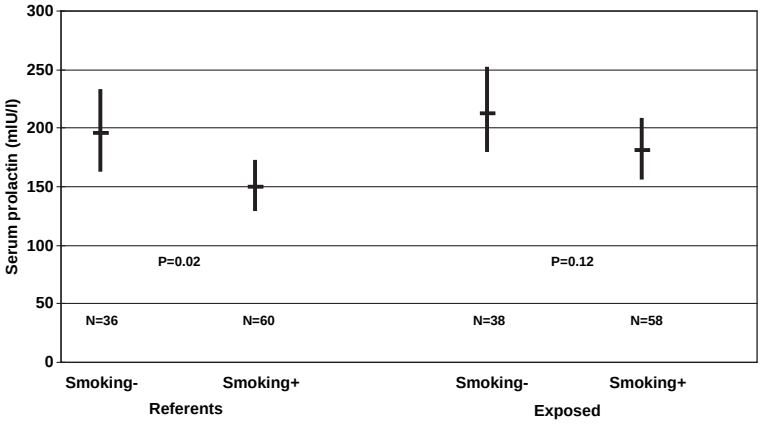

Figure 1. The geometric mean (and 95% confidence interval) concentrations of serum prolactin in 96 welders (exposed) and 96 referents according to smoking habits. The p-values refer to the group differences between smokers and non-smokers in the two groups, respectively.

lowest exposed welders and their age-matched referents only ($p=0.03$), while the PRL levels were similar in the highest exposed welders and the age-matched referents ($p=0.57$) (not shown). No dose–response pattern was found between the concentration of inhibin B in serum and any of the applied markers of exposure.

The univariate association found between the serum PRL concentration and B-Mn in non-smoking welders was of borderline significance (log PRL $=2.14+0.001$ B-Mn; $r=0.32$, $p=0.051$) (Figure 3), while the association found in smoking welders was far from being statistically significant.

Because all male patients were at least 41 years old, they were compared with all currently exposed welders and referents from the cross-sectional study who were at least 41 years of age. The patients (all former welders) were slightly older than the subjects in the other two subgroups (Table II). They had been exposed as welders for on average 22.7 years. They were, as a group, relatively young at the time they received

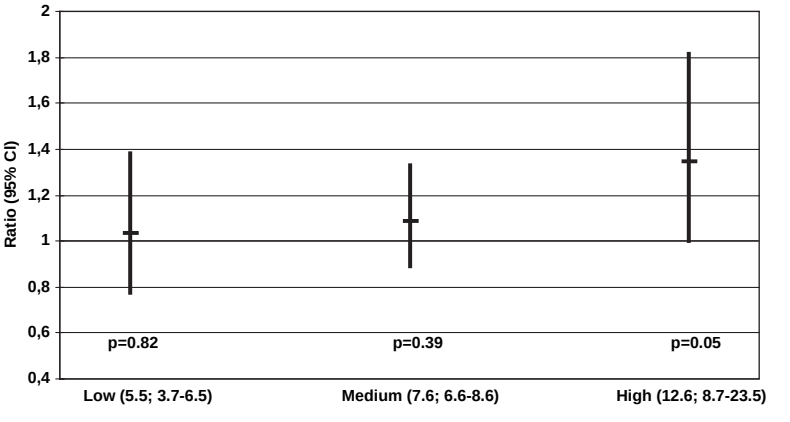

Figure 2. The difference of serum prolactin (expressed as ratio and 95% confidence interval (CI)) between welders stratified into three equally large subgroups according to their whole blood manganese concentration and their age-matched referents. The p-values refer to the difference between exposed and referents in the different strata after adjustment for age and the amount of current smoking.

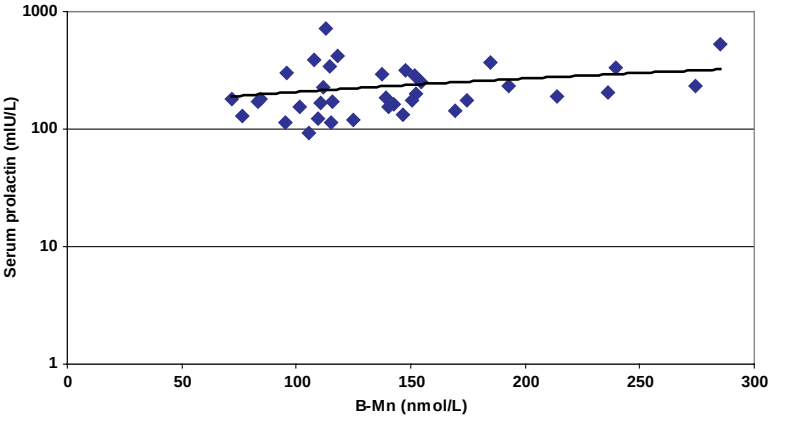

Figure 3. The association between the concentration of manganese in whole blood (B-Mn) and the serum prolactin concentration in 37 non-smoking welders.

their diagnosis (mean 44.5 years, range 34–51) (not tabulated). Their AM concentration of inhibin B was statistically significantly lower than in the referents and the current welders. The GM serum PRL concentrations, however, were comparable in the three groups under scrutiny.

Discussion

These biomarkers related to male reproductive functions were investigated for the first time in current and former welders exposed to welding fume particles that are generally measured in the respirable aerosol fraction. The GM concentration of Mn in the workroom air for the current welders was 121 μg m^{-3}, but the range in the individual exposures was substantial. Reliable data on the exposure levels of the former welders were not available.

The participation rates were high in the studied groups, thus reducing bias in the selection of the subjects. The current welders and the referents were selected from the same plants, where they all are blue collar workers, and the comparability of their sociodemographic background variables is good. This statement is also valid for the patients because they are all former welders and recruited from the same geographical area.

In accordance with the a priori hypothesis, the current welders had higher serum PRL concentrations than the referents. The concentrations, however, were generally well within the reference range of the laboratory. Higher B-Mn was associated with higher serum PRL concentrations, thus suggesting the existence of a dose–response relationship. This has previously not been shown in welders, but one previous small study of Mn alloy production workers reported associations between serum PRL and U-Mn and B-Mn (Mutti et al. 1996). The group of welders with the highest B-Mn concentrations had significantly higher serum PRL and on average 91 nmol l^{-1} higher mean B-Mn than their age-matched referents. The negative association between serum PRL and air-Mn that we observed was related to the difference between the lowest exposed welders and their age-matched referents, while the highest exposed subgroup of welders had similar GM PRL concentration compared to their

age-matched referents. This may not be biologically plausible, and could represent a random association.

The 23 male patients, who are all former welders, did not have increased serum PRL levels. This has previously not been shown. They were diagnosed as having manganism when they were on average 44.5 years (range 34–51) old, and as a group they have as expected severely impaired motor functions (results not published). It is reasonable to speculate that they had previously been highly exposed to Mn, and experienced increased PRL levels during their exposure. If this is correct, our results could suggest that increased PRL levels during ongoing exposure to Mn may be a reversible phenomenon after exposure cessation. The observation that serum PRL is a biomarker that is stable over time provided that the ongoing exposure is sufficiently high supports this view (Smargiassi & Mutti 1999).

Animal studies have indicated the presence of biochemical lesions of the dopaminergic system during Mn exposure that do not affect the structural integrity of dopaminergic nigrostriatal neurons (Bird et al. 1984, Normandin & Hazell 2002). Also there is evidence that Mn may interfere with TH (Bonilla 1980, Daniels & Abarca 1991). This rate-limiting enzyme of the dopamine synthesis requires Fe to be catalytically active (Ramsey et al. 1996). It is well known that Fe and Mn interact in humans (Roth & Garrick 2003). It is tempting to speculate that an interaction may occur at the level of the dopamine synthesis, thus reducing dopamine synthesis in the pituitary with the consequence of a reduced inhibition of synthesis and the release of PRL into the systemic circulation. Inhibited tyrosine hydroxylation with a concomitant reduction of dopamine that was reversible after exposure cessation was observed in rats after intranigral injection of $MnCl_2$ (Daniels & Abarca 1991). Increased Mn signal intensities observed in welders examined with magnetic resonance imaging points to the pituitary as one of the high Mn-accumulating tissues upon exposure by inhalation (Kim et al. 1999). Thus PRL production and secretion may constitute sensitive targets for Mn toxicity.

However, manganism is regarded as an irreversible disease that is mainly related to atrophic changes in the globus pallidus and nucleus caudatus, although some studies have reported signs of partial improvement in neurological functions upon removal from exposure (Olanow et al. 1996, Normandin & Hazell 2002). Thus, it is possible that high exposure to Mn may impact the human body through at least two different mechanisms. One component may be related to disturbances of the dopamine synthesis, that may be reversible upon cessation of exposure in a yet unknown time frame. The other component is irreversible, and the well-known severe movement disorders of manganism may be a consequence of a dysfunction in non-dopaminergic structures in the basal ganglia.

Nicotine has a depressive effect on the serum PRL concentration (Fuxe et al. 1989). This is compatible with our observation that the smokers had lower serum PRL concentrations than the non-smokers. Similar results were found in Mn alloy production workers (Ellingsen et al. 2003). Chronic nicotine administration suppresses the pituitary PRL secretion by increasing the hypothalamic tuberoinfundibular dopaminergic activity. The suppression is reversible in male Sprague–Dawley rats, the PRL level being normalized between 3 and 7 days post-exposure (Rasmussen 1998). Smoking habits can act as an effect modifier resulting in false estimation of the effect on the PRL levels attributable to exposure, and should be considered in epidemiological research.

To our knowledge, there are no previous investigations of inhibin B in occupationally Mn-exposed populations. Our a priori hypothesis was that the inhibin B levels should be lower in the welders as a possible sign of Sertoli cell dysfunction of the testes. The serum inhibin B concentrations were accordingly substantially lower in the patients than in the current welders or the referents of similar age. These patients are regarded as having welding-induced manganism, for which they have been financially compensated according to the Russian insurance system. They have quite severely reduced motor performance (results not published), and thus we assume that their exposure has been quite extensive during their average employment as welders of 22.7 years. The differential diagnosis between manganism and idiopathic Parkinson's disease is difficult, but we have no information of impaired Sertoli cell function or lower serum inhibin B levels in the latter group of patients. This may indicate that the Sertoli cells of the testes may also be a target for Mn toxicity, when exposure has been sufficiently high to induce severe motor disturbances. A complete absence of spermatocytes in the seminferous tubules in rats upon excessive Mn exposure has been reported, but no data exist in humans (Singh et al. 1974, Chandra et al. 1975). However, welding fumes contain a range of different elements, and one cannot exclude the possibility that welding fume components other than Mn (e.g. chromium), gases or physical factors may be involved. Men with normal fertility have higher levels of inhibin B than men with reduced fertility (Eckardstein et al. 1999, Ballescá et al. 2000, Brugo-Olmedo et al. 2001), and our findings may indicate reduced fertility in at least some of the patients in the present study.

However, the finding that the current welders had higher levels of inhibin B than the referents is not in accordance with our a priori hypothesis. Inhibin B has only rarely been investigated in any populations exposed to environmental contaminants, probably because this is a comparatively 'new' biomarker. It is therefore of considerable interest that increased inhibin B levels were found in workers with ongoing exposure to lead which also is known to affect the testicular Sertoli cells (Mahmoud et al. 2005). It has been suggested that reactive oxygen species may stimulate the secretion of inhibin B by the Sertoli cells (Comhaire & Mahmoud 2003). If such mechanisms are operating, this could explain our finding of higher inhibin B in the welders. Potential mechanisms, however, remain to be elucidated.

In conclusion, current welders had higher serum PRL concentrations than their referents, and a possible association between serum PRL and B-Mn was observed. The increase may be reversible as suggested by the results from the examinations of former welders. Former welders diagnosed as having manganism had substantially lower inhibin B concentrations in serum, which could suggest a Sertoli cell dysfunction. However, the finding of higher inhibin B concentrations in the current welders as compared to the referents should be confirmed in future studies, as this result was not in accordance with our a priori hypothesis.

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References

Alessio L, Apostoli P, Ferioli A, Lombardi S. 1989. Interference of manganese on neuroendocrinal system in exposed workers. Biological Trace Elemement Research 21:249–53.

Anderson RA, Wallace EM, Groome NP, Bellis AJ, Wu FCW. 1997. Physiological relationships between inhibin B, follicle stimulating hormone secretion and spermatogenesis in normal men and response to gonadotrophin suppression by exogenous testosterone. Human Reproduction 12:746–751.

Baader EW. 1932. Manganvergiftungen in Elementefabriken. Archiv für Gewerbepathologie und Gewerbehygiene 4:101–116.

Ballescá JL, Balasch J, Calafell JM, Alvarez R, Fábregues F, De Osaba MJM, Ascaso C., Vanrell JA. 2000. Serum inhibin B determination is predictive of successful testicular sperm extraction in men with non-obstructive azoospermia. Human Reproduction 15:1734–1738.

Barbeau A, Inoué N, Cloutier T. 1976. Role of manganese in dystonia. In: Eldridge R, Fahn S, editors. Advances in Neurology. New York: Raven Press. p. 339–352.

Bird ED, Anton AH, Bullock B. 1984. The effect of manganese inhalation on basal ganglia dopamine concentrations in Rhesus monkey. Neurotoxicology 5:59–66.

Bonde JPE. 1990a. Subfertility in relation to welding: A case referent study among male welders. Danish Medical Bulletin 37:105–108.

Bonde JP. 1990b. Semen quality and sex hormones among mild steel and stainless steel welders: a cross sectional study. British Journal of Industrial Medicine 47:508–514.

Bonde JP, Ernst E. 1992. Sex hormones and semen quality in welders exposed to hexavalent chromium. Human & Experimental Toxicology 11:259–263.

Bonilla E. 1980. L-tyrosine hydroxylase activity in the rat brain after chronic oral administration of manganese chloride. Neurobehavioral Toxicology 2:37–41.

Brugo-Olmedo S, Vincentiis SD, Calamera JC, Urrutia F, Nodar F, Acosta AA. 2001. Serum inhibin B may be a reliable marker of testicular spermatozoa in patients with nonobstructive azoospermia. Fertility and Sterility 76:1124–1129.

Calne DB, Chu N-S, Huang C-C, Lu C-S, Olanow W. 1994. Manganism and idiopathic parkinsonism: Similarities and differences. Neurology 44:1583–1586.

Chandra SV, Ara R, Nagar N, Seth PK. 1973. Sterility in experimental manganese toxicity. Acta Biologica et Medica Germanica 30:857–862.

Chandra SV, Saxena DK, Hasan MZ. 1975. Effects of zinc on manganese induced testicular injury in rats. Industrial Health 13:51–56.

Comhaire FH, Mahmoud A. 2003. The role of food supplements in the treatment of the infertile man. Reproductive Biomedicine Online 7:385–391.

Daniels AJ, Abarca J. 1991. Effect of intranigral Mn2+ on striatal and nigral synthesis and levels of dopamine and cofactor. Neurotoxicology and Teratology 13:483–487.

Deskin R, Bursian SJ, Edens FW. 1980. Neurochemical alterations induced by manganese chloride in neonatal rats. Neurotoxicology 2:65–73.

Eckardstein Von S, Simoni M, Bergmann M, Weinbauer GF, Gassner P, Schepers AG, Nieschlag E. 1999. Serum Inhibin B in combination with serum follicle-stimulating hormone (FSH) is a more sensitive marker than serum FSH alone for impaired spermatogenesis in men, but cannot predict the presence of sperm in testicular tissue samples. The Journal of Clinical Endocrinology & Metabolism 84:2496–2501.

Elbetieha A, Bataineh H, Darmani H, Al-Hamood MH. 2001. Effects of long-term exposure to manganese chloride on fertility of male and female mice. Toxicology Letters 119:193–2001.

Ellingsen DG, Haug E, Gaarder PI, Bast-Pettersen R, Thomassen Y. 2003. Endocrine and immunologic markers in manganese alloy production workers. Scandinavian Journal of Work Environment & Health 29:230–238.

Ellingsen DG, Dubeiskovskaya L, Dahl K, Chashchin M, Chashchin V, Zibarev E, Thomassen Y. 2006. Air exposure assessment and biological monitoring of manganese and other major welding fume components in welders. Journal of Environmental Monitoring 8:1078–1086.

Fuxe K, Andersson K, Eneroth P, Härfstrand A, Agnati LF. 1989. Neuroendocrine actions of nicotine and of exposure to cigarette smoke: medical implications. Psychoneuroendocrinology 14:19–41.

Griffin KP, Ward DT, Liu W, Stuart G, Morris ID, Smith CP. 2005. Differential expression of divalent metal transporter 1 (Slc1 1a2) in the spermatogenic epithelium of the developing and adult rat testis. American Journal of Physiology-Cell Physiology 288:176–184.

Hjollund NHI, Bonde JPE, Jensen TK, Ernst E, Henriksen TB, Kolstad HA, Giwercman A, Skakkebæk NE, Olsen J. 1998. Semen quality and sex hormones with reference to metal welding. Reproductive Toxicology 12:91–95.

Kim Y, Kim KS, Yang JS, Park IJ, Kim E, Jin Y, et al. 1999. Increase in signal intensities on T1-weighted magnetic resonance images in asymptomatic manganese-exposed workers. Neurotoxicology 20:901–908.

Kumanov P, Nandipati KC, Tomova A, Robeva R, Agarwal A. 2005. Significance of inhibin in reproductive pathophysiology and current clinical applications. Reproductive Biomedicine Online 10:786–796.

Laskey JW, Rehnberg GL, Hein JF, Carter SD. 1982. Effects of chronic manganese (Mn3O4) exposure on selected reproductive parameters in rats. Journal of Toxicology and Environmental Health 9:677–687.

Mahmoud AM, Comhaire F, Depuydt CE. 1998. The clinical and biological significance of serum inhibin in subfertile men. Reproductive Toxicology 12:591–599.

Mahmoud A, Kiss P, Vanhoorne M, De Bacquer D, Comhaire F. 2005. Is inhibin B involved in the toxic effect of lead on male reproduction? International Journal of Andrology 28:150–155.

Melmed S, Kleinberg D. 2003. Anterior pituitary. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, editors. Williams Textbook of Endocrinology. 10th ed. Philadelphia, USA: Elsevier Science, p. 177–279.

Mortensen JT. 1988. Risk for reduced sperm quality among metal workers, with special reference to welders. Scandinavian Journal of Work Environment & Health 14:27–30.

Murthy RC, Srivastava RS, Gupta SK, Chandra SV. 1980. Manganese induced testicular changes in monkeys. Experimentelle Pathologie 18:240–244.

Mutti A, Bergamaschi E, Alinovi R, Lucchini R, Vittori MV, Franchini I. 1996. Serum prolactin in subjects occupationally exposed to manganese. Annals of Clinical and Laboratory Science 26:10–17.

Normandin L, Hazell AS. 2002. Manganese neurotoxicity: an update of pathophysiologic mechanisms. Metabolic Brain Disease 17:375–387.

Olanow CW, Good PF, Shinotoh H, Hewitt KA, Vingerhoets F, Snow BJ, Beal MF, Calne DB, Perl DP. 1996. Manganese intoxication in the rhesus monkey: A clinical, imaging, pathologic, and biochemical study. Neurology 46:492–498.

Petrie JR, RG, Morales CR. 1992. Receptor-mediated endocytosis of testicular transferring by germinal cells of the rat testis. Cell & Tissue Research 267:45–55.

Ramsey AJ, Hillas PJ, Fitzpatrick PF. 1996. Characterization of the active site iron in tyrosine hydroxylase. Journal of Biological Chemistry 271:24395–24400.

Rasmussen DD. 1998. Effects of chronic nicotine treatment and withdrawal on hypothalamic propiomelanocortin gene expression and neuroendocrine regulation. Psychoneuroendocrinology 23:245–259.

Rodier J. 1955. Manganese poisoning in Morroccan miners. British Journal of Industrial Medicine 12:21–35.

Roth JA, Garrick MD. 2003. Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese. Biochemical Pharmacology 66:1–13.

Schuler P, Oyanguren H, Maturana V, Valenzuela A, Cruz E, Plaza V, Schmidt E, Haddad R. 1957. Manganese poisoning. Industrial Medicine and Surgery 26:167–73.

Singh J, Husain R, Tandon SK, Seth PK, Chandra SV. 1974. Biochemical and histopathological alterations in early manganese toxicity in rats. Environmental Physiology & Biochemistry 4:16–23.

Smargiassi A, Mutti A. 1999. Peripheral biomarkers and exposure to manganese. Neurotoxicology 20:401–406.

Thorner MO, Vance ML, Horvath E, Kovacs K. 1992. The anterior pituitary. In: Wilson JD, Foster DW, editors. Williams Textbook of Endocrinology. 8th ed. Philadelphia: WB Saunders. p. 221–310.