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The time-trend and the relation between smoking and circulating selenium concentrations in Norway

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Abstract

Objectives: The objectives of this study were to investigate biomarkers of selenium status in relation to smoking habits and to analyze the time-trend of selenium in serum (S-Se) in Norway during the time period 1995–2006.

Methods: The impact of smoking habits was investigated in a population recruited to a cross-sectional study of blue-collar workers in the southern part of the country ($n = 98$). The time-trend was studied in all subjects who delivered blood samples for the determination of S-Se to a large commercial clinical chemistry laboratory in Norway.

Results: Smokers had 0.14 and 0.20 μmol/L lower concentrations of selenium in whole blood (B-Se) and serum, respectively, than non-smokers. The amount of smoking, as assessed by the serum cotinine concentration, was negatively associated with the B-Se concentration (Pearson's $r = -0.43$). The 1/3 of the blue-collar workers with the lowest concentrations of B-Se or S-Se had lower activity of glutathione peroxidase in serum (S-GSHpx) than the remaining subjects. Snuff users had about the same levels of B-Se and S-Se as the non-smokers, although they had about the same amount of nicotine metabolites in urine and serum as the smokers. A decreasing trend of S-Se was observed during the observation period from 1995 to 2006. The mean concentration was 1.26 μmol/L in 1995, while the lowest mean concentration was measured in 2003 (1.01 μmol/L).

Conclusion: Smoking, but not snuffing, is associated with lower concentrations of B-Se and S-Se. The reduction of B-Se is negatively associated with the nicotine biomarker cotinine in serum. A substantial proportion of blue-collar workers had not maximized the activity of S-GSHpx. Selenium status may have become poorer since 1995.

Keywords: Selenium; Smoking; Cotinine; Glutathione peroxidase; Time-trend

Introduction

Selenium (Se) is an essential trace element and numerous seleno-proteins in mammalian organisms have been identified, but their functional roles are not yet fully understood [1]. Most seleno-proteins are involved in anti-oxidative defence and redox metabolism [1]. There is strong evidence that selenium-containing deiodinases are important for normal thyroid function [1,2]. The identification of sperm nuclei glutathione peroxidase (GSHpx) points to a role of Se in normal male fertility [3,4]. A heart-specific knockout of thioredoxin reductase 2 resulted in dilatative cardiomyopathy, suggesting the importance of Se to normal heart function [1,5]. Whether Se deficiency is a risk factor for coronary heart disease remains to be elucidated [6]. Also impaired immune system functions may result from Se

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deficiency [7]. Animal studies indicate that Se also may act antagonistic toward some heavy metal pollutants [8]. A recent review suggested that there is evidence of Se giving protection against cancer of the prostate and possibly also against the lung cancer [9].

A dietary daily intake of 55 μg Se in adults has been recommended in order to balance the intake and excretion. The recommendation is based on the intake required to maximize the GSHpx activity in plasma [10,11]. However, recommended intakes of Se differ throughout the world [12]. Blood Se concentrations are often used as an indicator of the dietary Se intake, and several reports support its usefulness [13]. Another biomarker of Se status is the activity of GSHpx, which is involved in the intracellular defence against oxidative damage by preventing the production of reactive oxygen species [14].

Studies conducted before 1990 indicated that the serum Se concentrations in Norwegians were higher than in many other European countries [13]. We have determined the concentrations of B-Se in several cross-sectional studies of blue-collar workers, and have observed decreasing concentrations during the 1990s [15–17]. Generally, smokers had lower concentrations than non-smokers *in these studies*. These observations have led us to perform this study.

Reports have suggested that the concentrations of Se in blood are lower in tobacco smokers than in non-smokers [11]. The activity of GSHpx has been much less studied. We have only found one previous study that has quantitatively assessed tobacco consumption by measuring nicotine-specific biomarkers [18]. Thus, data on dose–response associations between biomarkers of Se status and nicotine consumption are sparse. The habit of cigarette smoking is prevalent, but in recent years the habit of sucking smokeless tobacco (snuff) has increased in some Scandinavian countries. The most widely used biomarkers of tobacco use are degradation products of the nicotine metabolism, and cotinine is considered to be a reliable biomarker for the total nicotine intake [19].

The aims of this study were to investigate the impact of tobacco consumption on selenium status biomarkers by quantitatively assessing biomarkers of tobacco consumption in a cross-sectional study. Because cadmium (Cd) may be a constituent of tobacco smoke, Cd in whole blood was also determined. Also the association between Se in blood and GSHpx was explored. Further, extensive follow-up data of S-Se in Norway are presented for the time period 1995–2006.

Material and methods

Cross-sectional study

The study of the relationship between smoking habits and selenium status parameters is based on a cross-sectional study that was originally carried out to study long-term health effects in male chloralkali workers. They had ceased being exposed to mercury vapour (Hg^0) for on average 4.8 years prior to the study. Details on the design and the inclusion of subjects into that study have been described [20]. In short, all male workers who had been exposed for at least one year were eligible for inclusion. Sixty-three previously exposed subjects fulfilled the inclusion criteria, but five were excluded owing to medical conditions. Forty-nine subjects attended the examinations, because nine subjects declined to participate. The referents were recruited from men working in other facilities of the same industrial complex. Four out of 57 potential referents who were invited for comparison declined to participate. Four referents were not included because they did not fulfil the criterion for age-matching. Identical exclusion criteria, including alcohol abuse, major head injuries, metabolic diseases, major psychiatric or neurological diseases causing severe disability, were applied to all subjects.

The personnel of the Occupational Health Service (OHS) of the industrial complex performed a structured interview. They also collected the biological samples. Information on self-reported alcohol intake during the year preceding the examinations and smoking/snuff using habits were obtained by means of a questionnaire [21].

By coincidence, half of the subjects were current smokers or snuff users ($n = 49$), while the other half were current non-smokers/non-snuff users ($n = 49$). Two subjects that were both smokers and snuffers were categorized as smokers. The prevalence of smoking or use of snuff was higher in the previously exposed workers (55% vs. 45%; $p = 0.32$).

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

Laboratory measurements

First voided morning urine samples were collected directly in 30 mL SARSTEDT® polypropylene tubes (Sarstedt, Nümbrecht, Germany). Heparinized whole blood samples were collected the same day from the cubital vein in 10 mL Venoject® tubes (Terumo Corp., Belgium). The serum was stored in NUNC® 1.8 mL cryotubes after being centrifuged at 1500 rpm for 10 min. All biological samples were stored in the dark at −20 °C prior to analysis.

Determination of trace elements

For the determination of cadmium (B-Cd) and selenium (B-Se) in whole blood, 1.5 mL of 65% ultrapure nitric acid (Chemscan Ltd., Elverum, Norway) was added to 1.0 mL of whole blood, which had been

transferred to an acid pre-washed polypropylene tube that was kept at room temperature overnight and heated at 95 °C for 1 h in a laboratory oven. After cooling to room temperature, 100 μL of a 100 μg/L internal standard solution (for Cd) was added and the digest was diluted to a final volume of 10 mL with deionised water. Since serum and whole blood normally contain precipitates, it is of advantage to homogenize the samples by a nitric acid dissolution procedure.

Details on the determination of B-Cd have been presented [20]. Cadmium was determined in the digested sample by use of Thermo Electron Element 2 inductively coupled plasma high resolution mass spectrometer programmed to determine Cd by use of the $^{114}Cd^+$ ion with automatic mass correction caused by the $^{114}Sn^+$ ionic interference. The $^{115}In^+$ ion was used as the internal standard. The instrument was calibrated (two-point) by use of whole blood matched standard solutions. Selenium was determined by electrothermal atomic absorption spectrometry (ETAAS) using a Perkin Elmer Model SIMAA 6000/THGA system calibrated (three-point, calibration concentrations 1.3, 2.2 and 3.0 μmol/L of Se) with whole blood matrix-matched standards under stabilized atomiser conditions using palladium (as nitrate) chemical modification. The detection limits (DLs) of the methods were 0.9 nmol/L of Cd and 0.04 μmol/L of Se in undiluted whole blood. Seronorm human whole blood quality control samples, batches OK0336 (recommended values 6.3 nmol/L of Cd and 0.95 μmol/L of Se) and MR9067 (recommended values 52 nmol/L of Cd and 1.52 μmol/L of Se), were used for daily quality assurance (Sero Ltd., Asker, Norway). The measured concentrations in these two quality control samples were 6.3 ($n = 5$, RSD = 9%) and 54 ($n = 5$, RSD = 5%) nmol/L of Cd and 0.97 ($n = 5$, RSD = 4%) and 1.55 ($n = 5$, RSD = 3%) μmol/L of Se, respectively.

Details on the determination of Se in serum (S-Se) have been presented [22]. The samples were analyzed for S-Se by ETAAS calibrated with serum-matched standard solutions (three-point, calibration concentrations 1.3, 2.2 and 3.0 μmol/L of Se). The DL was 0.05 μmol Se/L. The accuracy and the reproducibility of the measurements were assessed using Seronorm™ human serum STE 605113. The reproducibility was $\leqslant 5\%$ and the measured average Se concentration within $\pm 3\%$ when compared to the recommended value of 0.99 μmol/L. Seronorm human serum batches 102, 103 and 105 have also been used on a regular basis until 2005 to ensure the accuracy of the method. The Se content in these sera has been established in several large inter-laboratory exercises. In addition, our laboratory participates routinely in an external proficiency testing scheme organized by Wadsworth Centre (New York, USA) for measurements of trace elements in urine and whole blood.

Glutathione peroxidase in serum (S-GSHpx)

The determination of S-GSHpx has been described previously [22]. In short, S-GSHpx was determined by an automated assay [23] on an Axon autoanalyzer (Technicon Instruments, Bayer Corp., New York). Enzyme activity was determined in a two-step reaction. The disappearance of NADP was measured spectrophotometrically by reduced absorbance at 340 nm. The standard curve was linear up to a concentration of 500 U/L. The samples were analyzed with a within-assay CV of 3.5%.

Determination of nicotine and cotinine

The methods for determination of nicotine and cotinine in serum and urine have been presented [24]. In short, 0.5 mL of serum and 1.5 mL of urine were added in the internal standard (2-phenylimidazole) and prepared at room temperature by liquid–liquid extraction on Extrelut® NT 1 and 3 solid supports (VWR, Darmstadt, Germany), respectively. Nicotine and cotinine were determined by microbore reversed phase liquid chromatography with gradient elution (Waters Cap LC, Milford, MA, USA) coupled to a triple quadropole mass spectrometer operated in positive electrospray ionization and multiple reaction monitoring mode (Quattro LC-MS, Micromass, Manchester, UK). The quantification was obtained by using the internal standard and the relative comparisons to spiked serum and urine blank samples. The methods were validated over the concentration ranges 18–18×10^3 and 0.9–4.5×10^3 ng/mL for nicotine and cotinine in serum, and 10–1×10^4 and 0.5–1×10^3 ng/mL for nicotine and cotinine in urine, displaying coefficients of correlations >0.998 and within-assay ($n = 6$) and between-assay ($n = 6$) precisions $<10\%$ and 14%, respectively. The DLs were 6.0 and 0.3 ng/mL for nicotine and cotinine in serum, and 3.3 and 0.2 ng/mL for nicotine and cotinine in urine.

Time-trend study

Subjects, sampling and the determination of selenium in serum (S-Se)

The samples for the determination of S-Se were collected from patients visiting clinical practices all over Norway. Samples were collected mainly from the southeast part of the country, i.e. the surroundings of Oslo, and sent for analysis to a large commercial laboratory in Norway. To the best of our knowledge, no subjects were occupationally exposed to Se. Whole blood was collected in sterile Venoject or Vacutainer tubes without addition according to the recommendation of the laboratory. Serum was separated by

centrifugation at 3000*g* for minimum 12 min within 2 h after collection and stored at 4–8 °C for maximum 3 days before analysis. About 10–20% of the samples were sent by post at ambient temperature to the laboratory. The number of measured samples was known for 30 different months of the time period under study, the numbers ranging from 457 to 1153. The mean number of samples was 824/month (95% CI 733–915).

The serum samples were determined as routine measurements from 1995 to 2006 at the commercial laboratory by electrothermal graphite furnace atomic absorption (using Pd as matrix modifier) with Zeeman correction for unspecific absorption. From 1995 Perkin Elmer 5100/HGA 500 was used, while a Solaar M6 (Thermo Electron) was applied from 2002.

This laboratory participates regularly in an external proficiency testing scheme organized by Laval University, Canada. The average deviation from the target Se concentrations in the period February 1999 to March 2002 was +11%, while the deviation was reduced by the introduction of a new method to +4% from March 2002 to October 2003. Therefore the presented concentrations of the time-trend study were reduced by 11% before March 2002 and by 4% from March 2002. In addition to participating in an external proficiency testing scheme, the laboratory on a daily base measures selenium in in-house quality control serum samples (e.g. animal serum, Autonorm from Sero Ltd., Asker, Norway). In the period March 1996–December 1997 the reproducibility of the monthly mean values was 2.8% and 4.4% at concentration levels of 1.3 and 0.89 μmol/L of selenium, respectively. The reproducibility of the monthly mean values in the period January 2000–December 2006 was 4.5% and 6.3% at concentration levels of 1.0 and 0.74 μmol/L of selenium, respectively. During these periods no systematic changes in the mean concentration of the in-house materials could be observed.

Statistics

Distributions of continuous variables with skewness that exceeded 2.0 were log-transformed to achieve normalisation. Thus, the concentrations of urinary nicotine and serum cotinine were log-transformed, and the geometric means (GM) are presented. Otherwise, the arithmetic mean (AM) values are given. Analysis of variance (ANOVA) was applied for group comparisons of continuous variables, the least square difference (LSD) being calculated when more than two groups were compared. Dunnett's test was used to assess the difference in S-GSHpx between the subgroups with the highest concentrations of B-Se or S-Se and the subgroups with the lower concentrations. Univariate associations were based on least square regression, calculating Pearson's correlation coefficient (Pearson's *r*).

Stepwise multiple linear regression analysis (backward procedure) was performed by including all subjects in order to assess associations between the Se status parameters as dependent variables and the smoking habits (being a current smoker or not (1/0)) by taking into account the potential confounders as independent variables: previously exposed to Hg^0/not exposed to Hg^0 (1/0), age (in years), alcohol consumption (L/year), the number of fish meals/week and the use of prescribed medication (1/0).

For the time-trend study only median values for each month were known. Thus, the calculated annual mean concentrations are based on these monthly medians. Concentrations of cotinine and B-Cd below the DL were set to $\frac{1}{2}$ DL. The level of significance was set at 0.05 (two-tailed). The statistics were calculated with the data package SPSS 11.5 on a PC.

Results

Table 1 shows background data and the concentrations of biomarkers of selenium status and tobacco consumption according to the habits of tobacco use. The non-smokers were older than the snuff users and the smokers. A statistically significant difference of 0.14 μmol/L between the AM B-Se concentrations of 49 non-smokers/non-snuff users and 38 smokers was found, while the corresponding difference for S-Se was 0.20 μmol/L. Eleven subjects who reported to be snuff users only had about the same B-Se and S-Se concentrations as the non-smokers/non-snuff users. The mean B-Cd concentration of the snuff users was also comparable to that of the non-smokers. The activity of S-GSHpx was statistically significantly lower in the smokers (but not in the snuff users) as compared to the non-smokers/non-snuff users. One snuff user and one smoker had S-Cotinine below the DL and their U-Cotinine concentrations were 8.2 and 139 μg/mmol creatinine, respectively.

The consumption of alcohol, number of fish meals/week, the use of prescribed medication, being a smoker or not, age and having been exposed in the chloralkali plant were included as independent variables in a multiple linear regression analysis to assess their impact on the dependent variables B-Se, S-Se and S-GSHpx (results not tabulated). None of these dependent variables were statistically associated with previous exposure in the chloralkali plant. While S-GSHpx was not significantly associated with smoking, the B-Se and the S-Se concentrations were associated with smoking habits. The concentration of B-Se was also associated with the number of fish meals/week. The calculated regression equation (with the 95% confidence intervals of the regression coefficients in brackets) was "B-Se

Table 1. Background data, selenium status biomarkers and data related to the consumption of tobacco.

	Non-smokers/non-snuff users ($n = 49$) AM (range)	Smokers ($n = 38$) AM (range)	Snuff users ($n = 11$) AM (range)	P_{ANOVA}
Age (years)[b]	49.1 (29.9–68.8)	44.7 (28.2–68.7)	40.1 (31.8–53.7)	0.02
Fish meals/week (no)	1.4 (0–5)	1.1 (0–3)	1.0 (0–2)	0.30
Alcohol consumption (L/year)	2.7 (0–11.7)	3.9 (0.2–17.3)	2.9 (0.3–7.3)	0.22
B-Se (μmol/L)[a]	1.52 (1.0–2.3)	1.38 (0.9–2.1)	1.50 (1.3–1.9)	0.02
S-Se (μmol/L)[1,a]	1.54 (0.9–2.7)	1.34 (0.7–2.0)	1.55 (1.1–2.1)	0.02
S-GSHpx (U/L)[a]	146 (105–203)	137 (103–201)	140 (106–182)	0.12
Smoking tobacco (g/week)[a,c]	0 (–)	77 (5–150)	0 (–)	<0.001
Snuffing tobacco (g/week)[b]	0 (–)	2.6 (0–50)	75 (2–200)	<0.001
B-Cd (nmol/L)[a,c]	3.3 (0.9–11.2)	15.8 (4.5–35.8)	2.9 (DL-7.4)	<0.001
S-Cotinine (μg/L)[2,a,b]	<DL	110 (<DL-447)	137 (<DL-1312)	<0.001
U-Cotinine (μg/mmol cr.)[a,b]	1.2 (<DL-49)	161 (5.1–302)	159 (8.2–428)	<0.001
U-Nicotine (μg/mmol cr.)[2,a,b]	0.4 (0.2–1.6)	41 (0.3–752)	26 (0.4–560)	<0.001

[1]48 non-smokers/non-snuff users and 37 smokers.
[2]Geometric mean.
[a]$p < 0.05$ between non-smokers/non-snuff users and smokers.
[b]$p < 0.05$ between non-smokers/non-snuff users and snuff users.
[c]$p < 0.05$ between smokers and snuff users.

(μmol/L) = 1.42 (1.33–1.51) +0.07 (0.02–0.12) fish meals/week −0.12 (−0.21 to −0.03) tobacco smoking" (multiple $r = 0.38$; $p = 0.001$). Based on the calculated point estimates of the β-coefficients in the regression equation, a current smoker has on average 0.12 μmol/L lower B-Se concentration than a current non-smoker. Eating one fish meal/week results in an average B-Se increase of 0.07 μmol/L. The corresponding regression equation for S-Se was "S-Se (μmol/L) = 1.55 (1.46–1.64) −0.21 (−0.36 to −0.07) tobacco smoking" ($r = 0.29$; $p = 0.005$). When all subjects, including smokers, snuff users and non-smokers, were considered, all markers of tobacco consumption were associated both with the concentration of B-Se and with the activity of S-GSHpx (Table 2), while the associations were generally weaker when S-Se was considered. When only smokers were considered, a negative association was observed between the concentration of B-Se and cotinine in serum (Fig. 1). No statistically significant correlation between self-reported tobacco consumption and any of the biomarkers of Se status was observed among the smokers.

The activity of S-GSHpx was associated with the concentration of B-Se and S-Se (Table 2). Thus, the subjects were stratified in to three groups according to B-Se. The three groups did not contain exactly the same number of subjects, because some subjects had identical B-Se. When comparing the group of subjects with the highest concentrations of B-Se with the two groups with the lower concentrations of B-Se using Dunnett's test, statistically significantly lower activities of S-GSHpx were observed in the group of subjects with B-Se $\leqslant$ 1.3 μmol/L (Table 3). Using the same approach of stratifying the subjects into three subgroups according to S-Se showed that the subjects with the highest S-Se had significantly higher S-GSHpx than the group with the lowest S-Se concentrations ($p = 0.006$).

Fig. 2 shows the trend in the mean S-Se concentrations during the time period from 1995 to 2006. The concentrations are adjusted according to the average deviation from the target S-Se concentration before and after March 2002. No data were available for the year 1998, while no confidence interval was calculated for 1996 because the median concentration was 1.25 μmol/L for all 12 months of that year. A continuing decrease in the mean concentrations from 1.26 μmol/L in 1995 to 1.01 μmol/L in 2003 was observed. There is apparently a slight increase in the mean S-Se concentrations up to 2006 (AM = 1.08 μmol/L).

Discussion

The main results of this study are the observations of significantly lower mean concentrations of B-Se and S-Se in smokers as compared to non-smokers and the significant negative association between the concentrations of cotinine in serum and B-Se. Further, about 1/3 of the studied blue-collar workers with the lowest concentrations of B-Se (or S-Se) had lower activities of S-GSHpx than the subjects with the highest levels, indicating that a substantial number of the blue-collar workers have S-GSHpx activities which are not optimized. Finally the S-Se concentrations have apparently decreased significantly during the 1990s.

Table 2. Pearson's correlation coefficients between smoking related data, B-Se, S-Se and S-GSHpx.

	All subjects ($n = 98$)			Smokers ($n = 38$)		
	S-GSHpx	B-Se	S-Se	S-GSHpx	B-Se	S-Se
U-Cotinine	−0.19	−0.25*	−0.07	0.02	−0.14	0.32
S-Cotinine (log)	−0.27**	−0.30**	−0.20*	−0.21	−0.43**	−0.06
U-Nicotine (log)	−0.24*	−0.30**	−0.18	−0.11	−0.29	0.02
Smoking tobacco	−0.22*	−0.28**	−0.26*	−0.20	−0.10	−0.05
B-Cd	−0.14	−0.23*	−0.25*	−0.03	−0.09	−0.16
B-Se	0.28**	–	0.47***	0.43**	–	0.46**
S-Se	0.33**	–	–	0.42**	–	–

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

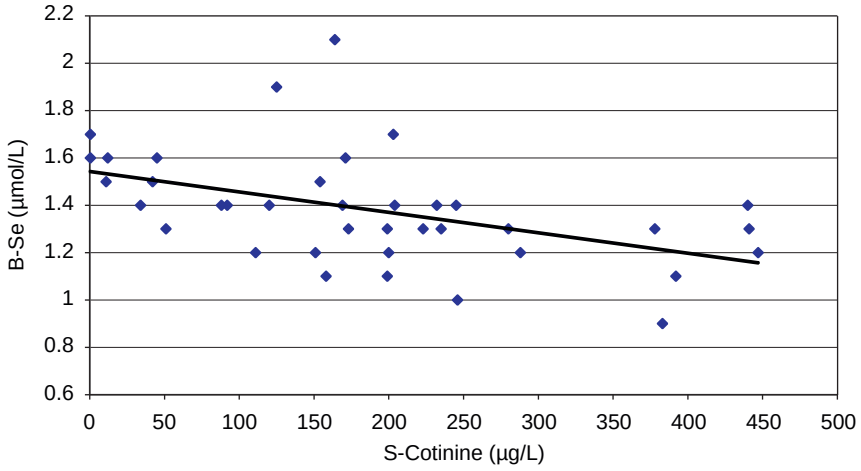

Fig. 1. The association between the concentration of selenium in whole blood and cotinine in serum in 38 smokers. The calculated regression line has the equation: B-Se = 1.54 (SE = 0.06; $p < 0.001$)−0.001 (SE < 0.0001; $p = 0.003$) S-Cotinine.

Table 3. The activity of S-GSHpx in 98 subjects stratified into three groups according to the B-Se concentration.

	Low B-Se 0.9–1.3 µmol/L ($n = 30$) AM (95% CI)	Medium B-Se 1.4–1.5 µmol/L ($n = 37$) AM (95% CI)	High B-Se 1.6–2.3 µmol/L ($n = 31$) AM (95% CI)
S-GSHpx (U/L)[a,b]	134 (126–142)	143 (136–150)	147 (139–156)
B-Se (µmol/L)	1.22 (1.18–1.26)	1.44 (1.43–1.46)	1.73 (1.67–1.79)
Prevalence of smokers (%)	60	32	26

The p-values are calculated using Dunnett's test.
[a] $p = 0.03$ between the high B-Se group and the low B-Se group
[b] $p = 0.61$ between the high B-Se group and the medium B-Se group.

The magnitude of 0.12 µmol/L lower means B-Se concentration in current smokers (after adjustment for fish consumption) compared to current non-smokers is about the same as we have observed in previous cross-sectional studies of blue-collar workers [15,16]. Lower plasma Se concentrations have been reported in smokers compared to non-smokers, which is similar to our observation of lower S-Se concentrations in the current smokers [25,26]. Poorer selenium status appears to be dependent on the amount of smoking tobacco consumption, as suggested by the significant correlation between serum cotinine and B-Se (Fig. 1). A dose–response association based on individual measurements of cotinine and B-Se has to the best of our knowledge not previously been reported, but it strengthens the evidence that current smoking actually has an impact on

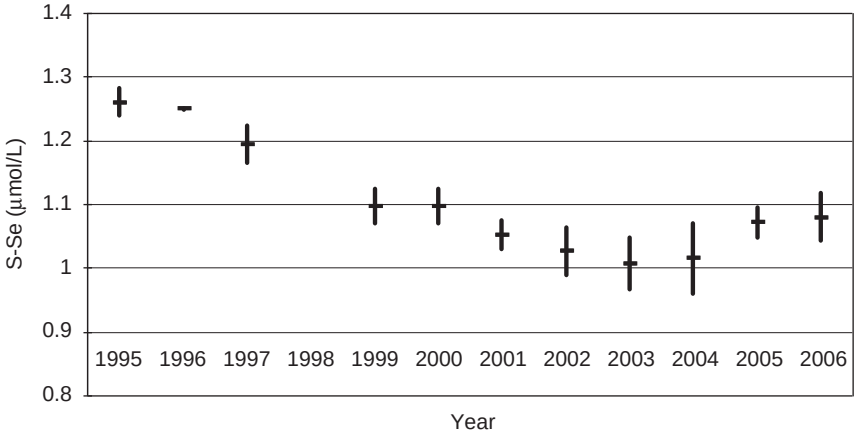

Fig. 2. The mean concentrations of selenium in serum from 1995–2006 determined at a commercial laboratory. The concentrations are adjusted according to the average deviation from the target S-Se concentration before and after March 2002.

the Se status. However, the cross-sectional design of the study is a limitation with respect to assess causal relations. One study has reported an association between the two biomarkers based on grouped data previously [18]. That study reported nearly 4% lower S-Se in the men with the highest S-Cotinine levels as compared to those with the lowest levels. In contrast, the smokers in the present study had around 13% lower S-Se as the non-smokers.

The activity of S-GSHpx was not significantly associated with any of the nicotine-related biomarkers, but smokers had significantly lower mean S-GSHpx activity when compared to non-smokers, suggesting that this biomarker is also influenced by smoking habits. The habit of using smoke-free tobacco, as a substitute for smoking tobacco, is rapidly increasing in Norway. It is notable that the snuff users in the present study had about the same mean concentrations of B-Se and S-Se as the non-smokers, although their self-reported tobacco consumption and their concentrations of the nicotine-related biomarkers were similar to those of the smokers. This could indicate that the route of administration may be important for the observed effect on the Se concentrations, or that smoking tobacco is combusted whereas smokeless tobacco is not. It has been proposed that tobacco smoking causes inflammation and that the lower B-Se concentration may be a consequence of the inflammatory process [11]. Also the mean B-Cd concentration of the snuffers was similar to that of the non-smokers, while the smokers, as expected, had much higher B-Cd concentrations, indicating that the route of tobacco administration is of importance for the determined concentrations of B-Cd. Other potential causes besides inflammation that have been suggested as reasons why smokers have lower Se status than non-smokers include that smokers eat less or that their simultaneous exposure to Cd decreases the bioavailability of Se [11]. We do not have any data on food consumption, but the body mass index of the smokers was about the same as the snuffers. Also no significant association between B-Cd and the Se status biomarkers was observed in the study.

About 1/3 of the blue-collar workers with the lowest levels of B-Se (or S-Se) had a lower activity of S-GSHpx than the subjects with the highest levels. Nutritional recommendations are often based on the maximation of the S-GSHpx activity as a guideline for the recommended intake of Se [10]. Thus, this could imply that a significant proportion of the studied blue-collar workers has an intake of Se that may be too low. The intake considered to maximize the activity of GSHpx in blood plasma is about 55–60 µg/day or somewhat higher [10,27]. If the requirement of saturation of the GPX activity in the platelets, rather than in the plasma, is used as a basis for the recommendation, then the higher level of intake of around 80–100 µg/day is required [28].

Although relatively more current smokers were found in the group with the lowest concentrations of B-Se, many non-smokers belonged to that group, suggesting a lower intake of selenium in non-smokers as well. It should be emphasized that the studied subjects were recruited from a population currently at work, and they were apparently healthy at the time of the examination. Thus, it is likely that they are on average in a better nutritional condition than the average Norwegian population.

The study presents results which are indicative of a decreasing trend in the S-Se concentrations during the years from 1995 to 2003 (Fig. 2). The number of measurements taken each year is substantial, but it still cannot be decided if the measured concentrations are representative for the population mean. However, if the selection of subjects delivering blood samples for Se measurements has not changed substantially during the studied time period, one can assume that the decreasing trend is likely to be the same as in the population as a

whole, and thus the Norwegian population may have a lower intake of Se today compared to the mid-1990s. Previous cross-sectional studies in Norway indicate that the levels of S-Se were even higher during the 1980s. In a population-based study of 273 men and 194 women, the S-Se concentrations were 1.63 and 1.51 μmol/L in samples collected in 1980 [29]. Other, smaller studies revealed mean S-Se concentrations between 1.45 and 1.53 μmol/L in samples collected during the 1980s [30–32]. These studies suggest that the population mean concentrations of Se in serum may have been in the range from 1.45 μmol/L to around 1.60 μmol/L, which is compatible with the compiled data from various European countries [13]. Thus, the available evidence could allow us to suggest a reduction in the S-Se concentrations of around 30% between the 1980s and 2003. The mean concentration of S-Se in the range between 1.0 and 1.1 μmol/L between the end of the 1990s and 2006 (Fig. 2) is comparable to levels measured in several countries on the European continent [33]. However, it should be emphasized that the mean S-Se in the blue-collar workers in the present study was higher than the levels measured in the time-trend study. This may be because the blue-collar workers constitute a highly selected population of particularly healthy individuals, while the subjects in the time-trend study are patients visiting clinical practices.

About 1/3 of the blue-collar workers of the cross-sectional study belonged to the subgroup with lower activity of S-GSHpx as compared to the remaining subjects. The cut-point for B-Se in this group was 1.3 μmol/L, while the cut-point for S-Se was 1.22 μmol/L. The latter cut-point could suggest that a substantial proportion of the subjects included in the time-trend study do not have optimal saturation of S-GSHpx. However, sample preparation and measurement methods are not completely identical, and thus some caution in the interpretation is warranted.

While a sub-optimalized activity of S-GSHpx is observed in several of the subjects, the B-Se levels are much higher than those associated with the cardiomyopathy related to Se deficiency (Keshan's disease). The lowest B-Se concentrations in the cross-sectional study was 0.9 μmol/L, which may also be sufficient to maintain a normal function of the iodothyronine 5′ deiodinase that is involved in the deiodination of thyroxin to tri-iodothyronine [12]. Whether the measured concentrations are so low that the risk of acquiring certain cancers, alterations in immune system functions or cardiovascular diseases increases, cannot be determined from this study.

One important factor determining the content of Se in food is the content of Se in the soil where plants are grown or animals are raised [10]. The soil in the Scandinavian countries is in general poor in Se. The population Se intake of most European countries is low, and much lower than the intakes in North and also South America [34,35]. The situation in Norway with apparently decreasing S-Se concentrations is notable. In the decades before 1990 as much as about 70% of the grain consumed in Norway was imported from Se-rich areas in the Americas. The lower blood Se levels observed in Norway in recent years could reflect the increased consumption of domestic grain with a low content of Se, although this possibility has to be elucidated further. Whether the consumption of Se-rich grain can account for the slight increase in S-Se observed in 2005 and 2006 cannot be determined in this study. It is unlikely that the decreasing prevalence of smoking in Norway can account for this increase.

In conclusion, the average S-Se concentrations in a selected population in Norway appear to have decreased during the 1990s and are now comparable to those in Sweden, Denmark and some other European countries. The recruitment of these subjects could imply that they do not represent the population as a whole. However, this result could eventually indicate that a number of people have a sub-optimal activity of S-GSHpx. Smokers are apparently more prone to have lower levels of Se, but at present the biological reasons are not sufficiently understood. Further studies are needed on a population level, in order to assess the possibility that some subjects may be in a Se deficiency state.

References

[1] Gromer S, Eubel JK, Lee BL, Jacob J. Human selenoproteins at a glance. Cell Mol Life Sci 2005;62:2414–37.
[2] Olivieri O, Girelli D, Azzini M, Stanzial AM, Russo C, Ferroni M, et al. Low selenium status in the elderly influences thyroid hormones. Clin Sci 1995;89:637–42.
[3] Hawkes WC, Turek PJ. Effects of dietary selenium on sperm motility in healthy men. J Androl 2001;22:764–72.
[4] Pfeifer H, Conrad M, Roethlein D, Kyriakopoulos A, Brielmeier M, Bornkamm GW, et al. Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. FASEB J 2001;15:1236–8.
[5] Navarro-Alarcòn M, Lopez-Garcia Serana H, Pèrez-Valero V, López-Martinez C. Serum and urine selenium concentrations in patients with cardiovascular diseases and relationship to other nutritional indexes. Ann Nutr Metab 1999;43:30–6.
[6] Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E. Selenium and coronary heart disease: a meta-analysis. Am J Clin Nutr 2006;84:762–73.
[7] Cunningham-Rundles S, McNeeley DF, Moon A. Mechanisms of nutrient modulation of the immune response. J Allerg Clin Immun 2005;115:1119–28.
[8] WHO. Safety evaluation of certain food additives and contaminants. Geneva: WHO Food Additives Series 44, IPSC, WHO; 2000.

[9] Silvera SAN, Rohan TE. Trace elements and cancer risk: a review of the epidemiologic evidence. Cancer Causes Control 2007;18:7–27.
[10] Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. A report of the panel on dietary antioxidants and related compounds, subcommittees on upper reference levels of nutrients and interpretation and uses of dietary reference intakes, and the standing committees on the scientific evaluation of dietary reference intakes. Washington, DC: National Academy Press; 2000. p. 283–324.
[11] Northrop-Clewes CA, Thurnham DI. Monitoring micronutrients in cigarette smokers. Clin Chim Acta 2007;377: 14–38.
[12] Thomson CD. Assessment of requirements for selenium and adequacy of selenium status: a review. Eur J Clin Nutr 2004;58:391–402.
[13] Néve J. Methods in determination of selenium status. J Trace Elem Electrolytes Health Dis 1991;5:1–17.
[14] Tinggi U. Essentiality and toxicity of selenium and its status in Australia: a review. Toxicol Lett 2003;137: 103–10.
[15] Ellingsen DG, Holland RI, Thomassen Y, Olstad ML, Frech W, Kjuus H. Mercury and selenium in workers previously exposed to mercury vapor at a chloralkali plant. Br J Ind Med 1993;50:745–52.
[16] Ellingsen DG, Efskind J, Berg KJ, Gaarder PI, Thomassen Y. Renal and immunologic markers for chloralkali workers with low exposure to mercury vapour. Scand J Work Environ Health 2000;26:427–35.
[17] Ellingsen DG, Haug E, Gaarder PI, Bast-Pettersen R, Thomassen Y. Endocrine and immunologic markers in manganese alloy production workers. Scand J Work Environ Health 2003;29:230–8.
[18] Kafai MR, Ganji V. Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third national health and nutrition examination survey, 1988–1994. J Trace Elem Med Biol 2003;17:13–8.
[19] Boswell C, Curvall M, Elswick Jr RK, Leyden D. Modelling nicotine intake in smokers and snuff users using biological fluid nicotine metabolites. Biomarkers 2000;5:341–54.
[20] Bast-Pettersen R, Ellingsen DG, Efskind J, Jordskogen R, Thomassen Y. A neurobehavioral study of chloralkali workers after the cessation of exposure to mercury vapor. Neurotoxicology 2005;26:427–37.
[21] Hauge R, Irgens-Jensen O. Alcohol in the Nordic countries. Tidsskr Nord Alkoholforskn 1987;4(Suppl.): 48–9 [in Norwegian].
[22] Efskind J, Ellingsen DG, Hartman A, Thomassen Y, Ulvik RJ, Gaarder PI, et al. Renal function in chloralkali workers after the cessation of exposure to mercury vapour. Scand J Work Environ Health 2006;32:241–9.
[23] Wheeler CR, Salzman JA, Elsayed NM, Omaye ST, Korte Jr DW. Automated assays for superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activity. Anal Biochem 1990;184:193–9.
[24] Ellingsen DG, Bast-Pettersen R, Efskind J, Gjølstad M, Olsen R, Thomassen Y, et al. Hand tremor related to smoking habits and the consumption of caffeine in male industrial workers. Neurotoxicology 2006;27:525–33.
[25] Kocyigit A, Erel O, Gur S. Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities. Clin Biochem 2001;34(8):629–33.
[26] Kosanovic M, Jokanovic M, Jevremovic M, Dobric S, Bokonjic D. Maternal and fetal cadmium and selenium status in normotensive and hypertensive pregnancy. Biol Trace Elem Res 2002;89:97–103.
[27] MacPherson A, Barclay MNI, Scott R, Yates RWS. Loss of Canadian wheat lowers selenium intake and status of the Scottish population. In: Fischer PWF, L'Abbé MR, Cockel KA, Gibson RS, editors. Trace elements in man and animals 9. Ottawa: NRC Research Press; 1997. p. 203–5.
[28] Néve J. New indices for assessment of trace element status and requirement, with a special focus on selenium. In: Roussel AM, Favier A, Anderson RA, editors. Trace elements in man and animals 10. New York: Plenum Press; 2000. p. 317–22.
[29] Ringstad J, Jacobsen BK, Thomassen Y. The Tromsø heart study: relationships between the concentration of selenium in serum and risk factors for coronary heart disease. J Trace Elem Electrolytes Health Dis 1987;1: 27–31.
[30] Aaseth J, Smith-Kielland A, Thomassen Y. Selenium, alcohol and liver diseases. Ann Clin Res 1986;18:43–7.
[31] Aaseth J, Thomassen Y, Aadland E, Fausa O, Schrumpf E. Hepatic retention of copper and selenium in primary sclerosing cholangitis. Scand J Gastroenterol 1995;30: 1200–3.
[32] Glattre E, Thomassen Y, Thoresen SØ, Haldorsen T, Lund-Larsen PG, Theodorsen L, et al. Prediagnostic serum selenium in a case-control study of thyroid cancer. Int J Epidemiol 1989;18:45–9.
[33] Alfthan G, Neve J. Reference values for serum selenium in various areas evaluated according to the TRACY protocol. J Trace Elem Med Biol 1996;10:77–87.
[34] Jackson MJ, Broome CS, McArdle F. Marginal dietary selenium intakes in the UK: are there functional consequences? J Nutr 2003;133:1557S–9S.
[35] Rayman MP. The importance of selenium to human health. Lancet 2000;356:233–41.