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Circulating levels of metals are related to carotid atherosclerosis in elderly

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ABSTRACT

The aim of this study was to investigate if blood levels of trace and/or heavy metals are related to atherosclerosis in a cross-sectional study in elderly.
In the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (1016 subjects, all aged 70), the prevalence of carotid artery plaques was recorded by ultrasound. The numbers of carotid arteries with plaques (0, 1 or 2) were recorded. Also the thickness (IMT) and gray scale (IM-GSM) of the intima-media complex were measured together with plaque echogenicity. Eleven heavy metals and trace elements were analyzed in whole blood, using inductively coupled plasma-sector field mass spectrometry. Nickel levels were related to the number of carotid arteries with plaques in an inverted U-shaped manner after multiple adjustment for gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use ($p = 0.026$). IM-GSM and plaque echogenicity were both inversely related to chromium in a linear fashion, and to aluminum in an inverted U-shaped manner (both $p<0.0001$ for IM-GSM). The relationships between metals and IMT were modest.
Circulating levels of some metals, like nickel, aluminum and chromium, were related to atherosclerotic plaques or the echogenicity of the IM-GSM and overt plaques independently of cardiovascular risk factors, including lipids.

1. Introduction

Metals, both trace elements and so called heavy metals, can be measured in a variety of body compartments, such as whole blood, serum, plasma, urine, hair and toenails in the epidemiological setting. Using metal determinations, it has been shown in different studies that Pb, Cd, Ni, Co and Hg levels are increased in patients with coronary artery disease, or in subjects developing myocardial infarction during a certain follow-up in cohort studies. In contrast, Cu, Zn and Cr were reported to be reduced in such studies (Afridi et al., 2010; Afridi et al., 2008: Alissa et al., 2009; Everett and Frithsen, 2008; Giannoglou et al., 2010: Guallar et al., 2002; Kazemi-Bajestani et al., 2007; Kazi et al., 2008; Krachler et al., 1997; Leach et al., 1985; Menke et al., 2009; Oster et al., 1989; Salonen et al., 2000; Virtanen et al., 2005; Vlad et al., 1994). Some essential metals can give adverse effects if the levels are decreased, but can also accumulate in the body, yielding higher levels, especially in the elderly (Afridi et al., 2010; Ashraf et al., 1994; Dar et al., 2008; Kazi et al., 2008; Khalique et al., 2005; Lopes et al., 2004). The hallmark of myocardial infarction is atherosclerosis. In the case of myocardial infarction, an atherosclerotic plaque in the coronary arteries ruptures and gives rise to an occluding thrombus. A number of studies have reported associations between metals and atherosclerosis, evaluated by carotid artery intima-media thickness (IMT).

In those studies, increased levels of Cd and Hg, and reduced levels of Zn, were related to a thickened IMT (Messner et al., 2009: Salonen et al., 2000: Skoczyńska et al., 2009) or severity of coronary lesions at angiography (Giannoglou et al., 2010). Furthermore, exposure to Cd increased, while administration of Zn and Cr reduced atherosclerosis development in the hypercholesterolemic rabbit and ApoE knock-out mice (Messner et al., 2009: Price Evans et al., 2009; Subramanyam et al., 1992), giving further support to the theory that metals might be involved in the pathogenesis of atherosclerosis.

Since coronary and carotid artery atherosclerosis often go hand in hand (Hulthe, et al., 1997), we hypothesized that subjects with high circulating levels of heavy metals and low levels of trace elements more often show carotid atherosclerotic plaques. Furthermore, since we recently found that the echogenicity of the intima-media complex (IM-GSM) in the carotid artery – a possible marker of lipid infiltration

Abbreviations: Al, aluminum; BMI, body mass index; Cd, cadmium; Co, cobalt; Cu, copper; Cr, chromium; CV, cardiovascular; DBP, diastolic blood pressure; HDL, high-density lipoprotein; Hg, mercury; ICP-SFMS, inductively coupled plasma-sector field mass spectrometry; IM-GSM, intima-media complex; IMT, intima-media thickness; LDL, low-density lipoprotein; Mn, manganese; Mo, molybdenum; Ni, nickel; Pb, lead; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; SBP, systolic blood pressure; Zn, zinc.

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in the vascular wall – is a powerful predictor of future cardiovascular (CV) death (Wohlin et al., 2009), we also investigated if metal levels were associated with IM-GSM. For these aims, we used the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (Lind et al., 2005) in which we have data on atherosclerosis and circulating metal levels in almost 1000 subjects.

2. Material and methods

2.1. Subjects

Eligible to the study were all subjects aged 70 living in the community of Uppsala, Sweden. The subjects were randomly chosen from the register of community residents. A total of 1016 subjects participated, giving a participation rate of 50.1%. The primary aim of the collection of data in the PIVUS study was to evaluate if measures of endothelium-dependent vasodilatation were independent predictors of future myocardial infarction and stroke. Since many cases of myocardial and stroke occur between 70 and 80 years of age, we did chose to investigate subjects at a baseline of 70 years, which would give us a good power to detect about 100 stroke cases and 100 myocardial infarction cases during 10 years follow-up with the present sample size. The study was approved by the Ethics Committee of the University of Uppsala.

All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. An arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators. The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to nearest mm Hg after at least 30 min of rest, whereupon the average of three recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques (Carlsson et al., 2010; Lind et al., 2005). Basic characteristics medical history, and regular medication are given in Tables 1 and 2.

2.2. Carotid artery ultrasound evaluation

The carotid artery was assessed by external B-mode ultrasound imaging (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, California, USA).

The common carotid artery, the bulb and the internal carotid artery at both sides were visually investigated for the presence of plaque. A plaque was judged to be present in a particular carotid artery if a local thickening of the IMT was seen that was more than 50% thicker than the surrounding IMT in any part of the carotid artery investigated. We recorded whether carotid plaques were present in none, one or both of the carotid arteries.

The images were digitized and imported into the AMS (Artery Measurement Software) automated software (Liang et al., 2000) for dedicated analysis of IMT and the gray scale median of the IM-GSM. A maximal 10 mm segment with good image quality was chosen for IMT analysis from the carotid artery. The program automatically identifies the borders of the IMT of the far wall and the inner diameter of the vessel, and calculates IMT and the diameter from around 100 discrete measurements through the 10 mm long segment. This automated analysis could be manually corrected if not found appropriate at visual inspection. The given value for carotid artery IMT is the mean value from both sides.

A region of interest (ROI) was placed manually around the intima-media segment that was evaluated for IMT, whereby the program calculates the echogenicity in the IM-GSM from an analysis of the individual pixels within the region of interest on a scale from 0 (black) to 256 (white). The blood was used as the reference for black and the adventitia was the reference for white. The given GSM value is the mean value from both sides.

An ROI was also placed manually around plaques for measurement of plaque GSM.

GSM of the plaques was evaluated with the same software as used for IM-GSM. This measurement was repeated in 25 random subjects, giving a coefficient of variation of 8.3% for GSM in the plaques. The mean length of the evaluated intima-media segments was 9.0 (SD 2.1) mm when subjects with a segment recording less than 5 mm were excluded, leaving 946 subjects with valid recordings. The measurements of the intima-media were repeated in 30 random subjects, giving a coefficient of variation of carotid artery IMT of 7.2% and 7.5% for echogenicity in the IM-GSM.

2.3. Analysis of metals in whole blood

All 11 elements (Al, Cd, Co, Cu, Cr, Hg Mn, Mo, Ni, Pb, and Zn) in this study were determined in whole blood. The analysis was performed using inductively coupled plasma-sector field mass spectrometry, ICP-SFMS, after microwave assisted digestion with nitric acid (Rodushkin et al., 2000) according to a method accredited for 10 of

Table 1
Basic characteristics in the investigated sample, with means and standard deviation (SD) in parenthesis. SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; LDL = low density lipoprotein; HDL = high density lipoprotein.

n	1016
Females (%)	50.2
Height (cm)	169 (9.1)
Weight (kg)	77 (14)
Waist circumference (cm)	91 (12)
BMI (kg/m2)	27.0 (4.3)
Waist/hip ratio	0.90 (0.075)
SBP (mm Hg)	150 (23)
DBP (mm Hg)	79 (10)
Serum cholesterol (mmol/L)	5.4 (1.0)
LDL cholesterol (mmol/L)	3.3 (0.88)
HDL cholesterol (mmol/L)	1.5 (0.42)
Serum triglycerides (mmol/L)	1.3 (0.60)
Fasting blood glucose (mmol/L)	5.3 (1.6)
Current smoking (%)	11

Table 2
Self-reported history of cardiovascular (CV) disorders and regular drug intake given in percentage (%) in the investigated sample. CABG/PTCA indicates coronary revascularization; GTN, any nitroglycerine preparation.

n	1016
Myocardial infarction	7.1
Stroke	3.7
Angina pectoris	8.1
CABG/PTCA	5.3
Congestive heart failure	3.8
Diabetes	8.7
Any regular drug	70
Any CV drug	45
Any antihypertensive medication	32
Beta-blockers	22
Calcium antagonists	11
Diuretics	13
ACE-inhibitors	8.5
Angiotensin II-blockers	8.3
GTN	3.0
Digoxin	2.1
Statins	15
Other antihyperlipidemic drugs	1.2
Insulin	1.8
Oral antidiabetic drugs	6.1
Warfarin	3.2
Aspirin/Clopidogrel	18
Other antiarrythmic drugs	0.2

the 11 elements tested, Al being unaccredited. All testing follows the stringent QA/QC demands required by the Swedish National Accreditation Body, SWEDAC, in accordance with ISO 17025.

The performing laboratory, ALS Scandinavia AB in Luleå, is registered with the FDA and has a certificate of GMP compliance issued by the Swedish Medical Products Agency.

2.4. Statistical analysis

All variables were evaluated regarding non-normality, and variables with a skewed distribution, such as fasting glucose, serum triglycerides and all metals besides Zn, were ln-transformed. The metals were also divided into quintiles to evaluate potential non-linear relationships.

For all metals and for all outcomes, interactions between the metal levels and gender were evaluated. Since no such interactions were found, a gender adjustment was performed in the first set of models. In the second set, we adjusted for multiple CV risk factors used as continuous variables (gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use).

In all those models, analysis was first run with the metals as continuous variables and also using quintiles. If the quintile analysis suggested a non-linear relationship this was tested by adding the quadratic term of the metal to the model.

Ordinal logistic regression models were used when the outcome was the number of carotid arteries with plaques (grouped as 0, 1 or 2). Linear regression was used when the outcomes were the continuous variables IMT or IM-GSM. Fractional polynomial regression and generalized additive models were used to generate the figure plots.

3. Results

The levels of metals for men and women and reference values for the country are presented in Table 3.

3.1. Metals versus carotid plaques

In the total sample, 273 subjects (27%) showed bilateral plaque, 343 (34%) showed unilateral plaque and 327 (32%) were free of carotid plaque.

When relating the metal levels as continuous variables to the number of arteries with plaque, only Cd was significantly related to the number of carotid arteries with plaques in a highly significant manner ($p=0.001$, Table 4). However, when adjusting for gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use this association lost significance. It was mainly adjustment for smoking that attenuated the association between Cd and carotid plaques. Excluding the smokers (11% of the sample) did not change the results described above in any essential way nor did further adjustment for history of diabetes mellitus and coronary heart disease.

When analyzed according to quintiles, both Cu and Ni showed an inverted U-shape association with the number of carotid arteries with plaque even following adjustment of multiple traditional risk factors (highest ORs in the fourth and third quintiles, respectively). However, when evaluating this in a more formal way by inclusion of the quadratic terms of Cu or Ni in the models, the quadratic term of Ni (OR 0.92, $p=0.034$, Fig. 1), but not that of Cu (OR 0.64, $p=0.81$), was significant.

No significant interactions were seen between the levels of metals and gender regarding carotid plaque, and therefore we adjusted for rather than stratified the sample by gender.

Table 3
Median (inter quartile range) levels of metals in men and women for the investigated sample and reference values for the country. The reference values are from ALS Scandinavias website (http://www.alsglobal.se/hem2005/sv/medicin/jamforelse_medicin.asp) and made by analyses from ALS Scandinavia and published research results and represents levels in human blood from subjects that are not occupationally exposed.

	Men	Women	Both sexes
	Median (IQ)	Median (IQ)	Reference values
Al (μmol/L)	0.637 (0.296)	0.637 (0.286)	<0.1
Cd (nmol/L)	2.18 (1.70)	2.61(2.21)	2–7 (non-smoker) /12–40 (smoker)
Co (nmol/L)	1.41 (0.78)	1.44 (0.80)	–
Cr (nmol/L)	11.9 (6.64)	11.7 (6.20)	–
Cu (nmol/L)	12.0 (2.0)	13.7 (2.5)	–
Hg (nmol/L)	9.42 (8.17)	8.62 (7.72)	5–35
Mn (nmol/L)	133 (48)	143 (52)	≤350
Mo (nmol/L)	9.90 (3.95)	9.56 (3.49)	–
Ni (nmol/L)	92.25 (43.90)	87.70 (37.80)	–
Pb (μmol/L)	0.091 (0.054)	0.074 (0.049)	≤0.5
Zn (μmol/L)	100.0 (15.6)	92.6 (15.6)	70–102

3.2. Metals versus IMT

The mean value for IMT was 0.88 mm (0.16 SD). When relating the metal levels as continuous variables to IMT, only Cd was significantly related to IMT in a significant manner ($p=0.005$, Table 5). However, when adjusting for gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use this association lost significance. A quintile analysis did not reveal any obvious curvilinear relationships.

No significant interactions were seen between the levels of metals and gender regarding IMT, and therefore we adjusted for rather than stratified the sample by gender.

3.3. Metals versus IM-GSM

The mean value for IM-GSM was 79 (SD 23). When relating the metal levels as continuous variables to IM-GSM, Al was positively related to IM-GSM, while both Cr and Mo were inversely related to IM-GSM in a significant manner even after adjustment for gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use ($p=0.03$–0.0001, see Table 5 for details). However, when examining those relationships more closely, Al showed an inverted U-shape relationship vs. IM-GSM (regression coefficient -6.8, $p<0.0001$ for the quadratic term of Al, Fig. 2). For Cr, a fairly inverse linear relationship vs. IM-GSM was seen (Fig. 3). Regarding Mo, an irregular pattern was seen when the quintiles were analyzed. Further adjustment for history of diabetes mellitus and coronary heart disease did not change the above presented results in any substantial way.

No significant interactions were seen between the levels of metals and gender regarding IM-GSM, and therefore we adjusted for rather than stratified the sample by gender.

3.4. Metals versus plaque GSM

The relationships that were significantly related to IM-GSM were also evaluated vs. plaque GSM (mean 74, SD 32, $n=593$). Cr was inversely related to plaque GSM in a linear fashion also following adjustment for multiple risk factors (regression coefficient -6.5, $p=0.009$, see Table 4). Similarly, just like regarding IM-GSM, Al was related to plaque GSM in an inverted U-shaped manner ($p=0.032$ following adjustment for multiple risk factors). Further adjustment for history of diabetes mellitus and coronary heart disease did not change the above presented results in any substantial way.

Table 4
Relationships between metal levels in whole blood and the number of arteries with plaque or the gray scale median of the plaques (plaque GSM) in the common carotid artery. Relationships are given both for the metals as continuous variables (regression coefficient for ln-transformed values) and for quintiles (Q1-Q5) of the variables. Median values for IMT or IM-GSM in the different quintiles are given. Regarding the quintiles, p-values are given for each quintile in comparison with the first quintile. P-values are given for two levels of adjustment; P1 is for gender adjustment only. P2 is for adjustment for multiple CV risk factors (gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use). Also p-values for interactions between gender and metals regarding the two vascular phenotypes are given.

	Plaque prevalence					Plaque GSM				
	OR for continuous (95%CI)	OR for quintiles	P1	P2	Gender interaction	Regression coefficient	Quintile median	P1	P2	Gender interaction
Al continuous	1.28 (0.94–1.74)		0.12	0.068	0.16	4.3		0.027	0.018	0.86
Q1		Ref	Ref	Ref			71	Ref	Ref	
Q2		0.78	0.20	0.35			76	0.007	0.005	
Q3		0.93	0.71	0.73			79	0.0001	0.0001	
Q4		1.19	0.34	0.42			78	0.0001	0.0001	
Q5		1.21	0.31	0.18			75	0.005	0.002	
Cd continuous	1.38 (1.14–1.68)		0.001	0.21	0.14	0.84		0.48	0.72	0.99
Q1		Ref	Ref	Ref			72	Ref	Ref	
Q2		1.04	0.83	0.50			74	0.41	0.47	
Q3		1.18	0.39	0.32			78	0.074	0.14	
Q4		1.67	0.008	0.004			77	0.19	0.29	
Q5		1.57	0.022	0.72			76	0.19	0.48	
Co continuous	1.12 (0.94–1.35)		0.20	0.25	0.18	−0.99		0.40	0.36	0.85
Q1		Ref	Ref	Ref			81	Ref	Ref	
Q2		0.97	0.87	0.44			69	0.0001	0.001	
Q3		0.95	0.79	0.73			76	0.046	0.031	
Q4		1.16	0.42	0.53			75	0.001	0.0001	
Q5		1.43	0.057	0.22			78	0.18	0.17	
Cr continuous	0.96 (0.77–1.19)		0.71	0.88	0.030	−6.0		0.0001	0.0001	0.07
Q1		Ref	Ref	Ref			79	Ref	Ref	
Q2		0.94	0.77	0.85			81	0.63	0.59	
Q3		0.95	0.82	0.88			76	0.082	0.11	
Q4		0.85	0.41	0.54			73	0.057	0.08	
Q5		0.99	0.96	0.78			69	0.0001	0.0001	
Cu continuous	2.53 (1.03–6.20		0.042	0.060	0.50	8.6		0.14	0.047	0.15
Q1		Ref	Ref	Ref			74	Ref	Ref	
Q2		1.15	0.45	0.46			73	0.32	0.30	
Q3		1.30	0.17	0.15			75	0.74	0.92	
Q4		1.70	0.010	0.017			81	0.045	0.008	
Q5		1.38	0.11	0.13			76	0.78	0.41	
Hg continuous	0.95 (0.80–1.14)		0.62	0.99	0.16	0.10		0.93	0.47	0.86
Q1		Ref	Ref	Ref			76	Ref	Ref	
Q2		0.78	0.19	0.27			78	0.35	0.33	
Q3		1.03	0.84	0.79			78	0.23	0.41	
Q4		0.91	0.62	0.95			74	0.97	0.97	
Q5		0.87	0.48	0.77			73	0.99	0.44	
Mn continuous	0.99 (0.66–1.49)		0.97	0.77	0.14	−0.57		0.83	0.99	0.99
Q1		Ref	Ref	Ref			72	Ref	Ref	
Q2		1.08	0.65	0.77			81	0.060	0.052	
Q3		1.15	0.45	0.44			74	0.43	0.47	
Q4		0.94	0.75	0.61			77	0.34	0.24	
Q5		1.10	0.59	0.48			75	0.77	0.92	
Mo continuous	0.89 (0.65–1.22)		0.50	0.33	0.18	−4.8		0.016	0.028	0.85
Q1		Ref	Ref	Ref			76	Ref	Ref	
Q2		0.87	0.44	0.83			72	0.83	0.65	
Q3		0.73	0.10	0.48			80	0.22	0.19	
Q4		0.83	0.34	0.72			78	0.57	0.43	
Q5		0.78	0.22	0.21			71	0.13	0.25	
Ni continuous	1.03 (0.91–1.16)		0.62	0.34	0.030	0.50		0.52	0.53	0.07
Q1		Ref	Ref	Ref			71	Ref	Ref	
Q2		1.24	0.24	0.51			73	0.67	0.54	
Q3		1.62	0.012	0.026			79	0.036	0.020	
Q4		1.46	0.046	0.19			80	0.008	0.009	
Q5		1.08	0.69	0.50			76	0.62	0.51	
Pb continuous	0.96 (0.75–1.23)		0.77	0.41	0.50	2.7		0.087	0.34	0.15
Q1		Ref	Ref	Ref			77	Ref	Ref	
Q2		0.81	0.29	0.74			73	0.78	0.56	
Q3		0.93	0.71	0.87			75	0.46	0.71	
Q4		0.97	0.88	0.76			76	0.14	0.37	
Q5		0.90	0.58	0.60			77	0.20	0.45	
Zn continuous	0.99 (0.98–1.008)		0.76	0.62	0.50	0.017		0.78	0.38	0.15
Q1		Ref	Ref	Ref			75	Ref	Ref	
Q2		0.97	0.86	0.89			77	0.73	0.68	
Q3		0.78	0.21	0.12			77	0.88	0.76	
Q4		1.06	0.74	0.87			72	0.45	0.45	
Q5		0.87	0.50	0.56			78	0.77	0.40	

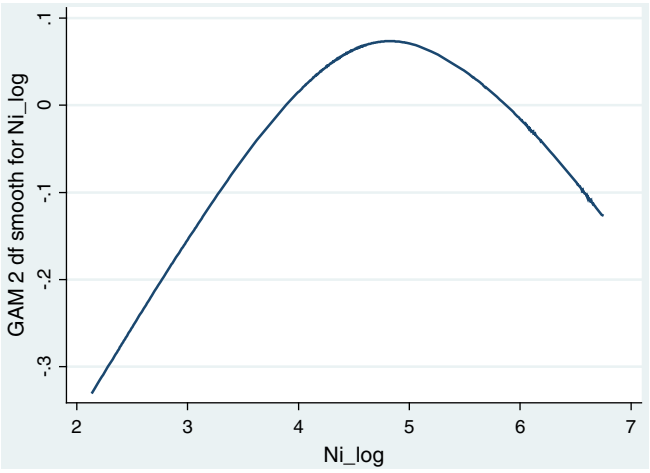

Fig. 1. Relationship between nickel (Ni, log-transformed) and the number of arteries with carotid plaques (log odds on the y axis). A general additive model was used for calculation of the relationship. $P<0.0001$ for the curvilinear relationship.

4. Discussion

The present cross-sectional study showed that several of the investigated metals were related to carotid artery atherosclerosis characteristics. Nickel levels were related to the number of carotid arteries with plaques in an inverted U-shaped manner after adjustment for multiple CV risk factors. IM-GSM and plaque echogenicity were both inversely related to chromium in a linear fashion and to aluminum in an inverted U-shaped manner, while the relationships between metals and IMT were modest.

The levels of metals in the investigated sample are comparable to the available reference values in the area. Previous studies have showed that the levels of metals could be associated with age or age and gender. Increasing levels of Cu and decreasing levels of Zn and Mn have been found (Rahil-Khazen et al., 2000; Menditto et al., 1993) but no such associations were found for Cu and Zn levels by Mártin-Lagos et al. (1997). Cd and Pb increase with age, whereas Cr, Mn and Ni tended to decrease, smoking induced elevation both in Cd and in Pb (Davies et al., 1997; Ikeda et al., 2011).

4.1. Carotid artery plaque

Presence of atherosclerotic plaques in the carotid arteries is a known predictor of future CV events (O'Leary et al., 1999). Atherosclerosis in the carotid arteries is also known to be predictive for plaques in the coronary circulation (Hulthe et al., 1997). Thus, subjects with carotid artery plaques are at increased risk also for myocardial infarction. Furthermore, since it has been shown that subjects with bilateral carotid plaques are at higher risk for future CV disorders than subjects with unilateral plaques (Davidsson et al., 2010), we used a graded response for carotid plaques (0, 1 or 2 carotid arteries with plaques) rather than a binary outcome (plaque or not plaque) to utilize the variation in carotid plaque prevalence in a more powerful fashion.

Evidence in humans for a link between metal exposure and disease may be found either in samples that have been exposed to very high levels of a certain compound – either by accident or through occupational exposure – or by measuring circulating levels of metals in representative populations and relating the levels to the outcome of interest. In the case of myocardial infarction, a link between metal exposure and myocardial infarction has been documented by both of these approaches (Afridi et al., 2008: Alissa et al., 2009; Everett and Frithsen, 2008; Leach et al., 1985; Menke et al., 2009; Oster et al., 1989; Salonen et al., 2000; Virtanen et al., 2005; Vlad et al., 1994). However, since metal exposure has also been linked to several of the well known CV risk factors, including lipid oxidation, which is a crucial step in atherosclerosis development (Kapiotis et al., 2002; Elis et al., 2001), it is unclear by which route metals could induce myocardial infarction.

First it was found that Cd levels related to the number of atherosclerotic plaques in a fairly linear and highly significant manner. However, when adding traditional risk factors to the model, Cd lost significance and a further analysis showed that it was mainly the inclusion of smoking that markedly attenuated the impact of Cd on plaque. It is well known that smoking is an important risk factor for atherosclerosis, and it has also been shown that smoking is an important source of Cd exposure (Galazyn-Sidorczuk et al., 2008; Kazi et al., 2010a; Kromhout et al., 1985; Nordberg et al., 2000; O'Connor et al., 2010; Tulley and Lehmann, 1982). This was found in the present study as well. Increased Cd accumulation was observed in patients who smoked as compared to patients who were non-smokers, while low Zn levels were associated with both smoker and non-smoker CV disease patients (Afridi et al., 2011). Lower Zn levels in smokers have been shown (Ghayour-Mobarhan et al., 2005; Kazi et al., 2010a; Kazi et al., 2010b; Uz et al., 2003) while others have found that Zn levels were not significantly affected by smoking (Rahil-Khazen et al., 2000; Tulley and Lehmann, 1982).

The toxic mechanisms of Cd are not clear, as it is unable to generate free radicals directly. Indirect formation of ROS and RNS involving the superoxide radical, hydroxyl radical and nitric oxide has been reported (Waisberg et al., 2003) and it is known to act intracellularly, mainly via free radical-induced damage, particularly to the heart, lungs, kidneys, bone, central nervous system and reproductive organs and (Waalkes, 2000). Cd can replace Fe and Cu in various cytoplasmic and membrane proteins (e.g. ferritin, apoferritin), thus increasing the amount of unbound free or poorly chelated Cu and Fe ions participating in oxidative stress via Fenton reactions (Price and Joshi, 1983; Watjen and Beyersmann, 2004).

The relationship between Ni and carotid plaques persisted even after adjustment for multiple risk factors. Only a few previous studies relating metals to coronary heart disease (CHD) or atherosclerosis have evaluated Ni, but two independent studies have reported increased levels of Ni in hair and serum of CHD patients (Leach et al., 1985; Afridi et al., 2008).

The relationship between Ni and carotid artery plaques however was non-linear (as could be seen in Fig. 1), but tended to show an inverted U-shaped curve both when evaluated by quintile analysis and a general additive model.

It has previously been shown that some other environmental pollutants, such as persistent organic pollutants (POPs), could have deleterious health effects even in low dosages, an effect that was abolished at higher levels of exposure, resembling the pattern seen for Ni in the present study (Lee et al., 2010). Similar results where environmental pollutants affecting CV diseases and different CV risk factors are also shown by our research group recently (Hong et al., 2011; Lee et al., 2011a, 2011b; Lind and Lind, 2011; Rönn et al., 2011).

The mechanism whereby Ni might promote atherosclerosis development at low dosages independently of traditional risk factors is largely unknown, but a recent study showed that inhalation of Ni nanoparticles exacerbated atherosclerosis in apoE knock-out mice in parallel with an increased inflammation and oxidative stress (Kang et al., 2011).

Also Cu tended to show an inverted U-shaped relationship vs. carotid plaque following adjustment for multiple risk factors, but in this case the quadratic term of Cu was not significant. Several studies on Cu and CHD have shown low Cu levels in serum and hair compared to healthy controls (Oster et al., 1989; Aalbers and Houtman, 1985; Kazemi-Bajestani et al., 2007), supporting the finding that Cu might be linked to atherosclerosis.

The relationship between coronary risk factors and serum Cu and Zn was assessed in a large Iranian population sample and showed that the ten-year coronary risk was positively correlated with serum Cu

Table 5
Relationships between metals and intima-media thickness (IMT) or gray scale median of the intima-media complex (IM-GSM) in the common carotid artery. Relationships are given both for the metals as continuous variables (regression coefficient for ln-transformed values) and for quintiles (Q1-Q5) of the variables. Median values for IMT or IM-GSM in the different quintiles are given. Regarding the quintiles, p-values are given for each quintile in comparison with the first quintile. P-values are given for two levels of adjustment; P1 is for gender adjustment only. P2 is for adjustment for multiple CV risk factors (gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use). Also p-values for interactions between gender and metals regarding the two vascular phenotypes are given.

	IMT					IM-GSM				
	Regression coefficient	Quintile median	P1	P2	Gender interaction	Regression coefficient	Quintile median	P1	P2	Gender interaction
Al continuous	−0.0011		0.93	0.68	0.16	4.3		0.027	0.018	0.86
Q1		0.87	Ref	Ref			71	Ref	Ref	
Q2		0.84	0.41	0.74			76	0.007	0.005	
Q3		0.86	0.42	0.45			79	0.0001	0.0001	
Q4		0.88	0.44	0.43			78	0.0001	0.0001	
Q5		0.89	0.84	0.70			75	0.005	0.002	
Cd continuous	0.023		0.005	0.22	0.14	0.84		0.48	0.72	0.99
Q1		0.87	Ref	Ref			72	Ref	Ref	
Q2		0.86	0.97	0.98			74	0.41	0.47	
Q3		0.85	0.92	0.43			78	0.074	0.14	
Q4		0.87	0.65	0.63			77	0.19	0.29	
Q5		0.91	0.016	0.10			76	0.19	0.48	
Co continuous	0.0079		0.33	0.13	0.18	−0.99		0.40	0.36	0.85
Q1		0.87	Ref	Ref			81	Ref	Ref	
Q2		0.85	0.85	0.49			69	0.0001	0.001	
Q3		0.85	0.62	0.73			76	0.046	0.031	
Q4		0.91	0.23	0.14			75	0.001	0.0001	
Q5		0.88	0.44	0.43			78	0.18	0.17	
Cr continuous	−0.0064		0.53	0.70	0.030	−6.0		0.0001	0.0001	0.07
Q1		0.89	Ref	Ref			79	Ref	Ref	
Q2		0.87	0.38	0.60			81	0.63	0.59	
Q3		0.87	0.39	0.52			76	0.082	0.11	
Q4		0.84	0.13	0.26			73	0.057	0.08	
Q5		0.87	0.62	0.79			69	0.0001	0.0001	
Cu continuous	0.095		0.018	0.11	0.50	8.6		0.14	0.047	0.15
Q1		0.87	Ref	Ref			74	Ref	Ref	
Q2		0.87	0.82	0.68			73	0.32	0.30	
Q3		0.87	0.36	0.64			75	0.74	0.92	
Q4		0.87	0.29	0.84			81	0.045	0.008	
Q5		0.87	0.047	0.073			76	0.78	0.41	
Hg continuous	0.0061		0.44	0.18	0.16	0.10		0.93	0.47	0.86
Q1		0.86	Ref	Ref			76	Ref	Ref	
Q2		0.88	0.11	0.13			78	0.35	0.33	
Q3		0.86	0.33	0.30			78	0.23	0.41	
Q4		0.88	0.18	0.20			74	0.97	0.97	
Q5		0.87	0.44	0.21			73	0.99	0.44	
Mn continuous	0.00019		0.99	0.96	0.14	−0.57		0.83	0.99	0.99
Q1		0.87	Ref	Ref			72	Ref	Ref	
Q2		0.87	0.59	0.89			81	0.060	0.052	
Q3		0.87	0.55	0.89			74	0.43	0.47	
Q4		0.87	0.92	0.55			77	0.34	0.24	
Q5		0.87	0.81	0.92			75	0.77	0.92	
Mo continuous	0.00021		0.99	0.74	0.18	−4.8		0.016	0.028	0.85
Q1		0.88	Ref	Ref			76	Ref	Ref	
Q2		0.87	0.93	0.99			72	0.83	0.65	
Q3		0.87	0.33	0.73			80	0.22	0.19	
Q4		0.87	0.91	0.75			78	0.57	0.43	
Q5		0.87	0.92	0.90			71	0.13	0.25	
Ni continuous	0.0027		0.61	0.57	0.030	0.50		0.52	0.53	0.07
Q1		0.88	Ref	Ref			71	Ref	Ref	
Q2		0.87	0.32	0.60			73	0.67	0.54	
Q3		0.87	0.19	0.53			79	0.036	0.020	
Q4		0.87	0.93	0.15			80	0.008	0.009	
Q5		0.87	0.36	0.40			76	0.62	0.51	
Pb continuous	0.0061		0.58	0.48	0.50	2.7		0.087	0.34	0.15
Q1		0.85	Ref	Ref			77	Ref	Ref	
Q2		0.87	0.22	0.037			73	0.78	0.56	
Q3		0.86	0.68	0.33			75	0.46	0.71	
Q4		0.88	0.49	0.22			76	0.14	0.37	
Q5		0.88	0.45	0.20			77	0.20	0.45	
Zn continuous	0.00026		0.55	0.85	0.50	0.017		0.78	0.38	0.15
Q1		0.87	Ref	Ref			75	Ref	Ref	
Q2		0.86	0.99	0.84			77	0.73	0.68	
Q3		0.86	0.34	0.14			77	0.88	0.76	
Q4		0.88	0.81	0.84			72	0.45	0.45	
Q5		0.88	0.82	0.96			78	0.77	0.40	

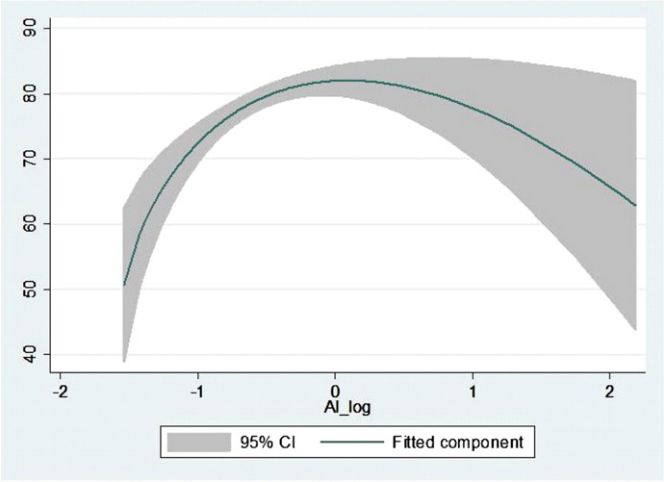

Fig. 2. Relationship between aluminum (Al) levels and gray scale median of the intima-media complex (IM-GSM). P<0.0001 for the quadratic term of log-transformed Al levels, indicating a non-linear relationship.

and Zn in men and Cu in women (Ghayour-Mobarhan et al., 2009). Low serum Zn levels have been shown to be a risk factor for heart diseases in healthy subjects (Lee et al., 2005; Reunanen et al., 1996; Singh et al., 1997, 1998). High serum Cu values gave a 30% increase for cardiovascular mortality compared with low values (and subjects with a combination of low Zn and high Cu levels had synergistically increased all-cause mortality risks) in a study of 4035 middle-aged men (Leone et al., 2006). One study of Saudi men showed that the characteristics differentiating patients with CV disease from controls in odds ratios were serum Zn and Cu/Zn but the levels of Cu and Zn were not significant different (Alissa et al., 2006). Other epidemiological studies and reviews, suggest that high serum Cu concentrations promote heart diseases (Elcarte Lopez et al., 1997; Ferns et al., 1997; Iskra et al., 1993; Magalova et al., 1994; Reunanen et al., 1996; Salonen et al., 1991a, 1991b). Too little Cu can also affect the homeostasis, as dietary Cu deficiency is suggested to impair cardiovascular health (Saari, 2000; Saari and Schuschke, 1999).

4.2. Echogenicity of the intima-media complex

The echogenicity of the IM-GSM is closely related to the echogenicity on overt plaques (Lind et al., 2007), and has been shown to be a powerful predictor of future CV mortality (Wohlin et al., 2009). The echogenicity of overt plaques has been shown to be related to the composition of the plaque (El-Barghouty et al., 1996), and it is therefore likely that also the echogenicity of the IM-GSM is a marker of composition of the vascular wall.

In the present study, Al and Cr were both related to the echogenicity of the IM-GSM as well as to the echogenicity of overt plaques, even after adjustment for CV risk factors, including lipids. However, while the relationship between Cr and echogenicity was linear in an inverse way, an inverted U-shape relationship was seen for Al. Low Cr levels have previously been found in serum in CHD patients (Vlad et al., 1994) and Cr supplementation reduced the degree of atherosclerosis in rabbits given a hypercholesterolemic diet (Price Evans et al., 2009). However, previous data on the influence of Cr on vascular wall and plaque composition is lacking.

Very few studies have investigated the impact of Al on atherosclerosis or its consequences. It has been shown, however, that Al increases the oxidation of LDL, the main driver of atherosclerosis development. How this should be interpreted in the found non-linear relationship is unclear.

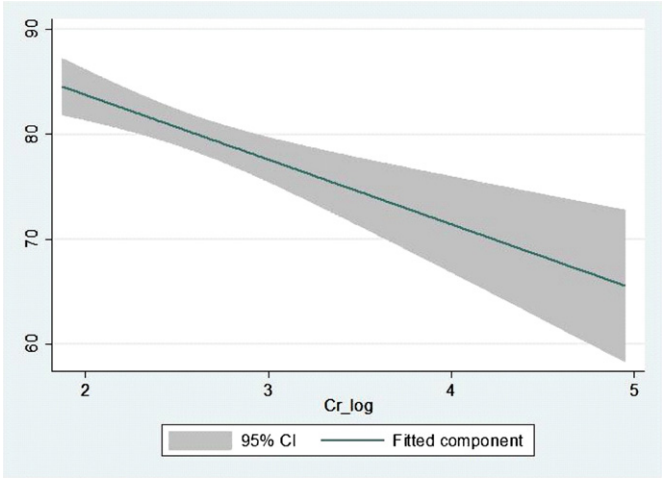

Fig. 3. Relationship between chromium (Cr) levels and gray scale median of the intima-media complex (IM-GSM, P<0.0001 for log-transformed Cr levels).

4.3. Intima-media thickness

IMT is known as a predictor of CV events, and is usually regarded as a marker of atherosclerosis (O'Leary et al., 1999). However, atherosclerosis is a disorder of the intima, while IMT also incorporates the media in the measurement. In the present study, no independent associations between metals and IMT could be found. The previously reported associations between increased levels of Hg and Cd and IMT could not be replicated (Salonen et al., 2000; Skoczyńska et al., 2009; Messner et al., 2009).

4.3.1. Limitation of the study

The present sample is limited to Caucasians aged 70. So, caution should be made to draw conclusions to other ethnic and age groups.

The present study had a moderate participation rate. However, an analysis of non-participants showed the present sample to be fairly representative of the total population regarding most CV disorders and drug intake.

In the present study many statistical tests were performed. Applying a strict Bonferroni correction would result in a critical P-value of 0.0001. Thus any reported association with a P-value above this level should be regarded with caution. The major findings in the study however, IM-GSM and plaque echogenicity were both inversely related to chromium in a linear fashion, and to aluminum in an inverted U-shaped manner, both showed a p<0.0001.

5. Conclusion

Circulating levels of some metals, like nickel, aluminum and chromium, were related to atherosclerotic plaques or the echogenicity of the IM-GSM and overt plaques independently of CV risk factors, including lipids.

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