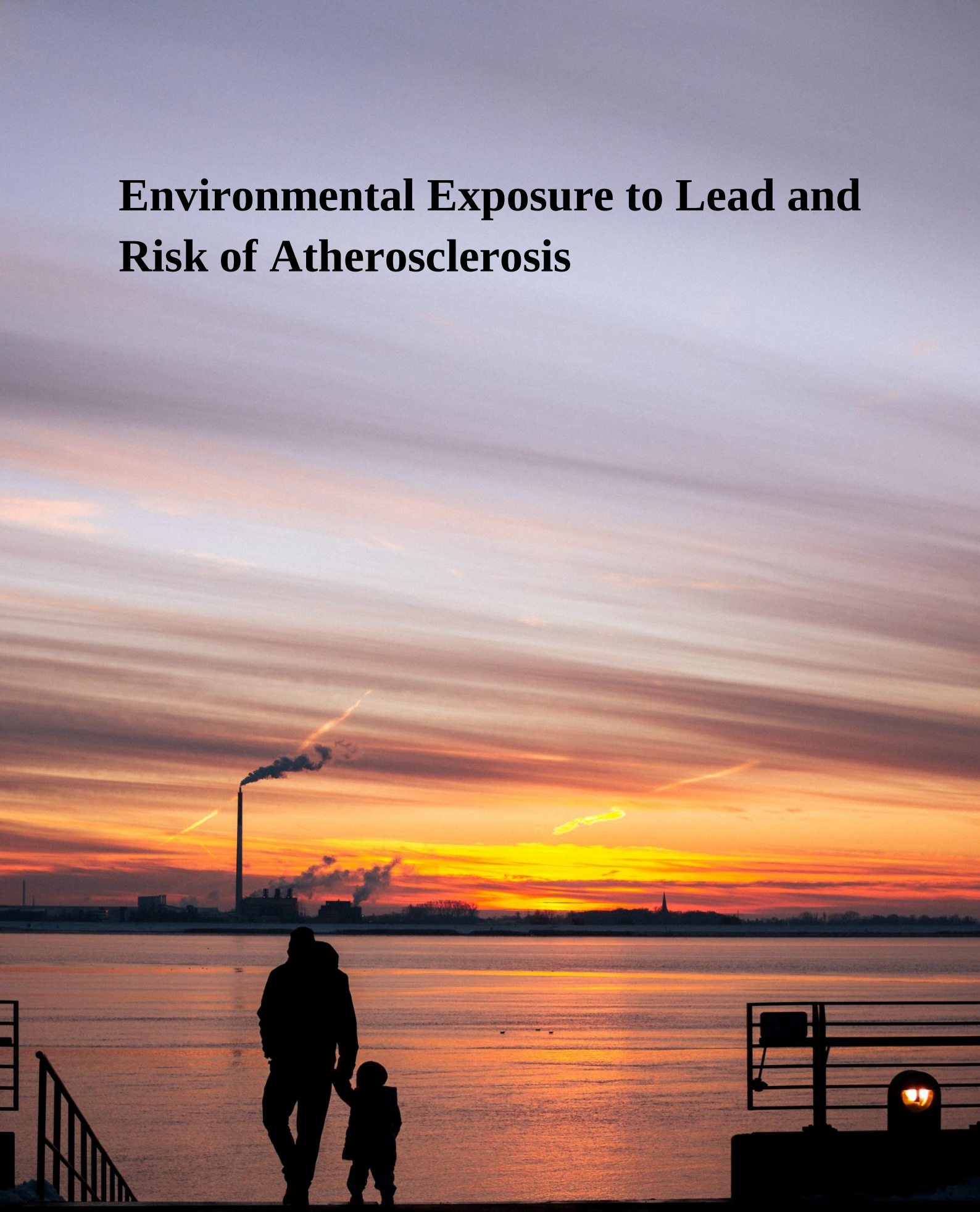
Environmental Exposure to Lead and Risk of Atherosclerosis



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Degree Project in Medicine

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Abstract in English

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Background: Lead exposure is associated with an increased risk of cardiovascular mortality. Atherosclerosis has been hypothesized to be one mechanism underlying this association.

Aim: To assess whether lead exposure, assessed as the concentrations in blood, is associated with an increased risk of atherosclerosis in the carotid arteries in a large Swedish population-based cohort.

Methods: We performed a cross-sectional study using data from the population-based Swedish CardioPulmonary bioImage Study (SCAPIS), including 5622 men and women (50-64 years of age), enrolled 2013-2018. Blood lead (B-Pb), measured by inductively coupled plasma mass spectrometry, was used as exposure biomarker (in quartiles). The presence of atherosclerotic plaque in the carotid arteries was determined by ultrasonography. Associations between B-Pb and the risk of plaque was analysed using Poisson regression to estimate prevalence ratios (PR) in models adjusted for age, sex, LDL/HDL ratio, diabetes mellitus, waist circumference and smoking status.

Results: The prevalence of atherosclerotic plaques was 57% and the median B-Pb concentration was 14 µg/L (range: 0.75-203). Individuals in the fourth quartile of B-Pb (Q4) as compared with the first quartile (Q1), had a PR for plaque of 1.08 (95%CI: 1.01, 1.16), which was slightly higher among men (PR 1.11; 95%CI: 1.02, 1.20). PRs were weaker and non-significant among women (PR 1.06; 95%CI: 0.95, 1.17). Among never-smokers,

associations were mostly non-significant, except for women in Q3 (PR 1.29; 95%CI: 1.09, 1.53).

Conclusions: Individuals in the highest quartile of blood lead had a slightly increased risk of carotid plaque in this sample of the general population with lead concentrations comparable to those in other European countries and the U.S. Our study provides some support for the hypothesis that atherosclerosis is one of the mechanisms underlying the association between lead and cardiovascular disease.

Keywords: lead, atherosclerosis, cardiovascular disease, environmental epidemiology

Introduction

Lead

Lead is a metallic element with the symbol Pb, atomic number 82, atomic weight 207.2 and density 11350 kg/m^3 (20 ^{o}C). The melting point and boiling points are 327.5 ^{o}C and 1740 ^{o}C, respectively. The oxidation states are 0, +2 and +4. [1] There are two types of lead: organic and inorganic. In nature, the inorganic forms are the most prevalent. [2]

In total, there is about 1.5 billion tons of lead on Earth. There are approximately 13 grams of lead per ton of mass in the Earth's crust, which makes it the 36th most common element. Lead can be extracted from different lead minerals. The most common mineral is lead sulphide (PbS). Anglesite ($PbSO_4$) and cerussite ($PbCO_3$) are also mined, but to a lesser extent. Lead sulphide often contains silver, silver monosulphide (AgS) and zinc sulphide (ZnS). [1]

Along with gold, silver and copper, lead is one of the first metals used by humans. It was probably known by Egyptians as early as 5000 B.C. Water pipes, cooking pots, coins and weights are examples of products in which lead was utilized in Rome and Greece. Additionally, lead acetate was used as a sweetener during ancient times, which is believed to have been a possible contributing factor to the high prevalence of lead poisoning during this period. [1]

Since electrification and further modernization of society became reality new areas of lead use have been discovered, such as batteries, solders and cable coverings. In the medical field, lead was utilized due to its protective properties in regards to radiation. Also, tetraethyl lead was used in gasoline due to its capacity to reduce engine knocking, boost octane ratings, and help with wear and tear on valve seats within the motor. However, increasing evidence on toxic health effects of lead resulted in a decreased use and ban of lead in gasoline, starting in the 70's. Health risks of lead exposure have also been a concern regarding leaded paints,

which has led to a decreased use of lead in paint over the years in many countries, although in a slower pace. Batteries and power sources used in hospitals, industrial equipment and vehicles, are products where lead is still used. [3]

The global production of lead has varied throughout the years. The yearly production was about 100 000 tons in 1850. By year 1950, it had increased to 1.8 million tons. In 2009, 8.7 million tons were produced. However, 55% of the lead produced in 2009 was obtained through recycling. Since 1979 the production of primary lead has remained on a constant level, but the recycling of lead products has been intensified to meet the increased consumption. [1]

In a global perspective, 75% of lead mining is conducted in Australia, China, USA and Peru, who are the largest producers of lead ore (2009). Lead production is however conducted in many countries. [1]

In Sweden, lead sulphide (PbS) has been mined since the end of the 15th century. Initially, this was because the ores contained silver. The lead production was launched during the 18th century. [1] The yearly lead production in Sweden is about 100 000 tons. A large majority, 75%, is obtained by recycling. In regards to primary lead, the Swedish extraction corresponds to 2% of the global production. Lead mines are located in Zinkgruvan (Askersund), Boliden (Västerbotten) and Garpenberg (Dalarna). Processing of the ores is carried out in Rönnskärsverken in Skelleftehamn. Recycling is conducted at Boliden Bergsöes smelter in Landskrona. [1]

Exposure to Lead

Lead occurs naturally in the environment but also as a result of pollution, mainly due to mining and battery production. [2] The general population is exposed to lead mainly through food, drinking water and smoking. Regarding food, vegetables and cereal products are the

main contributors to lead exposure through diet. A German study [4] using consumption data from approximately 15 000 individuals aged 14-80 years showed that the primary food groups contributing the most to lead exposure were beverages (for example wine, beer and mineral water) followed by vegetables, as a result of substantial consumption volume. Fruits and nuts, cereals and dairy products were also significant contributors. However, meat was top ranked of primary food groups regarding mean concentration of lead. There was also a large intrinsic variation within the meat fraction. For instance, pork had a low lead concentration in comparison to meat from wild duck; the concentrations were 0.038 mg kg^{-1} and 3.18 mg kg^{-1} respectively. [4] Drinking water may also contribute to substantial lead exposure. [2] Although lead concentrations in drinking water vary, a US study examining blood lead levels in over 60,000 children found an association between increased lead levels and lead service lines in the household. [5] Another study reported flushing the water before use, and thereby getting rid of stagnant water, as a way to reduce exposure. [6] As of today, the limit for lead in drinking water in Sweden is 10 µg/L. [7]

Additional sources to lead are contaminated soil, dust and air. [2] It has been reported that coal combustion is a possible contributor to lead exposure, where children come in contact with emitted particles via inhalation and ingestion of soil or dust. [8] However, the exposure to lead has decreased during the last two decades as a result of the ban on lead in gasoline. A study from 2012 investigated the blood lead levels in children in Skåne enrolled in 2009 and 2011. A large decrease was observed in comparison to year 1978: from almost 60 µg/L to 11 µg/L in 2011.[9] In a study measuring blood lead concentrations in about 1000 Swedish adolescents during 2016-17, the geometric mean value of blood lead levels was 7.3 µg/L. [10]

There are several ways of assessing lead exposure. The most common exposure biomarker is the concentrations in whole blood (B-Pb) and represents the exposure during the last 2-3

months but it is also a marker of body burden of lead. Another exposure biomarker for lead is the concentrations in bone, as lead is stored in the skeleton. Lead concentrations in bone decreases at a slower rate than in blood which makes bone lead a more reliable biomarker of cumulative exposure. However, the turnover rate has a certain variation depending on type of bone. Because the largest portion of lead in the body is found in the skeleton, bone lead reflects the total body burden of lead as well. The usual locations to determine lead levels are the patella, tibia, finger bone and calcaneus using X-ray fluorescence. Lead can also be measured in plasma/serum (P-Pb/S-Pb) and P-Pb and B-Pb have shown a curvilinear relation. However, more than 99% of lead in blood is found in erythrocytes and there is limited data regarding evaluation of lead exposure by using P-Pb and S-Pb as biomarkers. There might be certain areas where P-Pb is more useful than B-Pb, for example when studying lead effects on blood formation. This is due to the effect of anaemia on B-Pb measurements, where the values may appear to be falsely low. Finally, lead can be measured in urine (U-Pb) but its utility as exposure biomarker in relation to inorganic lead is limited. U-Pb has been used to estimate the index of body burden of lead and risk when administrating chelating agents. [11]

Health Effects of Lead

Lead is known to cause a large range of negative health effects in adults and children. The target organs and systems include the central and peripheral nervous system, blood-forming organs, kidneys, cardiovascular system, endocrine system, gastrointestinal tract and skeleton. [11]

The gastrointestinal tract is a common location for the manifestations of lead poisoning. Painful convulsions in the abdomen along with extended constipation, digestion difficulties and vomiting belong to the clinical picture. For a period of time, a patient with lead poisoning might be helped by being given intravenous calcium. The explanation is that calcium in

smooth muscle cells is thought to interact with lead and thereby mediate the clinical expression. [11]

The central nervous system has been observed to be affected by lead, where exposure may result in encephalopathy. Convulsions, ataxia and coma are clinical manifestations associated with extensive lead toxicity. [11] Adverse effects have also been seen in cognitive functions. A study conducted on Canadian lead workers found association between neuropsychological performance and time integrated blood lead levels. In contrast to the cumulative levels, the participants' current blood lead levels did not show association to the cognitive performance. [12] A meta-analysis from 2019, examining 22 articles from 1976-2014, concluded that there is association between reduced cognitive ability in adults and lead exposure but further investigation is required. [13] Lead exposure is known to affect the developing central nervous system in children and has been associated with lower IQ. [14] Lead can also affect the peripheral nervous system, where peripheral paralysis ("ankle drop", "wrist drop") is a possible clinical manifestation of heavy exposure. [11]

Lead can also cause anaemia. Lead inflicts disturbances to the synthesis of heme, as well as alpha and beta chains of globulin. In addition to blood production, there are also effects on already existing erythrocytes. A reduced life length of red blood cells has been observed. This is believed to result from effects on cell membrane proteins leading to hemolysis. It has also been suggested that lead disrupts transportation of iron which may leave an adverse impact on the heme metabolism. Furthermore, renal effects are considered possible contributors to anaemia through causing inadequate levels of erythropoietin. [11]

In adults, blood lead levels below 100 µg/L have been associated with reduced estimated glomerular filtration rate (eGFR), lower creatinine clearance and increased risk of chronic kidney disease. [14] Individuals with diabetes and hypertension seem to be more susceptible.

[15, 16] According to the United States National Toxicology Program (NTP), there is sufficient evidence of an impaired renal function in relation to lead exposure even below 50 µg/L. However, it is difficult to draw the same conclusion in regards to children due to insufficient or limited data. [14]

Health Effects of Lead on the Cardiovascular System

Lead exposure has been associated with health effects on the cardiovascular system in different ways. Two important aspects are hypertension and cardiovascular disease. Hypertension or elevated blood pressure is a risk factor for cardiovascular disease. [11, 17] Several studies involving individuals with blood levels below 100 µg/L, have reported an association between bone lead concentrations and hypertension or elevated blood pressure. In pregnant women, blood lead levels below 100 µg/L have been associated with a higher risk of hypertension during pregnancy. [14] Moreover, it is critical to specify that the association with hypertension is stronger to bone lead rather than blood lead [14, 18]. This may imply that the cumulative exposure to lead is of larger importance in this instance. [11, 14] Despite all studies investigating the association between blood pressure and lead, it is still unclear what the underlying mechanisms are. Because of the toxic effects of lead on the kidneys, it is possible that the impairment of the renal function results in hypertension. However, the opposite may also be true; hypertension induces decreased renal function. [14] A review from 2006 concluded that there are enough data to suggest a causal association between lead exposure and hypertension. [19]

Lead exposure has been associated with an increased risk of cardiovascular disease and mortality in several studies. [20-22] Even at blood lead levels below 100 µg/L, lead exposure has been associated with cardiovascular mortality. However, according to The United States

National Toxicology Program (NTP) there is only limited data to support this correlation. [14]

A study including about 14 000 American adults recruited in 1988-1994 showed a hazard ratio of 1.55 (95% CI: 1.08, 2.24) for cardiovascular disease mortality, 1.89 (95% CI: 1.04, 3.43) for myocardial infarction mortality, 2.51 (95% CI: 1.20, 5.26) for stroke mortality and 1.25 (95% CI: 1.04, 1.51) for all-cause mortality among individuals in the highest tertile of exposure (≥36.3 µg/L) compared with the lowest tertile (<19.3 µg/L). [21]

A review article from 2006 concludes that although there is data indicating a causal association between lead exposure and clinical cardiovascular outcomes, there is still not adequate evidence. [19]

A population-based cohort study from 2018 investigated the number of deaths in the United States attributable to environmental lead exposure, using a study population consisting of 14 289 adult participants from the Third National Health and Nutrition Examination Survey (NHANES-III). An increase in blood lead level from 10 to 67 µg/L was associated with a hazard ratio of 1.70 (95% CI: 1.30, 2.22) for cardiovascular disease mortality, 2.08 (95% CI: 1.52, 2.85) for ischemic heart disease mortality and 1.37 (95% CI: 1.17, 1.60) for all-cause mortality and the corresponding attributable fractions were 28.7% (95% CI: 15.5, 39.5), 37.4% (95% CI: 23.4, 48.6) and 18.0 % (95% CI: 10.9, 26.1), respectively. In addition, it has been estimated that lead exposure accounts for approximately 400 000 deaths yearly in the United States. [23] A comparative study from 2016 estimated that 495 000 deaths and 9 287 000 disability-adjusted life-years (DALYs) in 2015 were attributable to lead exposure. In comparison to 2005, the number of deaths and DALYs had increased with 7.3% and 1.1% respectively. [24]

It has been proposed that atherosclerosis may be one of the mechanisms mediating the development of cardiovascular disease resulting from lead exposure. The underlying processes seem to be several, including impaired angiogenesis and endothelial repair. [25] There is some experimental support for this [26, 27]. An experimental study using bovine aortic smooth-muscle cells showed that lead stimulates DNA synthesis and the growth factor bFGF. [26] Another study showed that lead stimulates the activity of interleukin 8 emitted by vascular endothelial cells, resulting in amplified migration of smooth muscle cells and increased width of the intima. [27]

There is some epidemiological evidence for an association between lead exposure and atherosclerosis but results are divergent. A recently published cross-sectional Korean study consisting of 2193 adult men and women recruited during 2011-2018 showed an odds ratio of 1.14 (95% CI: 1.02, 1.26) for moderate-to-severe coronary artery stenosis (MSCAS) for every 10 µg/L increment in blood lead levels. When stratified by sex, men had a 14% increased risk (95% CI: 1.01, 1.28) for MSCAS while the results for women were weaker and non-significant (OR 1.10; 95% CI: 0.86, 1.41). [28] A Swedish cross-sectional study including 4172 middle-aged men and women enrolled 1991-1994 showed an odds ratio of 1.35 (95% CI: 1.09, 1.66) for atherosclerosis in the carotid artery among individuals in the highest quartile of blood lead (median 42 µg/L) compared with the lowest quartile (median 15 µg/L). In models stratified by sex, women had a 58% increased risk of atherosclerosis (95% CI 1.20, 2.08), while the association for men was weaker and non-significant (OR 1.18, 95% CI: 0.83, 1.69). [29] However, another cross-sectional Swedish study involving 1016 subjects aged 70 years found no associations between lead exposure and increased risk of carotid plaque nor with intima-media thickness. [30] In addition, a study using Mendelian randomization involving 13 single nucleotide polymorphisms (SNPs) independently

associated with blood lead, concluded that lead was not associated with coronary artery disease, blood pressure or diabetes. [31]

Aim

To assess whether lead exposure, assessed as the concentrations in blood, is associated with an increased risk of atherosclerosis in the carotid arteries in a large Swedish population-based cohort.

Material and Methods

Study Design and Population

This cross-sectional study used data from the Swedish CardioPulmonary bioImage Study (SCAPIS) cohort, a population-based cohort with the overall aim to improve risk prediction of cardiopulmonary diseases and optimize the ability to study disease mechanisms. The original cohort consisted of 30 154 participants, ranging from 50 to 64 years of age, from six cities in Sweden – Umeå, Uppsala, Stockholm, Linköping, Gothenburg and Malmö. Participants recruited in SCAPIS were chosen at random from the general population. With the purpose of being capable to give informed consent, individuals unable to understand written and spoken Swedish were not included in the study.

The present thesis is based on data from Malmö and Gothenburg only. In these two study sites, participants were consecutively recruited during 2013-2018, in total 5903 individuals. There was a 52% participation rate of those invited. All participants received detailed information about the study and gave their informed consent before the start of the study.

This study was approved by the Ethics Committee in Gothenburg (2013.05-05, 2013-70-32M).

Outcome Measurements

A Siemens Acuson S2000 ultrasound scanner provided with a 9L4 linear transducer (both from Siemens, Forchheim, Germany) was utilized to assess atherosclerotic carotid plaque according to standard practice, resulting in a two-dimensional greyscale image. The images were further examined in order to evaluate a number of vascular characteristics: size and number of plaques in left and right carotid artery, intima-media thickness (IMT), echogenicity of plaques as well as heterogeneity and homogeneity of plaque formations [32, 33]. The definition of plaque used in this study is as set by the Mannheim consensus: Focal

structures encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or demonstrates a thickness >1.5 mm as measured from the intima-lumen interface to the media-adventitia interface. [34]

For the purpose of this thesis, we used the presence of plaque (yes/no) in either one or both carotid arteries as outcome.

Cardiovascular Risk Factors and Confounders

The study participants underwent thorough clinical examinations during two or three days within a 2-week period, including a questionnaire on medical history, heredity for cardiovascular diseases, medication (including lipid-lowering treatment and antihypertensive treatment), occupational and environmental exposures, lifestyle (including smoking habits), socio-economic status, educational level, level of physical activity and country of birth [35].

Heredity for stroke or acute myocardial infarction was considered to be present if a parent had been affected <65 years of age or <60 years of age respectively. To obtain the number of individuals with lipid-lowering treatment, the participants were asked to list all medications they used continually. Afterwards, medical staff evaluated the lists in regards to lipid-lowering treatments. Education level was categorized as low if the educational timeframe was shorter than nine years or consisted solely of primary school. Low physical activity was defined as having less than two hours of physical activity per week. Country of birth was categorized as being born in or outside Sweden.

Smoking was classified in three categories – current, former and never smokers. “Do you smoke?” was the phrasing of the question in the survey and had five answer options – “Yes, I smoke regularly”; “Yes, I smoke sometimes”; “No, I stopped smoking”; “No, I have never smoked”; “I don’t want to/cannot answer”. Participants were defined as current smokers if they answered either of the first two options (“regularly” or “sometimes”). Timeframe in

regards of smoking was not taken into consideration for this classification. If the respondents answered the third option ("stopped smoking") they were categorized as former smokers. Information on pack-years was also collected through the questionnaire.

Blood pressure was measured using an automatic blood pressure monitor (Omron M10-IT, Omron Health care Co, Kyoto, Japan). The pressure in each arm was measured twice, resulting in a total of four measurements, from which the mean value was determined the final result. The examination was conducted with the participants in supine position. Prior to the measurement they had five minutes of rest.

Presence of hypertension (yes/no) was established if at least one of three conditions were fulfilled: systolic blood pressure >140 mmHg alternatively diastolic blood pressure >90 mmHg measured at physical examination, or stating to have hypertension diagnosed by a physician in the survey forms.

During physical examination data about waist circumference, body weight and height was collected.

Prevalence of diabetes mellitus (no/yes) was determined using three methods. A previously known diagnosis of diabetes mellitus could be confirmed by participants in survey forms and interviews. Information concerning possible new diagnoses was assembled by analysing fasting blood glucose and HbA1c collected from blood samples. A diagnosis was set if p-glucose ≥7 mmol/L or HbA1c ≥48 mmol/mol.

A venous blood sample (100 mL) was collected from participants after an overnight fast for immediate analysis of plasma glucose, HbA1c, high-sensitivity C-reactive protein (CRP) as well as low-density cholesterol (LDL) and high-density cholesterol (HDL), from which LDL/HDL ratio was calculated.

Blood Lead Analysis

At the same time as the clinical examinations, whole blood samples were collected in a metal free tube for metal analysis. An alkaline solution was used to dilute the blood samples 20 times before they were examined. Lead concentrations in blood were measured by coupled plasma mass spectrometry with kinetic energy discrimination provided by a collision cell. Helium was utilized as collision gas. Both analytical accuracy and inter-laboratory comparison displayed good agreement. In terms of testing the analytical accuracy, certified reference materials were used – Seronorm Trace elements whole blood L-1 and L-2 (SERO AS, Billingstad, Norway). Cadmium in blood was also measured because it has been shown to be associated with atherosclerosis in several studies.

Ethics

All data was already collected and coded before the start of this project and has been handled according to the GDPR (General Data Protection Regulation). All participants received detailed information about the study and gave their informed consent before the start of the study. The Declaration of Helsinki has been followed.

During this project the data has been stored in a password protected computer owned by the University of Gothenburg. The data is presented on population level.

This study was approved by the Ethics Committee in Gothenburg (2013.05-05, 2013-70-32M).

Statistical Methods

Stata (version 16.1; StataCorp) was used to perform the statistical analyses. Bivariate analyses were conducted to examine the association between blood lead quartiles (Q1-Q4) and plaque occurrence with known risk factors for cardiovascular disease and potential confounders. Differences among quartiles of blood lead was evaluated using the Kruskal Wallis H test for continuous variables while chi square test was used for categorical variables. For continuous variables following normal distribution average values and standard deviations were calculated while median (range) was reported for asymmetric or skewed variables. Categorical variables were reported as specified in absolute numbers and percentages (%). A p-value <0.05 was considered statistically significant.

Poisson regression was performed to estimate prevalence ratios (PR) and 95% confidence intervals (CI) for plaque occurrence in relation to blood lead quartiles. Two models were performed: Model 1 was adjusted for sex (male/female) and age (years) and the final model, Model 2, was additionally adjusted for cardiovascular risk factors associated with both blood lead concentration and plaque occurrence in this study population and included LDL/HDL ratio, diabetes mellitus (yes/no), waist circumference (cm) and smoking status (never/former/current). We additionally performed a fully-adjusted model (Model 2) estimating PRs and 95% CI for occurrence of unilateral and bilateral plaque separately in relation to blood lead quartiles.

Several sensitivity analyses were performed. Analyses were performed among never smokers and were adjusted for the same variables as in Model 2. Also, blood lead was used as continuous variable instead of a categorical (quartiles) and analyses were also limited to individuals with B-Pb <100 µg/L in order to assess whether associations were driven by extreme values. In addition, plaque occurrence was assessed in relation to blood lead

quartiles in models adjusted for the same variables as in Model 2, and additionally for other well-known cardiovascular risk factors (brachial diastolic blood pressure, physical activity, education level, nationality, heredity for acute myocardial infarction/stroke and logCRP) that were not associated with blood lead or plaque occurrence in the bivariate analyses and also for cadmium. Finally, odds ratios were calculated for comparison.

Results

Characteristics of the Study Cohort

Characteristics of the population for all individuals as well as in relation to blood lead quartiles (Q1-Q4) are displayed in table 1. The median blood lead level was 14 µg/L. Plaque was more frequent in higher quartiles in comparison to lower, ranging from 53% in Q1 to 61% in Q4. When categorized by plaque location, a progressive increase was seen regarding bilateral plaque with 30% of individuals with bilateral plaque in Q4 compared with 25% in Q1. Blood cadmium levels and number of pack years increased in a similar pattern. For women, blood lead levels were higher among those in the postmenopausal group. Overall, the mean age was 57 years. There was a slight difference in mean age across quartiles, where the participants in the higher quartiles were on average one year older. Body mass index, waist circumference and LDL/HDL ratio were higher in Q1 than in remaining quartiles. Diabetes mellitus was least prevalent in Q1 but similar in Q2-Q4. The proportion of participants born outside Sweden was slightly larger in Q4 in comparison to Q1.

Characteristics of the population in relation to plaque occurrence are displayed in table S1. Presence of plaque was associated with male sex, higher blood lead concentration, age, blood cadmium, number of pack years, LDL/HDL ratio, systolic and diastolic blood pressure. Plaque prevalence was also higher among postmenopausal women as well as in participants with hypertension, diabetes mellitus or low education level.

Association Between Blood Lead Concentrations and Plaque

Table 2 shows the prevalence ratios for atherosclerotic plaque in relation to blood lead quartiles among all individuals as well as by sex. In model 1, adjusted for age and sex, individuals in Q4 had a 10% increased PR (95% CI: 1.03, 1.17) compared to individuals in Q1. In the models stratified by sex, men in Q4 showed a PR of 1.12 (95% CI: 1.03, 1.21)

compared to men in Q1. Women in Q4, however, showed a weaker and non-significant estimate. Interestingly, women in Q3 showed a PR of 1.12 (95% CI: 1.01, 1.24) compared to women in Q1. The fully adjusted Model 2 showed similar results with a PR of 1.08 (95% CI: 1.01, 1.16) for individuals in Q4 vs. Q1, as well as for men in Q4 vs Q1 (PR: 1.11, 95% CI: 1.02, 1.20) but weaker and non-significant results for women in Q4 vs Q1 (PR: 1.06, 95% CI: 0.95, 1.17).

Table 3 shows prevalence ratios for unilateral and bilateral plaques in relation to blood lead quartiles. Statistically significant results were only observed in regards to unilateral plaque. The PR for unilateral plaque when considering all individuals was 1.15 (95% CI: 1.03, 1.28) and for men 1.22 (95% CI: 1.05, 1.42) when comparing Q4 with Q1. A weaker and non-significant result was seen for women in Q4 compared with those in Q1 (PR: 1.09, 95% CI: 0.93, 1.27). PRs for bilateral plaque were weaker and did not reach significance.

Table 4 shows the results among never-smokers and except for a statistically significant PR for women in Q3 vs Q1 (PR: 1.29, 95% CI: 1.09, 1.53), no associations were found.

Subgroup and Sensitivity Analyses

When using blood lead as continuous variable, a 3% increased risk was found for every 10 µg/L increase in blood lead concentrations (95% CI: 1.02, 1.05) and associations for men were also significant (PR: 1.04, 95% CI: 1.02, 1.05) (Table 5). Associations for women remained non-significant. After excluding the participants with relatively extreme blood lead concentrations (B-Pb ≥100) the confidence intervals widened but PRs remained essentially the same.

PRs were similar even after further adjustment of model 2 for variables that were not associated with plaque or blood lead but that are well-known cardiovascular risk factors (brachial diastolic blood pressure, physical activity, education level, nationality, heredity for

acute myocardial infarction/stroke and logCRP) (Table S2). Prevalence ratios for individuals in Q4 vs Q1 were 1.09 (95% CI: 1.02, 1.16) when considering all individuals. Prevalence ratios for men in Q4 vs Q1 were 1.10 (95% CI: 1.01, 1.20) while they were non-significant for women (PR: 1.07, 95% CI: 0.96, 1.19). Estimates remained essentially unchanged after further adjustment for blood cadmium (Table S2).

Tables S3-S6 shows odds ratios for comparison. In all the different models, ORs were generally 2 to 3-fold higher than the estimated prevalence ratios in the previous models. For example, in Table S3, individuals in Q4 showed an OR of 1.20 (95% CI: 1.03, 1.41) compared with Q1. For men in Q4 vs Q1, ORs were 1.34 (95% CI: 1.05, 1.69). Associations among non-smokers remained non-significant when estimating ORs (Table S4).

Discussion with Conclusions and Implications

This study provides some support for the hypothesis that atherosclerosis may be one of the main mechanisms underlying the association between lead exposure and cardiovascular disease. After adjusting for known risk factors and confounders, blood lead in the highest quartile (median: 25 µg/L) was associated with a slightly increased risk of carotid plaque (8%) compared with the lowest quartile (median: 8.4 µg/L). Associations were stronger among men and non-significant among women. No associations were found among never smokers. To note, this study is a sample of the general population with lead concentrations comparable to those in other European countries and the United States.

The association between blood lead concentration and risk of plaque in the carotid arteries found in this study was consistent even after adjustment for several known risk factors and confounders. However, the estimated risk appears to be quite modest. The degree of relevance on an individual level is thus debatable but the relevance at population level remains.

When stratifying the models by sex, the associations remained significant for men in Q4 vs Q1 but no associations were found for women in Q4 vs Q1. However, women in Q3 showed statistically significant PRs compared with Q1 (PR 1.12, 95% CI 1.01, 1.24). This was also the case among female non-smokers. The reason behind this finding is unclear. When examining the characteristics of the female study population across quartiles, variables such as age, body mass index, waist circumference, smoking, pack years, diabetes mellitus and LDL/HDL ratio were similar across quartiles. In a previous Swedish study using data from the well-known Malmö Cancer and Diet study, associations between blood lead and carotid plaque were observed mainly among women and this was particularly restricted to postmenopausal women [29]. In the present study, we did not examine the associations

among post- and premenopausal women, however, the prevalence of postmenopausal women was not different among women with or without plaque. Although the increased risks found among women in Q3 might be caused by other potential factors, which could have been different among women in the different quartiles but were not available when this study was performed, these findings may well be due to chance.

Cadmium exposure has previously been suggested to increase the risk of atherosclerosis [36]. When additional adjustments were made for other well-known cardiovascular risk factors, and also for cadmium, PRs remained essentially the same [PR: 1.08; 95% CI: 1.01, 1.15].

When performing separate analyses to estimate the risk for unilateral and bilateral plaques, statistically significant increased risks were only seen for unilateral plaques while PRs for bilateral plaques were weaker and non-significant. Previous studies have only measured the presence of atherosclerotic plaque in one side so this is the first study to assess the occurrence of plaque in both carotid arteries. However, our findings were unexpected as we hypothesised that there would be a gradual increment in the risk of plaque and thus, a higher risk of developing plaques in both carotid arteries rather than only in one. The reason for finding the opposite might be that other well-known cardiovascular risk factors are of greater importance for the development of plaque and, thus, a greater plaque burden, than lead exposure.

In general, the results act in accordance with those from several other studies investigating environmental lead exposure and atherosclerosis [28, 29], giving support for the hypothesis that atherosclerosis is one of the mechanisms underlying the association between lead and cardiovascular disease. Lead exposure has been reported to be associated with cardiovascular disease [20-23] even at blood lead levels below 100 µg/L [14, 21, 23]. Additionally, associations to peripheral artery disease [37, 38] and hypertension [14, 18] have been observed. It has been estimated that 495 000 deaths and 9 287 000 disability-adjusted life-

years (DALYs) in 2015 were attributable to lead exposure [24]. Another study concluded that lead exposure is responsible for around 400 000 deaths yearly in the United States [23].

A number of mechanisms through which lead affects the vascular system have been suggested. These mechanisms include stimulation of inflammation and oxidative stress, disturbing nitric oxide signalling and availability, interference with vascular smooth muscle signalling, alteration of levels of prostaglandins regulating vasoconstriction and vasodilation as well as disruption of endothelial healing and induction of lesions in the endothelium. [25] An article from 1995 reported that a stepwise increase of lead concentration (0.5-10 μM) resulted in a progressive increase of [^{3}H]thymidine incorporation in cultured bovine aortic smooth muscle cells, thus suggesting that lead stimulates DNA synthesis and proliferation in cultured vascular smooth muscle cells. [26] A study from 2010 observed an amplified migration of smooth muscle cells and increased intimal width consequently to lead-induced elevation of activity of interleukin 8 emitted by vascular endothelial cells. [27]

Despite the number of publications indicating lead-induced effects on the cardiovascular system, some articles report diverging results. A cross-sectional study from 2012 examined prevalence of carotid plaque in 1016 70-year old men and women and did not find association between blood lead concentration and plaque in the carotid artery. [30] A meta-analysis from 2019 used genetic instruments (Mendelian randomization) to investigate the relationship between blood lead and coronary artery disease, blood pressure and diabetes, but concluded that lead did not have association with these pathologies. [31] In a prospective study from 1992 including 1000 men and women that were followed up 11 and 5 years after baseline no associations were found. [39]

There are multiple strengths in this study. The ultrasonography used to evaluate presence of atherosclerotic plaque was performed using a standardized method with a highly-sensitive

ultrasonography. Regarding blood lead analyses, both analytical accuracy and inter-laboratory comparison displayed good agreement. The large study population contributes to an increased power in regards to results. Due to the high prevalence of atherosclerotic plaque, the use of Poisson regression to estimate prevalence ratios for carotid plaque allowed more accurate and precise estimates compared with logistic regression. However, the results calculated with logistic regression may facilitate comparability with other studies. Availability of population data on cardiovascular risk factors and confounders allowed adequate adjustments of the models, which contributed to reliable risk estimates.

There are also several weaknesses and limitations in this study. Firstly, this is an observational cross-sectional study. Therefore, it is not possible to draw conclusions about causality between lead exposure and atherosclerosis. Prospective studies are needed to further investigate potential causality. The necessity of supplementary prospective studies exploring the potential causal association between lead exposure and clinical cardiovascular outcomes has been reported previously as well [19]. Secondly, only a binary classification (yes/no) of carotid plaque prevalence was used in the models. Additional analyses using number of plaques, plaque area and degree of stenosis would provide greater insight into the relationship between lead exposure and carotid artery atherosclerosis. It should also be noted that plaque prevalence was only measured in the carotid arteries. Although this may be a proxy of atherosclerosis in the whole body, examination of the presence of plaque in other parts of the vascular tree would have been relevant, such as in the coronary arteries, due to the observed association between blood lead and ischemic heart disease mortality [23].

Conclusions and Implications

In summary, this study give some support for the hypothesis that atherosclerosis is one of the mechanisms underlying the association between lead and cardiovascular disease. Cardiovascular disease is a leading cause of death globally and in Sweden. Many people are exposed to lead through food and drinking water to a degree associated with increased cardiovascular mortality. Prevention of this exposure and morbidity is possible, for example by instituting low exposure limits and decreasing environmental contamination. This project aims to contribute with knowledge to future risk assessments in regards to lead toxicity and is therefore of significance to the matter of public health.

Populärvetenskaplig sammanfattning på svenska

Titel: Miljöexponering för bly och risk för ateroskleros
Examensarbete, läkarprogrammet
Författare: Carl Guldbrand
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2021

Exponering för bly har visats ha samband med ökad risk för hjärt-kärlsjukdom. En möjlig underliggande mekanism är åderförkalkning. En tidigare studie, som undersökte drygt 4000 män och kvinnor, visade att individer med högst blyhalter i blodet hade 35% större risk att ha förträngningar i halskärlen, i jämförelse med dem med lägst halter. Detta nya projekt syftar till att undersöka om sambandet mellan blynivåer i blod och förträngningar i halskärlen kan bekräftas i en studiepopulation med lägre blod-blyhalter jämfört med den tidigare studien.

I denna nya undersökning användes ett urval av personer från projektet Swedish CardioPulmonary bioImage Study (SCAPIS), bestående av 5622 medelålders män och kvinnor som rekryterats 2013-2018. Blyhalter i blodet mättes med hjälp av så kallad masspektrometri, ett instrument med hög känslighet för att mäta spårelement. Ultraljud användes för att undersöka förträngningar i halskärlen. Därefter användes biostatistiska analysmodeller för att uppskatta risken för förträngningar i halskärlen vid blyexponering, där hänsyn togs till andra faktorer såsom ålder, kön, blodfetter, diabetes, midjeomfång och rökvanor.

Resultatet visade att förträngningar i halskärlen (”plack”) förekom hos 57% av alla studiedeltagare. De med högst blyhalter i blodet hade 8% större risk för plack i halskärlen, i jämförelse med dem med lägst halter. Delades deltagarna in efter kön, hade män med högt blodbly 11% ökad risk och kvinnor 8% ökad risk. Resultatet för kvinnor var dock inte statistiskt säkerställt. Vi fann inga samband hos icke-rökare.

Slutsatsen är att höga blynivåer i blodet var associerade med en något ökad risk för förträngningar i halskärlen i denna studiepopulation. Därmed ger denna studie visst stöd för hypotesen att åderförkalkning är en av de mekanismer som förmedlar kopplingen mellan blyexponering och hjärt-och kärlsjukdom. Resultatet från detta projekt bidrar till kunskapsunderlaget för framtida riskbedömningar gällande blys hälsoeffekter.

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Tables, Figures and Appendices

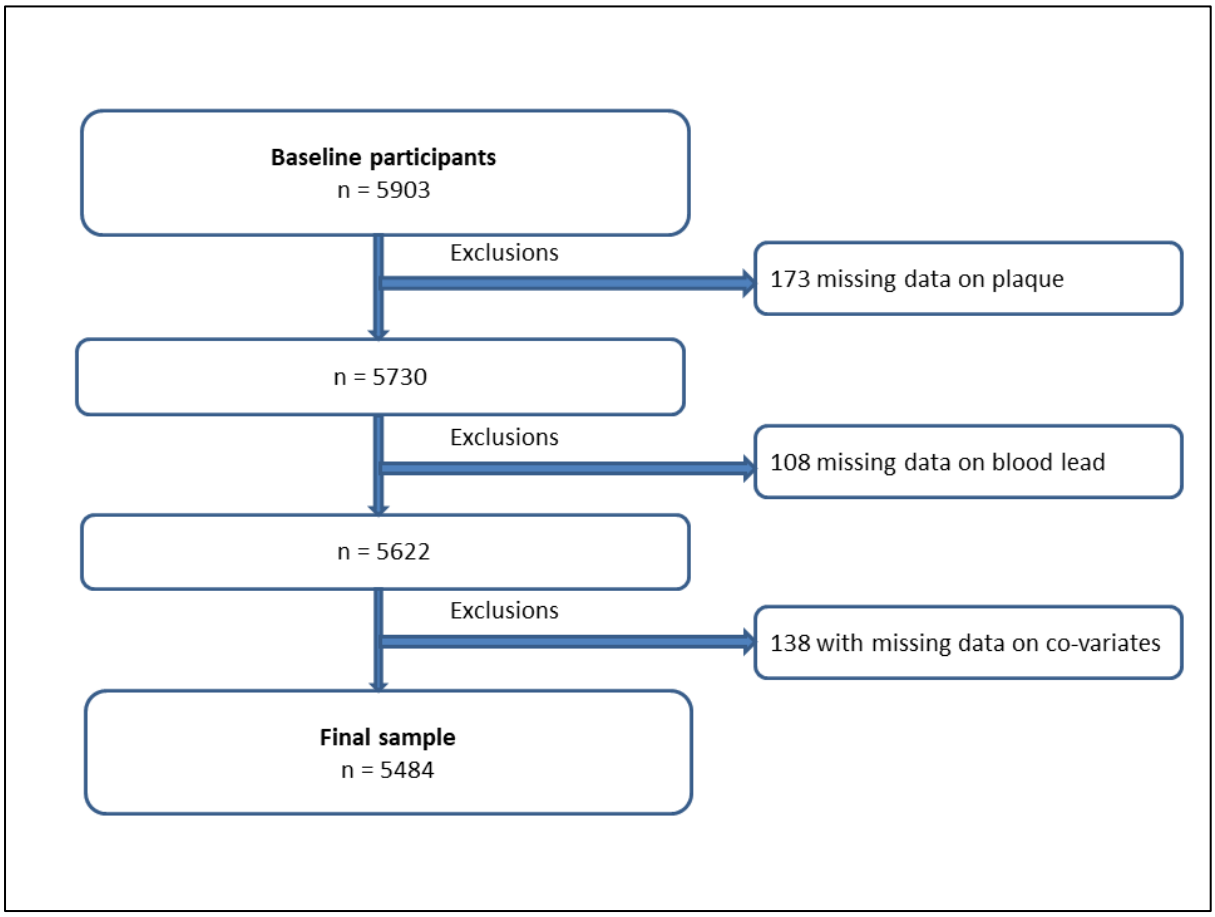

Figure 1. Diagram of the study population in the Swedish CardioPulmonary bioImage Study (SCAPIS) cohort.

Table 1. Characteristics of the study subjects in the Swedish CardioPulmonary bioImage Study (SCAPIS) cohort on all individuals as well as by quartiles of blood lead.

	Overall	**Q1**	**Q2**	**Q3**	**Q4**	
Characteristics	n=5622	n=1404	n=1406	n=1406	n=1406	**p-Value**[a]
Plaque occurrence in carotid artery						<0.001
No	2393 (43)	664 (47)	616 (44)	563 (40)	550 (39)	
Yes	3229 (57)	740 (53)	790 (56)	843 (60)	856 (61)	
Plaque occurrence categorized						<0.001
Neither side	2393 (43)	664 (47)	616 (44)	563 (40)	550 (39)	
One side	1728 (31)	388 (28)	443 (32)	457 (33)	440 (31)	
Both sides	1501 (27)	352 (25)	347 (25)	386 (27)	416 (30)	
Blood lead (µg/L)	14 (0.75-203)	8.4 (0.75-11)	12 (11-14)	17 (14-20)	25 (20-203)	<0.001
Age (y)	57 ± 4.3	57 ± 4.4	57 ± 4.2	58 ± 4.2	58 ± 4.2	<0.001
Sex						0.12
Male	2731 (49)	645 (46)	692 (49)	707 (50)	687 (49)	
Female	2891 (51)	759 (54)	714 (51)	699 (50)	719 (51)	
Menopausal status						<0.001
Premenopausal	834 (29)	276 (36)	204 (29)	184 (26)	170 (24)	
Postmenopausal	2057 (71)	483 (64)	510 (71)	515 (74)	549 (76)	
Blood cadmium (µg/L)	0.24 (0.0098-8.5)	0.21 (0.0098-4.3)	0.22 (0.055-7.3)	0.25 (0.037-4.4)	0.29 (0.047-8.5)	<0.001
Low education level						0.66
No	4964 (90)	1251 (91)	1253 (91)	1237 (90)	1223 (90)	
Yes	526 (10)	123 (9)	130 (9)	132 (10)	141 (10)	
Low physical activity						0.20
No	4785 (88)	1172 (87)	1206 (89)	1214 (90)	1193 (89)	
Yes	622 (12)	175 (13)	152 (11)	141 (10)	154 (11)	
Body mass index (mg/m2)	27 ± 4.4	28 ± 4.8	27 ± 4.4	27 ± 4.2	27 ± 4.0	<0.001
Waist circumference (cm)	94 ± 13	95 ± 13	94 ± 13	94 ± 12	93 ± 12	0.0087
Smoking status[b]						<0.001
Never-smokers	2517 (45)	731 (52)	666 (47)	623 (44)	497 (35)	
Former smokers	2100 (37)	489 (35)	518 (37)	509 (36)	584 (42)	
Current smokers	881 (16)	159 (11)	188 (13)	240 (17)	294 (21)	
Pack years[c]	13.5 (0.05-104)	11.6 (0.1-82)	13.0 (0.05-72)	14.4 (0.1-78)	15.0 (0.1-104)	0.019
Hypertension						0.84
No	4320 (79)	1087 (79)	1089 (80)	1086 (79)	1058 (78)	
Yes	1130 (21)	281 (21)	276 (20)	282 (21)	291 (22)	
Systolic blood pressure (mmHg)	122 ± 16	121 ± 16	121 ± 16	122 ± 17	122 ± 17	0.15
Diastolic blood pressure (mmHg)	73 ± 10	73 ± 10	72 ± 9.8	73 ± 10	73 ± 10	0.066
Diabetes mellitus						<0.001
No	5247 (93)	1274 (91)	1327 (94)	1322 (94)	1324 (94)	
Yes	375 (7)	130 (9)	79 (6)	84 (6)	82 (6)	

LDL/HDL ratio	2.4 ± 1.1	2.5 ± 1.0	2.4 ± 1.0	2.4 ± 1.1	2.4 ± 1.0	<0.001
hsCRP (mg/L)	1.0 (0.15-83)	1.0 (0.15-83)	0.96 (0.15-47)	0.98 (0.15-63)	1.1 (0.15-83)	0.0098
Nationality (born in Sweden)						0.038
No	1144 (21)	281 (20)	258 (19)	287 (21)	318 (23)	
Yes	4380 (79)	1103 (80)	1124 (81)	1094 (79)	1059 (77)	
Heredity for AMI and stroke						0.18
No	4909 (89)	1212 (88)	1233 (89)	1244 (90)	1220 (89)	
Yes	592 (11)	167 (12)	145 (11)	131 (10)	149 (11)	
HRT						0.85
No	2062 (73)	540 (73)	514 (74)	504 (74)	504 (72)	
Yes	745 (27)	196 (27)	178 (26)	178 (26)	193 (28)	

[a]P-values were obtained using Kruskal Wallis H test for continuous variables and chi-squared test for categorical variables.

[b]There was missing data for smokers (n=124).

[c]Pack-year was calculated only among former or current smokers (n=2981).

Table 2. Prevalence ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (in quartiles) in SCAPIS, in all individuals as well as stratified by sex.

	Model 1[a]			**Model 2**[b]		
Quartiles of blood lead [range (µg/L)]	**With plaque [n (%)]**	**Without plaque [n (%)]**	**PR (95% CI)**	**With plaque [n (%)]**	**Without plaque [n (%)]**	**PR (95% CI)**
All						
Q1 (0.75-11)	740 (53)	664 (47)	Ref	721 (52)	653 (48)	Ref
Q2 (11-14)	790 (56)	616 (44)	1.04 (0.97, 1.11)	767 (56)	603 (44)	1.04 (0.97, 1.11)
Q3 (14-20)	843 (60)	563 (40)	1.10 (1.03, 1.17)	821 (60)	551 (40)	1.09 (1.02, 1.17)
Q4 (20-203)	856 (61)	550 (39)	1.10 (1.03, 1.17)	831 (61)	540 (39)	1.08 (1.01, 1.16)
Men						
Q1 (1.1-11)	384 (60)	261 (40)	Ref	374 (59)	256 (41)	Ref
Q2 (11-14)	436 (63)	256 (37)	1.04 (0.96, 1.14)	422 (63)	251 (37)	1.04 (0.96, 1.14)
Q3 (14-20)	464 (66)	243 (34)	1.08 (0.99, 1.17)	454 (66)	238 (34)	1.08 (0.99, 1.17)
Q4 (20-178)	473 (69)	214 (31)	1.12 (1.03, 1.21)	455 (69)	208 (31)	1.11 (1.02, 1.20)
Women						
Q1 (0.75-11)	356 (47)	403 (53)	Ref	347 (47)	397 (53)	Ref
Q2 (11-14)	354 (50)	360 (50)	1.04 (0.93, 1.15)	345 (49)	352 (51)	1.04 (0.93, 1.15)
Q3 (14-20)	379 (54)	320 (46)	1.12 (1.01, 1.24)	367 (54)	313 (46)	1.12 (1.01, 1.24)
Q4 (20-203)	383 (53)	336 (47)	1.08 (0.97, 1.19)	376 (53)	332 (47)	1.06 (0.95, 1.17)

[a]Model 1 was adjusted for age and sex.
[b]Model 2 was additionally adjusted for LDL/HDL ratio, diabetes mellitus, waist circumference and smoking status.

Table 3. Prevalence ratios (95% confidence intervals) for unilateral and bilateral plaques in the carotid arteries in relation to blood lead concentrations (in quartiles) in SCAPIS, in all individuals as well as stratified by sex.

Quartiles of blood lead [range (µg/L)]	**Without plaque [n (%)]**	**With unilateral plaques [n (%)]**	**PR (95% CI)**	**With bilateral plaques [n (%)]**	**PR (95% CI)**
All					
Q1 (0.75-11)	653 (48)	375 (27)	Ref	346 (25)	Ref
Q2 (11-14)	603 (44)	428 (31)	1.10 (0.99, 1.22)	339 (25)	1.02 (0.91, 1.14)
Q3 (14-20)	551 (40)	443 (32)	1.18 (1.06, 1.31)	378 (28)	1.07 (0.96, 1.20)
Q4 (20-203)	540 (39)	429 (31)	1.15 (1.03, 1.28)	402 (29)	1.09 (0.98, 1.21)
Men					
Q1 (1.1-11)	256 (41)	173 (27)	Ref	201 (32)	Ref
Q2 (11-14)	251 (37)	227 (34)	1.16 (1.00, 1.35)	195 (29)	0.99 (0.85, 1.14)
Q3 (14-20)	238 (34)	225 (33)	1.19 (1.02, 1.38)	229 (33)	1.05 (0.92, 1.20)
Q4 (20-178)	208 (31)	210 (32)	1.22 (1.05, 1.42)	245 (37)	1.13 (0.99, 1.29)
Women					
Q1 (0.75-11)	397 (53)	202 (27)	Ref	145 (19)	Ref
Q2 (11-14)	352 (51)	201 (29)	1.04 (0.89, 1.22)	144 (21)	1.05 (0.87, 1.27)
Q3 (14-20)	313 (46)	218 (32)	1.16 (1.00, 1.36)	149 (22)	1.10 (0.91, 1.33)
Q4 (20-203)	332 (47)	219 (31)	1.09 (0.93, 1.27)	157 (22)	1.02 (0.85, 1.23)

Adjusted for age, sex, LDL/HDL ratio, diabetes mellitus, waist circumference and smoking status.

Table 4. Prevalence ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (in quartiles) in SCAPIS, among non-smokers and stratified by sex.

Quartiles of blood lead [range (µg/L)]	**With plaque [n (%)]**	**Without plaque [n (%)]**	**PR (95% CI)**
All			
Q1 (0.75-11)	364 (50)	365 (50)	Ref
Q2 (11-14)	346 (52)	320 (48)	1.02 (0.93, 1.13)
Q3 (14-20)	353 (57)	270 (43)	1.11 (1.00, 1.22)
Q4 (20-203)	268 (54)	226 (46)	1.05 (0.94, 1.16)
Men			
Q1 (1.1-11)	218 (60)	144 (40)	Ref
Q2 (11-14)	209 (60)	142 (40)	0.98 (0.87, 1.10)
Q3 (14-20)	201 (61)	130 (39)	1.00 (0.89, 1.13)
Q4 (20-178)	154 (63)	91 (37)	1.01 (0.89, 1.14)
Women			
Q1 (0.75-11)	146 (40)	221 (60)	Ref
Q2 (11-14)	137 (43)	178 (57)	1.09 (0.92, 1.30)
Q3 (14-20)	152 (52)	140 (48)	1.29 (1.09, 1.53)
Q4 (20-203)	114 (46)	135 (54)	1.10 (0.91, 1.32)

Adjusted for age, sex, LDL/HDL ratio, diabetes mellitus and waist circumference in never smokers.

Table 5. Prevalence ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (as continuous variable, B-Pb) in SCAPIS, in all individuals and among those with B-Pb <100 µ/L as well as stratified by sex.

	All individuals			**Individuals with B-Pb <100 µg/L**		
	With plaque [n (%)]	**Without plaque [n (%)]**	**PR (95% CI)**	**With plaque [n (%)]**	**Without plaque [n (%)]**	**PR (95% CI)**
All	3140 (57)	2347 (43)	1.03 (1.02, 1.05)	3133 (57)	2346 (43)	1.03 (1.01, 1.06)
Men	1705 (64)	953 (36)	1.04 (1.02, 1.05)	1699 (64)	952 (36)	1.04 (1.02, 1.07)
Women	1435 (51)	1394 (49)	1.03 (0.99, 1.06)	1434 (51)	1394 (49)	1.03 (0.99, 1.07)

Prevalence ratios (PRs) are displayed in the context of 10 µg/L advancements of blood lead concentration.
Adjusted for age, sex, LDL/HDL ratio, diabetes mellitus, waist circumference and smoking status.

Supplemental Tables

Table S1. Characteristics of the study population in the Swedish CardioPulmonary bioImage Study (SCAPIS) cohort by prevalence of plaque (yes/no).

	Overall	**No plaque**	**Plaque**	
Characteristics	n=5622	n=2393	n=3229	**p-Value**[a]
Plaque occurrence categorized				<0.001
Neither side	2393 (43)	2393 (100)	0 (0)	
One side	1728 (31)	0 (0)	1728 (54)	
Both sides	1501 (27)	0 (0)	1501 (46)	
Blood lead (µg/L)	14 (0.75-203)	14 (2.8-116)	15 (0.75-203)	<0.001
Blood lead quartile				<0.001
Q1	1404 (25)	664 (28)	740 (23)	
Q2	1406 (25)	616 (26)	790 (24)	
Q3	1406 (25)	563 (24)	843 (26)	
Q4	1406 (25)	550 (23)	856 (27)	
Age (y)	57 ± 4.3	57 ± 4.2	58 ± 4.2	<0.001
Sex				<0.001
Male	2731 (49)	974 (41)	1757 (54)	
Female	2891 (51)	1419 (59)	1472 (46)	
Menopausal status				0.001
Premenopausal	834 (29)	451 (32)	383 (26)	
Postmenopausal	2057 (71)	968 (68)	1089 (74)	
Blood cadmium (µg/L)	0.24 (0.0098-8.5)	0.23 (0.043-4.4)	0.25 (0.0098-8.5)	<0.001
Low education level				<0.001
No	4964 (90)	2161 (92)	2803 (89)	
Yes	526 (10)	182 (8)	344 (11)	
Low physical activity				0.28
No	4785 (88)	2038 (88)	2747 (89)	
Yes	622 (12)	279 (12)	343 (11)	
Body mass index (mg/m2)	27 ± 4.4	27 ± 4.5	27 ± 4.3	0.14
Waist circumference (cm)	94 ± 13	93 ± 13	95 ± 13	<0.001
Smoking status[b]				<0.001
Never-smokers	2517 (45)	1186 (50)	1331 (41)	
Former smokers	2100 (37)	858 (36)	1242 (38)	
Current smokers	881 (16)	309 (13)	572 (18)	
Pack years[c]	13.5 (0.05-104)	11.4 (0.05-88)	15.2 (0.1-104)	<0.001
Hypertension				<0.001
No	4320 (79)	1958 (84)	2362 (76)	
Yes	1130 (21)	369 (16)	761 (24)	
Systolic blood pressure (mmHg)	122 ± 16	119 ± 16	124 ± 16	<0.001
Diastolic blood pressure (mmHg)	73 ± 10	72 ± 10	74 ± 10	<0.001
Diabetes mellitus				<0.001
No	5247 (93)	2266 (95)	2981 (92)	

Yes	375 (7)	127 (5)	248 (8)	
LDL/HDL ratio	2.4 ± 1.0	2.3 ± 1.0	2.5 ± 1.1	<0.001
hsCRP (mg/L)	1.0 (0.15-83)	1.0 (0.15-47)	1.0 (0.15-83)	0.26
Nationality (born in Sweden)				0.27
No	1144 (21)	472 (20)	672 (21)	
Yes	4380 (79)	1886 (80)	2494 (79)	
Heredity for AMI and stroke				0.031
No	4909 (89)	2118 (90)	2791 (88)	
Yes	592 (11)	228 (10)	364 (12)	
HRT				0.30
No	2062 (73)	1023 (74)	1039 (73)	
Yes	745 (27)	353 (26)	392 (27)	

[a]P-values were obtained using Kruskal Wallis H test for continuous variables and chi-squared test for categorical variables.

[b]There was missing data for smokers (n=124).

[c]Pack-year was calculated only among former or current smokers (n=2983).

Table S2. Prevalence ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (in quartiles) in SCAPIS, in all individuals as well as stratified by sex, in models additionally adjusted for well-known cardiovascular risk factors and blood cadmium.

	Model 1[a]			**Model 2**[b]		
Quartiles of blood lead [range (µg/L)]	**With plaque [n (%)]**	**Without plaque [n (%)]**	**PR (95% CI)**	**With plaque [n (%)]**	**Without plaque [n (%)]**	**PR (95% CI)**
All						
Q1 (0.75-11)	683 (52)	629 (48)	Ref	683 (52)	629 (48)	Ref
Q2 (11-14)	745 (56)	586 (44)	1.05 (0.98, 1.12)	745 (56)	586 (44)	1.05 (0.98, 1.12)
Q3 (14-20)	785 (60)	529 (40)	1.10 (1.03, 1.18)	785 (60)	529 (40)	1.09 (1.02, 1.17)
Q4 (20-203)	789 (61)	511 (39)	1.09 (1.02, 1.16)	789 (61)	511 (39)	1.08 (1.01, 1.15)
Men						
Q1 (1.1-11)	358 (60)	242 (40)	Ref	358 (60)	242 (40)	Ref
Q2 (11-14)	409 (62)	247 (38)	1.04 (0.95, 1.13)	409 (62)	247 (38)	1.03 (0.95, 1.13)
Q3 (14-20)	426 (66)	224 (34)	1.07 (0.98, 1.17)	426 (66)	224 (34)	1.06 (0.98, 1.16)
Q4 (20-178)	432 (69)	196 (31)	1.10 (1.01, 1.20)	432 (69)	196 (31)	1.09 (1.00, 1.19)
Women						
Q1 (0.75-11)	325 (46)	387 (54)	Ref	325 (46)	387 (54)	Ref
Q2 (11-14)	336 (50)	339 (50)	1.07 (0.96, 1.19)	336 (50)	339 (50)	1.06 (0.95, 1.18)
Q3 (14-20)	359 (54)	305 (46)	1.14 (1.02, 1.26)	359 (54)	305 (46)	1.13 (1.02, 1.26)
Q4 (20-203)	357 (53)	315 (47)	1.07 (0.96, 1.19)	357 (53)	315 (47)	1.06 (0.95, 1.18)

[a]Model 1 was adjusted for age, sex, LDL/HDL ratio, diabetes mellitus, waist circumference, brachial diastolic blood pressure, smoking status, physical activity, education level, nationality, heredity for acute myocardial infarction/stroke and logCRP.
[b]Model 2 was additionally adjusted for cadmium level.

Table S3. Odds ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (in quartiles) in SCAPIS, in all individuals as well as stratified by sex.

	Model 1[a]			**Model 2**[b]		
Quartiles of blood lead [range (µg/L)]	**With plaque [n (%)]**	**Without plaque [n (%)]**	**OR (95% CI)**	**With plaque [n (%)]**	**Without plaque [n (%)]**	**OR (95% CI)**
All						
Q1 (0.75-11)	740 (53)	664 (47)	Ref	721 (52)	653 (48)	Ref
Q2 (11-14)	790 (56)	616 (44)	1.09 (0.94, 1.27)	767 (56)	603 (44)	1.09 (0.93, 1.27)
Q3 (14-20)	843 (60)	563 (40)	1.24 (1.07, 1.45)	821 (60)	551 (40)	1.23 (1.05, 1.44)
Q4 (20-203)	856 (61)	550 (39)	1.25 (1.07, 1.46)	831 (61)	540 (39)	1.20 (1.03, 1.41)
Men						
Q1 (1.1-11)	384 (60)	261 (40)	Ref	374 (59)	256 (41)	Ref
Q2 (11-14)	436 (63)	256 (37)	1.12 (0.90, 1.40)	422 (63)	251 (37)	1.11 (0.88, 1.39)
Q3 (14-20)	464 (66)	243 (34)	1.23 (0.98, 1.53)	454 (66)	238 (34)	1.22 (0.97, 1.53)
Q4 (20-178)	473 (69)	214 (31)	1.37 (1.09, 1.72)	455 (69)	208 (31)	1.34 (1.05, 1.69)
Women						
Q1 (0.75-11)	356 (47)	403 (53)	Ref	347 (47)	397 (53)	Ref
Q2 (11-14)	354 (50)	360 (50)	1.07 (0.87, 1.31)	345 (49)	352 (51)	1.07 (0.87, 1.33)
Q3 (14-20)	379 (54)	320 (46)	1.26 (1.03, 1.56)	367 (54)	313 (46)	1.26 (1.02, 1.56)
Q4 (20-203)	383 (53)	336 (47)	1.16 (0.94, 1.43)	376 (53)	332 (47)	1.11 (0.90, 1.38)

[a]Model 1 was adjusted for age and sex.
[b]Model 2 was additionally adjusted for LDL/HDL ratio, diabetes mellitus, waist circumference and smoking status.

Table S4. Odds ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (in quartiles) in SCAPIS, among all non-smokers and stratified by sex.

Quartiles of blood lead [range (µg/L)]	**With plaque [n (%)]**	**Without plaque [n (%)]**	**OR (95% CI)**
All			
Q1 (0.75-11)	364 (50)	365 (50)	Ref
Q2 (11-14)	346 (52)	320 (48)	1.05 (0.85, 1.31)
Q3 (14-20)	353 (57)	270 (43)	1.26 (1.01, 1.57)
Q4 (20-203)	268 (54)	226 (46)	1.11 (0.87, 1.40)
Men			
Q1 (1.1-11)	218 (60)	144 (40)	Ref
Q2 (11-14)	209 (60)	142 (40)	0.95 (0.70, 1.29)
Q3 (14-20)	201 (61)	130 (39)	1.00 (0.73, 1.36)
Q4 (20-178)	154 (63)	91 (37)	1.03 (0.73, 1.45)
Women			
Q1 (0.75-11)	146 (40)	221 (60)	Ref
Q2 (11-14)	137 (43)	178 (57)	1.17 (0.85, 1.59)
Q3 (14-20)	152 (52)	140 (48)	1.62 (1.18, 2.23)
Q4 (20-203)	114 (46)	135 (54)	1.18 (0.84, 1.65)

Adjusted for age, sex, LDL/HDL ratio, diabetes mellitus and waist circumference in never smokers.

Table S5. Odds ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (as continuous variable, B-Pb) in SCAPIS, in all individuals and among those with B-Pb <100 µg/L as well as stratified by sex.

	With plaque [n (%)]	**Without plaque [n (%)]**	**OR (95% CI)**	**With plaque [n (%)]**	**Without plaque [n (%)]**	**OR (95% CI)**
All	3140 (57)	2347 (43)	1.10 (1.03, 1.17)	3133 (57)	2346 (43)	1.10 (1.03, 1.17)
Men	1705 (64)	953 (36)	1.14 (1.05, 1.24)	1699 (64)	952 (36)	1.15 (1.05, 1.25)
Women	1435 (51)	1394 (49)	1.07 (0.98, 1.16)	1434 (51)	1394 (49)	1.06 (0.96, 1.15)

Odds ratios (ORs) are displayed in the context of 10 µg/L advancements of blood lead concentration.
Adjusted for age, sex, LDL/HDL ratio, diabetes mellitus, waist circumference and smoking status.

Table S6. Odds ratios (95% confidence intervals) for plaque in the carotid arteries (yes/no) in relation to blood lead concentrations (in quartiles) in SCAPIS, in all individuals as well as stratified by sex, in models additionally adjusted for well-known cardiovascular risk factors and blood cadmium.

	Model 1[a]			**Model 2**[b]		
Quartiles of blood lead [range (µg/L)]	**With plaque [n (%)]**	**Without plaque [n (%)]**	**OR (95% CI)**	**With plaque [n (%)]**	**Without plaque [n (%)]**	**OR (95% CI)**
All						
Q1 (0.75-11)	683 (52)	629 (48)	Ref	683 (52)	629 (48)	Ref
Q2 (11-14)	745 (56)	586 (44)	1.11 (0.95, 1.30)	745 (56)	586 (44)	1.10 (0.94, 1.29)
Q3 (14-20)	785 (60)	529 (40)	1.26 (1.07, 1.48)	785 (60)	529 (40)	1.23 (1.05, 1.45)
Q4 (20-203)	789 (61)	511 (39)	1.22 (1.03, 1.43)	789 (61)	511 (39)	1.18 (1.00, 1.39)
Men						
Q1 (1.1-11)	358 (60)	242 (40)	Ref	358 (60)	242 (40)	Ref
Q2 (11-14)	409 (62)	247 (38)	1.09 (0.87, 1.38)	409 (62)	247 (38)	1.08 (0.85, 1.36)
Q3 (14-20)	426 (66)	224 (34)	1.21 (0.95, 1.53)	426 (66)	224 (34)	1.17 (0.92, 1.49)
Q4 (20-178)	432 (69)	196 (31)	1.33 (1.04, 1.70)	432 (69)	196 (31)	1.28 (1.00, 1.63)
Women						
Q1 (0.75-11)	325 (46)	387 (54)	Ref	325 (46)	387 (54)	Ref
Q2 (11-14)	336 (50)	339 (50)	1.13 (0.91, 1.41)	336 (50)	339 (50)	1.12 (0.90, 1.40)
Q3 (14-20)	359 (54)	305 (46)	1.31 (1.05, 1.63)	359 (54)	305 (46)	1.29 (1.04, 1.61)
Q4 (20-203)	357 (53)	315 (47)	1.14 (0.91, 1.42)	357 (53)	315 (47)	1.11 (0.88, 1.38)

[a]Model 1 was adjusted for age, sex, LDL/HDL ratio, diabetes mellitus, waist circumference, brachial diastolic blood pressure, smoking status, physical activity, education level, nationality, heredity for acute myocardial infarction/stroke and logCRP.
[b]Model 2 was additionally adjusted for cadmium level.