

Bone Lead Levels and Risk of Incident Primary Open-Angle Glaucoma: The VA Normative Aging Study

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BACKGROUND: Oxidative stress may play an important role in the etiology of primary open-angle glaucoma (POAG). The association between risk of POAG and lead exposure, which is an environmental source of oxidative stress, has not been fully investigated yet.

OBJECTIVE: Our objective was to determine the association between bone lead—a biomarker of cumulative lead dose (tibia lead) or an endogenous source of stored lead (patella lead)—and incident POAG.

METHODS: We examined a prospective cohort of 634 POAG-free men [mean baseline age = 66.8 y of age (SD = 6.7)] from the Normative Aging Study (NAS) who had tibia and patella K X-ray fluorescence lead measurements between 1 January 1991 and 31 December 1999. They also had standard ocular evaluations by NAS optometrists until 31 December 2014. POAG cases were identified by consistent reports of enlarged or asymmetric cup-to-disc ratio together with visual field defect or existence of disc hemorrhage. We used Cox proportional hazards regressions to estimate hazard ratios (HRs) of incident POAG and adjusted survival curves to examine changes in the risk of POAG during follow-up according to bone lead quartiles.

RESULTS: We identified 44 incident cases of POAG by the end of follow-up (incidence rate = 74 per 10,000 person-years; median follow-up = 10.6 y). In fully adjusted models, 10-fold increases in patella lead and tibia lead were associated with HRs of 5.06 (95% CI: 1.61, 15.88, $p = 0.005$) and 3.07 (95% CI: 0.94, 10.0, $p = 0.06$), respectively. The HRs comparing participants in the third and fourth quartiles with the lowest quartile were 3.41 (95% CI: 1.34, 8.66) and 3.24 (95% CI: 1.22, 8.62) for patella lead (p -for-trend = 0.01), and 3.84 (95% CI: 1.54, 9.55) and 2.61 (95% CI: 0.95, 7.21) for tibia lead (p -for-trend = 0.02).

CONCLUSIONS: Our study provides longitudinal evidence that bone lead may be an important risk factor for POAG in the U.S. population. <https://doi.org/10.1289/EHP3442>

Introduction

Glaucoma accounts for approximately 8% of global blindness according to the 2010 World Health Organization report (Pascolini and Mariotti 2012). It is the second leading cause of blindness in the world after cataracts, and the leading cause of irreversible loss of vision (Pascolini and Mariotti 2012). Despite the large patient population and severe consequences, the exact etiology of glaucoma is still unclear. Based on glaucoma clinical trials, the established risk factors for glaucoma include older age, intraocular pressure, race, myopia, optic nerve susceptibility, and positive family history (Jonas et al. 2017). Other clinical risk factors, such as various systemic diseases (e.g., diabetes, hypertension, ischemic vascular diseases) and unhealthy behaviors (e.g., smoking, alcohol consumption), remain inconsistent among different studies (Cioffi

and American Academy of Ophthalmology 2014; Doshi et al. 2008; Fan et al. 2004; Ko et al. 2016; Renard et al. 2013). Although there is a large population burden and severe consequence to quality of life, there is a gap in knowledge to advance our understanding beyond the established clinical risk factors for glaucoma.

In addition to clinical risk factors, genetic risk factors for glaucoma have been established through the Mendelian studies and genome-wide association studies (GWAS) (Cioffi and American Academy of Ophthalmology 2014; Mabuchi et al. 2015; Sakurada and Mabuchi 2015; Wiggs 2015; Wiggs et al. 2013). Adult-onset glaucoma occurs mostly among individuals >40 y of age. Primary open-angle glaucoma (POAG) is the major form of adult-onset glaucoma in the United States (prevalence: 1.9%) (Friedman et al. 2004). A recent heritability estimate to quantify the proportion of genetic attribution on the total phenotype variation of POAG was about 42%, which was lower than its proportion for age-related macular degeneration (AMD) (>70%) (Cuellar-Partida et al. 2016). Although the various genetic risk alleles for specific forms of glaucoma have been successfully identified by linkage and GWAS approaches, the environmental risk factors for glaucoma have proven difficult to identify.

Oxidative stress plays a role in glaucoma pathogenesis (Babizhayev 2012; Goyal et al. 2014; Majsterek et al. 2011; Zhao et al. 2016). The pathophysiology of glaucoma involves complex tissues in the anterior segment that regulate aqueous humor fluid dynamics and determine intraocular pressure and posterior segment end organ damage of the optic nerve, which was recently reviewed by Jonas et al. (2017). The complex relationships among the delicate ganglion cells that contribute to the axonal fibers of

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the optic nerve, the vascular supply, the glial support tissue, and the connective support tissues in the optic nerve canal as well as counter-pressure from the cerebral spinal fluid are active areas of research (Jonas et al. 2017). Within these tissues, markers of oxidative stress—such as superoxide dismutase, glutathione peroxidase, and catalase levels—are elevated in the aqueous humor of patients with POAG (Babizhayev 2012; Goyal et al. 2014; Majsterek et al. 2011). Oxidative stress can disrupt the normal function of trabecular meshwork cells, block the outflow of aqueous humor, and increase the intraocular pressure (Babizhayev 2012; Saccà et al. 2016; Zhao et al. 2016). In the posterior segment, elevated 4-hydroxy-2-nonenal adducts generated by free radicals have been detected in the glaucomatous retina cases, implying that oxidative stress plays a pathogenic role damaging the retina/optic nerve (Tezel et al. 2010).

As a key environmental source of oxidative stress, heavy metals may be an important risk factor for glaucoma. As early as 1990, a study reported higher copper levels in the aqueous humor of glaucoma patients (Akyol et al. 1990). Recent studies have also indicated a significant association between heavy metal and glaucoma. A cross-sectional analysis of the Korean National Health and Nutrition Examination Survey (KNHANES) found that higher blood mercury and lower blood manganese levels were associated with higher prevalence of glaucoma (Lin et al. 2015). Another KNHANES study found that higher blood cadmium levels were associated with higher glaucoma risk, particularly in men with intraocular pressures within the normal range (Lee et al. 2016). A case-control study conducted in Japan reported that higher hair lead level was associated with POAG, especially normal tension glaucoma in females (Yuki et al. 2009).

The threat of nonoccupational cumulative exposure to low-dose lead has been reported since lead was banned from gasoline and paint in the United States in the 1990s. As there is a gap in knowledge on heavy metals as potential environmental risk factors for glaucoma, we propose an epidemiological study to test the hypothesis that cumulative lead exposure increases the risk of POAG. To the best of our knowledge, no epidemiologic study has ever tested the association between cumulative lead exposure and risk of POAG. Results of previous studies, which were mostly based on Asian populations, may not be generalizable to the U.S. population. Moreover, no previous lead-glaucoma study has ever utilized bone lead levels as a biomarker of cumulative lead dose (tibia lead) or an endogenous source of stored lead (patella lead) (Hu et al. 2007). Bone lead, which represents the majority of the body's lead burden with a half-life spanning years to decades, is known to be a better biomarker for assessing chronic health effects than blood or urinary lead (Hu et al. 2007). Further, cross-sectional studies have raised causal inferences and reverse causality concerns. In this study, we aim to examine the association between bone lead levels and incident POAG in a male population in the Boston area, the Normative Aging Study (NAS).

Method

Study Population

The NAS is a longitudinal study of aging started in 1963 by the U.S. Department of Veterans Affairs (Glynn et al. 1982). The study recruited 2,280 healthy male participants who were predominantly whites and free of systemic disease at the time of enrollment. Participants underwent a comprehensive physical examination, including a standard ocular evaluation, every 3–5 y (Schaumburg et al. 2004). Informed consent was provided by participants at each visit. From 1991 to 1999, 868 participants underwent bone lead measurements via K X-ray fluorescence (KXRF). We set the date of the first bone lead measurement as the baseline of the longitudinal study. Because the cohort had a long follow-up

time which may have accumulated survival bias, we restricted the study cohort follow-up to within 15 y. The inclusion criteria for this project were a pre-cohort ophthalmology examination prior to the KXRF measurement, a minimum of one ophthalmology examination after the baseline, and no missing covariate data. Seven hundred two participants had both complete ophthalmology evaluations and bone lead measurements. After excluding those who were not eligible [8 for no ophthalmology examination after bone lead measurement; 30 for missing covariate data; 18 for missing information on inverse probability weighting (more details described below); 8 for preexisting diagnosis of either open-angle glaucoma, secondary glaucoma, or narrow angle glaucoma; 1 for unacceptable uncertainty for patella lead; and 3 for follow-up of <2 y], we included a total of 634 individuals into this study (Figure 1). The current study was reviewed and approved by the institutional review boards of each participating institute: the University of Michigan School of Public Health, the Harvard School of Public Health, and the Department of Veterans Affairs Boston Healthcare System.

Bone Lead Measurements

Bone lead levels (micrograms of lead per gram of bone mineral) at the mid-tibial shaft and patella were measured for the NAS using a KXRF instrument. Tibia and patella are representative of two typical bone structures: cortical bone, and trabecular bone (Hu et al. 1995). Tibia lead is a biomarker of past lifetime exposure, and patella lead is a biomarker of an endogenous source of lead body burden (Hu et al. 1995, 2007; Wilker et al. 2011). The KXRF instrument utilizes low-dose gamma radiation to provoke the release of X-rays that are specific and proportional to the lead level in bones (Hu et al. 1995). It provides a noninvasive and safe method to

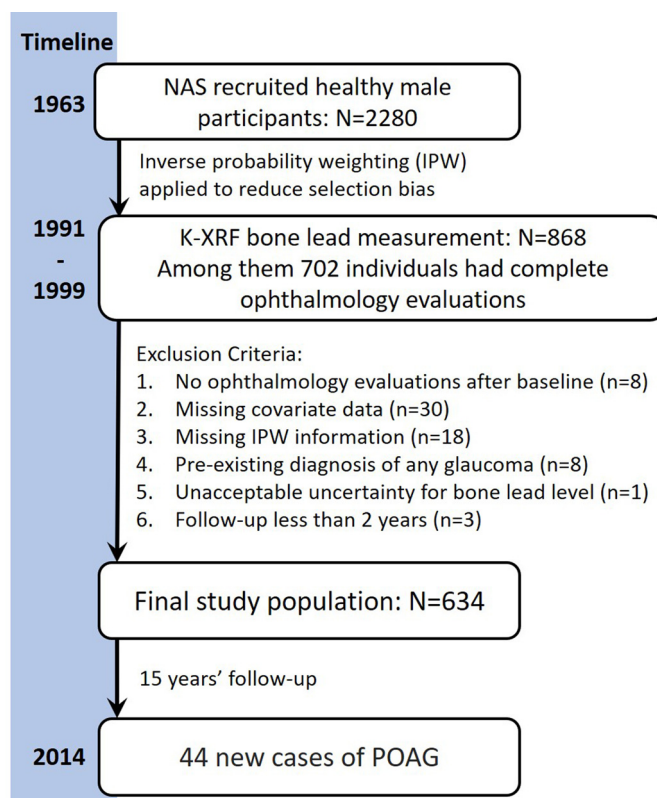


Figure 1. Diagram illustrating the establishment cohort structure of the study population, from the NAS original recruitment in 1963 to the KXRF measurement in the 1990s, which is the baseline of our study, until the end of the 15-y follow-up. Note: KXRF, K X-ray fluorescence; NAS, Normative Aging Study.

precisely evaluate bone lead concentrations. The physical principles, technical specifications, and validation of this instrument have been described in detail elsewhere (Burger et al. 1990).

We have multiple measurements of bone lead over time in this cohort. Instead of time-varying bone lead levels, we used the first measurements in our study. One reason is that not all subjects had multiple measurements. Besides, a previous study with repeatedly measured bone lead levels in the same population had shown that tibia lead decreased slightly over 11 y (1.4% annual decline) after cessation of exposure, whereas patella lead had an initial decline of 5% per year during the first 5 y but then did not change much (0.4% annual decline) after 5 y (Wilker et al. 2011). We assumed that baseline tibia lead levels did not change much during follow-up and that baseline patella lead levels could reasonably capture the average of exogenous exposures that had gradually decreased since the phaseout of lead from gasoline and paint.

A subset of bone lead levels measured by KXRF had negative values (3 for tibia lead levels and 5 for patella lead levels) because the instrument provides an unbiased point estimate that may oscillate around the true value (Kim et al. 1995). In order to better present the true distribution of bone lead levels, we used original values, including negative values, rather than using a substitution method. As a quality control procedure, we adopted the measurement uncertainty for each bone lead measurement to evaluate the chance of estimated level corresponding to a true level (Hu et al. 1995). The measurement uncertainty is equal to 1 standard deviation (SD) of replicated measurements; the higher uncertainty a bone lead measurement has, the lower reliability this value possesses. We only included those participants who had bone lead levels within an acceptable uncertainty (10 µg/g for tibia and 15 µg/g for patella).

Glaucoma Identification

The NAS standard ocular evaluation includes family and personal ocular and systemic disease history, medical history, visual acuity data, biomicroscopy, tonometry, and ophthalmoscopy (Schaumburg et al. 2004). A staff optometrist performed examinations at the NAS examination facility and results were reviewed and cosigned by a second qualified person. For the current study, we reviewed the de-identified medical records spanning the years 1991 to 2014. Variables were extracted for glaucoma identification, including personal and family history of glaucoma, medication, visual acuity, intraocular pressures of each eye (pre-dilated, measured in the morning), the vertical cup-to-disc ratios (CDRs) of each eye and other descriptions from the fundus exam, additional testing that included visual field, and ocular diagnoses made by the NAS optometrists. Central cornea thickness was not part of the NAS standard ophthalmology examination.

The ascertainment of incident POAG cases were adopted from the glaucoma phenotype description defined from the National Eye Institute Glaucoma Human genetics collaBORation (NEIGHBOR) consortium (Wiggs et al. 2013). POAG cases were identified in participants who showed one of the following characteristics (Table 1): a) either eye having a CDR ≥ 0.7 ; b) the difference of two eyes' CDRs ≥ 0.2 , which indicates an asymmetric cup-to-disc ratio; c) any eye's CDR ≥ 0.6 , with either disc hemorrhage or visual field defect; or d) vision loss due to nerve fiber layer loss. In addition, an open angle was assumed based upon biomicroscopic description of the deep chamber and the lack of an NAS optometric description of narrow angles. Those who had glaucoma prior to the baseline were defined as baseline glaucoma cases and were excluded from the longitudinal analysis ($n = 8$, prevalence at baseline = 1.1%). All eligible participants were followed until the end of the 15 y since baseline, the last recorded visit if lost to follow-up, or the date of

Table 1. Identification of primary open-angle glaucoma cases.

Diseases	Criteria
POAG patients	
POAG ^a	1) Either eye CDR ≥ 0.7 , open angle ^b 2) The difference between two eyes' CDRs ≥ 0.2 , open angle 3) Either eye CDR ≥ 0.6 , together with disc hemorrhage or visual field defect, open angle 4) Vision loss of either eye together with nerve fiber layer loss, open angle
Non-POAG	
PACG	Same as criteria of POAG, but angle narrowed or closed (angle $\leq 1/4$, or being diagnosed as PACG by NAS optometrist)
Secondary glaucoma	1) Pseudoexfoliation glaucoma 2) Pigment dispersion glaucoma 3) Glaucoma secondary to other diseases or accidents (e.g., trauma, stroke, surgery)
Glaucoma suspects	Being diagnosed as a glaucoma suspect by NAS optometrist, without any of the above characteristics

Note: CDR, cup-to-disc ratio; NAS, the Normative Aging Study; PACG, primary angle closure glaucoma; POAG, primary open-angle glaucoma.

^aCriteria of POAG were adopted from the NEI Glaucoma Human genetics collaBORation (NEIGHBOR) consortium (Wiggs et al. 2013) and were modified to be more applicable to the NAS population.

^bAngle was defined as the angle between the cornea and iris in the anterior chamber of eye; an open angle was assumed based upon biomicroscopic description of deep chamber and lack of NAS optometric description of narrow angles.

the first vision test identifying the onset of POAG or other types of glaucoma (Table 1).

Other Variables

Established risk factors for POAG include older age, elevated intraocular pressure (IOP) defined as greater than or equal to 23 mmHg, and myopia (Jonas et al. 2017). Given the extensive data on this NAS cohort, the following variables were analyzed: age at baseline (years), race/ethnicity (white or other), body mass index (BMI, varying at each follow-up visit, kg/m²), educational attainment ($\leq$ high school, high school, some college, and ≥ 4 y of college), and job types (blue collar, white collar, or mixed). Cigarette smoking status is an inconsistent risk factor for POAG, but meta-analyses and systemic reviews show heavy smoking, not simply a positive smoking status, is associated with POAG (Bonovas et al. 2004; Cioffi and American Academy of Ophthalmology 2014; Jain et al. 2017; Zhou et al. 2016). Thus, we used categorized cigarette consumption data based on pack-years (0, 0–19 pack-years, and ≥ 20 pack-years) to adjust for smoking behavior. In addition, we also controlled for diabetes mellitus status (yes/no; identified by either having been diagnosed as having diabetes mellitus, or having used insulin or other diabetes medication/treatment, or ever having a blood fasting glucose level ≥ 126 mg/dL), systemic hypertension (yes/no; identified by systolic blood pressure ≥ 140 mm/Hg or diastolic blood pressure ≥ 90 mm/Hg or ever having used hypertension medication/treatment), and ocular hypertension (yes/no; identified by either the highest intraocular pressure (untreated) value ≥ 23 mm/Hg at that visit or the highest intraocular pressure (treated) value ≥ 23 mm/Hg after being divided by 0.7 at that visit; criteria was made according to NEIGHBOR Consortium's IOP GWAS study (Ozel et al. 2014). The covariates were collected by the time of bone lead measurement.

Handling Selection Bias and Inverse Probability Weighting

Because our bone lead study was conducted several decades after the inception of the NAS, it was subject to selection bias due to loss to follow-up (Weisskopf et al. 2015), which is common to

observational prospective cohort studies (Howe et al. 2016). We had two types of selection bias: selection bias due to restriction of analysis to the KXRF substudy, and selection bias due to survivorship from glaucoma diagnosis (i.e., no development of glaucoma) at the later follow-up period or loss to follow-up that could have been influenced by lead exposure (for the directed acyclic graphs depicting these two types of selection bias, see Figure S1).

Among the original 2,280 NAS participants enrolled in the 1960s, nonparticipation in the subsequent KXRF bone lead substudy in the 1990s was likely related to past lead exposure and other confounders (e.g., socioeconomic status) that could affect participation. Restricting to the subset of those who participated in the bone lead substudy (i.e., conditioning on a collider) could therefore bias the exposure–outcome association (Hernán et al. 2004). To reduce this potential selection bias, we applied inverse probability weighting (IPW) to our models (Weisskopf et al. 2015). Briefly, we ran a logistic regression model to predict the probability of KXRF enrollment for all NAS participants (see Table S1), and calculated IPW from this probability. For those in our substudy, in this model we used all observations from NAS recruitment to the time of KXRF measurement and each visit was treated as a single observation. For those who were not in the substudy, we used observations until the last visit before year 1999, which is the last year of bone lead measurement in our study. IPW of our study population ranged from 1.0 to 6.1, with the mean of 1.18 (data not shown). This approach simulates a pseudo-population similar to the original NAS population and therefore can account for potential selection bias that may have happened before our bone lead substudy.

Selection bias due to survivorship from glaucoma diagnosis at the later follow-up period or loss to follow-up was also likely to occur. Those who were more susceptible to lead toxicity could have developed POAG earlier or have dropped out earlier. Again, IPW is a standard recommendation used to account for this selection bias, but because we had already included the aforementioned IPW and it is challenging to include two IPWs in the analysis, we needed another way to address this possible bias. Such selection bias may result in time-varying hazard ratios (HRs), which have been commonly reported in prospective observational studies (Hernán 2010). Simply reporting the average HR during the whole follow-up time may result in underestimating the association. Thus, instead of IPW, we created adjusted survival curves as the solution. This approach was suggested to address two key limitations of the use of average HR using Cox proportional hazard modeling: the time-varying HR, and a built-in selection bias (Hernán 2010). See analytical approach described below.

Statistical Analysis

We compared baseline population characteristics [means (SDs)] for continuous variables and frequencies for categorical variables by POAG status. We also performed bivariate analysis between baseline covariates and bone lead concentrations.

We used Cox proportional hazard models to evaluate the association between bone lead and incident POAG. Three sequential covariate models were performed: Model 1 was adjusted for age; Model 2 was further adjusted for BMI, educational levels, job types, and categorical pack-years—all of which are known risk factors for POAG; Model 3 only further adjusted for three components: diabetes mellitus, systemic hypertension, and ocular hypertension. All these three components are either systemic or ocular diseases associated with the pathogenesis of POAG. We show Model 3 as a separate model because those diseases may act as mediators rather than confounders in the lead–POAG association.

The proportional hazard assumption was tested by creating Schoenfeld residual plots. Because the assumption is often violated and HRs are not constant over time in prospective observational studies (Hernán 2010), we evaluated whether HRs were time-varying in our study by using the adjusted survival curves. We illustrated the risks of POAG of participants in different bone lead quartiles throughout the entire 23-y follow-up using adjusted survival curves. The procedures of creating adjusted survival curves was adopted from Hernán, briefly fitted discrete-time models with adjustment of covariates, and then estimated the conditional survivals under different exposure levels using manipulated counterfactual data (Hernán 2010).

In the Cox regression, we restricted our analysis with the follow-up visits to 15 y after baseline because selection bias may have increased with longer follow-ups, given that those who tended to live longer were healthier than the baseline population and less susceptible to the lead toxicity. We chose 15 y because Schoenfeld residual plots for bone lead versus time using the entire follow-ups of up to 23 y showed flat fit lines centered at zero during the 15-y follow-up and then declined afterwards, suggesting that the HRs were consistent across the first 15 y and then decreased over time (see Figure S2); such characteristics of time-varying HRs were confirmed by the adjusted survival curves.

We treated the lead variables in two ways in the Cox proportional hazard models: *a*) we log-transformed the lead variables on the natural scale and calculated HRs together with 95% confidence intervals (CIs) for the occurrence of POAG for a 10-fold increase in each lead variable (five participants for tibia lead and three participants for patella lead were excluded due to negative values); and *b*) we categorized the lead variables into four quartiles, calculated HRs for POAG by each quartile, and tested the significance of a linear trend across the quartiles (each quartile was ordinally coded 1, 2, 3, or 4). To evaluate nonlinear dose–response relationships, we fit the lead variable using natural splines with knots at the 25th, 50th, and 75th percentiles.

As a sensitivity analysis, we additionally ran all models without the application of IPW. We also restricted the models to within the white race, or extended the follow-up time beyond 15 y by using all follow-ups (range = 1 to 23 y) to test the robustness of the association.

All analyses were performed using SAS (version 9.4; SAS Institute Inc.) and RStudio (version 1.0.136).

Results

In total, 634 individuals with 1,868 observations were eligible to be included in the study after excluding those who had missing covariate data. During follow-up, (median = 10.6 y, range = 2–15), 44 incident POAG cases were identified (incidence rate = 74 per 10,000 person-years). The mean baseline age at the date of bone lead measurement was 66.8 y (SD 6.7, range = 49.9–94.0 y) (Table 2). The concentration of tibia lead ranged from –5 to 126 µg/g (median was 19 µg/g), while patella lead ranged from –10 to 165 µg/g (median = 27 µg/g). The Pearson correlation coefficient comparing the two bone lead measures was 0.78 ($p < 0.001$). Baseline ocular hypertension ($p < 0.001$) was associated with POAG identification (Table 2).

Higher tibia lead levels were associated with older baseline age ($p < 0.001$), nonwhite ($p = 0.03$), baseline diabetes history ($p = 0.04$), baseline systemic hypertension history ($p = 0.05$), lower education levels ($p < 0.001$), and blue-collar jobs ($p < 0.001$) (Table 3). Higher patella lead levels were associated with older baseline age ($p < 0.001$), nonwhite ($p = 0.04$), history of systemic hypertension ($p = 0.02$), history of ocular hypertension ($p = 0.02$), lower educational attainment ($p < 0.001$), a greater

Table 2. Baseline characteristics of study population comparing participants with POAG vs. participants with non-POAG.

Characteristics	Total population (<i>n</i> = 634)	Non-POAG (<i>n</i> = 590)	POAG (<i>n</i> = 44)	<i>p</i> -Value ^a
Bone lead levels				
Tibia lead, mean ± SD (μg/g)	21.7 ± 13.7	21.6 ± 13.8	23.5 ± 12.4	0.37
Patella lead, mean ± SD (μg/g)	31.0 ± 20.2	30.6 ± 20.1	36.3 ± 21.4	0.08
Age at baseline, mean ± SD (years)	66.8 ± 6.7	66.8 ± 6.8	67.7 ± 6.1	0.36
Age at end of 15-y follow-up, mean ± SD (years)	76.8 ± 6.7	76.8 ± 6.7	75.8 ± 6.4	0.30
BMI, mean ± SD (kg/m ²)	27.9 ± 3.7	27.9 ± 3.8	27.5 ± 3.4	0.44
Diabetes mellitus [<i>n</i> (%)]	89 (14.0)	81 (13.7)	8 (18.2)	0.41
Systemic hypertension [<i>n</i> (%)]	346 (54.6)	320 (54.2)	26 (59.1)	0.53
Ocular hypertension [<i>n</i> (%)]	21 (3.3)	13 (2.2)	8 (18.2)	<0.001
White population [<i>n</i> (%)]	616 (97.2)	575 (97.5)	41 (93.2)	0.11
Educational levels [<i>n</i> (%)]				
≤High school	65 (10.3)	62 (10.5)	3 (6.8)	0.71
High school	230 (36.3)	214 (36.3)	16 (36.4)	
Some college	157 (24.8)	144 (24.4)	13 (29.6)	
≥4 y college	182 (28.7)	170 (28.8)	12 (27.3)	
Pack-years [<i>n</i> (%)]				
0	204 (32.2)	190 (32.2)	14 (31.8)	0.97
1–19	171 (27.0)	159 (27.0)	12 (27.3)	
≥20	259 (40.9)	241 (40.9)	18 (40.9)	
Job type [<i>n</i> (%)]				
Blue collar	265 (41.8)	247 (41.9)	18 (40.9)	0.99
Mix	139 (21.9)	129 (21.9)	10 (22.7)	
White collar	230 (36.3)	214 (36.3)	16 (36.4)	

Note: BMI, body mass index; POAG, primary open-angle glaucoma; SD, standard deviation.

^a*p*-Values were calculated using logistic regression; educational levels and pack-years were treated as ordinal variables.

number of baseline pack-years of cigarette smoking ($p = 0.01$), and blue-collar jobs ($p < 0.001$) (Table 3).

Log-transformed bone lead was associated with incident POAG (Table 4). After adjustment for age, educational level, job types, BMI, and cumulative cigarette smoke, a 10-fold increase in patella lead was significantly associated with an HR of 5.30 (95% CI: 1.71, 16.43, $p = 0.004$), and a 10-fold increase in tibia lead was positively, but not significantly, associated with an HR of 2.78 (95% CI: 0.83, 9.31, $p = 0.10$) (Table 4, Model 2). The HRs comparing participants in the third and fourth quartiles with the lowest quartile were 3.90 (95% CI: 1.52, 9.97) and 3.60 (95% CI: 1.34, 9.65) with a positive linear trend (p -for-trend = 0.007) for patella lead; and 3.95 (95% CI: 1.59, 9.86) and 2.44 (95% CI: 0.87, 6.83) for tibia lead (p -for-trend = 0.03) (Table 4, Model 2). The associations remained significant even after further controlling for ocular hypertension, diabetes mellitus, and systemic hypertension. A 10-fold increase in patella lead was significantly associated with an HR of 5.06 (95% CI: 1.61, 15.88, $p = 0.005$), and a 10-fold increase in tibia lead was positively, but not significantly, associated with an HR of 3.07 (95% CI: 0.94, 10.0, $p = 0.06$) (Table 4, Model 3). The HRs comparing participants in the third and fourth quartiles with the lowest quartile were 3.41 (95% CI: 1.34, 8.66) and 3.24 (95% CI: 1.22, 8.62) for patella lead (p -for-trend = 0.01); and 3.84 (95% CI: 1.54, 9.55) and 2.61 (95% CI: 0.95, 7.21) for tibia lead (p -for-trend = 0.02) (Table 4, Model 3). Smoothing plots based on natural splines supported these findings that the associations linearly increased until the third quartile and plateaued in the range of the fourth quartile (see Figure S3).

The survival curves comparing the four quartiles of bone lead illustrated that the absolute risks started to get closer and cross over between 15–20 y of age. This suggests that the HRs in our study were not constant and changed over time (Figure 2). This observation is consistent with the Schoenfeld residual plots, the assessment of proportional hazard assumption.

We performed several sensitivity analyses to assess the robustness of the findings (see Table S2). In fully adjusted models, restricting the study population to whites only ($n = 613$ for patella lead and $n = 611$ for tibia lead) did not change main findings, with a 10-fold increase in patella lead significantly associated with an HR of 4.18 (95% CI: 1.29, 13.57, $p = 0.02$), and a 10-fold increase

in tibia lead positively, but not significantly, associated with an HR of 3.00 (95% CI: 0.89, 10.15, $p = 0.08$). The association was attenuated when follow-up time extended up to 23 y: A 10-fold HR for a fully adjusted model for patella lead became 2.59 (95% CI: 1.00, 6.68, $p = 0.049$) and the association between tibia lead and POAG became insignificant. Results were similar without the application of IPW: The associations were attenuated, with a 10-fold HR of 4.29 for a fully adjusted model for patella lead (95% CI: 1.18, 15.55, $p = 0.03$) and nonsignificant association for tibia lead.

Discussion

Our study provided longitudinal evidence that bone lead may be an important risk factor of POAG. Men in the third and fourth quartiles of patella lead levels had a more than 3-fold higher risk of POAG compared to those in the lowest quartile during the 15 y of follow-up. A 10-fold increase in patella lead level was associated with more than 5-fold higher risk of POAG during the 15 y of follow-up. Similar, but slightly weak, associations were observed for tibia lead.

Previous studies suggested that lead and other heavy metals may be associated with glaucoma pathogenesis in different Asian populations. Although the end organ damage of glaucoma is at the level of the optic nerve, there are diverse phenotypes based on anatomy and clinical findings that vary widely based on different populations (Jonas et al. 2017). The various phenotypes include POAG, normal tension glaucoma, and primary angle closure glaucoma. As the epidemiology for the various forms of glaucoma varies among different populations (Chan et al. 2016; Cheng et al. 2014; Kapetanakis et al. 2016; Tham et al. 2014), it is essential that epidemiology studies be interpreted in the context of the study population and not generalized to different populations. In addition, it is important to not overinterpret findings from cross-sectional study designs regarding causal inferences and reverse causality. Two major strengths of our study are the longitudinal study design and a predominantly white study population.

Another strength of our study was the utilization of bone lead levels as biomarkers. Tibia bone lead can better indicate cumulative lead dose compared with blood or hair lead levels as measured in previous studies, while patella bone lead mainly reflects a source of cumulatively stored lead that is bioavailable (Hu et al.

Table 3. Bivariate analysis of lead concentration by baseline characteristics.

Characteristics	n (%)	Bone lead concentration, mean (SD) (µg/g)		p-Value ^a	
		Tibia lead	Patella lead	Tibia lead	Patella lead
Overall	634 (100)	21.7 (13.7)	31.0 (20.2)	—	—
Age at baseline (years)					
45–59	95 (15.0)	14.7 (8.0)	22.5 (12.9)		
60–70	351 (55.4)	20.7 (11.8)	29.3 (17.6)		
>70	188 (29.7)	27.1 (16.9)	38.5 (24.9)	<0.001	<0.001
Race/ethnicity					
White	616 (97.2)	21.5 (13.5)	30.7 (19.7)		
Nonwhite	18 (2.8)	28.5 (19.2)	40.5 (33.2)	0.03	0.04
Diabetes mellitus					
Yes	89 (14.0)	24.5 (14.1)	34.1 (21.5)		
No	545 (86.0)	21.2 (13.6)	30.5 (20.0)	0.04	0.12
Systemic hypertension					
Yes	346 (54.6)	22.7 (15.3)	32.7 (22.8)		
No	288 (45.4)	20.5 (11.3)	28.9 (16.3)	0.05	0.02
Ocular hypertension					
Yes	21 (3.3)	25.8 (18.4)	41.0 (27.7)		
No	613 (96.7)	21.6 (13.5)	30.6 (19.8)	0.17	0.02
BMI (kg/m ²)					
<25	135 (21.3)	20.4 (11.5)	29.9 (15.6)		
25–30	342 (53.9)	22.1 (13.3)	31.1 (19.2)		
≥30	157 (24.8)	21.8 (16.1)	31.6 (25.3)	0.40	0.47
Educational levels					
≤High school	65 (10.3)	28.1 (17.9)	39.5 (24.3)		
High school	230 (36.3)	24.2 (15.4)	35.3 (23.0)		
Some college	157 (24.8)	20.6 (11.4)	28.7 (17.2)		
≥4 y of college	182 (28.7)	17.2 (9.4)	24.4 (14.0)	<0.001	<0.001
Pack-years					
0	204 (32.2)	21.2 (14.1)	29.6 (20.2)		
1–19	171 (27.0)	20.1 (12.4)	27.9 (17.0)		
≥20	259 (40.9)	23.2 (14.1)	34.1 (21.7)	0.10	0.01
Job type					
Blue collar	265 (41.8)	26.2 (16.4)	37.0 (4.0)		
Mix	139 (21.9)	19.1 (10.8)	27.3 (15.3)		
White collar	230 (36.3)	18.1 (9.8)	26.2 (15.8)	<0.001	<0.001

Note: —, data not available; BMI, body mass index; SD, standard deviation.

^ap-Values were calculated using linear regression; educational levels and pack-years were treated as ordinal variables.

2007). Blood lead reflects a combination of recent exogenous exposure and endogenous exposure by the cumulative lead body burden; it has a half-life of approximately 1 month, which limits inferences regarding chronic effects of cumulative exposure (Hu

et al. 1995, 2007). Because POAG is an age-related disease, any biomarkers with a relatively short half-life should be interpreted cautiously as a risk of chronic conditions (Lin et al. 2015). Hair lead levels used in the Japanese case-control study were also

Table 4. Hazard ratio (95% CI) of POAG by bone lead concentrations with application of IPW.

Exposure	Total (n)	Cases (n)	Range (µg/g)	Model 1 ^a		Model 2 ^b		Model 3 ^c	
				10-fold HR (95% CI)	p-Value	10-fold HR (95% CI)	p-Value	10-fold HR (95% CI)	p-Value
Tibia									
Continuous ^d	629	44	1–126	2.73 (0.92, 8.16)	0.07	2.78 (0.83, 9.31)	0.10	3.07 (0.94, 10.0)	0.06
Quartiles ^e									
1	148	6	–5 to 12	Reference		Reference		Reference	
2	154	7	13–18	1.48 (0.51, 4.24)		1.56 (0.54, 4.55)		1.76 (0.60, 5.13)	
3	169	20	19–27	3.75 (1.55, 9.05)		3.95 (1.59, 9.86)		3.84 (1.54, 9.55)	
4	163	11	28–126	2.34 (0.89, 6.19)	0.02	2.44 (0.87, 6.83)	0.03	2.61 (0.95, 7.21)	0.02
Patella									
Continuous ^d	631	44	1–165	4.68 (1.65, 13.30)	0.004	5.30 (1.71, 16.43)	0.004	5.06 (1.61, 15.88)	0.005
Quartiles ^e									
1	162	6	–10 to 18	Reference		Reference		Reference	
2	150	10	19–26	2.29 (0.88, 6.01)		2.52 (0.95, 6.73)		2.23 (0.83, 5.98)	
3	165	14	27–38	3.47 (1.40, 8.58)		3.90 (1.52, 9.97)		3.41 (1.34, 8.66)	
4	157	14	39–165	3.35 (1.32, 8.53)	0.006	3.60 (1.34, 9.65)	0.007	3.24 (1.22, 8.62)	0.01

Note: We applied unstabilized IPW into our models; IPW of all participants ranged from 1.0 to 6.1. CI, confidence interval; HR, hazard ratio; IPW, inverse probability weighting; POAG, primary open-angle glaucoma.

^aModel 1 was adjusted for age.

^bModel 2 was further adjusted for body mass index, educational levels, job types, and categorical pack-years.

^cModel 3 was further adjusted for diabetes mellitus, systemic hypertension, and ocular hypertension.

^dTo calculate 10-fold HR for POAG using continuous bone lead levels, we natural-log-transformed the values, excluded five participants for negative levels in tibia lead and three participants for negative levels in patella lead. Bone lead levels measured by KXRF can have negative values given that the instrument provided an unbiased point estimate that may oscillate around the true value.

^ep-Values represented trend p-values calculated by applying ordinal values (1, 2, 3, 4) to bone lead quartiles.

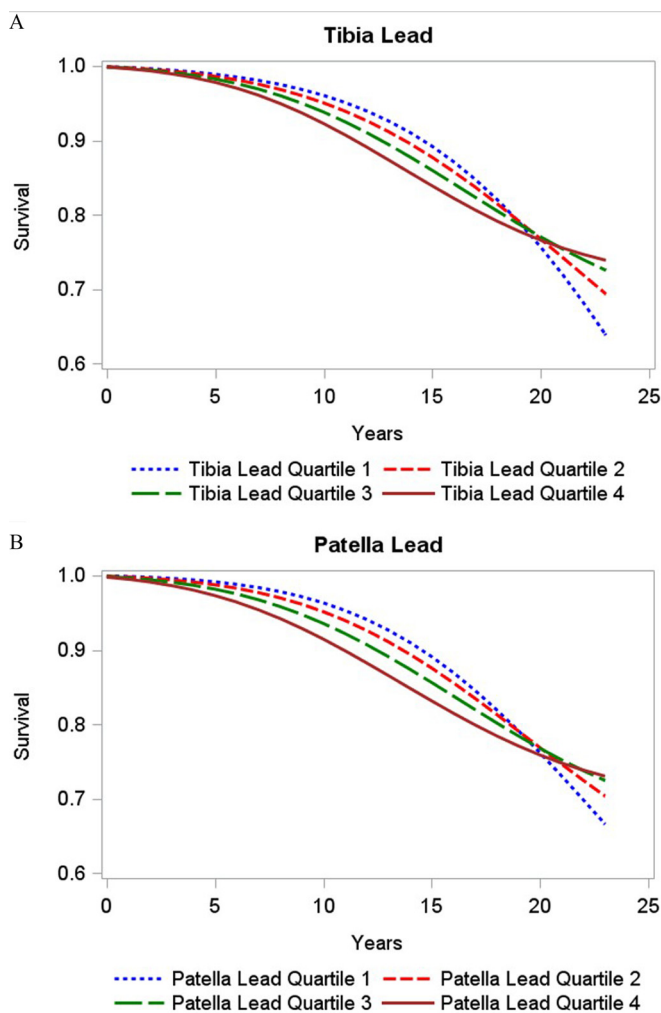


Figure 2. Adjusted survival curves illustrating changes of survival of different bone lead quartiles during follow-up. x-Axis indicates years since baseline, y-axis indicates the survival calculated by discrete-time hazard models with adjustment for baseline age, body mass index, educational levels, job types, smoking, diabetes mellitus, systemic hypertension, ocular hypertension. (A) Tibia lead; (B) patella lead.

poor indicators of cumulative lead exposure because hair lead can be greatly affected by the frequency and method of hair washing and cutting (Barbosa et al. 2005). Bone lead has a much longer half-life, which makes it a better indicator of cumulative exposure. Studies showed that more than 90% of the lead body burden is stored in bone with a half-life of years to decades: The half-life of tibia lead can be up to 48.6 y, assuming a constant decline rate (Wilker et al. 2011).

We observed a stronger association with patella lead than tibia lead. We hypothesize that this discordance may reflect the different metabolic activity of these two kinds of bones. Bones are the major storage site of lead in the body, but they are also an important source of endogenous lead (Hu et al. 1998). Lead in bone can be mobilized gradually into the plasma and transferred into other target tissues through the circulatory system (Wilker et al. 2011). Trabecular bone, such as patella bone, has a higher rate of metabolic activity compared with cortical bone, such as mid-tibia bone (Rabinowitz 1991; Wilker et al. 2011). Tibia lead slowly declines at about 1.4% per year after the cessation of exogenous exposure to lead, while patella lead follows a piecewise log-linear decline with a rapid initial rate more than twice as fast as tibia lead and then goes into a plateau (Wilker et al. 2011).

Thus patella lead may be more likely to reflect biologically available endogenous lead, which can affect the development of age-related diseases in other tissue, such as glaucoma in eyes.

The mechanisms of lead on the pathogenesis of glaucoma may involve oxidative stress. Lead can increase oxidative stress through the depletion of the glutathione and thiol pools, as well as by disrupting the antioxidant defense system (Ercal et al. 2001; Jomova and Valko 2011; Valko et al. 2016). Excessive oxidative stress and lipid peroxidation may lead to the accumulation of free radicals and their derivatives (reactive oxygen species, ROS), overwhelming the antioxidant defense system; cause the loss of cell adhesion and changes in the cytoskeletal structure of trabecular meshwork cells; induce the dysfunction of the aqueous humor drainage system; disrupt the outflow of aqueous humor from the eyeball; result in the increase of intraocular pressure; and finally, cause the development of glaucomatous neuropathy (Babizhayev 2012; Saccà et al. 2016; Zhao et al. 2016). In addition, oxidative stress may directly damage the head of optic nerves through a similar cell dysfunction mechanism that induces the development of glaucoma (Tezel et al. 2010). Our results showed that after controlling for ocular hypertension, the association between patella lead and POAG was attenuated but remained significant, suggesting that lead could directly affect glaucoma pathogenesis other than through the dysfunction of the aqueous humor drainage system.

Further investigation of the lead–gene interaction may help reveal the mechanisms of lead’s effect on POAG as well as the unclear pathogenesis of POAG. The heritability of POAG is polygenic, and usually related to genes having incomplete penetrance (Wiggs 2015). Currently known genes associated with POAG include Myocilin (*MYOC*), Atonal BHLH transcription Factor 7 (*ATOH7*), Transmembrane and Coiled-Coil Domains 1 (*TMC01*), SIX Homeobox 1/SIX Homeobox 6 (*SIX1/SIX6*), Growth Arrest Specific 7 (*GAS7*), (Abu-Amero et al. 2015), for example. These genes may interact with the lead metabolic pathways and affect the development of glaucoma. For instance, mutation in some genes such as *MYOC* may change the sensitivity of oxidative stress (Joe and Tomarev 2010), thus change the susceptibility of lead poisoning.

Our study has several limitations. Those who were eligible at the baseline might have been healthier than the original NAS cohort recruited in 1960s. We applied IPW to reduce such selection bias at the time of bone lead measurements (baseline). We also have another selection bias during the follow-up time. Follow-up time varied greatly among our participants, and as expected with an aging population, the rate of loss to follow-up was relatively high. Those who remained in the study for long follow-up may be even healthier than the baseline study sample and, consequently, may have been less susceptible to glaucoma. This was reflected by the time-varying HRs. Such selection bias could result in underestimation of the association, which means it would not change our conclusion. Besides, baseline bone lead levels may not capture the environmental lead exposure during the follow-up. Since the usage of lead in gasoline and paint has generally been banned in the United States since the 1990s, we assumed that the environmental exposure of lead largely decreased after our baseline. Thus, we hypothesized that participants’ tibia lead levels may not change a lot, while patella lead levels may reflect the gradually decreased exogenous exposures during the follow-up time. A natural limitation for costly cohort epidemiology studies is reduced power to detect age-related diseases, such as POAG; the sample size for incident cases was relatively small. We did not include family history of POAG in to our analysis, although it is an important risk factor of POAG. Family history of glaucoma was self-reported in NAS with lots of missing and uncertain descriptions. Given that no previous

study has reported that family history of POAG was associated with bone lead levels, it may not confound the lead–POAG association. NAS only recruited male veterans living in the Boston area (97% were white). Therefore, our results may not be generalizable to other populations, although the incident POAG rate in our population (15-y incidence of 7%) is comparable to other populations (i.e., the 26-y incidence of 9.7% in the Health Professional Follow-up Study) (Kang et al. 2018).

Lead is related to multiple age-related health problems such as cognitive decline (Fishbein et al. 2008), hearing loss (Park et al. 2010), cataract (Mosad et al. 2010; Schaumberg et al. 2004), and AMD (Erie et al. 2009; Hwang et al. 2015). Recent concerns about the widespread exposure of the residents of Flint, Michigan, to elevated lead levels in their drinking water was an important reminder that lead continues to be a dangerous environmental toxicant (Gómez et al. 2018; Hanna-Attisha et al. 2016; Zahran et al. 2017). Concerns are especially high in urban environments with aging infrastructure, and public awareness of lead exposure needs to be reinforced. Aging populations are at greater risk for lead toxicity given the cumulative nature of lead. Further, the older population is growing and age-related glaucoma-induced blindness will impose a huge economic burden on the whole society. In 2013, the number of glaucoma patients worldwide (40–80 y of age) was estimated to be 64.3 million, and this number is projected to increase to 76.0 million in 2020 and to 111.8 million in 2040 (Tham et al. 2014). In the United States, the National Eye Institute reported that approximately 1.9% of the population ≥40 y was suffering from POAG in 2010. The number of cases rose from 2.22 million in 2000 to 2.72 million in 2010 (National Eye Institute 2016). Glaucoma is a neurodegenerative disease; the loss of visual function is irreversible once symptomatic, and there is no cure (Cioffi and American Academy of Ophthalmology 2014; WHO 2004). In order to minimize the burden of glaucoma-related blindness, it is important to identify risk factors that can be implemented to clinical practice for presymptomatic prevention and earlier detection. Our finding contributes additional evidence on the chronic health effects of environmental lead exposure, which might help strengthen the public awareness of lead-related ocular diseases and blindness.

In conclusion, this is the first epidemiologic study indicating the association between bone lead levels and risk of POAG at a longitudinal scale. We show that bone lead may be an important risk factor of POAG in a U.S. population of men. Further studies for replication and studies in women are needed to validate our findings. We expect our study to increase the public awareness of cumulative environmental lead exposure, provide new points of view for the exploration of the pathogenesis of glaucoma, give new ideas for glaucoma interventions such as mitigating the oxidative stress consequence of lead in ocular tissues, and therefore provide new avenues to effectively decrease the global burden of blindness.

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