

**THE ASSOCIATION OF LEAD BIOMARKERS WITH HEALTH EFFECTS IN COMMUNITY
RESIDING WOMEN AND AN OCCUPATIONAL MALE COHORT**

by

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Although environmental and occupational lead exposure has decreased over the recent decades, the health outcomes associated with past lead exposure continue to be a significant clinical and public health issue. Lead is a multitargeted toxicant, that effect skeletal, cardiovascular and nervous system. Mounting evidence supports a link between lead at levels previously considered safe, to morbidity and mortality.

The objective of this dissertation was to examine association of lead biomarkers with changes in bone mineral density (BMD), incident fractures and falls, cognition and mortality. We utilized data from two epidemiological studies: a) The Study of Osteoporotic Fractures (SOF) that enrolled a population of elderly women (age 65-87), and b)The Lead Occupational study that followed a cohort of male lead exposed and control workers through middle age (40-76 years,63% exposed, 37% controls).

In the longitudinal SOF analysis, baseline total hip BMD was lower in women with high blood lead levels. The annualized rate of decline in hip BMD was greater among women with the high blood lead level, who also experienced a two-fold increased risk of fracture and falls.

In the longitudinal SOF mortality analysis, women with higher blood lead levels at baseline had increased risk of all cause, and cardiovascular mortality compared to women with

lower blood lead levels. No association with cancer mortality was found. These relationships were independent of age and shared risk factors between blood lead levels and BMD, fractures, falls and mortality.

In the Lead Occupational study, compared to controls, lead exposed workers had lower total cognitive scores cross sectionally. In longitudinal analysis, cognitive scores of lead exposed workers declined more in compared to controls. Age and important risk factors of lead exposure and cognitive change did not explain this association.

Overall, our findings provide epidemiological evidence of an association between lead exposure, morbidity, and mortality in community residing elderly women as well as a male occupational cohort. A more stringent control of lead exposure and better understanding of the mechanism of its effects may help reduce the public health burden of disease.

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1.0 DISSERTATION OVERVIEW AND OBJECTIVE

Lead exposure continues to be a public health concern. Lead is a multitargeted toxicant, effecting skeletal, cardiovascular, and nervous system. Mounting biological and epidemiological evidence supports a link between lead at previously considered safe levels to morbidity and mortality, in cross sectional as well as longitudinal studies.

The objective of this dissertation was to examine the association of lead biomarkers with changes in bone mineral density (BMD), incident fractures and falls, cognition and mortality. Specifically, the following research questions were addressed in a series of three research manuscripts:

- 1) Are blood lead levels associated with decline in BMD, incident falls, and fractures?
- 2) Are blood lead levels associated with all cause and cause specific mortality?
- 3) Is long term occupational lead exposures associated with progressive decrement in cognition, and if specific cognitive functions decline more with aging in lead exposed workers?

These research questions were investigated in two epidemiological studies: the Study of Osteoporotic Fractures (SOF) that enrolled a population of elderly women, and the Lead Occupational study that followed a cohort of male lead exposed and control workers through middle age.

2.0 INTRODUCTION

Lead is a metal and has been a pollutant for centuries[1]. Levels of lead in human skeleton have increased 1000 fold compared to pre-industrial time[2]. The first major increase in environmental lead levels occurred during the industrial revolution and the second increase occurred after leaded gasoline [1923] was introduced to improve automobile performance[3].

Over the last two decades, atmospheric lead has decreased around the globe as more nations have removed lead from gasoline[4, 5]. About 80% of gasoline sold worldwide is lead-free[4]. Other important lead sources are house paint, solders, ceramics, and water pipes[6-10]. In the United States(U.S.) although the use of lead in paint peaked in 1940 and was banned in 1978, 40% of the housing still contain leaded paint[8, 9].

Because of multiplicity of uses, lead persists in air, soil and water and enters the body mainly by ingestion or inhalation. The Centers for Disease Control and Prevention (CDC) lowered the “acceptable” level of lead in children’s blood to <10 µg/dl in 1991[11]. For workers, Occupational Safety and Health Administration(OSHA) mandates that blood lead levels be maintained below 50 µg/dl[12, 13].

2.1 BACKGROUND

Global burden of disease due to lead results in about 234 000 (0.4%) deaths and 12.9 million (0.9%) Disability Adjusted Life Years lost (DALYs)/year [14]. Worldwide, 120 million people are estimated to have lead levels of 5-10 g/dl, with similar numbers above 10 g/dl. 40% of children have blood lead levels above 5 g/dl[14]. Following control measures, lead levels have been steadily declining in industrialized countries[5, 15]. In some developing countries, where leaded gasoline is still used, rapidly increasing traffic loads have the potential to increase lead exposures[16-20]. Industrial exposure to lead, such as from smelters or battery recycling, presents occupational health burden[15, 21, 22].

The National Health and Nutrition Examination Survey (NHANES) II and the first phase of NHANES III documented decline in blood lead levels between 1976-1980 and 1988-1991[23-25]. Among individuals aged 20 to 74 years, the geometric mean blood lead level decreased from 13.1 to 3.0 $\mu\text{g}/\text{dl}$ [26]. A further decline of 41% was noted in the second phase of NHANES III conducted in 1988-1994 and the National Health and Nutrition Examination Survey conducted in 1999-2002[27, 28]. Overall, in US adults, geometric mean blood lead level have decreased from 13.1 $\mu\text{g}/\text{dl}$ in 1976-1980 to 1.6 $\mu\text{g}/\text{dl}$ in 1999-2002 [26, 27]. Currently, 99% of US adults have blood lead levels below 10 $\mu\text{g}/\text{dl}$ [27].

Blood lead levels are higher for older children, older adults, males, African Americans, and for central-city residents[26, 29]. Other correlates of higher blood lead level include low income, low educational attainment, and residence in the Northeast region of the United States[25, 26, 29]. A trend of rising mean BLL with age is reported, with an overall mean blood lead level of 2.7 $\mu\text{g}/\text{dl}$, but 3.7 $\mu\text{g}/\text{dl}$ in aged 70 and older[30].

2.1.1 Lead exposure and health effects

Lead is extremely toxic and causes metabolic, neurological and developmental damage to the body[31-36]. At high levels, it can cause convulsions, coma, or death[37]. Lower levels effects the cardiovascular, neuro-muscular and skeletal system[31-36]. Lead accumulates in the liver, kidneys and bones[38]. In women, lead has been linked to increased frequency of miscarriages, premature, low birth-weight pregnancy outcome and premature menopause[31-36] [39].

2.1.2 Lead Metabolism and Kinetics

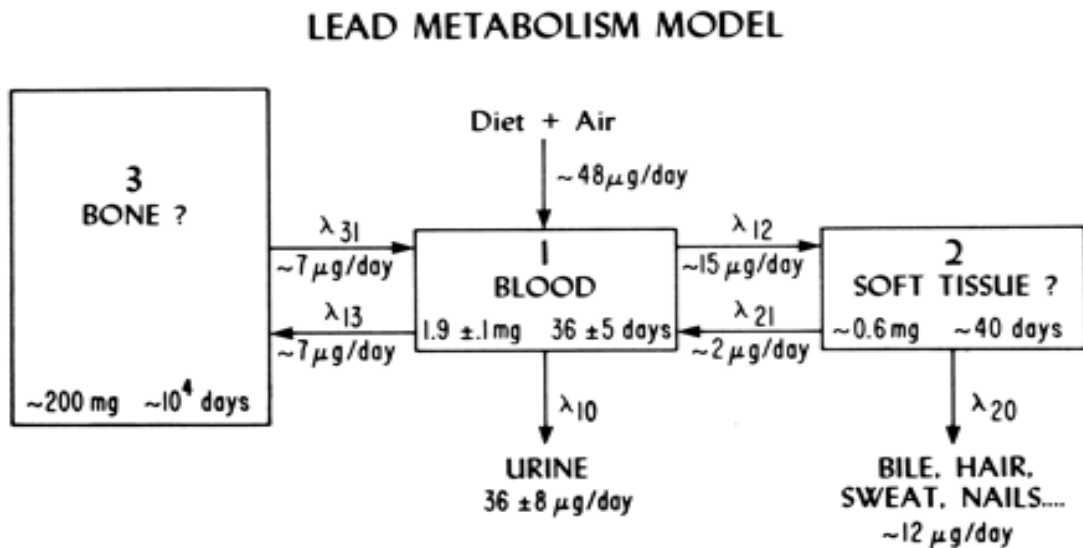


Figure 2-1 Three compartment model of lead metabolism -Journal of Clinical Investigations 1976;58:260-70(49)

After lead enters the body, it can travel along several pathways depending on subject's age, sex, nutritional status and genetic background[38]. There are three main compartments where lead distributes: blood, soft tissue, and bone (Fig 1). After being absorbed from the lungs or gastrointestinal tract, lead first enters the red blood cells

(RBCs), where it displaces zinc from the active site of various hematopoietic enzymes[40]. Blood lead represents only 1–5% of the total body burden, where it has a mean half-life of about 40 days in adult males[41]. At lower lead concentrations, 95–99% of blood lead is bound to RBCs and only 1% is in plasma. At higher concentrations, the RBCs become saturated and more lead is found in plasma.

Plasma lead can be easily absorbed into soft tissues, primarily the kidney and brain, where it exerts its most toxic effects[42]. Plasma lead can readily enter into bone, where it replaces calcium in hydroxyapatite crystals. The concentration of lead in blood reflects the equilibrium between current exposure, excretory loss and the movement of lead from bone and other deep compartments to blood[42]. Brito et al. (2005) examined exchange rates among compartments. The transfer of lead from blood to other compartments was much more rapid than the 1-month estimate reported previously, an overall half-life of 10-12 days. The blood level varies with current exposure and the magnitude of the total body burden. Mean endogenous contribution to blood lead is estimated as 10-20%, in currently employed lead workers, whereas in retired workers, all blood lead reflects past exposure[43].

2.1.3 Lead: Health Effects in Women

Lead from blood is incorporated into calcified tissues such as bone and teeth, where it can remain for 10 to 30 years[44]. Bone lead comprises 90-95% of the total body burden of lead in adults[45]. Lead is slowly released, depending on bone turnover rates, which in turn are a function of the type of bone, whether compact (slow turnover) or trabecular (rapid turnover)[46].

Bone remodeling in states of rapid turnover, such as, pregnancy, lactation, menopause and aging allow stored lead to recirculate: described as “endogenous contamination”[47]. Popovic et al. (2005) recently reported very different long-term lead kinetics between men and women, with premenopausal women appearing to retain lead more avidly or release lead more slowly compared to postmenopausal women and men[48-50].

Lead levels in bone accumulate progressively with age until middle or later life, when some decline occurs[51, 52]. The increase in blood lead levels, among recently menopausal women suggests that lead may be mobilized at rates consistent with the increase in bone loss[45, 52-54]. It has been estimated that trabecular bone loss is approximately 2-6 percent per year during the first 3 to 4 years after menopause. After that it decreases for 5-8 years and finally levels off at less than 1 percent per year. Cortical bone loss follows a similar but less rapid pattern[45, 52].

Increase in blood lead level is highest during early menopause, as bone turnover increases dramatically during the first three years, but then slows to a rate only slightly above the premenopausal bone loss rate in both Caucasian and African-American women[47]. As noted in NHANES II blood lead levels were 14.19 $\mu\text{g}/\text{dl}$ in nulliparous postmenopausal women and 12.97 $\mu\text{g}/\text{dl}$ in parous postmenopausal women as compared to 11.66 $\mu\text{g}/\text{dl}$ in premenopausal women[51, 52]. In NHANES II, age, black race, postmenopausal status, number of cigarettes smoked and alcohol use had positive association with blood lead [51].

Income and years since menopause were negatively associated with blood lead. In addition, number of pregnancies had little impact on blood lead levels among premenopausal women but among postmenopausal women, never-pregnant women had higher blood lead levels than ever-pregnant women[52, 55]. A three-way interaction was also observed where the difference between pre and postmenopausal women was

greatest in never-pregnant women who were current smokers. Postmenopausal Mexican-American women also had higher blood lead levels in the Hispanic Health and Nutritional Examination Survey (HHANES 1982-1984)[54].

2.2 OSTEOPOROSIS

Osteoporosis is the most common bone disease affecting humans, characterized by reduced bone mass with architectural deterioration of the skeleton, and increased risk of fracture[56].

In the US, approximately 20% of white women aged 50 or older have osteoporosis, which is defined as bone mineral density (BMD) greater than 2.5 standard deviations below the mean of young, healthy White women[57]. Another 35-50% have low bone mass, defined as BMD between 1 and 2.5 standard deviations below the mean[58]. Osteoporosis rates vary with ethnicity, with the highest rates in whites and those of Asian descent and the lowest rates in blacks[59]. Age increases the rates, which rise from 4% in women 50 to 59 years old to 52% for women 80 and older[57].

Osteoporosis can occur in both sexes at all ages, but often follows menopause in women[60, 61]. Although the process of bone loss begins during a woman's 30s and declines to 70% of its maximum value by the age of 80, osteoporosis occur in postmenopausal women, and the incidence increases with age[57]. Several lifestyle factors affect the risk of developing osteoporosis: nutrition, physical activity, cigarette smoking, and heavy alcohol consumption. Both calcium and vitamin D have a role in bone metabolism[62].

2.2.1 Osteoporotic Fractures

In the U.S., it has been estimated that for white women older than age 50, the risk of developing an osteoporotic fracture is nearly 40% in their remaining lifetime, with two-thirds of the fractures occurring after age 75[56]. Up to 90% of all hip and spine fractures in white women aged 65 to 84 years can be attributed to osteoporosis[63, 64]. The estimated lifetime risks of hip, vertebral, and forearm fracture for a 50-year-old white woman are 17.5%, 15.6%, and 16.0%, respectively[65].

Black women have about one-third the fracture rate of white women, a difference usually attributed to their higher bone mass[66]. Fractures have a devastating toll, resulting in higher cost and greater disability and mortality[56, 67]. Hip fractures cause up to a 20% increase in mortality within a year of the incident[67]. The pain and deformity can greatly restrict normal movement; including Activities of Daily Living (ADL).It takes a psychological toll as well: depression is common in women with osteoporosis[68, 69].

Bone remodeling is the process of bone resorption and bone formation. Osteoporosis ensues when there is an imbalance between bone removal and bone replacement.[70]. The osteoblasts promote bone formation by creating a protein matrix consisting of collagen, which is calcified, resulting in mineralized bone. Osteoclasts promote bone resorption by production of enzymes that dissolve bone mineral and proteins.[70].

Bone mass increases rapidly throughout childhood. After a slowing of bone mineral accumulation in the late teens, bone mass continues to increase during the 20s, and reaches a peak level and then starts declining. With menopause, bone loss begins to accelerate with the decline in circulating levels of 17[beta]-estradiol.

Bone loss at the spine begins about 1.5 years before the last menstrual period and totals approximately 10.5% over 8 years. Bone mass at the hip has a decline of about

0.5% per year before and after menopause, and it sustains an additional estrogen-related loss of approximately 5-7% across the menopause transition. Bone mass continues to decline after age 70 and approximately one-third BMD is lost between menopause and age 80.

The major factors influencing bone mass are age, genetics, lifestyle (including nutrition), and menopausal status. 80% of the variability in BMD is attributable to genetic factors[71]. Female children of women who have osteoporotic fractures have less bone mass than expected for their age[71]. Black women have higher BMD than white women[72]. Perimenopausal calcium requirements increase in women. By age 65, intestinal calcium absorption declines to less than 50% of that in adolescents and the renal enzymes for vitamin D metabolites (which control calcium absorption) decrease. Regular exercise is associated with increases BMD and reduced fracture risk. Prolonged bed rest, or inactivity, is associated with rapid bone loss[73].

Compared with nonsmokers, women smokers tend to lose bone more rapidly, have lower bone mass, and reach menopause up to 2 years earlier. Postmenopausal women smokers have higher fracture rates than nonsmokers[74]. Cigarette smoke interferes with calcium absorption and lowers endogenous 17[beta]-estradiol levels[75, 76]. Excessive alcohol consumption has detrimental effects on BMD and increases the risk for falls and hip fracture. Moderate alcohol consumption in women ≥ 65 years increases BMD [75].

BMD is a strong predictor of fracture risk because bone mass accounts for 75-85% of the variation in bone strength. BMD testing is the preferred method to diagnose osteoporosis and is recommended in all women 65 years of age. Dual-energy x-ray absorptiometry (DXA) is the technical standard for measuring BMD[77]. Results are reported as standard deviations, Z score or a T score. A Z score is based on the standard deviation (SD) from the mean BMD of a reference population of the same sex, ethnicity, and age[78]. A T score is based on the mean peak BMD of a normal, young adult

population and is expressed in terms of standard deviations from the average value of this reference population.

Every decrease of one standard deviation from age-adjusted bone density represents approximately a 10-12% change in BMD and an increase in the risk of fracture by a factor of approximately 1.5. In general, postmenopausal women lose about 0.5 in standard deviations from the mean in both T and Z scores every 5 years. In 1994, the World Health Organization (WHO) defined osteoporosis as a BMD T score below -2.5 SD based on measurements of any skeletal site[60].

2.3 LEAD AND BONE

2.3.1 Biological Evidence

Skeleton sequesters 95% of stored lead in different bone cells such as osteoclasts, osteoblasts, osteocytes, and growth plate chondrocyte[46]. However, most studies on the toxic effect of lead fail to address its effects on the skeleton itself[79]. The skeleton is maintained through a balance between osteoclastic resorption and osteoblastic bone formation[61]. Low serum level of calcium stimulates parathyroid hormone release which acts on osteoblasts to secrete a factor that activates osteoclasts[46]. Osteoclastic bone resorption restores the calcium levels; however, to maintain an appropriate skeletal mass, the lacunae created by the osteoclasts must ultimately be refilled with bone. Bone formation within the lacunae is mediated by osteoblastic activity[80].

Lead exposure can interfere with bone and calcium metabolism in several ways[46, 81]. Lead and calcium have similar physical and chemical characteristics and follow similar metabolic pathways[82]. In the skeleton, lead is contained in the mineral matrix, in close

chemical association with calcium and phosphate[44]. Lead can compete with calcium in intestinal absorption and deposition into bone, regulated by regulated by parathyroid hormone (PTH), 1, 25-dihydroxyvitamin D, and other factors[46]. Increases in serum PTH and 1, 25-dihydroxyvitamin D have been reported in occupational lead exposure[49, 79]. Bone resorption by PTH over a long period may lead to serious bone decalcification and may increase the risk for osteoporosis[83].

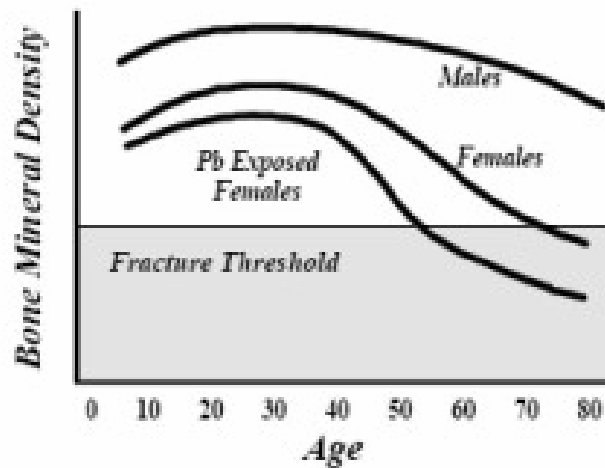
Studies indicate that lead exerts both direct (osteoblast and osteoclast function) and indirect (kidney dysfunction) actions on bone turnover[80]. Since skeletal mass is decreased in lead intoxicated animals and humans, bone formation must be decreased relative to the level of bone resorption. Studies on isolated normal cells and in vivo experiments show that lead inhibits secretion of osteonectin in ROS 17/2.8 cells and affects osteoblast cell proliferation[81, 84]. Lead intoxicated bone, is resistant to osteoclastic bone resorption[85].

There is evidence that lead can cause osteoporosis in animal models[86]. Animal studies have shown an association between increased bone lead concentration and lower bone mass and decreased mechanical strength[87, 88]. Lead induced inhibition of axial bone development and a decrease in bone mass, produced by enhanced resorption, and increase in bone mass, produced by lead accumulation in bone[89]. Osteopenia is induced by long-term, low level and high-level exposure of the adult rat to lead[90]. Findings show that lead is incorporated into bone mineral after only 1 month of exposure to low lead (0.01%, 100 ppm), with significant osteopenia after 12 months of exposure; high lead (0.5%, 5000 ppm) caused osteopenia at 1 months. No compensatory mechanism was elicited to maintain bone mass[90].

2.3.2 Epidemiological Evidence

In growing children, lead exposure can stunt bone growth by affecting endochondral bone formation[79, 91-93]. In the NHANES II blood lead level was inversely related to growth in height, weight, and chest circumference in children[94]. In a follow-up study of children who had elevated postnatal blood lead levels, lower height was attained at 18 months of age[95].

Endogenous lead release occurs during activation of the skeleton in menopause[52]. Cortical bone, which contains the greatest amounts of lead, its remodeling at the menopause could compromise mechanical support, predisposing to fractures. The effect of lead on growing bone and adult remodeling could predispose to osteoporotic fractures. A hypothetical "Lead-exposed female" never reaches the peak bone density of her unexposed female counterpart because of compromised growth plate chondrocyte activity. At menopause, activated bone turnover and an exposure to released lead would accelerate the decline in bone density such that the "Lead-exposed female" would cross over a fracture threshold earlier.



Source: Nutrition and Bone Health, 2004

Figure 2-2 Model of Lead exposure and BMD change and fracture threshold .Source Nutrition and Bone Health 2004

2.4 LEAD AND FALLS

One third of people aged 65 and older fall each year, and this proportion increases to one half by 80 years of age. Falls account for 87% of the fractures, and fall related injuries for 5.3% of all hospitalizations at age 65 and older[96]. Psychological impact of falls result in disability, fear of future falls, restriction of activities and mobility, and nursing home admissions[68, 97].

Contributing factors for risk of falls include female sex, selected chronic diseases, medications and disabilities[98-100]. Epidemiological studies in community dwelling elderly have shown higher prevalence rates of 22% in women, to 7% in men. Functional status indicators are also associated with falling. Slower walking speed is a risk factor for

recurrent falls and also a predictor of hip fracture[101]. Impairment of leg extensor muscle strength, grip strength, balance and gait are risk factors for falls[101].

Loss of pressure sensitivity (peripheral neuropathy) is associated with reduced balance. Impaired vision and hearing may increase the risk of falls in older individuals[96, 102]. Hearing sensitivity may be adversely affected by osteoporotic bone loss. Those with hearing loss may be more likely to have impaired balance and thus be more prone to falls and fractures. Hearing sensitivity has been associated with decreased vestibular function[103-108]. Poor balance and postural instability is increased in individuals with lead who also have peripheral neuropathy.[109]

Lead exposure could predispose to fractures because of increased risk of falls as it effects neuromuscular function and balance[110, 111]. Lead exposure in childhood has been associated with impaired maturation of postural balance and central auditory processing[112-114]. Lead slows the nerve conduction velocities in peripheral nerves which effects motor coordination, perception of vibration, light touch and position of the joints[113, 115, 116].

Muscle is one of the soft tissues where lead is deposited in addition to skeletal, nervous and renal tissues. Muscle weakness and easy fatigue occur early and may be the only symptoms of lead exposure[114]. The muscle groups usually involved are the most active ones, such as the extensors of the forearms, wrist and fingers[117]. Blood lead levels as low as 8 µg/dl were associated with poorer performance on tests of visual perception, manual dexterity and psychomotor speed[118].

2.5 LEAD AND MORBIDITY

Lead is a multitargeted toxicant, effecting cardiovascular, renal, and nervous systems and may contribute to a fraction of associated morbidity and mortality.

2.5.1 Cardiovascular morbidity

Biological evidence

Lead-fed animals have increased vascular reactivity to norepinephrine[119]. Picomolar concentrations of lead have been shown to activate protein kinase C, a major regulator of vascular tone. Increases in blood pressure and renal damage have been observed after induction of lead exposure in rodent models[120].[121] .

Epidemiological evidence

In adults, the cardiovascular (CVD)system is very sensitive to lead exposure[131]. Research indicates that blood lead levels <10 µg/dl are associated with peripheral arterial disease, impaired renal function, and elevated blood pressure[132, 133]. Hypertension associated with lead exposure has been documented in occupational cohorts as well as in general population[132].[134-141] In one study, blood lead levels were non-significantly higher in incident cases of ischemic heart disease and stroke than in non cases, although the number of events was smaller (n=316 and 66 cases of ischemic heart disease and stroke, respectively) than in the NHANES studies[140]. The average blood lead levels in the study were also relatively high (mean 15.3 µg/dl). In a meta-analysis of 15 studies, a 5-µg/dl increase in blood lead was associated with an increase in systolic blood pressure of 1.25 mm Hg[142]. An association of elevated blood pressure due to lead with risks for more serious cardiovascular events has not been evaluated.

2.5.2 Lead and Carcinogenesis: Biological evidence

Based on biological evidence, mechanisms of lead carcinogenesis include mutagenicity, tumor promotion and cellular proliferation[122]. Lead is a weak mutagen and can disturb DNA synthesis or repair[123, 124]. Lead may exert diverse toxic effects on cells, disrupting the ability of cells to develop appropriate response to genotoxic agents. Lead enhances the mutagenicity of radiation and other carcinogens[123, 124].

The National Toxicology Program classifies lead as “reasonably anticipated to be human carcinogens. “The International Agency for Research on Cancer (IARC) rates lead as possibly carcinogenic to humans (2004)(Group 2B)[125-128]. The Environmental Protection Agency uses a similar classification scheme to that of IARC for lead.

Epidemiological evidence

Most of the evidence on the relationship between lead exposure and cancer comes from review of 8 studies of lead exposed workers by Steenland and Boffetta in 2000[123, 124, 129, 130].The relative risk of developing cancers of brain, stomach, lung, colon and kidney were higher with higher lead exposure. Increased risk of cancer mortality was observed in individuals with >20 µg/dl blood lead levels in NHANESII[123], this risk was not observed at levels <10 µg/dl[123].

2.6 LEAD AND MORTALITY

2.6.1 Epidemiological evidence

Several occupational studies report the association of lead exposure and mortality. Gerhardsson et. al reported increased all-cause mortality in lead-exposed workers[143, 144]. McDonald and Potter reported increased all-cause, cardiovascular and cerebrovascular deaths in a study of 454 children longitudinally (hospitalized for lead poisoning between 1923 and 1966)[145]. Moller and Kristensen documented association of blood lead with all-cause mortality in a population-based survey in Copenhagen, Denmark[146].

Lustberg et al analyzed data from NHANES (1976-1980) and reported that individuals with blood lead level 20 to 29 µg/dl experienced 46 % increased all cause, 39% circulatory disease, and 68% mortality due to cancer relative to those with < 10 µg/dl. Analysis of cause specific cancer mortality indicated that blood lead was associated with mortality due to lung, stomach and kidney cancer(N=9250[123, 147].

Schober et al. analyzed mortality data for individual's ≥ 40 years of age in the NHANES III (1988-1994)[147]. Blood lead levels 5-9 µg/dL were associated with 24 % increased risk of death from all causes, 20% increased cardiovascular , and 44% increased cancer mortality(N=9757). Menke et al documented 25% increased all cause and 55% increased cardiovascular mortality in NHANES III (1988-1994) data at blood lead levels of >3.62 µg/dl as compared to those with <1.94 µg/dl[148]. However they did not observe an association between blood lead and cancer mortality in this range of exposure. This increased mortality effected non-Hispanic whites, non-Hispanic blacks, and Mexican Americans and both males and females (N=13,946)

2.7 LEAD AND COGNITIVE EFFECTS

Adults with occupational exposures have neurologic deficits at blood lead levels of 50 μ g/dl[149-151]. The older brain may be especially vulnerable to the effects of lead[30, 152]. Recent studies have begun to examine the relation between relatively low levels of lead exposure and cognitive function in community-dwelling older adults[153].

2.7.1 Peripheral nervous system

Epidemiological and biological research has associated lead with peripheral neuropathy, axonal degeneration and impaired nerve conduction velocity[154-156]. In the human peripheral nervous system, the motor axons are the principal target of lead[157-160]. Perception of vibration, light touch and position of the joints is effected when lead damages these fibers. Lead exposure may cause muscle weakness and easy fatigability. The most active fibers affected are the extensors of the forearms, wrist and fingers and the extractor muscles. Lead may be taken up from muscle by motor axons and transported back to nerve cell bodies in the spinal cord, where it may damage vital cellular processes. At lower levels of lead exposure, motor nerve conduction velocity, and coordination is decreased.

2.7.2 Central nervous system

Diminished neuropsychological performance has been observed in lead exposed workers[156]. Visual intelligence and visual motor coordination is affected[153, 161]. Lead disrupts the blood brain barrier and increases permeability to the free lead ion[162]. Lead accumulates in distinct anatomical regions: Hippocampus, frontal cortex or disturbs

neurotransmitters in brain. Lead effects upon the CNS may be mediated by disturbing mitochondrial energy metabolism, which then influences other metabolic processes[162, 163]. Lead disrupts oxidative phosphorylation in mitochondria also disrupts cytochrome metabolism[164-169].

Epidemiological studies report an inverse association between different measures of cumulative lead exposure and tests of cognitive function in both occupational cohorts and general population[35, 170-173]. One of the general population studies found that blood lead levels as low as 8 µg/dl were significantly associated with impairment on several neuropsychological tests among a cohort of rural women but not urban women[118].

Occupational studies have reported the relationship of cumulative exposure as measured by bone lead with cognitive decrement[153]. Acute exposures to high levels of lead among adults has been associated with deficits in performance on visuospatial skills, motor function, reaction time, memory, spatial, attention and concentration[174-177]. Fewer studies have examined the association between cumulative lead exposure over longer time and cognitive function in these populations partly because of difficulty in obtaining good biomarkers for cumulative exposure[150].

More recent studies have examined this issue using K-XRF technology to measure lead in bone such as tibia and patella[178-180]. In general, the cognitive effects persistently associated with higher bone lead levels are executive function/attention and visuospatial /visuomotor tasks, although associations with verbal and memory tasks are also noted[174, 180]. The studies that analyzed cognitive performance over time, document inverse association between tibia lead levels and, decreased performance on executive function/attention and manual dexterity[181, 182].

The mechanism underlying the effects of lead on nervous system have been of more recent interest. Lead accumulates in astroglia, which are essential for maintenance of neuronal environment[183]. Lead exposure can interfere with several calcium dependent processes[162].

Magnetic Resonance Imaging (MRI) studies have documented the association of higher tibia lead with increased prevalence and severity of white matter lesions and smaller structure-specific brain volume [184]. Cerebral white matter changes on MRI are associated with higher bone lead levels and psychomotor slowing as measured by Grooved Pegboard test [178, 184, 185]. Tibia lead has been associated with the strongest effects on verbal memory and learning, visual memory, and executive function which may indicate disruption of widely distributed neural networks involved in the integration of functions and would be consistent with lesions to cortical association areas[184].

Adult exposure to lead could accelerate age-associated changes in white matter[186]. Myelination continues into the fifth and possibly sixth decade of life in selective brain regions (e.g., inferior temporal, prefrontal, and temporoparietal regions)[187]. White matter produced later in life around cells with long but small caliber projections in cortico-cortical association areas may be sensitive to oxidative stress[188]. The significant associations of tibia lead with the parietal white and gray matter, temporal white matter, suggest that lead accelerates an age-associated process in selected brain regions[188, 189]. No significant associations were observed in regions where myelination occurs early in life (e.g., occipital lobe and cerebellum) and where short axonal projections are relatively common[189].

High blood pressure is also correlated with number of white matter lesions and total brain volume[184, 190]. Lead exposure is a risk factor for hypertension, which may cause cerebrovascular disease leading to poor performance on cognitive tests[191-193].

Decreased ratio of N-acetylaspartate (NAA) to creatine, a marker of neuronal density in hippocampus and frontal lobe as measured by Magnetic Resonance Spectroscopy (MRS) neuroimaging studies, is associated with higher bone lead levels and deficit on attention / executive function, visuospatial /visuomotor functioning and short term memory[194]. Lead induces neurofibrillary tangles in frontal cortex and hippocampus, and is selectively toxic to limbic system, that modulates behavior, emotions, learning and memory[195-197].

Lead and homocysteine are both associated with cardiovascular disease and cognitive dysfunction[186]. An association between blood lead and homocysteine has been documented which suggest that homocysteine could be a mechanism that underlies the effects of lead on the cardiovascular and central nervous systems, possibly offering new targets for intervention to prevent the long-term consequences of lead exposure[186, 198]. More advanced imaging techniques such as FLAIR, structural, functional, CT, MRI, SPECT,PET, MRS will likely advance understanding of how lead affects brain.

2.8 LEAD BIOMONITORING

The laboratory-based methods include direct determinations of lead in blood and in urine and components of the heme biosynthetic pathway, which is inhibited by lead. Assessment of recent exposure is achieved by direct measurement of lead in blood[199]. Blood lead levels can be reliably measured at concentrations as low as 1µg/dl using atomic absorption spectrophotometry[200-203]. Small hand held instruments for rapid direct measurement of blood lead in field are also available. The cumulative time-integrated blood lead index reflects both the duration and intensity of lead exposure.

Measurements of the heme precursors free erythrocyte protoporphyrin (FEP) and erythrocyte zinc protoporphyrin (ZnP) have also been used for screening purposes. A more accurate picture of the overall lead body burden can be provided by the provocative chelation test: calcium disodium ethylene-diamine-tetraacetic acid (CaNa₂EDTA), lead is mobilized from the soft tissues and excreted in the urine, where it is quantified by atomic absorption. Historically, wet chemical digestion of bone followed by atomic absorption-based measurements was done on cadaver or bone biopsy samples. Measurement of tooth lead is performed on shed primary teeth to assess lead exposure in children.

X-Ray Fluorescence

Over the past two decades, the technique of x-ray fluorescence (XRF) has emerged as a noninvasive method for bone lead determination, enabling direct in vivo measurements of skeletal lead. XRF occurs when absorption of a high-energy photon by a heavy metal atom induces the emission of a second x-ray photon of slightly lower energy. A typical x-ray fluorometer designed for in vivo measurements features a radioactive ¹⁰⁹Cd source, which emits at 88.035 keV, and a germanium crystal detector arranged in backscatter geometry (i.e., the detector is mounted behind the source). During a typical in vivo bone lead measurement, the bone is irradiated for 30–60 min, and the generated photons are collected and counted. This yields a measurement of bone lead in units of µg lead/g of bone mineral. The best site for assessment is a large dense bone with little overlying tissue; therefore, the tibia is preferred.

Bone lead as marker for cumulative exposure was established in heavily exposed workers. A time-integrated cumulative blood lead index was calculated from the recorded blood lead values and correlated to measurements of bone lead for four studies that ranged from 0.67 to 0.88. A time-weighted average blood lead was calculated to reflect the intensity of the exposure, and bone lead was measured in the tibia and calcaneus of

91 active lead workers: tibia lead concentration is related to both the duration and the intensity of exposure, whereas trabecular calcaneus bone reflects only the intensity. Trabecular bone lead is rapidly exchangeable bone lead compartment, in contrast to cortical lead.

2.9 OCCUPATIONAL LEAD EXPOSURE

Workers in smelters, refineries and other industries may be exposed to high levels of lead. Lead dust may be breathed in and can also cling to skin, hair, clothing, and vehicles, and be carried to the home, exposing workers' families. According to estimates made by the National Institute of Occupational Safety and Health (NIOSH), more than 3 million workers in the United States are potentially exposed to lead in the workplace.

Occupational exposure to lead in general industry is regulated by the 1978 Occupational Safety and Health Administration (OSHA) Lead Standard. The general industry standard specifies permissible limits on airborne lead exposure, as well as BLL. For workers in the United States who are covered by the OSHA lead standard, a detailed medical examination is delineated under specific conditions.

Any general industry worker (i.e., battery, foundry, smelting, mining, glass, ceramics) found to have a single blood lead level of 60 µg/dl or greater, or an average blood lead level of 50 µg/dl or greater must be removed from the high-exposure job (termed "medical removal protection"). The "removed" worker should subsequently receive more frequent medical evaluation and blood testing. A worker is not allowed to return to a job with the potential for high lead exposure until his or her blood lead level has fallen below 40 µg/dl on two successive tests.

As the skeleton is a major storage site for lead accumulation (90–95% of the total body burden), in lead workers, this fraction may be even higher. For example, skeletal lead content in lead workers is 1 g compared with 100 mg in non-occupationally exposed persons and a few milligrams in prehistoric persons. If this pool of bone lead is mobilized rapidly, it may constitute a health risk by impacting blood lead levels, especially among workers exposed long term to lead. Hence, bone lead is a valuable biomarker in epidemiologic studies.

2.10 LIMITATIONS OF THE EXISTING EPIDEMIOLOGICAL LITERATURE

Although several lines of evidence support a link between lead biomarkers and multiple health outcomes, the underlying mechanisms are not understood. Reports have generally focused on cognitive effects in children and occupational exposure in men. Less is known about the effects of lead exposure in women in occupational as well environmental settings and chronic disease outcomes. Epidemiological studies conducted at population level (NHANES) have not used the state of the art XRF-technology to associate the cumulative lead levels to health effects. In the existing literature, blood lead levels have been used, which may not measure cumulative exposure at population level.

Some of the methodological weaknesses in earlier research were sampling inadequacies, selection bias, and variation in data collection procedures, reliance on current indices of lead exposure rather than cumulative exposure indices, inaccurate measurement of exposure and / or dose, inadequate sensitivity of the neuropsychological tests used for detecting central nervous system effects.

Occupational studies grouped individuals as exposed or non –exposed, but the non exposed volunteers were often not screened for prior lead exposure. The exposed

and non-exposed were often not comparable with respect to demographic and pre-morbid characteristics. Bias could have been introduced in these occupational studies because of non blind examiners. Many studies did not include important confounders of lead exposure, e.g., socio-economic status, age, and education. Finally, inaccurate estimates of lead exposure, or about other confounding variables such as alcohol consumption, would bias the study towards finding association.

Studies of chronic lead exposure rely either on determinations of current blood level or historical reconstructions of exposures from past blood levels. To avoid the limitations of cross-sectional studies comparing BLL, a prospective repeated-measures design is needed. Specifically, baseline XRF analysis of lead in bone and BLL at baseline and at selected intervals should be performed. Bone densitometry should ideally be performed at the same intervals to determine bone turnover.

**3.0 THE RELATIONSHIP OF BLOOD LEAD LEVELS TO RATES OF DECLINE IN
BONE MINERAL DENSITY AND INCIDENT NON-SPINE FRACTURES AND FALLS IN
WOMEN: THE STUDY OF OSTEOPOROTIC FRACTURES**

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3.1 ABSTRACT

BACKGROUND: Lead disturbs neuromuscular functions, affects bone turnover, and decreases growth and stature in children. Lead is stored in the skeleton; post-menopausal women have higher Blood Lead Level (BLL) than pre-menopausal women. Whether lead is associated with bone loss and fracture and falls risk in older women is not established.

METHODS To test the hypothesis that women with higher BLL experience faster rates of decline in bone mineral density (BMD), and higher falls and fracture rates, we measured BLL in 533 white women aged 65-87 years in 1990-91 by atomic absorption spectrophotometry. Total hip BMD was measured twice by dual energy x-ray absorptiometry 3.3 years apart. Information on falls was collected every 4 months for 3 years. Incident non-spine fractures were identified over 10 years of >95% complete follow-up. Fractures were confirmed by radiographic report. We used ANCOVA to compare BMD and annualized (%) decline in BMD across three categories of BLL ($\mu\text{g}/\text{dl}$): low (≤ 3), medium (4-7) and high (≥ 8). Proportional hazards models were used to calculate the Hazard Ratio (HR) and 95% Confidence Intervals (CI) of fracture; the low BLL category formed the referent group. Poisson Regression with Generalized Estimating Equations was used to calculate multivariate adjusted fall Incidence Rate Ratios (IRR) and 95% CI. We adjusted for age, physical activity, calcium and Vitamin D use, history of fractures, maternal history of fractures, cognitive function, and baseline BMD.

RESULTS: The mean blood lead level was $5.3 \mu\text{g}/\text{dl} \pm 2.3$ and ranged from 1-21 $\mu\text{g}/\text{dl}$. Women in the high BLL category were older, had lived more years after menopause, and had lower body weight, height, and body mass index (BMI) than women with low BLL. Baseline total hip BMD was 7% lower in high BLL category compared to low BLL ($p < 0.02$). The annualized rate of decline in hip BMD was 3 times greater among women with the

high BLL. Women with high BLL had a 74% increased risk of fracture (HR = 1.74; 1.01-2.98) and 87% higher risk of falls (HR = 1.24; 1.24-2.82), compared to women with low BLL, independent of BMD and other covariates.

CONCLUSIONS: High BLL were associated with low baseline BMD, greater rate of decline in BMD, and increased risk of falls and fractures. This association was found at blood lead levels previously thought to be safe. Further reductions in BLL could lead to a decrease in falls and osteoporotic fractures.

3.2 INTRODUCTION

The skeleton is the major repository for lead within the body, sequestering up to 95 percent of lead and can be an endogenous source of lead for many years after exposure.[1] Lead may be mobilized from skeletal stores during conditions of high bone turnover, such as pregnancy, lactation, menopause and aging[2]. Indeed, blood lead levels are higher in postmenopausal women in comparison to premenopausal women[2, 3]. Lead is contained in the mineral matrix of bone in close association with calcium, sharing many physical and chemical characteristics and intracellular pathways. Lead can compete with calcium in intestinal absorption and deposition into bone. Analogous to calcium, lead absorption from the intestine is regulated by parathyroid hormone (PTH), 1, 25-dihydroxyvitamin D, and other factors. Studies indicate that lead may exert both direct (osteoblast and osteoclast function) and indirect (kidney dysfunction) actions on bone turnover[4]. In addition to effects on bone, lead exposure could predispose to fractures because of increased risk of falls as it affects neuromuscular function and balance. Lead

exposure in childhood has been associated with impaired maturation of postural balance and central auditory processing[5, 6].

In the current study, we examined the association between blood lead levels and rates of decline in BMD, falls and fractures in a subset of 533 women enrolled in the Study of Osteoporotic Fractures (SOF). We hypothesized that women with higher blood lead levels will experience faster rates of bone loss and a greater risk of falls and fractures.

3.3 METHODS

3.3.1 Study Population

The Study of Osteoporotic Fractures (SOF) is a longitudinal cohort study that enrolled 9704 white women from 1986 to 1988 using population-based listings in Baltimore, MD; Minneapolis, MN; Portland, OR; and the Monongahela Valley near Pittsburgh, PA. To be eligible to participate, women had to be aged 65 years or older and ambulatory.

The lead ancillary study was conducted in 1990-1991 in 533 white women aged 65-87 years enrolled in SOF at either the University of Pittsburgh or University of Maryland clinics. Initially, we examined the correlates of blood lead and the association of blood lead level to cognitive function [7]. We found a relationship between higher blood lead levels and worse cognitive function as measured by the part B of Trailmaking Test, but this association was confined to the rural SOF clinic [7, 8]. In the current paper, we extend the lead study to outcomes of bone mineral density, rates of decline in BMD and incident fractures and falls. The protocol and consent forms were approved by the institutional

review boards at the participating institutions. All women provided written informed consent.

3.3.2 Bone Mineral Density

Bone mineral density (BMD) was defined as the amount in grams of bone mineral content divided by the region of interest in centimeters squared. BMD of total hip and femoral neck were measured twice at the second (1988-1990) and fourth examination (1993-1994) by DXA using Hologic QDR 1000 scanners (Bedford, Mass) an average of 3.5 years apart. Calcaneal BMD was measured at baseline (1986-1988) and at fourth visit (1993-1994), with OsteoAnalyzers (Siemens-Osteon, Wahiawa, HI) using single photon absorptiometry at the baseline examination and single X-ray absorptiometry (Osteoanalyzer, Dove Medical Systems) at the fourth examination (mean follow-up=5.7years). Annual percent decline in BMD was estimated for the femoral neck, total hip, and calcaneus. Details of the measurement and densitometry quality-control procedures have been published [9]. A random sample of scans was reviewed by technicians at a quality-control center. To assess longitudinal performance of the scanners, a spine phantom was scanned daily and a hip phantom was scanned once per week at each clinic.

3.3.3 Other Measurements

Potential confounders of the association between blood lead levels and outcomes of interest (BMD, fractures and falls) were identified and adjusted for. Sociodemographic factors (age, study site/clinic, education), smoking history, alcohol consumption (past 30 days), and physical activity from walking, medication use [including hormone therapy, Thiazide diuretics, systemic corticosteroids, calcium supplements, vitamin D supplements,

Central Nervous System (CNS) active medications: benzodiazepines, antidepressants, anticonvulsants and narcotics], and time since menopause, number of children breastfed, health status compared to others, history of fractures after age 50 and history of maternal fractures, incident falls over the previous one year were determined by an interview-administered questionnaire.

Health impairment data included cognitive impairment, assessed as scores on Trailmaking Part B, functional status impairment, and depression as 6 or more depressive symptoms on the 15-item Geriatric Depression Scale Shortened. Prevalent chronic conditions such as diabetes, hypertension, arthritis, stroke, Parkinsonism, COPD, angina were defined as self-report of the condition previously diagnosed by a physician. Height and weight were obtained using a Harpenden stadiometer (Holtain Ltd, Crymych, UK) and a standard balance beam, respectively, and body mass index (BMI) was calculated as weight divided by height squared (Kg/m^2). Systolic and diastolic blood pressure was measured by manual mercury sphygmomanometer. Physical function was assessed including grip strength measured as average of right and left using an adjustable hand grip dynamometer in Kg (Preston Grip Dynamometer; Takei Kiki Kogyo, Tokyo, Japan), walking speed (second /6 meters), use of arms for 5 chair stands, corrected visual acuity, static balance as the ability to hold a standing tandem position for 10 seconds with eyes closed.

3.3.4 Fracture Ascertainment

After the initial enrollment visit, all women were contacted by either postcard or telephone every 4 months to determine whether any fractures had occurred in the preceding 4-months. After more than 10 years, fracture follow-up remains over 95% complete. Women who reported a fracture were interviewed by telephone about the circumstances

under which the fracture occurred. Fractures occurring because of a major trauma, e.g., motor vehicle crash were excluded. Women could report having more than 1 fracture. All fractures were confirmed by radiographic report. The time to first fracture was calculated as time from the baseline BMD measure to the event.

3.3.5 Falls

Data were prospectively collected on the number of falls using a series of postcards mailed every 4 months for 3 years following baseline visit. On each postcard, participants were asked if they had fallen in the past 4 months and, if so, how many times. Participants were contacted by phone when postcards were not returned. Follow-up on postcard mailings were 95% complete.

3.3.6 Blood Lead Measurements

A 5.0 ml sample of whole blood was drawn into Vacutainer tubes (BD Vacutainer Systems, Rutherford, New Jersey). Blood samples were analyzed at the Clinical Chemistry Laboratory of the University of Maryland, certified for the analysis of lead in blood by the Occupational Safety and Health Administration and Centers for Disease Control and Prevention, and documents a lower limit of detection for lead of 1µg/dL. BLL were determined by graphite furnace atomic absorption spectrometry (AAS model 5100, HGA with Zeeman Effect background correction: Perkin Elmer, Norwalk, Connecticut). To investigate intralaboratory variability in both the measurement of blood lead level and the stability of blood lead levels over 1year in this population, additional blood samples were collected from a random sample of participants in the Study of Osteoporotic Fractures[7].

The sample distribution was selected to represent the two clinic distributions of blood lead analyzed at the laboratory. Two tubes of blood were drawn from each of the 100 randomly selected women (50 women from each clinic) for this purpose during a later clinic visit. The laboratory was blind with respect to the second blood sample and therefore the intralaboratory variability in both the measurement of blood lead level and the stability of blood lead levels over time was determined. The intraclass correlation coefficient for the duplicates was 0.88. Mean values of 4.76 $\mu\text{g}/\text{dl}$ (range, 1-13 $\mu\text{g}/\text{dl}$) and 4.67 $\mu\text{g}/\text{dl}$ (range, 1-12 $\mu\text{g}/\text{dl}$) were obtained for the first and second determinations, respectively.

3.4 STATISTICAL ANALYSES

All analyses were performed by categorizing the study participants into three groups of blood lead level (BLL) corresponding to the upper and lower 15th percentiles of the distribution of blood lead variable. Thus the three groups were: low ($\leq 3 \mu\text{g}/\text{dl}$, n=122), medium (4-7 $\mu\text{g}/\text{dl}$, n=332), and high($\geq 8 \mu\text{g}/\text{dl}$, n=79)[7].

Chi-square test was used to test baseline characteristics for categorical variables by BLL status; ANOVA was used for continuous variables. Two-tailed p-values were used for all tests, at 5% statistical significance. Fracture incidence rate was calculated per 1,000 woman-years of follow-up for each BLL group. We used Cox proportional hazard regression analysis to estimate the relative risk (RR) of fracture and 95% confidence interval (CI). We plotted the cumulative hazard of fractures in the three BLL groups over the follow-up period by Kaplan- Meier Survival graphs. For each blood lead level category, the relative risk (RR) of falls was computed using Poisson regression models with generalized estimating equations (GEE) to adjust standard errors for correlated data

points at 4-month intervals. A Huber White Sandwich estimator of variance was used with GEE to construct valid standard errors. The RR was computed as fall rate (average number of falls per 4 months) in a specific category of blood lead level compared to the lowest or the referent category.

In multivariate models, we simultaneously analyzed blood lead levels and other potential risk factors. We included variables that were significantly associated with respective outcomes (BMD, fractures, and falls) or blood lead levels either reported in the literature or from preliminary univariate analysis. A forward stepwise selection process was used to add or remove potential covariates from the multivariate regression models (exit criteria: $p \geq .15$). We organized variables into 9 related groups, including 1) demographic factors :age, education, clinic, 2)anthropometric measures : height ,weight, BMI, weight change, weight loss since age 50, 3) lifestyle factors: smoking, alcohol intake past 30 days, walk for exercise, 4) physical functions: average grip strength, walk speed, use arms to stand up from chair, 5)health status: health compared to others, depression, functional limitation, 6)functional performance: Trailmaking part B, depression, 7) self reported medical history: hypertension, diabetes, stroke, angina, 8) medication and supplement use: hormone therapy, Vitamin D, calcium and thiazide diuretic, 9)reproductive factors: years after menopause, ever breastfed. Sequential models were analyzed in which all variables in one group were allowed to enter in a stepwise regression based on p- value at each step. The next model began with the significant variables remaining from the previous model. Then a new group of variables was allowed to enter, using the stepwise entry procedure, for successive groups of variables, until a final model of significant variables was selected. We computed overall and age-adjusted outcomes and 95% confidence intervals (CI).

In multivariate models, age, education, years since menopause, number of children breastfed, BMD, body mass index, height, weight, weight change/yr from v4 to baseline for calcaneus and from v4 to v2 for total hip and femoral neck, alcohol intake, walking speed, grip strength, calcium intake, total kcal/week from exercise, Trailmaking Part B, corrected visual acuity were entered into the models as continuous variables. All other variables were categorical: clinic, smoking (never/former/current), walking for exercise (yes/no), functional impairment (yes/no), two or more falls in the year before baseline (yes/no), oral estrogen, vitamin D use, thiazide diuretic, steroid use (current/former/never), and arthritis (yes/no), fractures after age 50(yes/no), health compared to other(good/excellent vs. fair/poor/very poor) and history of fracture in the mother(yes/no), tandem stand with eyes closed(poor/good) physician diagnosis of COPD, arthritis, hypertension, diabetes, stroke, angina (yes/no) and use of arms to stand up from the chair(yes/no), use of CNS active medication (yes/no), and depression score ≥ 6 (yes/no). The proportionality assumptions of the Cox models were evaluated with Schoenfeld residuals. Data was analyzed with Stata (edition 9, StataCorp, College Station, Texas)

3.5 RESULTS

The mean blood lead level (BLL) was 5.3 $\mu\text{g}/\text{dl}$ ($\pm\text{SD}$ 2.3) and ranged from 1-21 $\mu\text{g}/\text{dl}$. Women in the highest BLL category ($\geq 8 \mu\text{g}/\text{dl}$) had lived more years after menopause, had lower body weight and BMI, and were more likely to smoke and drink alcohol and less likely to take vitamin D supplements. Neither cognitive function nor functional status was significantly different across BLL categories. Women in highest BLL group had lower mean baseline total hip BMD compared to women in lower category ($p < 0.0211$; Table 3-1).

Femoral neck BMD was also lower in this group although it did not reach a statistical significance. Baseline calcaneal BMD did not differ across the three groups.

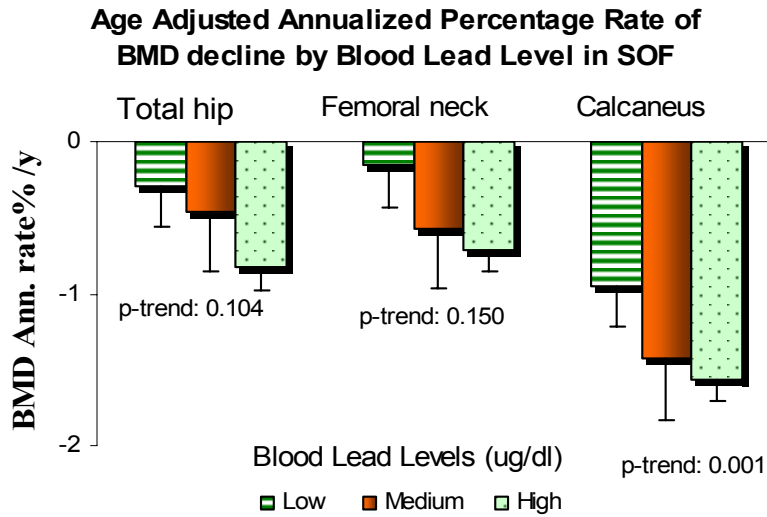


Figure 3-1 Age adjusted annualized percentage rate of BMD decline by blood lead levels in SOF

3.5.1 Change in Bone Mineral Density

Women with highest blood lead levels experienced a greater rate of decline in BMD (Table3-2). The annualized rate of decline in total hip BMD was 3 times higher for women in higher BLL category as compared to those in lower categories in age-adjusted models. When adjusted for other covariates, the difference in total hip BMD loss across three BLL categories persisted. Results were consistent at both the femoral neck and calcaneus. (Figure 3-1)

3.5.2 Fractures

After an average follow up of more than 10.5 years, 163 (23%) women reported an incident fracture. The incidence of non-traumatic non-spine fractures was higher among

women with higher blood lead levels (Figure 3-2). The relative risk of fracture was greatest among women with the blood lead levels $\geq 8\mu\text{g}/\text{dl}$ (Table3- 3: Figure 3-2). In age-adjusted models, women with the highest blood lead level had a 66% increased risk of fracture. The association remained significant in the multivariate model where women with the highest blood lead level had a 74% increased risk of fracture in comparison to women with the lowest blood lead levels. Women in the medium blood lead category were not at an increased risk of fracture. The incidence rate of fractures /1000 woman-years was highest in women with blood lead levels $\geq 8\mu\text{g}/\text{dl}$, who had 1.6 to 1.7 times higher rate of incident fractures when compared with women with the lowest and medium blood lead levels, respectively (Figure 3-4).

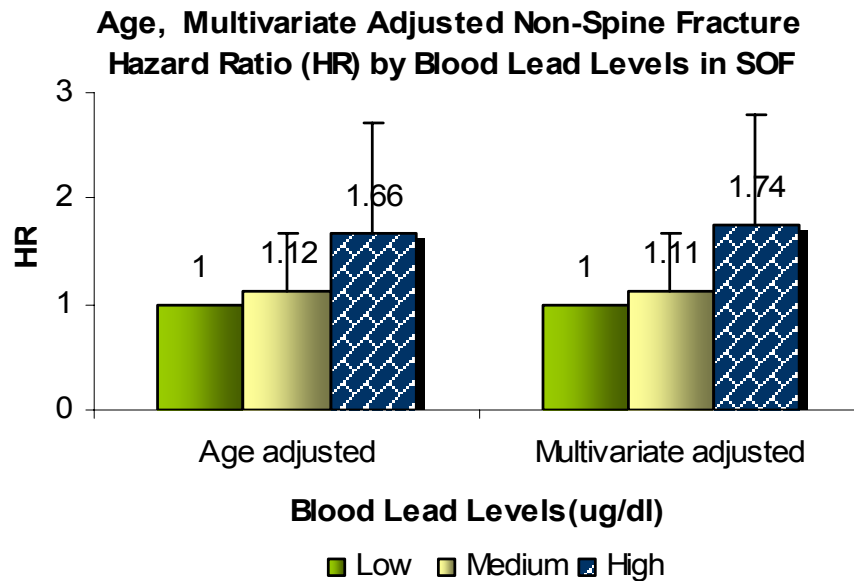


Figure 3-2 Age and multivariate adjusted non-spine fracture Hazard Ratio by blood lead levels.

Further analysis, using blood lead levels as a continuous variable, incident fractures were significantly higher, in unadjusted, age and multivariate adjusted models by 17%,13% and 18% respectively, per one standard deviation increase in blood lead levels.

The cumulative incidence of fracture as graphed by Kaplan Meier curves, increased over time in women with the highest blood lead level (Log rank test=0.038; Figure 3-5).

3.5.3 Falls

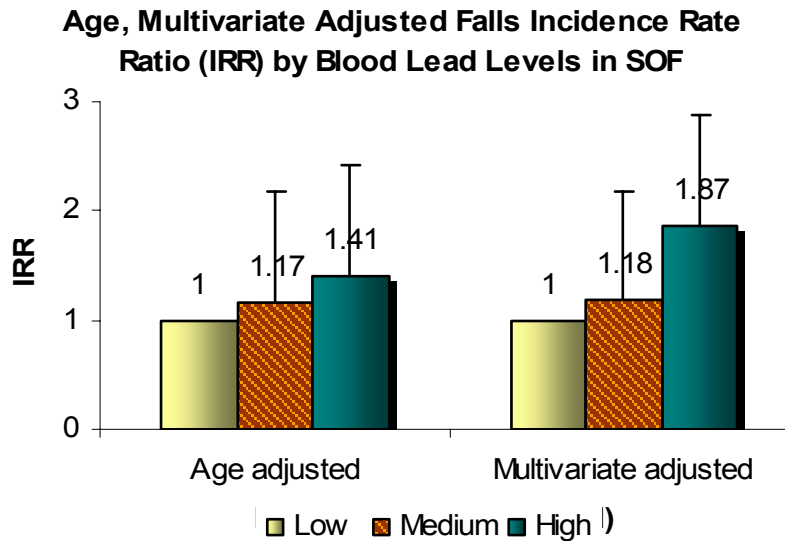


Figure 3-3 Age and multivariate adjusted falls Incidence Rate Ratio by blood lead levels in SOF

During the 3 year follow up period, the incidence of falls was higher among women with higher blood lead levels (Table 3). The relative risk of falls was greatest among women with the blood lead levels $\geq 8\mu\text{g} /\text{dl}$. In age-adjusted models, women with the highest blood lead level had a 40% increased risk of fall. The association was statistically significant in the multivariate model where women in the highest blood lead category were at 87% increased risk of falls as compared to women with the lowest blood lead level (p trend=0.006). Analyzing, blood lead levels as a continuous variable, incident falls were not significantly higher in unadjusted and age adjusted models. However after controlling

for shared risk factors, in multivariate adjusted models, risk of falls increased by 13% per one standard deviation increase in blood lead levels. (Figure 3-4)

3.6 DISCUSSION

In this cohort of older women, higher blood lead levels were associated with lower total hip BMD, greater rate of decline in BMD and an increased risk of falls and non-spine fractures. We found an association with fractures and decline in BMD at blood lead levels $\geq 8\mu\text{g/dl}$, a level previously thought to be safe. The association with non-spine fractures was not explained by baseline BMD or poor cognitive or physical function. Similarly, the association with falls was not explained by poor neuromuscular function. To our knowledge this is the first longitudinal study to explore the association of blood lead levels with osteoporotic fractures, falls, and rates of decline in BMD in older women.

Although mean U.S. national blood lead levels have decreased dramatically over the past 30 years, as documented by repeated National Health and Nutrition Examination Surveys from 1988 to 2002, lead toxicity continues to be a public health problem for older individuals with higher lifetime environmental lead exposure[10, 11]. More specifically, older women who grew up when environmental exposures were higher due to leaded gasoline, paint and unregulated industrial lead use, may have absorbed more lead from the environment in everyday life and thus have a higher body burden of stored lead[1].

Several large epidemiological studies have found that BLL are higher in older people than younger adults[1, 2] the difference being more pronounced in women across the menopausal transition, perhaps reflecting a greater release of lead from bone

secondary to increased menopausal bone loss. Research studies report increases in bone lead with older age [12-14].

Elevated blood lead levels may have a causative role in the pathogenesis of osteoporosis[15-17]. Animal studies have shown an association between increased bone lead concentration and lower bone mass, decreased mechanical strength and increase incidence of fractures[18-22]. Lead inhibits axial bone development and decreases bone mass due to enhanced resorption in animal models[19]. Histomorphometric studies have demonstrated a significant Pb-associated decrease in length of rat femoral growth plate cartilage[21].

An inverse relationship has also been documented between elevated BLL and skeletal development, chest circumference, and stature in children [23-25]. A longitudinal analysis of women with occupational lead exposure showed that vertebral BMD was inversely associated with BLL[26-28]. Bone mineral density was also significantly inversely associated with blood lead level in general population after adjusting for other factors associated with blood lead[29]. Hu, H. et al used K-X-ray fluorescence (K-XRF) to measure lead levels in the tibia and patella on a series of twelve lead exposed male subjects before and after chelation with EDTA (ethylenediamine tetraacetic acid). K-XRF levels in the patella were noted to decrease more rapidly than levels in the tibia after cessation of lead exposure, a finding that probably reflects the greater turnover of lead in trabecular bone than in cortical bone[30, 31]. We found significantly higher declines among the women with the highest lead levels. This observation was consistent across sites suggesting that lead influences both cortical and trabecular bone loss [30].

The effects of lead on growing bone as well as adult remodeling and age related osteoporosis can predispose to fractures[32]. Lead exposure has been reported to

accelerate bone maturation by inhibiting the effects of parathyroid hormone-related peptide[33]. Accelerated maturation of bone may ultimately result in a lower peak BMD, thus predisposing to osteoporosis in later life [33]. A hypothetical “Pb-exposed female” may never reach her peak bone mineral density because of compromised growth plate chondrocyte activity. At menopause, increased bone turnover would accelerate the loss of bone mineral such that the “Pb-exposed female” would cross over a fracture threshold earlier in life than an unaffected individual. In our study, baseline BMD levels were slightly lower among women with higher blood lead levels.

Lead exposure could predispose to fractures because of increased risk of falls as it affects neuromuscular function and balance[5, 6]. Lead exposure in childhood has been associated with impaired maturation of postural balance and central auditory processing[34-37]. Neuromotor dysfunction in lead workers has been reported at blood lead level as low as 12.1-17.3 (mean 14.4) $\mu\text{g}/\text{dl}$ [34]. Cerebellum which controls the postural balance is the second most frequently involved brain region for lead deposition, besides hippocampus. Cerebellar Purkinje cell damage in rats exposed to lead has been documented[38]. By computerized static posturography, the effect of lead on postural balance was examined in 49 male lead workers, with mean (range) BLL 18(7 to 36) $\mu\text{g}/100\text{ g}$. As compared to healthy non-lead exposed controls, the postural sway in lead exposed workers with eyes open, was significantly greater in the medio-lateral (right-left) and anterior-posterior directions. Similarly, the sway with eyes closed was significantly larger in lead workers than in controls in the medio-lateral direction. The pattern of sway suggests that the anterior cerebellar lobe, vestibulo-cerebellum, and spinocerebellar pathway were affected by lead[39].

Lead slows the nerve conduction velocities in peripheral nerves which could delay motor coordination, impair perception of vibration, light touch and position of the joints[34,

40]. Muscle is one of the soft tissues where lead is deposited in addition to skeletal, nervous, and renal tissues. Muscle weakness and easy fatigue occur early and may be the only symptoms of lead exposure. The muscle groups usually involved are the most active ones, such as the extensors of the forearms, wrist and fingers[41]. Blood lead levels as low as 8 µg/dl were associated with poorer performance on tests of visual perception, psychomotor speed, and manual dexterity[7].

A negative correlation has been observed between lead and Vitamin D[16]. Low Vitamin D has been linked to hip and non spine fractures and falls[42, 43]. Women with the highest blood lead levels in our study had higher fracture and fall risk. Women in this group had significantly lower Vitamin D intake at baseline in comparison with women with lower blood lead levels suggesting that circulating Vitamin D levels were also lower in this group (Table1). Genetic polymorphism has been identified in the vitamin D receptor (VDR), that can influence the accumulation of lead in bone[44].

There are a number of strengths to our study. We used a prospective study design and studied a well-characterized cohort of community-dwelling older women, using state-of-the-art measurements of BMD. Follow-up was nearly complete for important outcomes of falls and fractures, and fractures were validated by review of radiology reports and/or medical records. The analyses adjusted for a number of important covariates, including cognitive and neuromuscular function. However, this study does have some limitations. Our participants were likely to be healthier than average community-dwelling older women because they were volunteers. While we adjusted for a summary measure of cognitive function, it is possible that lead affects specific cognitive abilities. Finally, our data may not apply to older nonwhite women or men.

In conclusion, high BLL was associated with an increased risk of falls and fractures as well as greater rates of decline in BMD in older women independent of age and other

risk factors. This association was found at BLL previously thought to be safe. It is possible that treatment to reduce the elevated BLL could lead to a decrease in falls and osteoporotic fractures; clinical trials examining this hypothesis should be conducted.

3.7 ACKNOWLEDGEMENTS

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Table 3-1 Baseline characteristics in women in SOF by blood lead levels

Blood Lead Level	Low	Medium	High	
(µg/dl)	(≤3)	(4-7)	(≥8)	p -
(N=533)	N=122	N=332	N=79	value
Age (years), mean ±(SD)	72.3 (4.3)	72.4(4.5)	73(4.5)	0.411
Clinic, n (col %)				
Urban (n=205)	33(27)	131(39)	41(52)	0.002
Rural (N=328)	89(73)	201(61)	38(48)	
Education, years, mean±(SD)	12(3)	13(3)	13(3)	0.360
Weight (kg), mean ±(SD)	69(13)	67(12)	65(12)	0.089
Weight change, kg/yr mean ±(SD)				
(v4–v2,Total hip, femoral neck)	-0.14(1.0)	-0.12(1.2)	-0.03(1.0)	0.828
(v4–v1,Calcaneus)	-0.04(0.85)	-0.11(0.83)	-0.24(0.77)	0.388
Height (cm), mean ±(SD)	159(5.8)	159(5.6)	158(6)	0.653
Body mass index, kg/m², mean ± (SD)	27(4.8)	27 (4.6)	26 (4.6)	0.039
Years since menopause, mean ± (SD)	21.3(8.2)	23(7.3)	25 (8)	0.017
Age at menopause, yr, mean ± (SD)	49(6.3)	48(5.7)	46(6.2)	0.011
Ever pregnant, n (%)	103 (84)	280 (84)	64 (81)	0.757
Surgical Menopause: n (%)	20(13)	41 (13)	11(14)	0.485
Ever breastfed, n (col %)	66(64)	181(65)	29(45)	0.014
Smoke = n (%)				
Never	77(63)	199(60)	32(40)	0.000
Past	38(31)	94(28)	27 (34)	
Current	7 (6)	38 (12)	20 (25)	
Pack-years smoked, mean ±(SD)	18.3(18)	27(21)	31(22)	0.010

Table 3-1 continued

Alcohol (drinks /wk),mean ±(SD)	1.1 (3)	1.8 (4)	3.2(6)	0.002
Calcium intake, (mg/wk),mean ± (SD)	4720(2498)	4575 (2793)	4574 (3592)	0.886
Vit D supplement, n (%)				
Never	60(50)	195(60)	56(72)	0.052
Past	9(8)	17(5)	4 (5)	
Current	50 (42)	114(35)	18 (23)	
Total # Yrs Taken Vit. D, mean ± (SD)	13(15)	10(11)	5.0(7)	0.019
Current Estrogen use, n (%)	43(35)	113(34)	22(28)	0.511
Current Thiazide diuretic use, n (%)	35(29)	113(34)	24(30)	0.517
Diabetes, n (%)	14(12)	33(10)	7(9)	0.688
Hypertension, n (%)	28(23)	100 (30)	25(32)	0.289
Self reported health, n (%)				
excellent /good vs. fair /poor	99(81)	282(85)	66(83)	0.493
Functional status Impairments,				
mean±(SD)	0.77(1.3)	0.78 (1.3)	0.59(1.1)	0.506
Incident fracture, n (%)	43(20)	140(66)	29(14)	0.469
Any fracture after age 50, n (%)	40(33)	109(33)	22(28)	0.683
Maternal fracture history, n (%)	33(35)	82(32)	23(36)	0.763
Fallen in past year (≥ 1), n (%)	29(24)	75 (23)	20(25)	0.873
Walk for exercise: n (%)	44(23)	125 (64)	26(13)	0.795
Physical activity, past yr(kcal/week)	1044(1047)	1239(1368)	1104(1234)	0.311
mean ± (SD)				
Use arms to stand up from chair, n (%)	3(3)	10(3)	1(1)	0.671

Table 3-1 continued

Walking Speed Usual Pace (m/s) mean ± (SD)	1.01(0.2)	1.04(0.2)	1.03(0.2)	0.483
Grip strength (kg) mean ± (SD)	20.6(4)	20.9(4)	20.7(4)	0.756
Tandem stands (eyes closed), n (%)	20(16)	102(31)	26(33)	0.006
Current Estrogen use, n (%)	43(35)	113(34)	22(28)	0.511
Current Thiazide diuretic use, n (%)	35(29)	113(34)	24(30)	0.517
Trailmaking B(number of seconds) mean ± (SD)	116(36)	121(39)	126(44)	0.192
Bone Mineral Density (mg/cm²):				
Total Hip, mean ± (SD)	0.77(0.13)	0.76(0.13)	0.72 (0.12)	0.021
Femoral Neck, mean ± (SD)	0.65 (0.11)	0.66 (0.12)	0.62 (0.09)	0.071
Calcaneus, mean ± (SD)	0.41(0.09)	0.42(0.09)	0.39 (0.09)	0.145

Table 3-2 Multivariate adjusted Annualized Percentage Rate of bone mineral density decline by blood lead levels

BMD Decline (%/year)	Blood Lead Level						
	Low ($\leq 3\mu\text{g/dl}$) (n=122)		Medium (4-7 $\mu\text{g/dl}$) (n=332)		High ($\geq 8\mu\text{g/dl}$) (n=79)		P _{trend}
	Mean	95%CI	Mean	95%CI	Mean	95%CI	
Total Hip							
Age Adjusted	-0.29	-0.59,0.00	-0.46	-0.64,-0.28	-0.83	-1.23,-0.44	0.104
MV* Adjusted	-0.28	-0.57,-0.00	-0.41	-0.57,-0.24	-0.74	-1.11,-0.36	0.165
Femoral Neck							
Age Adjusted	-0.16	-0.55,-0.22	-0.57	-0.80,-0.34	-0.70	-1.21,-0.18	0.150
MV Adjusted	-0.21	-0.60,0.16	-0.59	-0.81,-0.36	-0.71	-1.21,-0.21	0.180
Calcaneus							
Age Adjusted	-0.95	-1.22, -0.68	-1.43	-1.60, -1.26	-1.55	-1.92, -1.18	0.001
MV Adjusted	-0.92	-1.23, -0.61	-1.32	-1.51, -1.12	-1.56	-2.02, -1.11	0.045

*MV=multivariate adjusted

*The Multivariate model included the following: age, weight, walk speed, weight change between v4 and baseline, smoking, walk for exercise, Thiazide diuretic use. Additional adjustment for clinic, education, height, age at menopause, consumed alcohol in the past 30 days, breastfed children, baseline BMD, total kilocalories /wk from walking ,any fractures since age 50, maternal history of fractures, calcium intake, Vitamin D use, hormone therapy, health compared to others, functional status, use of arms to stand up from a chair, Part B Trailmaking Test score, diabetes, hypertension, average grip strength (steroid use for calcaneus) had no effect on the BMD estimate .

Table 3-3 Age and shared risk factors adjusted Hazard Ratio(95%CI) of non-spine fractures and Incidence Rate Ratio of falls in women in SOF by blood lead level

	Blood Lead Levels ($\mu\text{g/dl}$)					
	Low(≤ 3)	Medium(4-7)	High(≥ 8)			
N=533	N=122	N=332	N=79		p-trend	
Hazard Ratio of Incident Non-spine Fracture**						
	Referent	HR	95%CI	HR	95%CI	
Age adjusted	1.0	1.12	0.75-1.66	1.66	1.00-2.72	0.061
MV [¶] adjusted	1.0	1.10	0.72-1.67	1.74	1.01-2.98	0.059
Incidence Rate Ratio of Falls‡						
	Referent	IRR	95%CI	IRR	95%CI	
Age adjusted	1.0	1.17	0.87-1.57	1.40	0.97-2.02	0.073
MV adjusted	1.0	1.18	0.87-1.58	1.87	1.24-2.82	0.006

¶ Multivariate Adjusted

**The Multivariate model included the following : age, BMI, years after menopause, baseline total hip BMD, fractures after age 50. Additional adjustment for clinic, height, weight, walk for exercise, total kilocalories /wk from walking, use arms to stand up from chair, fallen in the past year, consumed alcohol in the past 30 days, current smoking, current calcium use, hormone therapy, ever breastfed, maternal fracture history, average grip strength, osteoarthritis, fair or poor health, functional status, COPD, hypertension, diabetes, myocardial infarction, part B Trailmaking Test score had no effect on the HR estimate.

‡ Adjusted for age, weight, education, walk for exercise, use arms to stand up from chair, fallen in the past year, consumed alcohol in the past 30 days, current smoking, hormone therapy, current calcium use, average grip strength, fair or poor health, functional difficulty, COPD, arthritis, h/o stroke, hypertension, diabetes, Vit D use, part B Trailmaking Test score, use of benzodiazepines, antidepressants, narcotics and anticonvulsants, walking speed, balance impaired with eyes closed, corrected visual acuity, depression(GDSS ≥ 6), ever breastfed.

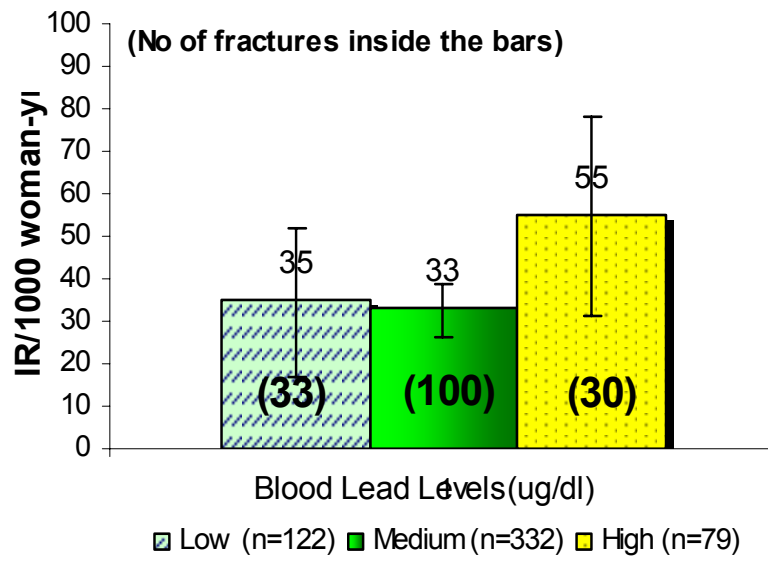
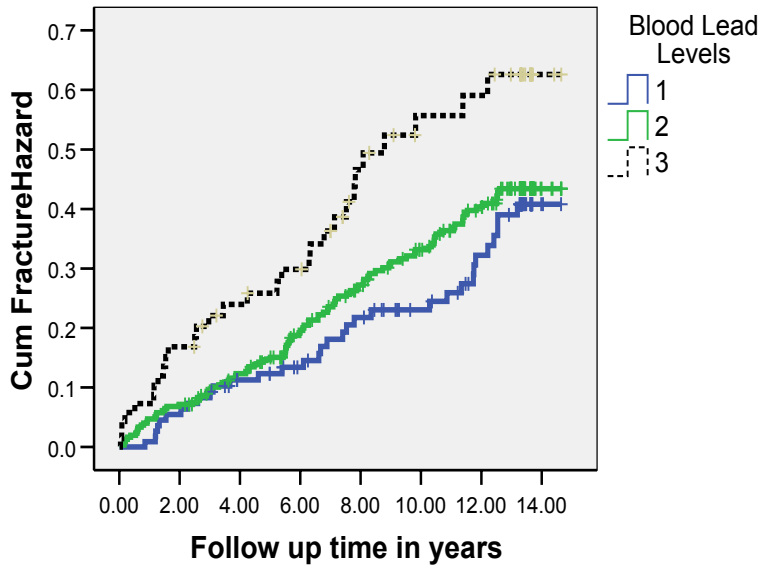


Figure 3-4 Incidence Rate of fractures/1000 woman-yr in SOF by blood lead levels

Hazard Function of Non-spinal, Non-traumatic Fractures in Women in SOF by Blood Lead Levels



(P -log rank test: 0.038)

Figure 3-5 Hazard Function of Non-spine Fractures in women in SOF by blood lead levels

4.0 THE ASSOCIATION OF BLOOD LEAD LEVELS AND MORTALITY IN OLDER WOMEN: THE STUDY OF OSTEOPOROTIC FRACTURES

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4.1 ABSTRACT

Background: Higher Blood Lead Levels (BLL) have been associated with increased all cause, and cardiovascular mortality in the general population and in exposed occupational cohorts. The objective of present study was to determine the associations of blood lead levels with mortality in older women.

Methods: 533 participants from the Study of Osteoporotic Fractures (SOF), with mean (\pm SD) age 72.5 (\pm 4.4) years were followed from 1986-1987. All deaths were confirmed by death certificates. Blood lead level was determined by graphite furnace atomic absorption spectrometry. Participants were divided into two categories based on distribution of blood lead level into low: ≤ 7 $\mu\text{g}/\text{dl}$ and high: ≥ 8 $\mu\text{g}/\text{dl}$. Relative hazard of total and cause specific mortality by blood lead categories was calculated using Cox proportional regression analyses.

Results: After 12.0 ± 2.9 years of $>95\%$ complete follow-up, women who died had slightly higher baseline mean (\pm SD) blood lead level 5.56 (± 3) $\mu\text{g}/\text{dl}$ than survivors: 5.17(± 2.0) $\mu\text{g}/\text{dl}$ ($p=0.09$). After adjustment for age, women with baseline blood lead levels of ≥ 8 $\mu\text{g}/\text{dl}$ had 68% increased risk of all cause mortality (Hazard Ratio[HR],1.68; 95% confidence interval [CI] 1.10, 2.60) compared to women in low blood lead level group. With multivariate adjustment, women in high blood lead group had 2 fold increased risk of all cause mortality (HR, 2.34; [CI] 1.41, 3.87), 3 fold higher risk of cardiovascular mortality (HR: 3.31[CI] (1.54, 7.12), compared to women in low blood lead group. There was no association of blood lead levels with mortality from cancer.

Conclusion: Women with blood lead levels of $\geq 8\mu\text{g}/\text{dl}$ experienced increased mortality, in particular from cardiovascular disease, as compared to those with lower blood lead levels.

4.2 INTRODUCTION

Lead is a multitargeted toxicant, effecting cardiovascular, and nervous systems. Lead may contribute to a fraction of morbidity and mortality associated with these affects[1],[2, 3].

An association between lead and mortality has been observed in both occupational and community based cohorts [4]. Results from the second National Health and Nutrition Examination Survey (1976-1980) and 2000 US Census and reported that blood lead level was an important predictor of mortality. Individuals with baseline blood lead level 20 to 29 $\mu\text{g}/\text{dl}$ experienced 46 % increased all cause mortality, relative to those with blood lead level less than 10 $\mu\text{g}/\text{dl}$ [1]. Furthermore an increased risk of death from all causes, cardiovascular disease, and cancer was associated with blood lead levels of 5–9 $\mu\text{g}/\text{dl}$ as compared to those with <5.0 $\mu\text{g}/\text{dl}$ in NHANES III (1988-1994) [5]. Menke et al documented 25% increased all cause and 55% increased cardiovascular mortality in NHANES III (1988-1994) at considerably lower blood lead levels: >3.62 $\mu\text{g}/\text{dl}$ as compared to those with <1.94 $\mu\text{g}/\text{dl}$ [6]. However they did not observe an association between blood lead and cancer mortality in this range of exposure.

Higher blood levels have also been associated with cognitive and neuromuscular decline[7-9], cardiovascular[10-14] and renal effects [3, 15-17] all of which could contribute to an increased risk of mortality. The effects of blood lead levels on cancer mortality however, are poorly understood [18]. In the current analysis we prospectively examined the association of blood lead levels to mortality in a cohort of 533 white women with mean age of 72.5(± 4.4) (range: 68-89) years and mean blood lead levels of 5.3 $\mu\text{g}/\text{dl}$ (± 2.3 SD) (range: 1-21 $\mu\text{g}/\text{dl}$). We hypothesized that women with higher blood lead levels will experience higher total and cause specific mortality.

4.3 METHODS

4.3.1 Study design and Population

The Study of Osteoporotic Fractures (SOF) is a longitudinal cohort study that enrolled 9704 white women from 1986 to 1988 using population-based listings in Baltimore, MD; Minneapolis, MN; Portland, OR; and the Monongahela Valley near Pittsburgh, PA. To be eligible to participate, women had to be aged 65 years or older and ambulatory.

The lead ancillary study was conducted in 1990-1991 in 533 white women aged 65-87 years enrolled in SOF at either the University of Pittsburgh or University of Maryland clinics. Initially, we examined the correlates of blood lead and the association of blood lead level to cognitive function [19]. We found a relationship between higher blood lead levels and worse cognitive function as measured by the part B of Trailmaking Test, but this association was confined to the rural SOF clinic [19, 20]. In the current paper, we extend the lead study to mortality outcome. The protocol and consent forms were approved by the institutional review boards at the participating institutions. All women provided written informed consent.

4.3.2 Other Measurements

Potential confounders of the association between blood lead levels and mortality were identified and adjusted for Sociodemographic factors (age, study site/clinic, education), smoking history, alcohol consumption (past 30 days), and physical activity from walking, medication use (including hormone therapy, thiazide diuretics, calcium supplements, vitamin D supplements), and time since menopause, children breastfed, weight loss since

age 50, health status compared to others, were determined by an interview-administered questionnaire.

Health impairment data included cognitive impairment, assessed as scores on Trailmaking Part B, functional status impairment, and depression as 6 or more depressive symptoms on the 15-item Geriatric Depression Scale Shortened. Prevalent chronic conditions such as diabetes, hypertension, arthritis, stroke, heart murmur, angina, EKG abnormality, congestive cardiac failure, enlarged heart, were defined as self-report of the condition previously diagnosed by a physician. Height and weight were obtained using a Harpenden stadiometer (Holtain Ltd, Crymych, UK) and a standard balance beam, respectively, and body mass index (BMI) was calculated as weight divided by height squared (Kg/m^2). Waist to hip ratio was determined. Systolic and diastolic blood pressure was measured by manual mercury sphygmomanometer. Physical function was assessed including grip strength measured as average of right and left using an adjustable hand grip dynamometer in Kg (Preston Grip Dynamometer; Takei Kiki Kogyo, Tokyo, Japan), walking speed (second /6 meters).

4.3.3 Mortality

The methods of determining deaths in SOF have been published[21, 22]. Participants were contacted every 4 months by postcard over 12(\pm 3) years of follow-up. These contacts are >95% complete. Deaths were confirmed by death certificates, and, when available, hospital discharge summaries were obtained. The underlying cause of death was coded by a clinical epidemiologist using the *International Classification of Diseases, Ninth Revision, Clinical Modification*, and categorized as due to cardiovascular disease (CVD) including all diseases of circulatory system except those involving veins and lymphatics

[ICD-9-CM codes 401 to <405, 410 to <415, 425,428, 429.2, 430 to <439, 440 to <445, and 798]; coronary heart disease (CHD) including deaths due to Ischemic heart disease, myocardial infarction and sudden death [ICD-9-CM 410-414]; atherosclerotic Cardiac disease [ICD-9-CM 410-415, 798, 427.5, 428, 429.2]; cancer [ICD-9-CM codes 140 to 239] and all other deaths.

4.3.4 Blood lead measurements

A 5.0 ml sample of whole blood was drawn into Vacutainer tubes (BD Vacutainer Systems, Rutherford, New Jersey). Blood samples were analyzed at the Clinical Chemistry Laboratory of the University of Maryland, certified for the analysis of lead in blood by the Occupational Safety and Health Administration and Centers for Disease Control and Prevention, and documents a lower limit of detection for lead of 1µg/dL. BLL were determined by graphite furnace atomic absorption spectrometry (AAS model 5100, HGA with Zeeman Effect background correction: Perkin Elmer, Norwalk, Connecticut). To determine intralaboratory measurement variability in lead level and the stability of samples over 1year, 100 samples (50 from each clinic) were drawn from randomly selected women during a clinic visit, one year later. The intraclass correlation coefficient for the duplicates was 0.88. Mean values of 4.76 µg/dl (range, 1-13 µg/dl) and 4.67µg/dl (range, 1-12 µg/dl) were obtained for the first and second determinations, respectively[19]

4.3.5 Statistical analyses

Participants were divided into two categories, corresponding to the upper 15th and lower 85th percentiles of the distribution of blood lead variable. Thus the two groups were : low (≤7 µg/dl, n=454), and high(≥8 µg/dl, n=79)[19]. To compare baseline characteristics by

mortality status, we used Chi-square tests for categorical variables and, t-tests for continuous variables. Two-tailed p-values were used for all tests, at 5 % statistical significance. We used Cox proportional hazard regression analysis to estimate the Hazard Ratio (HR) of mortality and 95% confidence interval (CI). Women in low category ($\leq 7\mu\text{g/dl}$) of blood lead level formed the reference group for all analyses. Separate models were analyzed for all cause and cause specific mortality. We classified the CVD associated mortality into two further subgroups: deaths due to coronary heart disease (CHD), and due to atherosclerosis.

In multivariate models, we simultaneously analyzed blood lead levels and other potential risk factors. We assessed variables for inclusion, based on documented association in the literature and biological plausibility. We organized variables into 9 related groups, including 1) demographic factors (age, education, clinic), 2) anthropometric measures (BMI, waist hip ratio, weight loss since age 50) 3) lifestyle factors (smoking, drink alcohol, walk for exercise), 4) physical functions (average grip strength, walk speed) 5) health status (health compared to others, depression, functional limitation), 6) functional performance (Trailmaking part B, depression), 7) self reported medical history (hypertension, diabetes, stroke, angina, heart enlargement, congestive cardiac failure, EKG abnormality), 8) medication and supplement use (hormone therapy, Vitamin D, calcium and thiazide diuretic), and 9) reproductive factors (years after menopause, ever breastfed). A forward stepwise selection process was used to add or remove potential covariates from the multivariate regression models (exit criteria: $p > .20$). Sequential models were analyzed in which all variables in one group were allowed to enter in a stepwise regression based on p- value at each step. The next model began with the significant variables remaining from the previous model. Then a new group of variables was allowed to enter, using the stepwise entry procedure, for successive groups of variables, until a final model of

significant variables was selected. We computed overall and age-adjusted mortality and 95% confidence intervals.

In multivariate models, age, education, years since menopause, clinic, children breastfed, BMD, body mass index, weight, weight change from age 50, alcohol intake, walking speed, grip strength, calcium intake, waist hip ratio, Trailmaking Part B, were entered into the models as continuous variables. All other variables were categorical: smoking (never/former/current), walking for exercise (yes/no), functional impairment (yes/no), oral estrogen, vitamin D use, thiazide diuretic, (current/former/never), health compared to other(good/excellent vs. fair/poor/very poor) and physician diagnosis of hypertension, diabetes, stroke, angina (yes/no) EKG abnormality, heart murmur, enlarged heart, congestive cardiac failure and depression score ≥ 6 (yes/no). The proportionality assumptions of the Cox models were evaluated with Schoenfeld residuals. We plotted the cumulative hazard of fractures in the two BLL groups over the follow-up period by Kaplan-Meier Survival graphs. Data was analyzed with Stata (edition 9, StataCorp, College Station, Texas)

4.4 RESULTS

The mean blood lead level was 5.3 $\mu\text{g}/\text{dl}$ (± 2.3 SD) and ranged from 1-21 $\mu\text{g}/\text{dl}$. The number of women who died was 123 (23% of the participants) over mean follow up time of 12.03 (± 3.0) years. Table 1 summarizes characteristics of this cohort by survival status. Women who died had slightly higher mean (\pm SD) blood lead levels; 5.56(± 3) $\mu\text{g}/\text{dl}$ than survivors: 5.17 (± 2.0) $\mu\text{g}/\text{dl}$ ($p=0.09$). Women who died were older and had lived more years after menopause. The same group had slower walking speed, lower grip strength,

worse functional status, and cognitive test scores and reported a higher proportion of hypertension and current smoking. A lower proportion of women who died reported current and past hormone use. Self reported history of angina, heart attack, electrocardiogram abnormalities and congestive heart failure all were higher in this group (Table4-1).

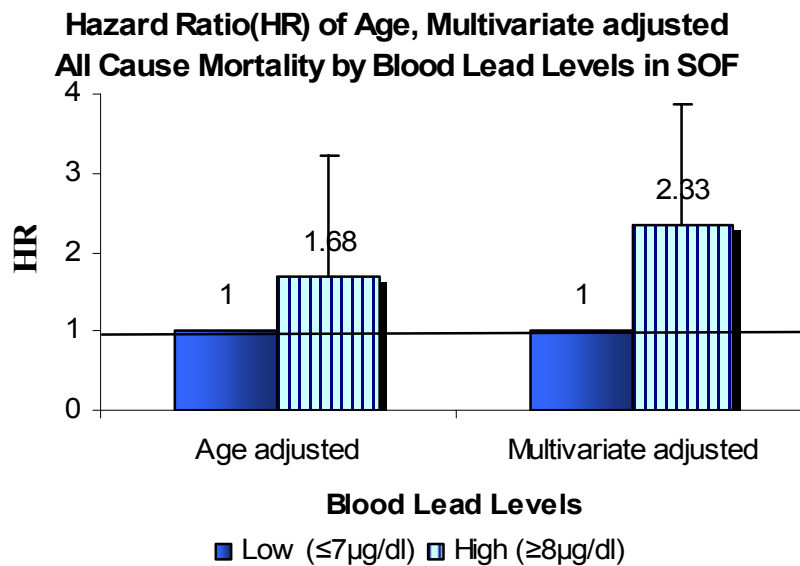


Figure 4-1 Age and multivariate adjusted all cause mortality in women in SOF by blood lead levels

High blood lead levels were associated with increased all cause mortality after age adjustment (Table 4-2). Women with baseline blood lead level of $\geq 8 \mu\text{g/dl}$ had a 68% increased risk of age-adjusted all cause mortality (Hazard Ratio [HR] =1.68; 95% confidence interval [CI] 1.10, 2.58) ($p=0.018$) compared to women in low blood lead level group. After multivariate adjustment, women in high blood lead group had a 2 fold increased risk of all cause mortality (HR=2.33; 1.41, 3.87) ($p=0.001$). The cumulative incidence of all cause mortality as graphed by Kaplan Meier curves, increased over time in women with blood lead level $\geq 8 \mu\text{g/dl}$. (Figure 4-3).

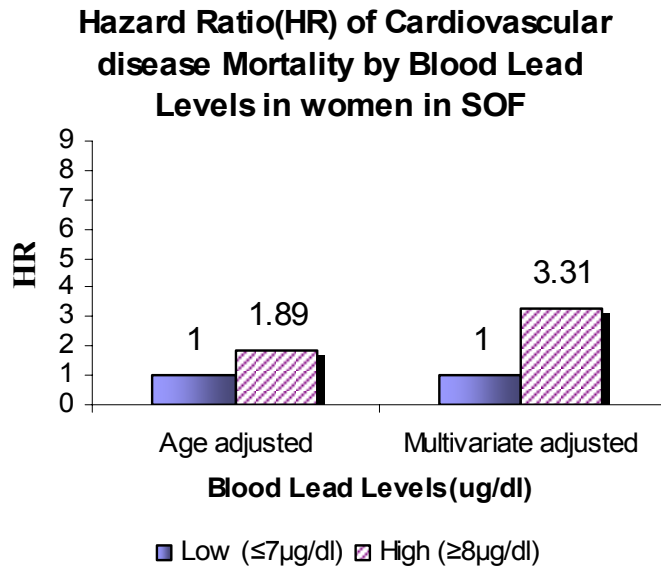


Figure 4-2 Age and multivariate adjusted Cardiovascular disease mortality in women in SOF by blood lead levels.

In cause-specific mortality analysis, increased mortality was predominantly due to cardiovascular disease. The multivariate adjusted hazard ratios (95% CI) for CVD mortality for high versus the low blood lead level were 3.31(1.54, 7.12), 8.66(2.82, 26.56), 3.93 (1.48, 10.44), respectively, for cardiovascular (CVD), coronary heart disease (CHD), and atherosclerotic disease mortality respectively. Association of blood lead and mortality from cancer and other causes was not significant. (Table 4-3).

4.5 DISCUSSION

Blood lead levels were an important predictor of all cause mortality in a cohort of community dwelling older women. Mortality was significantly higher in women with blood lead levels of $\geq 8 \mu\text{g/dl}$ as compared to those with lower blood lead level. Our results are

consistent with earlier studies based on occupational cohorts [4, 14, 23, 24] and NHANES II and III[5, 6, 12, 25].

Despite declines in blood lead levels during the past 30 years, environmental lead exposure continues to be a public health concern[3] . Initiated during the 1920s, the use of organic lead as gasoline additive has been phased out in US since 1976. Once released as combustion exhaust, particulate lead persists in air, water, and soil. Lead is a multitargeted toxicant, causing effects in cardiovascular, renal and nervous systems and may contribute to a fraction of morbidity and mortality associated with these effects. Lustberg and Silbergeld evaluated the association of lead exposure and mortality in the United States from analyses of NHANES II (1976-1980) [12]. Individuals aged 30 to 74 with blood lead levels of 20-29 $\mu\text{g}/\text{dl}$ experienced 46% increased all-cause, 39% increased cardiovascular and 68% increased cancer mortality compared with those with blood lead levels of less than 10 $\mu\text{g}/\text{dl}$. Individuals with blood lead levels 10-20 $\mu\text{g}/\text{dl}$ did not have higher risk of mortality.

Schober et al. analyzed mortality data for individual's ≥ 40 years of age in the NHANES III (1988-1994). Blood lead levels of 5-9 $\mu\text{g}/\text{dl}$ were associated with a 24 % increased risk of death from all causes, 20% increased cardiovascular , and 44% increased risk of cancer mortality compared to those with < 5 $\mu\text{g}/\text{dl}$ [5]. In a follow-up paper from the NHANES III data, participants in the highest tertile (≥ 3.6 $\mu\text{g}/\text{dl}$) of blood lead had 25% and 55% increased risk of all-cause and cardiovascular mortality, respectively compared with those in the lowest tertile (<1.94 $\mu\text{g}/\text{dl}$). However, no association between blood lead level and cancer mortality in this range of exposure was reported. We did not observe an association between all cause mortality and blood lead level until 8 $\mu\text{g}/\text{dl}$ [6]. Jemal et al (2002) reported a threshold level of blood lead levels at 24

µg/dl with cancer mortality, limited to white women in dose response analysis. The range of Blood lead level that we studied was 1-21 µg/dl.

The increased cardiovascular mortality risk may reflect an effect on sub-clinical cardiovascular risk factors for disease. As part of NHANES III, an increased risk of peripheral arterial disease, hypertension and renal dysfunction was observed in populations with an average blood lead level of 2 µg/dL [3, 11, 15, 26, 27]. For example, the odds ratio of diastolic hypertension was 8.1 comparing women with a blood lead level of 4.0-31.1 µg/dL to women with lower blood lead levels of 0.5-1.6 µg/dL. Other analyses support an association between blood lead levels and cardiovascular disease [10, 11, 28-31], renal function impairment [15, 32-37], increased blood pressure [27, 38, 39], an association which is biologically plausible [40-45]. Increase in blood pressure and renal damage have been observed after induction of lead exposure in rodent models [41-45]. Alterations in signal transduction that involve renal pathways (eg, angiotensin and vasopressin) have been reported in animal models [46-48]. Mechanisms, by which lead may increase cardiovascular risk, include effects on neuromuscular and neuro-humoral regulation of vascular function, alteration in sodium transport, and alterations in calcium regulation [6, 7, 11, 26, 30, 31, 49-52].

Lead is a toxic metal and categorized as probably carcinogenic to humans (Group 2A IARC 2004) [53, 54]. Association between occupational lead exposure and cancers of brain, stomach, kidney and lung have been reported [55-57]. However among non occupational cohorts, there has been little evidence of an association between blood lead and cancer mortality. Individuals with mean blood lead levels 10-19 µg/dl in the NHANES II (1976-1980) did not have increased risk of cancer mortality, when compared to those with blood lead levels <10 µg/dl. Our results are consistent with the observation. A higher risk of cancer deaths was only observed in with blood lead level > 20 µg/dl [12]. Individuals

with mean blood lead levels $<10 \mu\text{g/dl}$ in the NHANES III(1988-1994) did not have increased risk of cancer mortality[6].

There are several strengths to our study; we used almost 95% complete adjudicated mortality events. There was strict quality control in data collection as physician adjudicator confirmed all the events. The reliability of measurement of BLL was optimum and we controlled for a number of risk factors. This study has several limitations. Participants were limited to older Caucasian women, and the findings may not apply to men or nonwhite women. Indices of cumulative lead exposure (such as bone lead levels) were not available and thus we could not examine cumulative lead levels with mortality.

In conclusion, our study extends the findings of higher mortality from occupationally exposed cohorts to community dwelling elderly women. An increased mortality risk, especially cardiovascular disease was found at blood lead levels $>8 \mu\text{g/dL}$, well below the current OSHA action level of $50 \mu\text{g/dL}$. Our results, confirmed in other cohorts, suggest that the action levels of blood lead level need to be lowered.

4.6 ACKNOWLEDGEMENTS

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Table 4-1 Baseline Characteristics of Women in SOF by survival status

	Died	Survived	
Characteristic by survival status/mortality	N=123	N=410	P value
Blood lead level($\mu\text{g}/\text{dl}$), mean \pm(SD)	5.56 \pm 3	5.17 \pm 2	0.093
Age (years), mean \pm(SD)	72 \pm 4	70.0 \pm 4	0.000
Clinic, n (row %)			
Urban	38(19)	167(81)	0.002
Rural	85(26)	243(74)	
Body mass index(kg/m^2), mean \pm (SD)	26.2 \pm 5	26.8 \pm 5	0.164
Waist-hip ratio, mean \pm (SD)	0.82(0.07)	0.81(0.06)	0.074
Education(years), mean\pm(SD)	12.4 \pm 3	12.4 \pm 3	0.811
Alcohol (drinks /wk),mean \pm(SD)	1.92 \pm 5	1.84 \pm 4	0.840
Smoke, n (col %)			
Past	37(30)	122(30)	0.005
Current	25(20)	40(10)	
Years since menopause, mean \pm (SD)	25 \pm 7.6	22 \pm 7.6	0.001
Hypertension, n (%)	47(39)	106(26)	0.007
History of			
Abnormal EKG, n (%)	27(24)	58(15)	0.004
Stroke, n (%)	6(5)	11(3)	0.205
Angina, n (%)	21(18)	36(9)	0.011
Heart attack, n (%)	18(16)	21(5)	0.000
Congestive heart failure, n (%)	15(4)	4(1)	0.021
Diabetes, n (%)	14(12)	38(10)	0.407

Table 4-1. Continued

Estrogen use, n (%)			
Past	18(15)	93(23)	0.031
Current	9(8)	50(12)	
Vit D supplement use, n (%)			
Never	78(65)	233(58)	0.133
Past	9(8)	12(5)	
Current	33(28)	149(37)	
Self reported health, n (%)	27(22)	59(14)	0.046
(Excellent /good vs. fair /poor)			
Functional status Impairments,	1.1±2	0.66±1	0.003
mean±(SD)			

Table 4-2 Relative Hazard (95% CI) of All Cause Mortality in women in SOF by Blood Lead levels

Blood Lead Level	Age Adjusted			Multivariate Adjusted*		
	Hazard Ratio	95% CI	p-value	Hazard Ratio	95% CI	p-value
(≤7 µg/dl)n=96		1.0 (referent)		1.0 (referent)		
(≥8 µg/dl) n=27	1.68	(1.10, 2.6)	0.018	2.33	(1.41, 3.87)	0.001

†† Multivariate Adjusted

**The Multivariate model included the following: age, education, waist hip ratio, smoking, health compared to others, walk speed, hypertension, thiazide diuretic use, EKG abnormality, and history of congestive heart failure.

Additional adjustment for clinic, years after menopause, weight, weight loss since age 50, walk for exercise, consumed alcohol in the past 30 days, current calcium use, hormone therapy, ever breastfed, average grip strength, fair or poor health, functional status, diabetes, myocardial infarction, angina, enlarged heart, heart murmur, stroke, depression, part B Trailmaking Test score, vitamin D use, had no effect on the HR estimate.

Table 4-3 Relative Hazard (95% CI) of Cause Specific Mortality in women in SOF by Blood Lead levels

Cause of Death	Deaths	Blood lead level		
		($\leq 7 \mu\text{g/dl}$)	($\geq 8 \mu\text{g/dl}$)	<i>P</i> _{value}
1. Cardiovascular Disease	54			
Age Adjusted		1.0	1.89 (0.01-3.50)	0.046
**Multivariate Adjusted		1.0	3.31 (1.54-7.12)	0.002
Coronary Heart Disease	23			
Age Adjusted		1.0	3.16 (1.30-7.50)	0.009
§Multivariate Adjusted		1.0	8.66 (2.82-26.56)	0.000
Atherosclerosis	30			
Age Adjusted		1.0	2.51 (1.15-5.50)	0.021
‡Multivariate Adjusted		1.0	3.93 (1.48-10.44)	0.006
2. Cancer	38			
Age Adjusted		1.0	1.60(0.73-3.50)	0.236
§Multivariate Adjusted		1.0	2.13(0.88-5.18)	0.094
3. All Other Deaths	31			
Age Adjusted		1.0	1.43(0.59-3.50)	0.492
©Multivariate Adjusted		1.0	1.78(0.62-5.08)	0.283

Notes: ICD9 Code:

- 390-448: deaths due to CVD : Including all diseases of circulatory system except those involving veins and lymphatics
- 410-414: deaths due to CHD: Ischemic Heart Disease: Including MI and sudden death
- 410-415 ,798,427.5,428,429.2: deaths due to ASH: Atherosclerosis Cardio disease
- 140-239: deaths due to cancer.
- All other Deaths: Non CVD and non cancer deaths

****CVD:** The Multivariate model included the following: age, education, waist hip ratio, smoking, health compared to others, walk speed, hypertension, thiazide diuretic use, EKG abnormality and history of congestive heart failure.

Additional adjustment for clinic , years after menopause, weight, weight loss since age 50, walk for exercise, consumed alcohol in the past 30 days, current calcium use, hormone therapy, ever breastfed, average grip strength, fair or poor health, functional status, diabetes, myocardial infarction, angina, enlarged heart, heart murmur, stroke, depression, part B Trailmaking Test score, vitamin D use, had no effect on the HR estimate

§CHD: The Multivariate model included the following : age, weight, waist hip ratio, health compared to others, walk speed, diabetes hypertension, thiazide diuretic use, years since menopause, calcium use, and history of congestive heart failure, functional status

Table 4-3 Continued

Additional adjustment for clinic, years after menopause, weight loss since age 50, walk for exercise, consumed alcohol in the past 30 days, current calcium use, hormone therapy, ever breastfed, average grip strength, fair or poor health, functional status, diabetes, myocardial infarction, angina, enlarged heart, heart murmur, stroke, depression, part B Trailmaking Test score, vitamin D use, had no effect on the HR estimate

‡ **Atherosclerosis:** The Multivariate model included the following: age, weight loss since age 50, waist hip ratio, health compared to others, hypertension, diabetes, years since menopause, calcium use, hormone use, and history of heart attack, functional status. Additional adjustment for clinic, walk for exercise, consumed alcohol in the past 30 days, current calcium use, hormone therapy, ever breastfed, average grip strength, fair or poor health, functional status, diabetes, myocardial infarction, angina, enlarged heart, heart murmur, stroke, depression, part B Trailmaking Test score, vitamin D use, had no effect on the HR estimate

\$**Cancer Deaths:** The Multivariate model included the following : age, clinic, hypertension, diabetes, years since menopause, functional status, walk for exercise, part B Trailmaking Test score, smoking, hormone therapy.

© **All Other Deaths:** The Multivariate model included the following: age, clinic, hypertension, diabetes, years since menopause, functional status, and walk for exercise, part B Trailmaking Test score, smoking, hormone therapy.

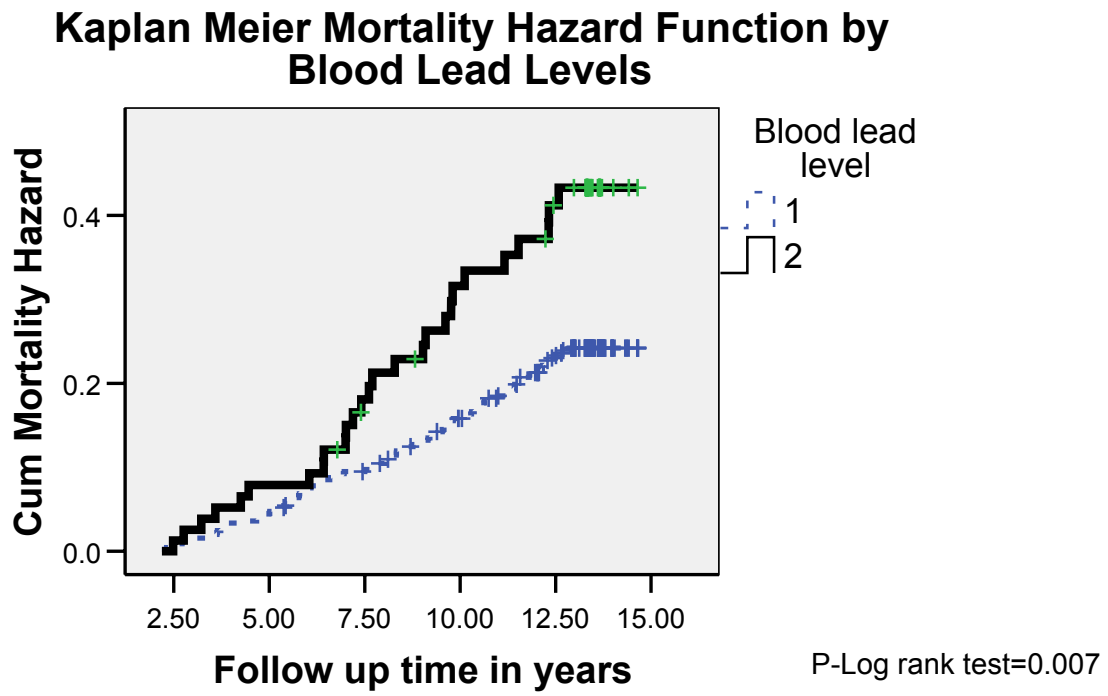


Figure 4-3 Kaplan-Meier Cumulative Mortality Hazard Function in women in SOF by Blood Lead Levels

5.0 THE ASSOCIATION OF CUMULATIVE LEAD EXPOSURE AND NEUROCOGNITIVE FUNCTION

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5.1 ABSTRACT

Background: Lead is known to alter performance on a variety of cognitive measures. Whether long term occupational lead exposure modifies age related cognitive decline, and if specific cognitive domains are affected, is of recent public health concern.

Methods: We completed a follow-up study to evaluate the association of lead biomarkers with cognitive function in 89 lead exposed and 52 un-exposed blue collar male workers from Eastern Pennsylvania. The workers had been previously assessed in 1982 as part of a larger cross-sectional study. For the current study, we measured tibia lead levels by x-ray fluorescence and collected blood lead levels. A battery of cognitive tests was completed in the original 1982 evaluation, and the current assessment repeated the same battery of tests. The test battery assesses five major cognitive domains: psychomotor speed, spatial function, executive function, general intelligence and memory as well as a composite score. Comparisons were made between workers. In addition, the effect of age, <55 years or greater, on cognition, exposure and lead biomarkers was analyzed, adjusting for education, income, marital status, years of employment, smoking and alcohol intake.

RESULTS: The mean (\pm sd) age of workers was 54(9) and they were employed for an average of 25(8) years. Controls had an average age of 55(9) years and were employed for an average of 27(7) years. For exposed workers, the median (\pm Inter quartile range) bone lead level was 57(20-86) μ g of lead/g of bone mineral (μ g/g) and 8(4-15) μ g/dl blood lead respectively. For controls, the median (Iqr) bone lead level was 12(-8 to 32) μ g/g, and 3(3-4) μ g/dl of blood lead. Compared to controls, exposed workers, had higher bone lead levels which were associated with lower total cognitive scores in 2004 ($p < 0.05$) as well as lower spatial function scores ($p < 0.001$). In exposed workers age 55 and older,

higher bone lead levels were associated with lower test scores for total score ($p < 0.001$) spatial function and general intelligence domains ($p < 0.001$ both). In exposed workers, higher bone lead levels also predicted cognitive decline in the overall total score, spatial ($p < 0.001$ for both), and executive function ($p < 0.05$) from 1982 to 2004. For total cognitive score in exposed workers, the decrement associated with an increase of 50 $\mu\text{g/g}$ bone lead was comparable in magnitude to the cognitive decline associated with 5 years of age.

Conclusions: Independent of recent lead dose, retained bone lead from cumulative exposure may have persistent effects on cognitive function. A portion of age related decrement in cognitive function in this population may be associated with earlier lead exposure.

5.2 INTRODUCTION

Lead is a highly prevalent neurotoxicant that bio-accumulates in bone with a half life of 25-30 years[1-4]. Lead is associated with decreased cognitive function in both children and adults[5-7]. In adults lead exposure has been associated with decreased performance in psychomotor speed, manual dexterity, memory, and learning ability[8]. Whether cumulative occupational exposure to lead causes progressive decrements in specific cognitive domains with aging, has been of recent concern[9]. The bio-markers that estimate content of lead in the body are, blood levels that estimates recent exposure or endogenous recirculation, and bone levels, which estimate cumulative dose[10-12].

The skeleton is the major repository for lead within the body, sequestering up to 95 percent of lead where it can be measured by X-ray fluorescence (XRF) [2, 3, 13-16]. Lead may be mobilized from skeletal stores when there is high bone turnover, such as in age related osteoporosis and can be an endogenous source of recirculation[4]. Blood lead concentrations are presumed to reflect exposure received in the most recent few months, but actually it is a composite index that reflects the equilibrium between ongoing exposure, excretory loss, and movement of lead from bone[3, 17].

Occupational studies have reported that lead adversely affects cognitive performance as a function of recent and cumulative dose[18, 19] Past cumulative absorption of lead has been associated with longitudinal declines in verbal memory and learning, visual memory, and executive function. A more recent study of organolead workers reported significant neurological and structural decrements with higher tibia lead, as long as 18 years after occupational exposure stopped[8]. It is postulated that neurological lesions associated with bone lead are persistent and progressive[20]. This is consistent with animal evidence suggesting that lead can cause cell death from oxidative

stress or changes in cell structure, such as neurofibrillary tangles[21-24]. Lead could disrupt the myelination that normally continues in the fifth to sixth decade of life in cortical association areas that have large axonal projections[25].

We report the results of a follow-up evaluation of an earlier occupational study conducted in 1982 where blood lead was predictive of poorer neurobehavioral test scores in psychomotor speed. To determine whether long term exposure to lead as an adult can cause progressive changes in cognitive function with aging, we conducted a follow up of 89 lead workers and 52 non-exposed controls (from the original cohort enrolled in 1982). We hypothesized that workers with higher bone and blood lead levels would have a greater risk of cognitive decline with age.

5.3 METHODS

5.3.1 Study population

The Lead Occupational Study was a cross sectional study conducted in 1982 with 288 white lead exposed male workers and 181 controls. Lead exposed workers were drawn from company lists at three lead battery plants located in Eastern Pennsylvania. Unexposed control workers were drawn from a neighboring factory that manufactured truck chassis. The control subjects had no documented exposure to lead or other neurotoxic chemicals[26]. Inclusion criteria for the initial evaluation were; employment for at least one year, English speaking Caucasian male, 18-60 years of age.

The 1982 Lead study compared cognitive function in exposed and control workers with the Pittsburgh Occupational Exposures Test (POET) battery [26-29]. The POET

consists of a battery of cognitive tests that was designed to assess performance across different cognitive domains. A factor analysis from the 1982 study revealed five primary cognitive domains: psychomotor speed, spatial function, executive function, general intelligence, learning, and memory.

At the initial evaluation in 1982 (v1), the exposed workers had a mean age, and education and employment of 35, 11 years, respectively. The control workers had a mean age, education of 40, 12 years respectively. The mean blood lead levels in exposed and controls workers was 40 $\mu\text{g}/\text{dl}$ and 7.2 $\mu\text{g}/\text{dl}$ respectively. After controlling for age, education, and income, the exposed workers scored significantly less on tests of psychomotor speed with the differences limited to older workers[26, 27]. The original income, alcohol use and blood lead levels data for these workers was de-identified and not available for the current.

In the current paper we present results on the cognitive measures for 89 of the original lead exposed participants (31% of the original exposed at v1) and 52 of the non-exposed controls (29% of the original non-exposed at v1). The same test battery that was administered in 1982 was re-administered in 2004. In addition to blood lead levels, tibia lead measurements with XRF were completed. The protocol and consent forms were approved by Institutional Review Board of the University of Pittsburgh, and all participants provided written informed consent. The University of Pittsburgh Radiation Safety Committee approved the XRF protocol. Subjects were paid a fee to travel to Pittsburgh for the current evaluation.

5.3.2 Data collection

Data was collected at the University of Pittsburgh. All subjects completed baseline testing in a standard order: standardized questionnaire to assess demographic and background information, blood pressure, height, weight, cognitive testing, and XRF measurement.

5.3.3 Cognitive test battery

The domains included **psychoMotor speed**: Trail making part A: Administered in standard fashion, Embedded Figures: Mean time to find correct solutions on the Boston Embedded Figures test (see below). Grooved Peg board: Time to insert 25 pegs in a pegboard (first with the dominant hand and then with the non dominant hand). **Spatial Function**: Visual Reproductions: Immediate reproductions of the Wechsler Memory Scale (WMS) in which subjects were asked to copy each of the designs. Delayed recall of the Visual Reproductions was assessed 30 min later when subjects were unexpectedly required to reproduce them from memory. Boston Embedded Figures: On each trial, a relatively simple line drawing was shown along with four complex patterns. The subject was asked to identify the matching pattern. Time to complete this test was coded under the Psychomotor Speed Domain (see above). The Wechsler Adult Intelligence Scale Revised (WAIS-R) Block design subtest: required subjects to reproduce a series of increasingly difficult geometric designs using red and white blocks. Scoring reflects both accuracy and speed in correctly reproducing the designs. **Executive Function**: Trail Making Part B was administered in the standard fashion. WAIS-R Digit Span and Digit Symbol Substitution subtests were administered in the usual manner. **General Intelligence**: WAIS-R Information, Picture Completion, and Similarities were administered in the standard fashion. **Learning and Memory**: Verbal Paired Associative Learning.

Each of the 10 pairs of unrelated nouns was read to the subject while presented visually. Subjects were tested by presenting the first word of each pair as a retrieval cue. Delayed verbal recall of the 10 pairs was assessed 30 min later by cueing subjects with the first word of each pair. Symbol Digit Paired Associate learning: seven symbols, each paired with a single digit, were presented one at a time for 3 seconds. Delayed symbol –digit recall was assessed 30 min later by presenting each of the 7 symbols and subjects were asked to recall the digit paired with the symbol. Incidental Recall of the symbols from the WAIS-R Digit Symbol Substitution Test was queried. A Recurring Words Test asked subjects to look at a series of four letter words and recall which ones were previously presented.

5.3.4 Biological measurements

Bone lead levels Bone lead concentration was obtained with the conventional K-XRF technique employing 88.025 KeVs from ^{109}Cd induced Pb K-shell x-ray fluorescence, measured with a backscatter counting geometry. A 30-minute measurement was taken at the midshaft of the left tibia after washing with a 50 percent solution of isopropyl alcohol. The tibial midshaft was taken as the midpoint between the tibial plateau and the medial malleolus. The K-XRF beam collimator was sited perpendicularly to the flat bony surface of the tibia. Tibia lead level was expressed in units of μg lead per gram of bone mineral, (hereafter referred to as $\mu\text{g/g}$). To maintain quality control, a check standard (Lucite encased lead target) was run prior to subject measurement. The University of Pittsburgh Radiation Safety Committee approved the XRF protocol.

Blood lead levels A blood sample for lead measurement was taken in a special lead-free tube containing ethylene-diamine-tetra-acetic acid and was determined using atomic absorption spectrophotometry conducted by the Central Laboratory Service Inc., an

affiliate of the University of Pittsburgh Medical Center. Detection limits for this laboratory are 3 ug/dl.

5.3.5 Covariates:

Information about age (years), cigarette smoking history (current, never), family income, and education was obtained from the interview. Information about height, weight, body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters) systolic and diastolic blood pressure and alcohol use (amount consumed per week) was obtained from the physical examination and examination-associated questionnaire, respectively. Income was categorized as >40,000 or ≤40,000\$/ year. A dichotomous education variable was created on the basis of the number of years of education reported by the participant (0-11 years: <high school; 12 years or greater = completed high school or higher).

5.3.6 Statistical analyses

Baseline characteristics by exposure status were compared by Chi-square test for categorical variables and either 2-sample t-test or Wilcoxon Mann Whitney-U test for continuous variables. Interquartile ranges were the difference between values at the 75th and 25th percentile levels of a given distribution for non-normal variables (bone lead, blood lead and drinks/wk). Two-tailed p-values were used for all tests, at 5 % statistical significance. Participants were analyzed by exposure status i.e., exposed (n=83) compared to controls (n=52). We further divided the exposed workers into two age groups: <55 years (n=54), ≥55 (n=29) for age stratified analysis. The 20 cognitive test scores were summarized into five cognitive domain scores (based on prior factor analysis)

to minimize multiple comparisons [26, 27]. All cognitive tests were z-transformed and were standardized for direction so that a negative regression coefficient indicated worse performance with increasing lead levels.

Linear regression was used to determine the association of cognitive function with lead biomarkers. To determine cross sectional association of lead biomarkers with cognition at v2, we used regression analysis stratified by exposure. The exposed group was stratified further by age into <55 and \geq 55 years. To predict cognitive decline from v1 to v2 we used linear regression analysis stratified only by exposure. We ran separate models for each cognitive domain first with covariates (Model1), sequential adjustment for bone lead (Model 2) , removed bone lead and added blood lead(Model 3), and blood and bone lead together (Model 4).

We also used linear regression to analyze association lead levels at v2 with change in scores from v1 to v2. This was based on the assumption that half life of tibia is 20-30 years and it would not substantially change between the two visits (22 years). A new change variable was created as a v2 minus v1 score for all the domains and the total cognitive score. In these models,

We controlled for baseline score for respective domains at v1 in addition to the covariates. We did not analyze association of blood lead levels in the change score models. A Huber White Sandwich estimator of variance was used with robust regression to construct valid standard errors. In multivariate models, we simultaneously analyzed lead biomarkers and other potential risk factors. We included variables which were significantly associated with respective outcomes or lead exposure either reported in literature or from preliminary univariate analysis. Age (years), BMI, alcohol intake (drinks/wk), duration of employment (years), years since last worked, blood pressure (systolic blood pressure divided by the diastolic blood pressure) were entered as

continuous variables. All other variables were categorical: income (≤ 40 k/yr vs. >40), smoking (yes/no) education (≥ 12 years, <12 years), and marital status (unmarried / married). To assess interactions between lead and other covariates, we created multiplicative interaction terms between the lead and other covariate variables and included them in the model along with the main effects. Examination of added variable plots and partial residual plots suggested five influential points which were statistically controlled. Data was analyzed with Stata (edition 9, StataCorp, College Station, Texas).

5.4 RESULTS

The mean (SD) age of exposed workers was 54(9) and was employed for 25(8) years. The mean age of controls was 55(9) years and they were employed for 27(7) years. The median (\pm Inter quartile range) of bone lead level was 57(20, 86) $\mu\text{g/g}$ in exposed and 12(-8, 32) $\mu\text{g/g}$ in controls, respectively ($p < 0.000$). The median (\pm Inter quartile range) of blood lead level was 12 (8, 19) $\mu\text{g/dl}$, in exposed and 3(3, 4) $\mu\text{g/dl}$ in controls, respectively ($p < 0.000$). BMI, income, marital status, blood pressure, smoking, alcohol intake were comparable in the two groups. However, exposed workers were less educated than controls. Bone lead levels were correlated to systolic blood pressure in exposed workers, spearman's correlation =0.27, ($p < 0.009$). In exposed workers, there was an inverse correlation between diastolic blood pressure and total cognitive score: ($r = 0.23$; $p < 0.03$), motor function($r = 0.25$; $p < 0.02$), spatial function($r = 0.25$; $p < 0.02$), and general intelligence ($r = 0.21$; $p < 0.05$).

Group Analysis (Stratified by Exposure)

At v2, unadjusted mean total cognitive score, spatial function and general intelligence scores were significantly lower in exposed than control workers ($p < 0.001$; Table 5-2). After controlling for covariates, higher bone lead levels were associated with lower scores in exposed workers than controls for all 5 cognitive domains achieving significance for total score ($p < 0.05$), spatial function ($p < 0.001$), learning and memory function ($p < 0.10$). (Table 5-3). In addition, in exposed workers higher blood lead levels were associated with decreased scores on learning and memory function (Model 3; $p < 0.10$). However, higher blood lead levels also predicted poorer performance for control workers in motor domain in addition to general intelligence and learning and memory (Model 3; $p < 0.10$ for all; Table 5-3).

When both blood and bone lead were analyzed together in exposed workers (Model 4) higher bone lead was a significant predictor of decreased total cognitive score, spatial function, and general intelligence ($p < 0.05$ for all). Blood lead levels and presence of bone lead level in Model 4, were inversely associated with general intelligence in control workers. ($p < 0.10$).

Exposed: Within group analysis (Stratified by age: ≥ 55 , < 55 years)

In age stratified analysis, for lead exposed workers age ≥ 55 , higher bone lead levels predicted lower test scores for total cognitive score ($p < 0.05$), spatial ($p < 0.01$) and learning and memory function ($p < 0.10$; Table 5-5). Blood lead levels in the age ≥ 55 models predicted negative association with learning and memory score ($p < 0.10$) and positive association with general intelligence ($p < 0.10$; 4). When both bone and blood lead were analyzed together in this model, in workers age ≥ 55 , higher tibia lead levels predicted decreased total cognitive score ($p < 0.05$), spatial function ($p < 0.01$) and general intelligence function ($p < 0.05$) and learning and memory ($p < 0.10$). In the same model blood lead levels

predicted a significant positive association with general intelligence ($p < 0.05$). The interactions between biomarkers, age and cognitive domains were not significant.

Change Score (v1 to v2)

The change score models were compared for exposed and control workers only with bone lead. After controlling for confounding variables, higher bone lead in exposed workers predicted cognitive decline over time for total cognitive score, executive function ($p < 0.05$ for both) and spatial function ($p < 0.01$; Table 5-7).

To interpret magnitude of decline in test scores, we describe the mean proportional difference that would be associated with an increase in lead biomarkers. Specifically, the β coefficients from models were multiplied with 50 for bone lead, 10 for blood lead, and 5, for age. For total cognitive score in exposed workers, the decrement associated with an increase of 50 $\mu\text{g/g}$ bone lead was comparable in magnitude to the cognitive decline associated with 5 years of age (Table 5-4., Col.1). In exposed workers, age 55 or older magnitude of cognitive change declined further, more than twice that of 5 years of age related change (Table 5-6, Col 1). Comparison of proportionate cognitive change, from v1 to v1, over 22 years was comparable to age associated cognitive decline by 5 years. (Table 5-8. Col.1)

5.5 DISCUSSION

In this cohort of middle aged men with an average occupational lead exposure of 25 years and 22 years between initial and follow-up cognitive testing, the presence of higher bone lead levels was predictive of lower total cognitive scores at the follow up evaluation. Cognitive function in the spatial, and learning and memory domains was also significantly

decreased. Regression coefficients were negative for psychomotor function, general intelligence and executive function. The association with cognitive decrement was not explained by education, income, lifestyle, and employment variables. Higher bone lead concentration was also associated with decline in performance over time from 1982 to 2004 for total cognitive score, spatial function and executive function [26, 27]. The cognitive decline was more pronounced in older lead workers. This association was seen even after controlling for other risk factors. To our knowledge this is the first study to explore the association of bone lead levels with the longest follow up time (average 22 years) for the same occupational cohort.

Our results corroborate and extend those suggested by several cross-sectional studies that found an inverse association between measures of cumulative lead exposure and tests of cognitive function in occupational cohorts and general population[30-32]. One of the population studies reported significant association of blood lead levels as low as 8 µg/dl with impairment on several neuropsychological tests among a cohort of rural women but not urban women [28].

Several occupational studies have reported the relationship of cross sectional(e.g., blood lead levels) as well as cumulative exposure(e.g., bone lead) with cognitive decrement[19, 33-37]. Acute exposures to high levels of lead among adults has been associated with deficits in performance on visuospatial skills, motor function, reaction time, memory, spatial function, and attention and concentration in lead exposed workers [34, 38-44]. Fewer studies have examined the association between cumulative lead exposure [32]. More recent studies have examined this issue using K-XRF technology to measure lead in bone.

A recent longitudinal study by Schwartz and colleagues compared and contrasted associations of blood lead and tibia lead with declines in cognitive function over the course of 2.2 years in subjects with current and past occupational exposure to inorganic lead[33]. There were consistent associations of blood lead with test scores at baseline and of tibia lead with declines in test scores over the next year, mainly in executive abilities, manual dexterity, and peripheral vibration threshold. The results support the inference that lead has an acute effect on neurobehavioral test scores as a function of recent dose and a longer-term (possibly progressive) effect on cognitive decline as a function of cumulative dose[33].

Recent research suggests that cognitive decline due to lead is progressive, even after the exposure ceases. A study of older former organolead workers(n=118; mean age of 56) who had not been exposed to lead for 16 years found that bone lead levels were associated with changes on cognitive tests over a 4 year span. Compared to controls, former lead workers performed worse over time for three tests of visuo-constructive ability and verbal memory and learning ($p < 0.05$). In the lead workers, bone lead also predicted declines for six tests of verbal memory and learning, visual memory, executive ability, and manual dexterity ($p < 0.05$ for four tests and < 0.10 for two additional tests)[18]. In general, the domains that have been consistently associated with bone lead concentration are executive function/attention and visuospatial/ visuomotor tasks, although associations with verbal and memory tasks has also been noted [19, 34, 36, 45, 46].

The mechanism underlying the effects of lead on the central nervous system have been of more recent interest [12, 18, 33, 34, 43, 47]. Lead is reported to effect specific areas in the brain such as the hippocampus, cerebellum, and frontal cortex [48-62]. Lead alters the permeability of the blood brain barrier[63], and accumulates in astroglia, which are essential for maintenance of neuronal environment[64]. Lead exposure can interfere

with several calcium dependent processes[65]. Lead activates protein kinase C (PKC)which has been implicated in neurotoxicity [65]. Magnetic Resonance Imaging (MRI) studies have reported an association of higher tibia lead with increased prevalence and severity of white matter lesions and smaller structure-specific brain volume [23]. Cerebral white matter changes on MRI are associated with higher bone lead levels and psychomotor slowing as measured by Grooved Pegboard test [23, 36, 66]. Tibia lead has been associated with the strongest effects on verbal memory and learning, visual memory, and executive function which may indicate disruption of widely distributed neural networks involved in the integration of functions and would be consistent with lesions to cortical association areas[23].

The studies by Stewart and colleagues suggest that adult exposure to lead may accelerate age-associated brain changes. Myelination continues into the fifth and possibly sixth decade of life in selective brain regions (e.g., inferior temporal, prefrontal, and temporoparietal regions). White matter produced later in life around cells with long but small caliber projections in cortico-cortical association areas may be sensitive to oxidative stress[66]. The significant associations of tibia lead with the parietal white and gray matter, and temporal white matter, suggest that lead accelerates an age-associated process in selected brain regions. No significant associations were observed in regions where myelination occurs early in life (e.g., occipital lobe and cerebellum) and where short axonal projections are relatively common[8].

Lead exposure is a risk factor for hypertension, which may cause cerebrovascular disease leading to poor performance on cognitive tests. [67-69]. High blood pressure also correlated with number of white matter lesions and total brain volume[23, 70]. We defined blood pressure as systolic blood pressure divided by diastolic blood pressure. A positive correlation was noted between systolic blood pressure and bone lead in lead exposed

male workers (spearman correlation: 0.28, $p < 0.009$). In exposed lead workers significant inverse correlation was observed between diastolic blood pressure and scores on several cognitive tests.

Decreased ratio of N-acetylaspartate (NAA) to creatine, a marker of neuronal density in hippocampus and frontal lobe as measured by Magnetic Resonance Spectroscopy (MRS) neuroimaging studies, is associated with higher bone lead levels and deficits on attention executive function, visuospatial /visuomotor functioning and short term memory [71]. Lead induces neurofibrillary tangles in frontal cortex and hippocampus [21, 22, 24] and is selectively toxic to limbic system, that modulates behavior, emotions, learning and memory[24].

Lead and homocysteine are both associated with cardiovascular disease and cognitive dysfunction[72]. An association between blood lead and homocysteine has been documented which suggest that homocysteine could be a mechanism that underlies the effects of lead on the cardiovascular and central nervous systems, possibly offering new targets for intervention to prevent the long-term consequences of lead exposure[72, 73].

We tried to overcome some of the methodological weaknesses in earlier research which included sampling inadequacies, selection bias, and variation in data collection procedures, and reliance on current indices of lead exposure rather than cumulative exposure indices. There are a number of strengths to our study. We studied a well-characterized cohort of lead exposed and control men, using state-of-the-art measurements of K-XRF and controlled for a number of important covariates. However there may be a bias in this study. At v1 the authors had reported no significant differences between exposed and non-exposed workers except psychomotor speed [26, 27]. However when we compared the v1 unadjusted mean scores between exposed and non-exposed for the current cohort , there were significant differences between spatial and general

intelligence function in addition to psychomotor scores. The workers that came back for v2 might be the ones who performed at the lowest range of cognition at initial evaluation.

A national public health objective for 2010 is to reduce the blood lead levels ≥ 25 $\mu\text{g}/\text{dL}$ among employed adults to zero[74]. The current blood lead levels ≥ 40 $\mu\text{g}/\text{dL}$, the level at which the Occupational Safety and Health Administration (OSHA) requires workers to have an annual medical evaluation, may need to be lowered. Projections using 1994-2004 ABLES (CDC's state-based Adult Blood Lead Epidemiology and Surveillance) data trends indicate that the national prevalence rate of adults with blood lead levels ≥ 25 $\mu\text{g}/\text{dL}$ will be approximately 5.7 per 100,000 employed adults in 2010. Increased prevention measures in work environments, will be necessary to reduce this rate to zero and decrease risk of cognitive decline in the workforce.

The magnitude of cognitive change associated with normal aging may be affected by environmental neurotoxicants like lead, effects which are persistent and progressive. Lead absorbed during occupational exposure induces neuronal structural damage which is nonreversible and may accelerate the age related structural and functional changes in brain function later in life. In summary, this analysis suggests that higher tibia lead concentrations predict a steeper decline over time in performance on the cognitive test in an occupational cohort of men; cognitive decrement was more pronounced in older workers. While circulating lead in blood may predict performance on some cognitive tests, the change in cognition over time is associated with cumulative exposure to lead.

Cognitive decline with aging is one of the common public health concerns. It impairs the quality of life of affected individuals and their caregivers. Impaired cognition among adults is associated with functional decline in activities of daily living, increased risk of injury to self and others, associated demands on caregivers, and an increased risk of mortality. Although not as severe, even mild cognitive impairment is recognized as a

transitional state between normal aging and dementia. The cognitive impairment associated with lead exposure may be preventable.

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Table 5-1 Socio-demographic characteristics in exposed and control male lead workers in 2004.

N=141	Controls n=52	Exposed n=89	P -value
Age (years) mean (sd)	55(9)	54(9)	0.458
BMI (kg/m ²) mean (sd)	31(6)	30.6(6)	0.739
Education (years) mean (sd)	12.2(1.8)	11.34(1.7)	0.005
Income (1000 \$/yr) n (%)			
> 40	28(54)	51(57)	0.690
≤ 40	24(46)	38(43)	
Marital Status, n (%)			
Married	44(85)	74(83)	0.820
Not Married	8(15)	15(17)	
Smoker, n (%)	10(19)	23(26)	0.371
Drinks (per wk), median*(Iqr)‡	2(0,3)	1(0,4)	0.342
Systolic Blood Pressure (mm Hg) mean (sd)	130(18)	127(17)	0.191
Diastolic Blood Pressures (mm Hg) mean (sd)	78.2(9)	77.9(9)	0.848
Systolic Hypertension§ n (col %)	14(27)	17(19)	0.279
Diastolic Hypertension	6(12)	9(10)	0.791
Blood lead (µg/dl) median (Iqr)	3(3,4)	12(8,19)	<0.000
Bone lead (µg/g bone mineral) median(Iqr)	12(-8,32)	57(20,86)	<0.000
Employment (years) mean (sd)	27(7)	25(8)	0.188
Since last worked (years) mean (sd)	0.04(0.3)	0.33(0.3)	0.007

*Non-parametric Wilcoxon, Mann Whitney U test

§ Defined as systolic blood pressure>140 or diastolic blood pressure >90 mm of Hg

‡Iqr=Inter quartile range

Table 5-2 Unadjusted Mean Cognitive scores in 1982 and 2004 in lead-exposed and control male workers.

Cognitive Domain	1982			2004		
	Mean score (sd)			Mean score (sd)		
	Controls n=52	Exposed n=89	p-value	Controls n=52	Exposed n=89	p-value
Total score	0.00(2)	-1.09(3)	0.012**	-0.00(2)	-1.35(3)	0.008***
Motor	0.00(3)	-1.21(3)	0.031**	-0.00(3)	-1.27(5)	0.105
Spatial	0.00(3)	-1.53(4)	0.013**	0.00(3)	-1.88(4)	0.003***
Executive	0.00(2)	-0.26(3)	0.545	-0.00(2)	-0.63(3)	0.139
Gen. Intel. ‡	0.00(2)	-1.24(2)	0.003***	0.00(3)	-1.54(3)	0.000***
Memory	0.03(4)	-1.25(6)	0.153	-0.00(5)	-1.43(6)	0.119

*significant p-value in bold letters, at *<0.10, **<0.05, ***<.01

‡ General Intelligence

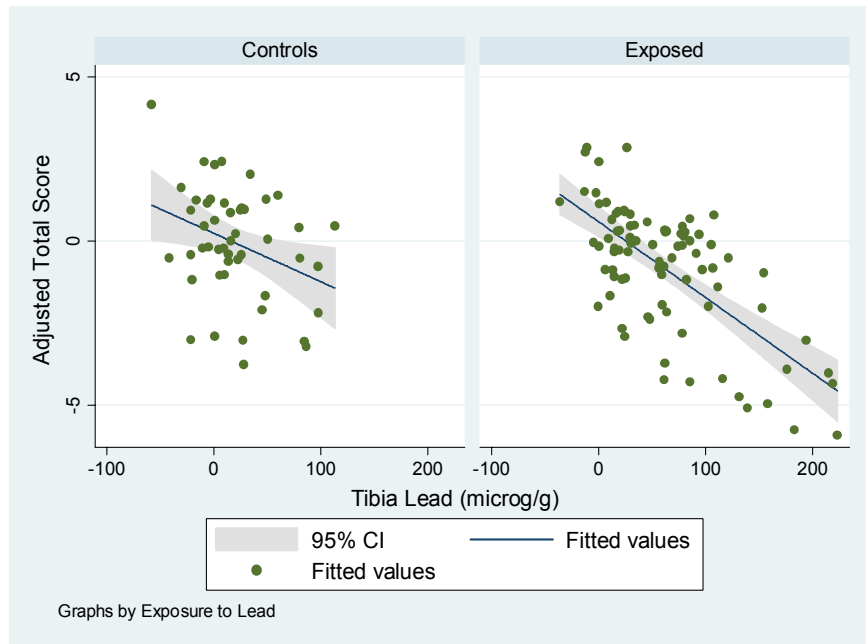


Figure 5-1 Adjusted total Z-score and bone lead levels in exposed and control male workers in 2004

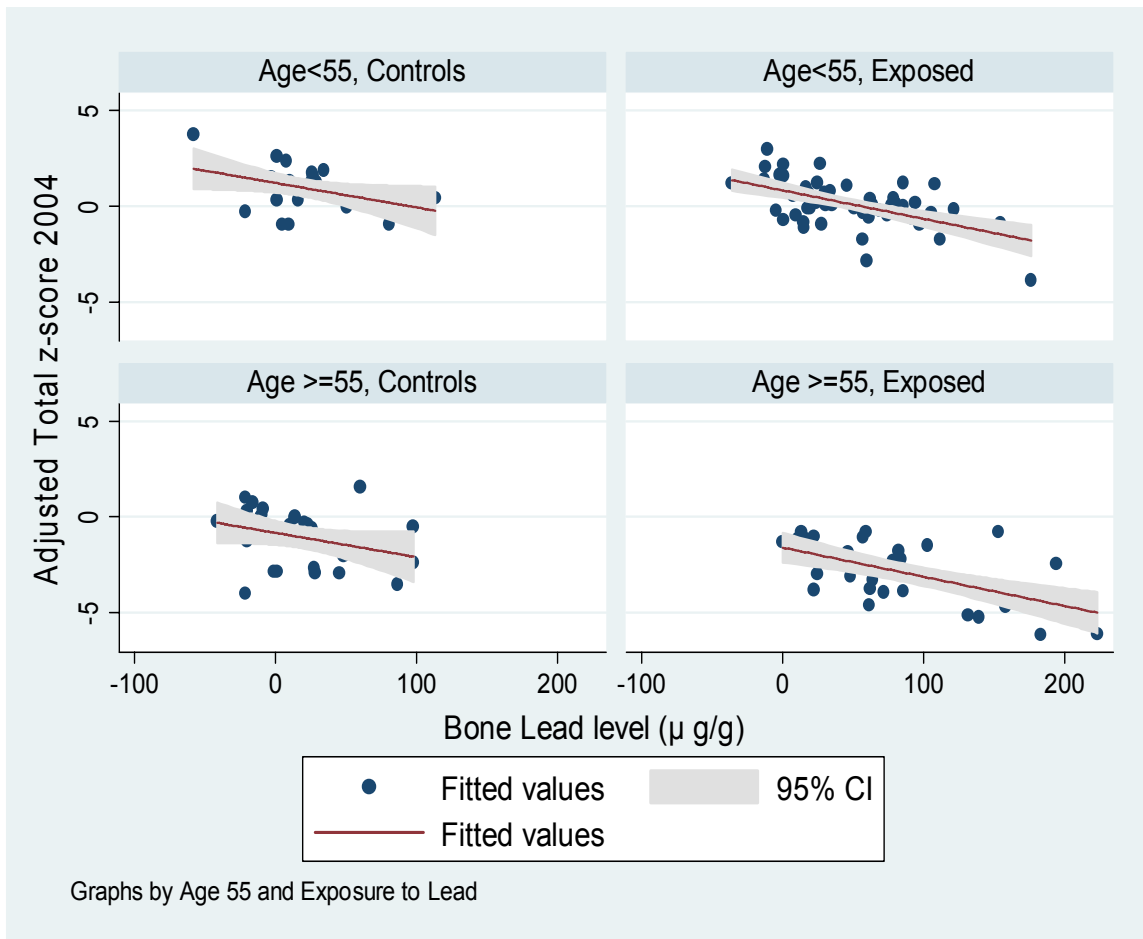


Figure 5-2 Adjusted total Z-scores and bone lead levels in control and exposed male workers by age

Table 5-3 Multiple Regression Models for Cognitive Domain Z-Scores in 2004 in Lead Study workers with Sequential Adjustment for Blood Lead and Bone Lead.

Cognitive Domain	Total Score		Motor		Spatial		Executive		Gen. Intel		Memory	
	Exp N=83	Con N=52	Exp	Con	Exp	Con	Exp	Con	Exp	Con	Exp	Con
Model 1@												
Model 2† Bone Lead	-0.01**	0.00	-0.00	-0.00	-0.02***	-0.00	-0.00	0.00	-0.01	0.00	-0.02*	0.00
Model 3‡ Blood Lead	-0.05	-0.53	-0.06	-1.15*	-0.05	0.41	-0.05	-0.47	0.06*	-0.48*	-0.13*	-0.92*
Model 4§ Bone Lead	-0.01**	0.00	-0.00	-0.00	-0.02**	-0.00	-0.01	-0.00	-0.01**	-0.00	-0.02	0.00
Blood Lead	-0.03	-0.53	-0.06	-1.15*	-0.03	0.41	-0.04	-0.02	0.07**	-0.48*	-0.11	-0.92*

Significant at *<0.10, **<0.05, ***<.01

@Model 1= Age, education, income, marital status, years of employment, years since last worked , smoking , drinks/wk, and BP

†Model 2= Model 1+ Bone lead ,

‡Model 3= Model 1+ Blood Lead ,

§Model 4= Model 1+Bone lead+ Blood lead .

Table 5-4 Cross-Sectional Analysis: Cognitive test scores and (95% CI) in Lead exposed workers in 2004

Estimate (95% C I) (N, Exposed=83)	Total Score	Motor	Spatial	Executive	Gen. Intel	Memory
Age Effect estimate per 5 yrs	-0.55*** (-0.91,-.19)	-1.22*** (-1.79,-.66)	-0.58*** (-1.00,-1.15)	0.03 (-0.40,0.46)	0.37 (-0.00,0.75)	-1.42*** (-2.12,-0.71)
Bone Lead, Effect estimate per 50 µg/g	-0.58** (-1.05,-0.11)	-0.13 (-0.84,0.58)	-0.88*** (-1.51,-0.25)	-0.46 (-1.02,0.09)	-0.39 (-0.88,0.09)	-1.03* (-2.15,0.08)
Blood Lead, Effect estimate per 10 µg/dl	-0.47 (-1.10,0.16)	-0.64 (-1.51,0.23)	-0.48 (-1.46,0.49)	-0.49 (-1.16,0.19)	0.59 (-0.01,1.20)	-1.34 (-2.90,0.22)
Bone Lead, Effect estimate per 50 µg/g	-0.54** (-1.00,-0.07)	-0.05 (-0.78,0.67)	-0.85*** (-1.49,-0.21)	-0.42 (-0.97,0.14)	-0.48** (-0.95,-0.02)	-0.90 (-1.99,0.21)
Blood Lead, Effect estimate per 10 µg/dl	-0.34 (-0.94,0.27)	-0.63 (-1.53,0.27)	-0.27 (-1.25,0.71)	-0.38 (-1.05,0.29)	0.71** (0.09,1.33)	-1.11 (-2.63,0.40)

Significant at *<0.10, **<0.05, ***<.01

@**Model 1**= Age, education, income, marital status, years of employment, years since last worked , smoking , drinks/wk, BP

†**Model 2**= Model 1+ Bone lead ,

‡**Model 3**= Model 1+ Blood Lead ,

§**Model 4**= Model 1+Bone lead+ Blood lead .

Table 5-5 Multiple Regression Models for Cognitive Domain Z-Scores in 2004 in Exposed Lead Study workers stratified by age

Cognitive Domain	Total Score		Motor		Spatial		Executive		Gen. Intel		Memory		
	≥ 55 yr N=29	<55 yr N=54	≥ 55 yr	<55 yr	≥ 55 yr	<55 yr	≥ 55 yr	<55 yr	≥ 55 yr	<55 yr	≥ 55 yr	<55 yr	
Model 1@													
Model 2† Bone Lead	-0.02**	0.00	-0.01	-0.00	-0.03***	-0.00	-0.02	-0.00	-0.01	-0.00	-0.03*	-0.01	
Model 3‡ Blood Lead	-0.08	-0.02	-0.07	-0.05	-0.06	0.01	-0.06	-0.02	0.13*	-0.05	-0.28*	-0.04	
Model 4§	Bone	-0.02**	-0.00	-0.00	-0.00	-0.03***	-0.01	-0.02	0.00	-0.01**	-0.00	-0.02*	-0.01
	Blood	0.03	-0.01	0.02	-0.05	-0.00	0.01	-0.03	-0.03	0.14**	0.04	-0.21*	-0.04

Significant at *<0.10, **<0.05, ***<0.01

@Model 1= Age, education, income, marital status, years of employment, years since last worked , smoking , drinks/wk, BP

†Model 2= Model 1+ Bone lead ,

‡Model 3= Model 1+ Blood Lead ,

§Model 4= Model 1+Bone lead+ Blood lead .

Table 5-6 . Cross-Sectional Analysis: Cognitive test scores and (95% CI) in Lead exposed workers in 2004 stratified by age (<55, ≥55 yrs) (Expressed per 50 µg/g increase of bone lead, per 10µg/dl increase of blood lead and per 5 increases of years in age).

Cognitive Domain	Total Score		Motor		Spatial		Executive		Gen. Intel		Memory	
	≥ 55 N=29	<55 N=54	≥ 55	<55	≥ 55	<55	≥ 55	<55	≥ 55	<55	≥ 55	<55
Age in years												
Model 2† Bone Lead Effect estimate per 50 µg/g	-0.88**	-0.22	-0.17	-0.11	-1.66***	-0.35	-0.65	-0.06	-0.50	-0.15	-1.48*	-0.48
Age Effect estimate per 5 yrs	-0.39	-0.20	-1.14	-0.81	0.06	-0.17	-0.65	0.26	0.48	0.24	-1.12	-0.48
Model 3‡ Blood Lead Effect estimate per 10 µg/dl	-0.79	-0.16	-0.69	-0.51	-0.83	-0.19	-1.00	-0.19	1.47*	0.57	-2.79*	-0.44
Model 4§ Bone	-0.84**	-0.20	-0.09	-0.03	-1.67***	-0.33	-0.56	-0.03	-0.75**	-0.24	-1.20*	-0.43
Blood	-0.33	-0.13	-0.65	-0.51	0.05	-0.13	-0.71	-0.18	1.87**	0.61	-2.14*	-0.36

Significant at *<0.10, **<0.05, ***<.01

©Model 1= Age, education, income, marital status, years of employment, years since last worked , smoking , drinks/wk and BP

†Model 2= Model 1+ Bone Lead ,

‡Model 3= Model 1+ Blood Lead ,

§Model 4= Model 1+Bone lead+ Blood lead .

Table 5-7 Multiple Regression Models for Cognitive Domain Z-Scores Change from 1982 to 2004 in Lead Exposed Workers with Sequential Adjustment for Bone Lead.

Cognitive Domain	Total Score	Motor	Spatial	Executive	Gen. Intel	Memory
Model 1[@]						
Model 2[†] Bone Lead	-0.0074^{**}	-0.0034	-0.0140^{***}	-0.0096^{**}	-0.0055	-0.0033

Significant at *<0.10, **<0.05, ***<.01

[@]**Model 1**= Age, education, income, marital status, years of employment, years since last worked, smoking, drinks/wk, BP

[†]**Model 2**= Model 1+ Bone Lead

Table 5-8 Cognitive test change scores and (95% CI) in Lead exposed and control workers from 1982 to 2004 (expressed per 50 µg/g increase of bone lead, per 10µg/dl increase of blood lead and per 5 increase of years in age).

Cognitive Domain	Total Score		Motor		Spatial		Executive		Gen. Intel		Memory	
	Exp	Con	Exp	Con	Exp	Con	Exp	Con	Exp	Con	Exp	Con
Model 1@												
Model 2† Bone Lead Effect estimate per 50 µg/g	-0.37**	0.18	-0.17	-0.07	-0.72***	-0.03	-0.48**	0.42	-0.28	-0.03	-0.16	0.61
Age Effect estimate per 5 yrs	-0.41***	-0.47**	-1.12***	-0.92**	-0.26	-0.24	0.01	-0.41	0.17	0.27	-0.82***	-0.73

Significant at *<0.10, **<0.05, ***<.01

@Model 1= Age ,education, income, marital status, years of employment, years since last worked , smoking , drinks/wk and BP

†Model 2= Model 1+ Bone Lead

6.0 DISCUSSION

6.1 SUMMARY OF FINDINGS

This prospective analysis examined the association of lead biomarkers with changes in bone mineral density (BMD), incident fractures and falls, cognition and mortality. In the SOF analysis, baseline total hip BMD was lower in women with high blood lead levels. The annualized rate of decline in hip BMD was greater among women with high blood lead level, who also experienced a two-fold increased risk of fracture and falls. Women with higher blood lead levels at baseline had increased risk of all cause, and cardiovascular mortality compared to women with lower blood lead levels. These relationships were independent of age and shared risk factors between blood lead levels and BMD, fractures, falls and mortality.

Our study findings in elderly women are an important addition to existing literature as this is the first longitudinal study to report an inverse relationship between lead levels and BMD decline in total hip and femoral neck. These associations are consistent with a recent report of vertebral BMD decline in women 52 years of age (range, 39–68 years) who had occupational lead exposure[48, 49, 204]. In a longitudinal analysis across 6 years, after controlling for baseline spine BMD, baseline blood lead, time since menopause, and occupational exposure, every log unit increase in blood lead measured at follow-up resulted in a decrease of -0.039 (g/cm²) in spine BMD. In NHANES II (1976-1980) in both black and white women there was a highly significant increase in blood and

calculated plasma lead concentration after menopause[52]. In another study both cortical and trabecular bone lead measures were significantly and positively associated with blood lead in among postmenopausal women not using estrogen[47]. The observed interaction of bone lead with estrogen status in determining blood lead supports the hypothesis that increased bone resorption, after menopause because of decreased estrogen production, results in heightened release of bone lead stores into blood. Women in our study with higher blood lead level had lowest intake of estrogen and Vitamin D.

In women we observed a two fold increased risk of non-spinal, non traumatic fractures that had higher blood lead levels. Notably this association was observed after controlling for age as well as other fractures risk factors. To our knowledge, no other study has explained the association of blood lead levels and fracture risk in older human subjects. In children stunted stature is reported after lead exposure. In one study of 7-15 years old children blood lead level was inversely related to reduced height, trunk, leg, and arm lengths. The estimated reduction in height was 5cm/ 10 ug /dl increase in blood lead levels. The reduction in height occurred primarily in leg length and was more marked in the female children [80, 205]. The inverse relationship of blood lead levels to stature was also evaluated in children aged 5-12 years, derived from the data sets of the Hispanic Health and Nutrition Examination Survey (HHANES) conducted in 1982-1984. There was an inverse relationship between blood lead with stature [93]. Animal models explain higher incidence of fractures with higher blood lead levels[87, 88].

There is paucity of literature on the association between lead exposure and risk of falls. We observed two fold higher risks of incident falls in women with higher blood lead levels even after controlling for age and risk factors. Reports of occupational lead exposure and impaired postural balance and vibration sensitivity are documented[113].

Children who survive acute lead encephalopathy suffer from ataxia and have difficulties maintaining postural equilibrium[206].

Growing children who had higher exposures to lead during first two years of life (CNS development) were observed to have more postural sway, and the body balance was affected, suggestive of, lead causing impairment in the functional capacities or interconnectivity of the vestibular systems and/or proprioception (position of joints) at 2 years of age [112, 116, 206-208]. Maintaining balance and proprioception are cues to physiological posture, and if disturbed may predispose to falls.

In mortality analysis, we found that elderly women with higher blood lead levels at baseline had increased risk of all cause, and cardiovascular mortality compared to women with lower blood lead levels. The cardiovascular risk factors such as hypertension, history of angina, stroke did not explain this association. An association between blood lead levels and mortality has been reported from analysis of data in NHANES surveys. In one study that reported inverse association of blood lead levels below 10 µg/dl with all-cause and cause-specific mortality from analysis of NHANES III (1988 to 1994), with participants followed up for up to 12 years. When cause-specific deaths were investigated, the increased mortality was concentrated in cardiovascular deaths, which is similar to our results. Women in SOF sub study had three to four fold higher CVD mortality risk at higher blood lead level[148]. We had much lower sample size as compared to this study (N=13,946) and therefore some of the

No association with cancer mortality was found in this range of blood lead. The finding of no increased cancer mortality risk in our study, and NHANES III study are consistent with experimental evidence in which increased numbers of tumors are induced in rodents only after relatively high doses. Accumulating evidence indicates that blood lead levels <10 µg/dl are associated with peripheral arterial disease, impaired renal

function, and elevated blood pressure[135-138, 209]. The present study adds important data on total and cardiovascular mortality, end points of unquestionable public health relevance.

In Lead Occupational study, compared to controls, lead exposed workers had lower total cognitive scores. Their performance was also decreased on spatial, memory and general intelligence domains cross-sectionally. In longitudinal analysis, cognitive scores of lead exposed workers declined more in total, spatial and executive domains, as compared to controls. Age and important risk factors between lead exposure and cognitive change did not explain this association. To our knowledge this is one of the longest follow up for cognitive function assessment in a cohort of lead exposed male workers. One of the strengths of this study was the availability of control group for comparison. The results are concurrent with studies that have reported association of tibia lead exposure with cognitive decline[153, 181, 184, 185, 190, 210, 211].

The findings from the SOF lead sub study need to be considered within the context of its limitations. An important limitation was the reliance on a single blood lead measurement to assess exposure. Blood lead, with a half-life of 30 days, reflects primarily recent external exposures, although it is also influenced by long-term exposures through endogenous recirculation of lead from skeleton. Thus, it is unclear whether the adverse health effects of lead observed here were associated with current or cumulative exposures. The findings of this study may not be generalizable, as men and women of other races were not included.

Despite these limitations, the study has several strengths. SOF data were collected by a rigorous study protocol with extensive quality control procedures and accurate follow up. To the best of our knowledge, the SOF BMD, fracture, and mortality study is the only prospective study besides the data analyses reported from NHANES.

One of the limitations of the lead occupational study is a bias. At the initial cognitive evaluation in 1982, 288 exposed and 181 control male lead workers were reported to perform equally well on measures of learning, memory, attention, visuospatial ability, and general intelligence. The exposed workers were reported to differ from controls only on one measure of psychomotor speed and manual dexterity, the Grooved Pegboard Test, and these between-group differences were restricted to the older lead workers [212, 213]. The records have been de-identified, except the cognitive scores from 1982. When we compared the 2004(v2) unadjusted scores to the 1982(v1) we found significant differences at both v1 and v2 for the current participants. There is the possibility that only the exposed workers who had decreased score at v1 came back for v2. Moreover, we did not have XRF assessment for v1.

In spite of these limitations, we were able to observe significant differences in several cognitive functions due to prolonged lead exposure. The range of blood lead levels (8-19µg/dl) reported at v2 is almost half of that reported from v1. The association detected at the present lower level provides new evidence of the adverse impact of lead at levels that is still considered by acceptable, particularly for occupational settings.

6.2 PUBLIC HEALTH SIGNIFICANCE

Current population blood lead levels are estimated to be substantially higher than blood lead levels in preindustrial period. Although a 10-fold decline in blood lead levels has occurred in the United States in recent decades, current levels remain higher for occupationally exposed, children and elderly. In one report of lead effects on mortality from NHANES, the association of blood lead with cardiovascular mortality was evident at

levels as low as 2 µg/dl. Because 38% of US adults had lead levels >2 µg/dl in NHANES 1999 to 2002, the public health implications of these findings are substantial. The health effects of current lead levels on adult populations, however, are not viewed as a pressing public health concern. The present study, in conjunction with previous data, indicates that this perception may be erroneous and that acceptable blood lead levels in adults need to be lowered further:

Overall, our findings provide epidemiological evidence for the presence of an association between lead exposure, morbidity and mortality in community residing elderly women as well as a male occupational cohort. A more stringent control of lead exposure and better understanding of the mechanism of its effects may help reduce the public health burden of disease.

6.3 FUTURE RESEARCH

The findings in our study for association of lead and multiple health effects could be validated in future studies, where bone lead levels in addition to blood lead levels would demonstrate cumulative environmental exposure assimilated in bones. Such a study would give a comprehensive picture of association of lead in the spectrum of changes seen in women's early growth, reproductive years, and menopausal transition. Women and men are respond differently to lead exposure across age periods, race and exposure levels. It would be of high scientific interest if future studies could look in the temporal association of lead exposure across these strata.

We have identified a possible association between bone mineral density, falls and fractures in addition to mortality and cognitive decline with lead. Future endeavors could

elucidate the common pathway between cardiovascular morbidity and cognitive decline, both though to be associated with homocysteine levels. The study of osteoporotic fractures has an invaluable array of data for anthropometric, biochemical measures across more than 20 years of follow-up, which could be used in future research to assess the common link between skeletal, cardiovascular and cognitive changes and lead.

The lead male cohort is the longest occupational follow up to date. Most of the participants are healthy. The state of the art neuroimaging technology being used in University of Pittsburgh's groundbreaking research could provide insight into the neurological effects of lead in this cohort.

6.4 CONCLUSION

Cardiovascular disease (CVD), cognitive decline, and osteoporotic fractures are major causes of morbidity, mortality, and disability which are commonly associated with normal aging. Recent research has indicated a link between these age related outcomes to environmental and occupational lead levels. Common biochemical processes such as calcium messenger system, has been implicated in skeletal, cardiovascular and neurological pathways. Homocysteine levels are associated with cardiovascular and neurological disturbance. Vitamin D has been associated with fractures, falls, and bone mineral density changes. All of these processes are disturbed by lead exposure. Future research could investigate the common underlying mechanism, these findings may help tailor targeted preventive and therapeutic approaches.

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