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ABSTRACT: Lead is a highly toxic metal that remains a public health concern worldwide. Even at historically low blood lead levels, lead exposure results in adverse health effects in adult populations, including mortality. The mode of action of lead in cardiovascular systems involves oxidative stress and calcium-dependent cellular processes. This paper reviews the literature on lead and cardiovascular function, including robust toxicological and epidemiological evidence of the impacts of lead exposure on morbidity and mortality. It also discusses evidence from a recent randomized controlled trial of chelation therapy in a high-risk population that suggests that reducing lead body burden can lead to immediate improvements in cardiovascular health.

KEYWORDS: Lead exposure, mortality, public health

JEL CODES: I18, Q53

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1. Lead remains a major public health concern in the 21st century

Lead, a highly toxic metal with numerous adverse health effects, was rare on the surface of the Earth during the early periods of human evolution. Extraction of underground lead through mining activity and the widespread use of lead in consumer products, especially during the 20th century, has made this toxic metal ubiquitous in the environment and a major contributor to cardiovascular disease, the leading cause of premature mortality in the United States.

Data on human exposure and knowledge about the harmful effects of lead are well known (Box 1).[1,2] The earliest evidence of human lead exposure, characterized by intermittent exposure over a no-lead background, comes from 250k year-old tooth samples from Neanderthal children.[3] In the Roman era, ice cores estimate that lead mining and mass production – for water pipes, utensils, pigments and other – increased atmospheric lead levels in Europe by a factor of 10.[4] In the middle-ages and the centuries that followed emissions were lower compared to Roman times, but certain workers and population subgroups remained affected by lead poisoning. The affected populations grew with the onset of the industrial revolution in the 18th and 19th centuries. The first published evidence on the cardiovascular effects of lead among chronically exposed workers dates from the 19th century (see details below).

These pre-20th century exposures were small compared to the massive exposures that would affect most populations in most countries during the 20th century.[2] This is particularly true in the United States, where industrial production and marketing of lead-based paint (banned in European countries as early as 1909), and, foremost, the expansion of the automotive industry contributed to markedly high lead exposure and to health consequences that persist today.[2] Lead-acid batteries (each one containing 15-20 pounds of lead), wheel balancing lead weights (still used in some states today), and the use of tetraethyl lead as an antiknock agent in gasoline, consumed and spread millions of tons of lead in the environment every year. Leaded gasoline, in particular, was the largest contributor to atmospheric lead emissions, affecting not just human exposure, but the entire Earth.[4,5] Lead in the body is stored in bone and bone lead levels of an average 20th century adult American were around 1000 times higher than those of pre-industrial humans.[6] Because bone lead has a long-half life,[7] most Americans born before the 1990s still have a body lead burden that is a result of their past exposures to leaded gasoline and other sources.

Despite the phase-out of leaded gasoline and lead-based paint during the 1970-1990s, and the declines in lead exposure among the general population, lead remains ubiquitous in the environment in the 21st century.[2,8] Current sources of lead include old paint, soil, tobacco products (conventional cigarettes and e-cigarettes), secondhand smoke, foods and drinks (including even baby formula), drinking water (water pipes), herbal remedies, toys, cosmetics, electronics, industrial emissions, and combustion sources. Because of its widespread exposure and hazardous health effects (with no threshold), lead remains

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classified as one of the most dangerous chemicals of concern for humans by the Agency for Toxic Substances and Disease Registry (ATSDR)[9] and the World Health Organization (WHO).[10]

Box 1. Lead exposure affecting human populations through past and recent times[1-3]

- Intermittent, short-term lead exposure is present in 250k year-old tooth samples from two Neanderthal children in Payre, France, an area with lead-rich ores nearby. No lead was found in tooth samples from a 100k year-old Neanderthal child in Belgium. Lead exposure was rare before mining developed around 10,000 years ago.
- Lead was one of the first metals mined and smelted from rocks during prehistorical times. First uses were in beads and statues, then pigments (first evidence 5,000 years ago), and as a by-product of silver mining and refining.
- Lead was extensively mined during the Roman era for use in water pipes, tanks, cisterns, roofing, construction materials, cooking utensils, cosmetics, and pigments. Written reports of the health effects of lead are at least 2,000 years old. Vitruvius, an architect, described it is healthier to drink water from clay pipes than lead pipes.
- In the middle-ages and following centuries, lead was used for pottery, roofing, stained glass, alchemists' experiments, and in bullets following the invention of fire-arms in China.
- During the 18th and 19th centuries, industrial uses started, including lead paint, food cans, soldering, and sealed joints in water pipes. The lead-acid battery was invented in 1859 in France. Occupational lead poisoning became a major problem and measures to protect workers from acute lead poisoning and death were slowly introduced.
- During the 20th century, the use of lead paint in the US and the explosion of the automobile industry worldwide (lead-acid batteries, lead wheel weights, and tetraethyl lead use in gasoline), led to a massive increase in the use of lead. The health effects of lead in the general population, in particular long-term neurotoxicity affecting children was described and contributed to the banning of tetraethyl lead in gasoline.
- 21st century: lead wheel weights and acid-lead batteries are still used today. Although they are recyclable, together with e-waste they remain a problem for many populations, especially in developing countries. Lead paints in toys, herbal products, cosmetics, water pipes, tobacco products, and leaded aviation fuel also remain a problem in many countries including the US. World lead production continues, rising from around 8 million tons in 2006 to almost 12 million tons in 2018.[11] China, Australia, Peru, the US, and Mexico were the major global producers in 2019.[11]

2. Why is lead so toxic for humans? How can lead affect so many organs and systems in the body, including the cardiovascular system?

Lead is probably the most studied toxic metal. Extensive research shows that even at levels considered low today (blood levels <5 µg/dL), lead is toxic to the nervous system, kidney, immune system, red blood cells, bones and teeth, and to the cardiovascular system.[12-16] The absence of lead exposure during the evolution of life on Earth (lack of opportunity to adapt to this toxic metal), its ability to form strong bonds with proteins in the body (lead is a cation and proteins are anions), and the resemblance of lead to essential metals such as calcium and zinc likely explain our inadequate response to the adverse molecular and cellular effects of lead. Indeed, through protein binding and the replacement of calcium and zinc in numerous proteins and enzymes, lead alters important pathways for normal cell function including antioxidant defense mechanisms and internal signaling.[13,17,18] Ultimately, chronic dysfunction of these pathways results in clinical disease, accelerated aging, and premature mortality. Epidemiologic and experimental studies support that these effects are independent of other risk factors. Relevant mechanisms for cardiovascular disease are summarized below.

Lead increases oxidative stress. Oxidative stress is caused by the imbalance between the production and accumulation of free radicals, which are toxic to cells and tissues, and the ability of these tissues to reduce free radicals through antioxidant defenses. Lead increases oxidative stress through several mechanisms. First, lead can directly inactivate a major buffer for antioxidant defense called glutathione (GSH).[17,19] Second, lead can inactivate enzymes needed for glutathione synthesis (e.g., glutathione

reductase, glutathione peroxidase, glutathione-S-transferase).[20] There is substantial experimental evidence on how lead inhibits these enzymes. Third, lead can replace zinc, an essential co-factor in antioxidant enzymes such as superoxide dismutase.[20,21] The reduction in glutathione levels and the impairment of antioxidant enzymes reduces the ability of the cells to dispose of free radicals. These free radicals in turn increase lipid peroxidation and oxidative stress, and reduce nitric oxide (NO) levels. Lipid peroxidation and the reduction of NO affect endothelial function (function of the lining of the arteries) and atherosclerosis (thickening and hardening of the walls of the arteries).[22,23] Atherosclerosis develops gradually over time, following endothelial dysfunction and resulting in the formation of plaque. The narrowing of the arteries, restriction of blood flow, and ultimately the rupture of the plaque can result in clinical symptoms, e.g., acute myocardial infarction (heart attack) when it affects the coronary arteries. While there are a number of risk factors that adversely impact endothelial function and atherosclerosis (e.g., diabetes, lipids, smoking (which effects could be at least partially mediated by lead)), increased lead exposure has been associated with atherosclerosis and cardiovascular disease independently of these risk factors (see section 3 below).

Lead impairs calcium-dependent intracellular signaling. Lead resembles calcium and binds to numerous proteins and enzymes that need calcium for their function, altering calcium-dependent internal signaling and interfering with many cellular processes critical for normal cell functioning.[17,24] One of those proteins is calmodulin, a calcium-dependent protein that mediates processes related to inflammation, apoptosis, and smooth muscle cell contraction.[25] These processes are critical for cardiovascular function. Calmodulin is also known to regulate short-term and long-term memory through the facilitation of inter-neuronal communication in the synapses (neuronal junctions). Extensive research has demonstrated that the binding of lead to calmodulin is one of the major mechanisms explaining the neurocognitive effects of lead.[17,24] By binding lead instead of calcium, the improper function of calmodulin could also explain the deleterious effects of lead on the cardiovascular system. Calmodulin is needed for the synthesis of NO, a soluble gas that is synthesized by nitric oxide synthase (NOS) in endothelial cells.[23,26] Multiple animal studies have established that chronic lead exposures reduce NO availability, resulting in endothelial dysfunction and lead-induced hypertension in the absence of other risk factors.[27-32] This reduction in NO could be mediated by improper functioning of calmodulin secondary to lead-binding. It could also be due to an increase in NO degradation because of increased oxidative stress (as explained above). NO is fundamental for the normal function of the endothelium, the monolayer of cells between the arterial lumen and the vascular smooth muscle cells.[22] Among other functions NO maintains vascular dilator tone (essential to maintaining blood pressure levels), regulates local cell growth, and protects the arterial wall from platelets and other cells in the blood.[23] Impaired NO function is a major established mechanism for endothelial dysfunction, which leads to atherosclerosis, clinical cardiovascular disease, and ultimately premature cardiovascular mortality. These processes take place over years and can be accelerated through increases in lead exposure levels from exogenous and endogenous sources, in an independent additive manner to other risk factors.

Lead in bone represents an endogenous source of exposure for decades. Because lead mimics calcium,[17,24] a large proportion of the absorbed lead is incorporated into bone tissue, where it binds to hydroxyapatite, which is the main bone mineral,[33] as well as to osteocalcin, a protein involved in bone mineralization.[34] In cortical bone (higher density bone, e.g. the tibia), the half-life of lead is 30 years or longer.[35,36] In trabecular bone (lower density bone, e.g. patella), the half-life of lead is about 5-10 years.[35,36] From the bone, lead is in recirculation with the blood serving as a continuous endogenous

source of lead to other tissues, including the endothelium. During periods of increased bone resorption (process through which the bone minerals are released and transferred to the blood, e.g. during menopause), lead levels in blood markedly increase. Endogenous lead exposure can thus occur for decades, many years after the main source of exposure has stopped. This persistence of lead in bone, and our inability to naturally eliminate it from the body, explains why so many individuals today are still suffering from the excessive exposure to lead that occurred in the United States during the 20th century. It also means that current exposures, for example such as those in Flint, Michigan or Newark, New Jersey or those in urban communities still affected by lead paint and contaminated soil, could result in health effects that will only manifest many decades down the road, unless something is done to remove those current exposures from the environment as well as from the internal body storage.

Lead health effects appear to be supra-linear. Epidemiologic data support that the health effects of lead are supra-linear (also called decelerating),[37] meaning that the estimated adverse health effects associated with a given increase in blood lead is greater at lower levels than at higher levels. This is well-established for the effects of lead on intellectual function. In a pooled analysis of 7 population-based cohort studies, the steepest declines in IQ were at blood lead levels < 10 µg/dL (3.9 IQ decrements), compared to 1.9 and 1.1 IQ decrements at blood lead levels between 10 and <20 µg/dL and ≥20 µg/dL, respectively.[15] For cardiovascular mortality, a pooled analysis of the dose-response relationship with blood lead levels is not available. The epidemiologic data (summarized in section 3), however, suggest this is possible, with increased risks that appear even stronger in populations exposed to lower lead levels. This supra-linear effect of lead and cardiovascular mortality would also be consistent with the association between particulate matter and cardiovascular disease.[38] These pooled analyses for lead and IQ and for particulate matter and cardiovascular mortality, together with the evidence summarized below for lead and cardiovascular mortality support the relatively strong cardiovascular impact of lead exposure even at currently low exposure levels.

3. Lead as a cardiovascular risk factor – associations with mortality and beyond

Early evidence of lead-induced atherosclerosis. The first known evidence of the association of lead with cardiovascular mortality and atherosclerosis was published in 1881 by E. Lancéreaux (from Paris, France), as part of the *Transactions* of the International Medical Congress taking place in London during August 2-9, 1881.[39] The author summarized data from 22 autopsies of patients with lead poisoning and kidney disease. The author indicated "*the renal effects coincide with a modification of the arterial system characterized by thickening of the intima with nodes more or less protruding"* and "*the heart is normally hypertrophied"* [translation from French]. In 1886, the *British Medical Journal* published the findings of G. Lorimer (from Buxton, England) of 107 autopsies of patients with lead poisoning and gout.[40] The author described that 69 of them showed "*sclerosis of the arterial walls, along with atheromatous changes".* The author interpreted these findings as "*premature ageing of the arterial system*" and attributed them "*to the action of lead, which causes contraction of the muscular walls of the arteries, and raises arterial tension"*. He also mentioned "*cardiac hypertrophy is observed in lead gout, especially at the advanced periods of the disease"*. These two autopsy series are remarkable. They report findings consistent with atherosclerosis at a time when lifestyle risk factors for cardiovascular disease were relatively rare. The descriptions, moreover, are consistent with numerous findings from epidemiological and experimental studies conducted in recent decades showing association of chronic low-level lead exposure with hypertension, atherosclerotic outcomes such as peripheral arterial disease, coronary heart disease and stroke, and left ventricular hypertrophy as measured by cardiac ultrasound.

They are also consistent with several autopsy studies published in the early 1980s reporting higher lead levels in the aorta (major artery in the body) of patients dying of cardiovascular disease compared to patients dying of other reasons.[41,42]

The 19th century autopsy studies, for unknown reasons, were overlooked for most of the 20th century, including the debates that took place in the early 1920s regarding the addition of tetraethyl lead to gasoline. Public health professionals, including the Surgeon General, Alice Hamilton, the first female professor at Harvard, and other public health professionals raised major concerns on the public consequences of the addition of tetraethyl lead to gasoline.[2] These concerns were mostly about neurotoxicity, anemia, infertility, and overt acute lead poisoning and death, particularly among workers. It is likely that the effects of lead on the cardiovascular system were not mentioned. It is not until 1957 that a reference to the Lorimer paper is found, this time in a paper in the *Archives of Internal Medicine* emphasizing the relationship between lead exposure and gout.[43] After 1957, the Lorimer study was referenced again in the 1980s, when additional recognition on the potential cardiovascular effects of lead resurfaced and an increasing number of studies started to evaluate the long-term consequences of lead exposure to the cardiovascular system using modern epidemiological methods, including the incorporation of lead biomarkers and cohort study designs to prospectively assess the role of lead exposure as a risk factor for cardiovascular mortality and other cardiovascular outcomes.

Lead Exposure and Cardiovascular Disease Mortality. A total of 15 prospective cohort studies conducted in Europe (4) and the United States (11) have evaluated the association between lead exposure and cardiovascular mortality, among other clinical cardiovascular outcomes, all of them with consistent findings (Table 1 - supplement). The studies in Europe included two studies conducted in the UK (Pocock et al 1988,[44] McElvenny et al 2015[45]), one from The Netherlands (Kromhout 1988),[46] and one from Denmark (Møller and Kristensen 1992).[47] The US studies included 7 studies from the National Health and Nutrition Examination Survey (NHANES) (Lustberg and Silbergeld 2002,[48] Menke et al 2006,[49] Schober et al 2006,[50] and Aoki et al 2016,[51] Ruiz-Hernandez 2017,[53] Lanphear 2018,[52] Wang et al 2019[54]), one study in white women recruited from Baltimore, MD, Minneapolis, MI, Portland, OR, and Pittsburgh, PA for the Study of Osteoporotic Fractures (SOF) (Khalil et al 2009),[55] two studies conducted in men participating in the Department of Veterans Affairs Normative Aging Study (VA-NAS) in Boston, MA (Jain et al 2007,[57] Weisskopf et al 2009[56]), and one study of participants in the Adult Blood Lead Surveillance Program (ABLES) in 11 US states, sponsored by NIOSH (Chowdhury et al 2014).[58]

The studies were published between 1988 and 2019.[51] The sample size ranged from 146 to 58,368 participants.[58] The participants were recruited between 1975-1979 (UK study)[44] and 1999-2012 (NHANES).[54] The mean age of the study participants at recruitment ranged from 39 years[51] to 70 years.[55] Four studies included only men, one study included only women, and six studies included both men and women. Participants recruited before 1980 showed higher blood lead levels compared to participants recruited after 1980, except for men in lead surveillance programs in the UK[45] and the US.[58] Blood lead levels were markedly lower for NHANES 1999-2010.[51] Despite differences in blood lead levels at recruitment across cohorts, all studies found that higher blood lead levels at the beginning (baseline) were associated with an increased risk of cardiovascular mortality at follow-up. In some studies, however, the associations were not statistically significant. Regarding the type of cardiovascular events evaluated, all the studies included fatal cardiovascular disease, either as a whole or for specific outcomes such as coronary heart disease and stroke; some studies also included non-fatal cardiovascular events.

The NHANES studies are of particular importance, as they include representative samples of the non-institutionalized US population in several waves of time. The first time that NHANES measured blood lead was NHANES II (cohort recruited in 1976-1980).[48] After adjustment for cardiovascular risk factors, NHANES II participants with blood lead levels between 20-29 μg/dL showed a 39% higher risk for total cardiovascular mortality compared to blood lead levels <10 μg/dL. In NHANES III (cohort recruited in 1988-1994), blood lead levels were lower but still associated with an increased risk for cardiovascular mortality.[49] Specifically, blood lead levels ≥ 3.63 μg/dL (highest tertile) compared to < 1.93 μg/dL (lowest tertile) were associated with a 89% higher risk of coronary heart disease mortality, 151% higher risk of stroke mortality, and 55% higher risk of overall cardiovascular disease mortality after adjustment. The findings from Menke et al. are particularly useful to understand the cardiovascular effects of lead, as hypertension and kidney function were adjusted progressively, showing that the associations remained even after adjustment for these potential mechanisms. In an accompanying editorial, Nawrot et al. concluded that blood lead levels as low as 2 μg/dL represent a cardiovascular health hazard.[59] These findings for NHANES III were replicated in an independent publication of the same data.[50] In NHANES 1999-2010, blood lead levels were modeled comparing a 10-fold increase (blood lead levels transformed as log 10, comparing for instance blood lead levels changing from 0.1 to 1 μg/dL, or 1 to 10 μg/dL).[51] A 10-fold increase in blood lead levels in NHANES 1999-2010 was associated with 44% higher risk for cardiovascular disease mortality. Using the most recent blood lead levels estimates in NHANES 1999-2012, cardiovascular mortality was 45% higher comparing participants at the 75th vs. 25th percentile (2.5 vs. 1.1 μg/dL) of blood lead levels, after adjustment for cardiovascular risk factors.[54]

In addition to these manuscripts on the association of lead exposure with cardiovascular mortality in the US,[48-51,54] NHANES data have been analyzed to assess attributable risk associated to lead exposure[52] and how reductions in lead exposure have contributed to recent reductions in cardiovascular mortality in the US.[53] Using NHANES III, Lanphear et al. estimated the population attributable fraction comparing blood lead levels 6.7 vs. 1 μg/dL (90th vs. 10th percentile) was 28.7% for cardiovascular disease mortality, corresponding to 256,000 deaths a year (185,000 deaths from ischemic heart disease). These figures are based on blood lead data from 1988-1994 and assume the population is first at the 90th and then at the 10th percentile of blood lead levels. Despite its limitations, the estimated population attributable fraction highlights lead exposure as a major risk factor for cardiovascular disease, at least in the 20th century. It is interesting to interpret these numbers in the context of the epidemic of cardiovascular disease, and more specifically coronary heart disease mortality, observed during the 20th century in the US. The age-adjusted coronary heart disease mortality rates increased until 1968 (500 deaths per 100,000 people, more than double today's rate) and started to decline sharply following that year. The National Heart, Lung, and Blood Institute (NHLBI) estimates that if the proportion of the population that died from cardiovascular diseases in 1968, the peak year for such deaths, were applied to 2010, there would have been 1,223,000 more cardiovascular deaths, showing remarkable rapid changes in cardiovascular mortality in the United States. The reasons for the sudden change in cardiovascular mortality rates after 1968 cannot be explained by sudden changes in clinical practice or lifestyle factors. Coincidentally, lead production in the US peaked in 1968, when more than 200K tons of lead were released into the atmosphere, and then started to decline sharply after that year.[60] These reductions in lead emissions have been reflected in lower blood lead levels in the NHANES participants over time. Using data from NHANES III (1988-1994) and NHANES 1999-2004, Ruiz-Hernandez et al have estimated there were 230.7 cardiovascular disease deaths / 100,000 person-years less in the US comparing the most recent to the earlier NHANES study.[53] After accounting for other changes in sociodemographic and

cardiovascular risk, 22.5% of those avoided deaths, or 52 deaths per 100,000 person-years, could be attributed to the reductions in blood lead levels observed between 1988-1994 and 1999-2004, when the geometric mean dropped from 3.2 to 1.9 μg/dL.[53] Overall, the NHANES data and the trends in cardiovascular and lead exposure levels occurring in the US population support that lead is an important risk factor for cardiovascular mortality and that benefits for cardiovascular health can be observed rapidly once lead is removed from the environment.

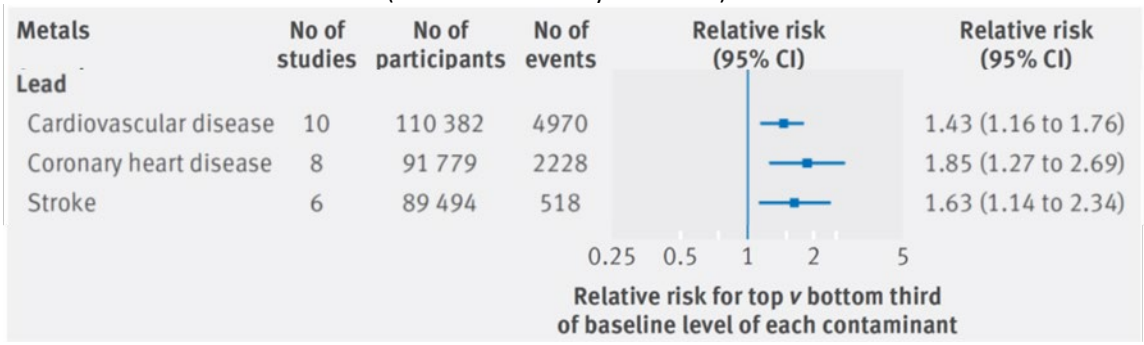
Beyond NHANES, four other studies have been conducted in the US, two of them published in the Veterans Affairs Normative Aging Study (VA-NAS), a prospective cohort study among community-based male veterans in the greater Boston area enrolled in 1963.[56,57] In these studies, during the 1990s, lead was measured in blood and also in bone using a non-invasive technology called X-ray fluorescence. The fully adjusted hazard ratios comparing tertiles 2 (4-6 μg/dL) and 3 (>6 μg/dL) of blood lead vs. the lowest tertile (<4 μg/dL) were 1.09 (0.69, 1.72) and 1.10 (0.67, 1.80) for all cardiovascular mortality, and 1.10 (0.54, 2.23) and 1.21 (0.57, 2.55) for coronary heart disease mortality.[56] The sample size in this study is much smaller (n=1,235) compared to the large sample sizes in NHANES, and these hazard ratios are not significant, however, the point estimates may support an increased risk of cardiovascular mortality with higher blood lead levels. The hazard ratio point estimates were larger and in some analyses were statistically significant for measures of bone lead, which included patella lead in 860 participants. Comparing tertiles of patella lead 2 (22-35 μg lead/g bone) and 3 (>35 μg/g) vs. tertile 1 (<22 μg/g), the hazard ratios were 1.04 (0.58, 1.86) and 1.42 (0.80, 2.51) for all cardiovascular mortality, and 1.08 (0.43, 2.70) and 1.87 (0.77, 4.53) for coronary heart disease mortality. In another analysis of the VA-NAS, the hazard ratio for incident coronary heart disease was 1.45 (1.01, 2.06) for a one log-unit change in blood lead and 2.64 (1.09, 6.37) for a one log-unit change in patella lead.[57]

The Study of Osteoporotic Fractures (SOF) recruited women from 4 US cities between 1986-1988.[55] Women with blood lead ≥ 8 μg/dL had 208% higher risk of coronary heart disease mortality compared to women with blood lead < 8 μg/dL after adjustment for cardiovascular risk factors. For stroke mortality, the corresponding increased risk was 13% but the association was not significant. In the Adult Blood Lead Surveillance (ABLES) program (n=58,368), the risk of coronary heart disease mortality was 13%, 46%, and 77% higher comparing participants with the highest blood lead level category between 5-<25, 25-<40, and ≥40 μg/dL compared to <5 μg/dL.[58] The corresponding figures for stroke mortality were 12%, 76%, and 88%. These US studies, together with evidence from NHANES and Europe support that lead is a risk factor for premature cardiovascular mortality, specifically for deaths due to coronary heart disease and stroke, in the US population.

Pooled estimates from meta-analyses. A recent systematic review has pooled 11 of the 15 studies with data on blood lead levels and cardiovascular mortality in a meta-analysis.[61] The study quality, based on the Newcastle-Ottawa scale for cohort studies, which has a score maximum of 9, ranged from 5 (Chowdhury et al. 2014, McElvenny et al. 2015)[45,58] to 8 (Aoki et al 2016, Lustberg and Silbergeld 2002, and Menke et al 2006),[48,49,51] one study scored a 6 (Weisskopf et al 2009),[56] and the remaining 5 studies received a 7. To enable a consistent approach to meta-analysis and interpretation of findings, relative risk estimates for the association of lead exposure and cardiovascular disease, coronary heart disease, and stroke were transformed to compare the top vs. bottom third of the distribution in each study using established methods. The summary relative risks were pooled using a random-effects model that included between study heterogeneity. Combining both fatal and non-fatal outcomes, the pooled relative risk was 1.43 (95% confidence interval 1.16, 1.76) for the 10 studies that included cardiovascular

outcomes, 1.85 (1.27, 2.69) for the 8 studies that included coronary heart disease, and 1.63 (1.14, 2.34) for the 6 studies that included stroke. Although some studies had non-fatal outcomes, all studies had data on mortality and these pooled estimates are likely to be more relevant for fatal outcomes. A limitation of the meta-analysis is that two studies from NHANES III (Menke et al 2006, Schober et al 2006)[49,50] were included. Ideally, only one of them should have been included. Despite this limitation, given the consistency of the effect estimates across studies, the pooled estimate would likely remain very similar after excluding one of the NHANES III studies.

Figure 1. Pooled relative risks and 95% confidence intervals (CI) for studies of blood lead levels and fatal and non-fatal clinical cardiovascular disease (Source: Chowdhury et al 2018).



In summary, the epidemiologic evidence, which includes 15 studies from populations in Europe and the US, consistently supports that lead exposure is associated with premature cardiovascular mortality in general populations. An important element in the available evidence is that the associations remained after adjustment for other cardiovascular risk factors including age, sex, smoking, body mass index, socioeconomic factors, physical activity, lipids, diabetes, and even after further adjustment for hypertension and kidney function (potential mechanisms for the cardiovascular effects of lead). This is an important finding that indicates that the role of lead in atherosclerotic cardiovascular disease is important in itself, and beyond other established risk factors for atherosclerosis, potentially explaining some of the risk for atherosclerosis that still remains when traditional risk factors have been taken into account or in individuals without established risk factors.

Indeed in the NHANES 1999-2012 study, lead together with other metals (cadmium and mercury) improved the ability to predict future risk of cardiovascular mortality beyond established risk factors.[54] This is an important finding as prediction models to predict cardiovascular disease beyond established risk factors have generally been unsuccessful, thus pointing out that lead and other metals might be distinct risk factors for cardiovascular disease. This is further supported by the early findings of atherosclerotic disease in workers exposed to lead at a time when lifestyle risk factors for cardiovascular disease were uncommon. While the evidence is compelling, the epidemiologic evidence is limited by a relatively small number of studies, especially compared to the large number of observational cohort studies that have evaluated the association of classical risk factors with clinical cardiovascular outcomes and cardiovascular mortality, including flagship cohorts such as the Framingham Heart Study. These classical cohorts, many of them funded by the National Heart Lung and Blood Institute, are currently missing information on lead, an important risk factor for cardiovascular disease in general populations.

Lead and other cardiovascular outcomes. Beyond cardiovascular mortality, there is also a large and consistent body of epidemiologic evidence on the association between lead and subclinical markers of

cardiovascular disease. This body of evidence provides additional support on the chronic adverse impact of lead exposure on the cardiovascular system.

For blood pressure, the association of higher blood lead levels with higher blood pressure levels has been identified in numerous studies in different settings, including prospective studies and in relatively homogenous groups.[12] The dose-response has also been established. The hypertensive effects of lead have also been confirmed in experimental models.[62,63] For these reasons, the evidence is sufficient to infer a causal association between lead exposure and blood pressure outcomes as indicated by the 2013 EPA's Integrated Science Assessment for Lead. With the evidence available from animal and epidemiologic studies, the subsequent cardiovascular consequences of lead-related hypertension are likely to be similar to other forms of hypertension. In the global burden of disease analysis conducted by the WHO, as well as past EPA regulatory analyses, the impact of lead exposure on cardiovascular mortality is estimated through the well-established effects of lead exposure on blood pressure levels. However, given the epidemiologic evidence that the cardiovascular disease effects of lead are not completely mediated by hypertension (i.e., lead is associated with increased cardiovascular mortality independent of blood pressure levels), the WHO approach of inferring the burden of cardiovascular disease due to lead through its effects on blood pressure, rather than directly, likely underestimates the overall impact of lead on cardiovascular disease.

The association of blood lead levels with left ventricular hypertrophy is a relatively specific finding observed in multiple studies. Five studies evaluating this association were included in a systematic review published in 2007, 4 of them conducted in lead workers and 1 study conducted in NHANES II.[12] Most studies found that lead was related to either higher prevalence of left ventricular hypertrophy (measured by electrocardiographic criteria in NHANES II) or increased left ventricular mass as measured by cardiac ultrasound. In a recent prospective study, baseline blood lead levels were associated with multiple markers of left ventricular function measured through ultrasound.[64] Left ventricular systolic function, but not diastolic function, was impaired with increased blood lead levels. The associations remained after adjustment for cardiovascular risk factors including blood pressure levels. These findings are important as impaired left ventricular function often precedes heart failure, a major form of clinical cardiovascular disease affecting the general population and for which, in many patients, the relevant risk factors are not always clear. While the exact mechanisms for these associations are unknown, interestingly the abnormal function of calmodulin and calcium-calmodulin protein kinases, whose function is likely impaired in the presence of lead, have been related to chronic heart failure.[65-67]

An increasing number of studies support that lead is associated with increased levels of atherosclerosis including peripheral arterial disease and carotid atherosclerosis.[68] Peripheral arterial disease was measured comparing blood pressure levels at the brachial artery vs. the ankle, in several studies in NHANES. A recent study has evaluated the association between environmental lead exposure, as measured in blood, with the prevalence atherosclerotic plaque in the carotid artery.[68] Although this is a cross-sectional study, carotid plaque is a subclinical measure, and thus participants are unlikely to modify their behavior and influence their blood lead levels. Blood lead levels were associated with higher prevalence of plaque, in particular among post-menopausal women. These studies on the association of lead with peripheral arterial disease and carotid plaque in the general population are important as they confirm that lead is atherogenic even at the low-level exposures that are currently relevant. Interestingly, these findings are consistent with the early 19th century autopsy studies conducted among lead exposed workers. These findings are also consistent with numerous

experimental studies evaluating endothelial dysfunction and atherogenesis in the aorta and other arteries in animal models exposed to lead.[28,32]

Lead has also been related to decreased heart rate variability. Heart rate variability is controlled by the autonomic nervous system, and high variability is a marker of good cardiovascular health. Lead is an established neurotoxicant and could affect heart rate variability by affecting autonomic control of the heart, resulting in decreased heart rate variability, a marker of cardiac autononomic dysfunction. Eleven studies, mostly cross-sectional were identified in the 2007 systematic review, 10 in occupationally exposed populations and one in public health officials not occupationally exposed to lead.[12] These studies are small and many of them did not adjust for confounders, but in general they support that increased lead exposure is associated with decreased heart rate variability. In a study of 140 children 9-11 years of age recruited from Pediatric clinics in New York State at very low lead levels (median 1.01 µg/dL), increasing blood lead levels were associated with reduced heart rate variability as well as with greater vascular resistance, reduced stroke volume and cardiac output during acute stress activities. These findings support that the cardiovascular effects of lead can start during childhood.[69]

Overall, the evidence on the association of lead exposure with subclinical cardiovascular outcomes supports that lead chronically affects key processes important for the development of cardiovascular disease, resulting ultimately in premature mortality. When investigated (6 of the epidemiological studies adjusted for hypertension or blood pressure levels, see Table 1), the evidence supports that the subclinical cardiovascular effects of lead are independent of other risk factors including hypertension. A limitation of this body of research is the observational nature and the possibility for confounding, selection bias, and information bias. However, a new body of human experimental evidence is emerging, further supporting that the removal of lead from the body can be beneficial for cardiovascular disease as described below.

4. Support for Causal Role from a Randomized Clinical Trial (RCT) of Metal Chelation

Over the last 20 years, clinical trials have addressed whether the use of a potent lead chelator can reduce cardiovascular outcomes. In participants from the general population without prior occupational lead exposure who had established coronary disease, Arenas et al showed that urinary excretion of lead increased by nearly 4000% after a single infusion of 3 grams of edetate disodium (EDTA),[70,71] a powerful but nonspecific chelator of lead and other cations.

The interventional body of evidence that relates active potential reduction in lead body burden to reduction in cardiovascular events comes from the Trial to Assess Chelation Therapy (TACT), an NIH-funded, placebo-controlled secondary prevention trial of 1708 post-myocardial infarction patients.[72,73] TACT was a $31M study carried out over 10 years and involved 134 sites in the US and Canada, with 55,222 infusions of edetate disodium or placebo administered. In TACT, repeated chelation with EDTA (up to 40 infusions) led to lower risk for a composite cardiovascular outcome in patients with a previous myocardial infarction as compared to repeated placebo infusions. In the highest risk patients, those with diabetes, the relative risk of death was reduced by 43% over a 5-year follow-up.[74] The benefit was observed immediately after starting the infusions, supporting there is a short-term benefit from reducing lead exposure, that continued to accrue after the infusions had stopped, supporting a potential persistent benefit once lead has been removed from the body. The participants for TACT were recruited

from the general population without any information on potential prior lead exposure. No data on lead biomarkers is available for TACT, either at baseline or during follow-up. All participants, however, were born before the 1980s and thus have likely been exposed to high lead levels throughout their lifetime.

The NIH is currently funding a replication study, TACT2, to confirm these findings and to assess whether the reduction of lead and cadmium body burden can explain the TACT findings. In a pilot study (n=10) extending chelation therapy to critical limb ischemia, a severe form of peripheral arterial disease, post-chelation urine lead levels dropped after 10 infusions, and improvement of vascular ischemia was remarkable for most participants rapidly after starting the treatment, with prevention of planned amputations in some.[75,76] Post-chelation urine lead is an established biomarker of the body burden of lead and correlates with the internal storage of lead in the bone.[36] EDTA can facilitate the excretion of many inorganic cations, in addition to lead. In the Arenas et al experiment mentioned above,[70] data from participants similar to those in TACT and receiving EDTA showed that lead is the metal with higher short-term increases comparing post-chelation urinary lead (3 hours post-initiation of EDTA) vs. pre-chelation urinary lead. The average changes show several fold increases in most individuals selected from the general population and not necessarily exposed to high lead levels. Moreover, post-chelation urine lead markedly declined with repeated infusions, supporting that repeated infusions of EDTA can reduce lead body burden. These results indicate that lead exposure in the population is widespread and that repeated chelation with an agent that removes lead can result in long-term cardiovascular benefits and prevent premature cardiovascular mortality in the population.

5. Conclusion

In summary, evidence on lead and cardiovascular mortality and other cardiovascular outcomes is strong. The epidemiologic evidence is robust, includes populations from the United States and Europe, and shows that the effects of lead are independent of cardiovascular risk factors. It is supported by experimental and epidemiologic evidence for lead and numerous mechanisms relevant for cardiovascular disease, as well as by randomized clinical trial evidence on the cardiovascular benefits of lead chelating agents. These cardiovascular benefits may appear short-term after lead is removed from the body. Overall, the evidence strongly indicates that lead is a risk factor for cardiovascular disease including subclinical atherosclerotic disease, with population-based implications for cardiovascular mortality and morbidity.

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Table 1. Epidemiologic studies of blood lead and clinical cardiovascular disease in general populations*

1st Author, year	Population (baseline)	Men% Age range	Range lead levels in µg/dL (method)	Outcome	Cases / Non-cases	Measure of association	Comparison	Adjusted for
				United States				
Lustberg 2002[48]	NHANES II (1976-1980)	47% 30 to 74 y	<10 to 29 (AAS)	CVD, fatal	424 / 3766	HR: 1.39 (1.01, 1.91)	20-29 vs. <10 µg/dL	Age, sex, race, educ., income, smoking, BMI, exercise, location
Menke 2006[49]	NHANES III (1988-1994)	47% ≥20 y	<1 to 10 (AAS)	CVD, fatal CHD, fatal Stroke, fatal	766 / 13198 367 / 13597 141 / 13823	HR: 1.55 (1.08, 2.24) HR: 1.89 (1.04, 3.43) HR: 2.51 (1.20, 5.26)	≥3.63 vs. <1.93 µg/dL	Age, sex, race, educ., income, smoking, alcohol, BMI, exercise, cholesterol, CRP, urban/rural, menopause, hypertension, kidney function
Schober 2006[50]	NHANES III (1988-1994)	48% ≥40 y	<5 to >10 (AAS)	CVD, fatal	1189 / 8568	HR: 1.55 (1.16, 2.07)	≥10 vs. <5 µg/dL	Age, sex, race, educ., smoking
Aoki 2016[51]	NHANES (1999-2010)	48% ≥40 y	<5 to >10 (ICPMS)	CVD, fatal	985 / 17617	HR: 1.44 (1.05, 1.98)	10-fold increase	Age, sex, race, educ., smoking, alcohol, CRP, cadmium, iron, calcium, hematocrit
Wang 2019[54]	NHANES (1999-2012)	48% ≥40 y	<0.3 to >2.49 (ICPMS)	CVD, fatal	261 / 950	HR: 1.45 (1.21, 1.74)	≥2.49 vs. <1.10 µg/dL	Age, sex, race, smoking, SBP, antihypertensive medication, lipids, diabetes, BMI
Jain 2007[57]	VA-NAS (1992-2001)	100% <60 to ≥70 y	Mean 6.3 (AAS)	CHD, fatal+NF	83 / 754	HR: 1.45 (1.01, 2.06)	Per log unit change	Age, race, smoking, alcohol, BMI, BP, lipids, family history hypertension
Weisskopf 2009[56]	VA-NAS (1991-1999)	100% Mean 67	Mean 5.7 (AAS)	CVD fatal CHD fatal	137 / 723 62 / 798	HR: 1.10 (0.67, 1.80) HR: 1.21 (0.57, 2.55)	>6.0 vs. <4.0 µg/dL	Age, smoking, education
Khalil 2009[55]	SOF - White women from 4 US-cities (1986-1989)	0% 65 to 87	1 to 21 (AAS)	CVD fatal CHD fatal Stroke fatal	54 / 479 23 / 510 21 / 512	HR: 1.78 (0.92, 3.45) HR: 3.08 (1.23, 7.70) HR: 1.13 (0.34, 3.81)	≥ 8.0 vs. < 8.0 µg/dL	Age, clinic, educ., smoking, alcohol, BMI, estrogen use, hypertension, exercise, diabetes, hip bone mineral density
Chowdhury 2014[58]	ABLES workers (1987-2012)	100%	<5 to >40 (NR)	CHD fatal Stroke fatal	569/57799 123/58245	RR: 1.77 (1.23, 2.56) RR: 1.88 (0.57, 6.28)	≥40 vs. <5.0 µg/dL	Age
				Europe				
Pocock 1988[44]	British Regional Heart Study (1978-1980)	100% 40 to 49 y	<6.2 to >35. 2 (AAS)	CHD, fatal+NF Stroke, fatal+NF	316/7063 66/7313	OR: 1.1 (0.4, 1.8) Mean: 16.7 vs. 15.3 µg/dL	>24.8 vs. <12.4 µg/dL Cases vs. non-cases	Age, smoking, location
Kromhout 1988[46]	Elderly men in Zutphen (1977-1978)	100% 57 to 76 y	<10.8 to >28.0 (AAS)	CHD, fatal+NF	26/115	HR: 1.34 (0.46, 3.94)	>23.8 vs. <13.0 µg/dL	Age, smoking, BMI, BP, cholesterol
Møller 1992[47]	4 cities Denmark (1976)	48% 40 y	2 to 60 (AAS)	CHD, fatal+NF CVD, fatal+NF	40/1005 54/991	HR: 1.58 (0.85, 2.95) HR: 1.10 (0.63, 1.93)	Per log unit change	Sex, smoking, alcohol, BP, cholesterol, exercise
McElvenny 2015[45]	UK lead workers (1975-1979)	100% Mean 35.2 y	2.3 to >322 (NR)	CVD, fatal CHD, fatal Stroke, fatal	1368/ 874/ 184/	HR: 1.30 (1.17, 1.44) HR: 1.30 (1.17, 1.43) HR: 1.25 (0.87, 1.62)	Per log unit change	Age, sex

ABLES: Adult Blood Lead Epidemiology and Surveillance, AAS: atomic absorption spectrometry; BMI: body mass index, BP: blood pressure, CHD: coronary heart disease; CRP: C-reactive protein, CVD: Cardiovascular disease, HR: hazard ratio; ICPMS: inductively coupled plasma mass spectrometry; NF: non-fatal, NHANES: National Health and Nutrition Examination Survey, NR: not reported, OR: odds ratio, RR: Risk Rate, SBP: systolic blood pressure SOF: Study of Osteoporotic Fractures. UK: United Kingdom, VA-NAS: Veterans Affairs Normative Aging Study.

*The studies by Ruiz-Hernandez et al. 2017[53] and Lanphear et al. 2018[52] are not listed in the table as their main findings are related to attributable risk and estimation of lead related-CVD reduction.