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Review

Effects of lead deposition on the musculoskeletal system

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ABSTRACT

In this treatise we will examine lead deposition and its effects on the musculoskeletal system. The population remains at risk of lead exposure due to its continued use, persistence in the environment, and the release of lead from skeletal repositories back into the body's soft tissues. Virtually all organ systems evaluated have proven susceptible to lead toxicity. Despite these findings, the skeleton was thought to be exempt from lead toxicity until very recently. Accumulating evidence shows that the musculoskeletal system is, in fact, susceptible to lead toxicity even at very low levels (5μg/dL). Lead-sensitive musculoskeletal components include: motor skills, bone growth and development, dentition, fracture healing, bone density, and joint maintenance. This organ system also seems to be vulnerable starting in utero through old age. Continued research in this area will identify novel strategies that may be used in the prevention and treatment of musculoskeletal disorders due to lead exposure.

KEYWORDS: lead (Pb), musculoskeletal system, osteoporosis, osteoarthritis, compartmentalization

INTRODUCTION

Lead has been widely used over the last 9000 years [1]. This usage stems from the ease of obtaining lead and its many beneficial properties. Ubiquitous application of lead in paint, plumbing, pesticide, gasoline etc. has resulted in a similarly ubiquitous dispersal [2-10]; environmental levels have risen rapidly over the past three centuries (in some areas by as much as 10,000 fold) [11]. This is unfortunate, however, given the toxicity of lead. When ingested, lead can attack virtually all organs and systems causing adverse effects in the reproductive system, kidneys, and exerting a wide variety of neurological and cognitive effects, as well as being a potential carcinogen [12].

In an attempt to reduce human exposure to environmental sources of lead, legislation has been passed. These laws limit lead use and set standards for acceptable environmental levels. Despite these measures, lead exposure remains a significant problem for several reasons. Firstly, while legislation has been effective in reducing lead use and deposition, it can only do so in countries that enact it. Numerous other countries either lack legislation regulating lead or don't enforce them stringently. In these countries, leaded gas [13-15] and improper remediation of lead containing material continue to contribute to lead exposure [16, 17]. Secondly, limiting input into the environment is an insufficient means of removing exposure. Once deposited in the environment, lead is not broken down and clings strongly to topsoil, where it may remain indefinitely [18, 19]. Therefore, despite efforts aimed at reducing lead usage, without remediation, environmental lead will persist. Thirdly, once lead enters the body, it is sequestered in the skeleton and may be slowly released back into the body's soft tissues. Documented findings have confirmed

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the skeleton as the major reservoir of lead in the body (harboring over 95% of the total lead burden) [20, 21]. Once stored in the skeleton the half-life of lead has been estimated to be from 20 to 30 years [22-24]. Experiments using lead isotopes show that in humans 40-70% of blood lead originates from re-release of skeletal repositories [25]. Furthermore, in times of excessive skeletal remodeling such as menopause, paraplegic immobilization, and endocrine diseases such as thyrotoxicosis, lead is increasingly released from bone back into circulation [26-29]. This secondary exposure may occur irrespective of present environmental lead levels.

Until very recently, lead sequestered in the skeleton was thought to be inert. However, new studies reveal that this complex and dynamic organ system is, in fact, susceptible to lead toxicity [30], often at very low-level exposure. Musculoskeletal diseases are far and away the number one cause of disability in the United States, and cost the country $849 billion annually [31]. In this review we will describe the compartmentalization of lead throughout the skeletal system. Additionally, the effects of lead on musculoskeletal formation, function, maintenance, and healing will be discussed.

Routes of exposure

Oral ingestion is the primary route by which lead absorption into the body occurs and in normal populations, comprises 99% of lead uptake, with inhalation responsible for the other 1% [32, 33]. Grasping, mouthing and other behaviors that increase lead exposure combine with physiological differences to make lead "the greatest environmental threat to the nation's children" with roughly 300,000 U.S. preschoolers above CDC recommended blood lead levels (BLLs) [34]. One such physiological difference is that the intestinal track of young children absorbs 30-40% of ingested lead, while adults absorb only 5-10% [35]. Deposition of lead in the lungs of children is also higher than for adults and 30-40% of inhaled lead is absorbed into the bloodstream [36].

Lead may also be absorbed through cuts in the skin; however, calculations based on findings of Moore *et al.* [37] show that an intact integument represents a barrier to elemental lead absorption (dermal absorption factor 0.0001µg Pb/dL blood/µg dermal Pb) [38]. Skin is, nevertheless, pervious to tetraethyl and tetramethyl lead species [39]. As discussed in detail below, despite reduced absorption in adults, lead bioaccumulates over time, due to its slow release from the body.

Upon absorption, lead moves into the blood stream.

Lead levels in body compartments

Bioaccumulation of lead

In general, lead bioaccumulates due to its long half-life in the body and persistence in nature, which provides continued exposure [19, 40, 41]; however, biomagnification up the food chain does not generally occur [42, 43]. Additionally, increases in environmental lead have given rise to increased body burden, with pre-industrial skeletons containing 50-200 fold lower lead levels than modern counterparts [44, 45]. As lead affects numerous aspects of the musculoskeletal system, its specific compartmentalization and deposition is of great interest.

Blood levels

The half-life of lead in blood is 30 days [46], therefore, blood levels constitute a poor surrogate for assessing long-term exposure. Blood lead levels are a sensitive metric, however, for the assessment of short-term exposure. Inhaled lead and ingested lead are absorbed into the blood stream, where 99% is bound to erythrocytes. While in the blood, lead is capable of passing through the blood brain barrier and placenta [47]. Blood lead sampling also represents one of the easiest metrics to obtain and has been used and studied extensively. Furthermore, blood lead levels reflect the interaction of environmental levels with human exposure; numerous studies have correlated the removal of lead from gasoline with reductions in blood lead levels [48-51].

Intracellular concentrations

Relatively little is known about intracellular lead concentrations. Cellular uptake of lead can vary drastically between cell types, with Dorsal root ganglia allowing no entry [52] and Chinese hamster ovary (CHO) cells taking up 97µM lead [53]. It seems that cells of the skeleton such as osteoblasts

and chondrocytes have intracellular lead levels hovering around 120-180nM (Ubayawardena unpublished data). These levels are comparable to intracellular calcium and it has been speculated that lead is transported by the same divalent cation transporter.

Skeleton and teeth

As a divalent cation, lead exhibits a strong affinity for calcified tissues of the skeleton and dentition. It has been shown that levels of lead in pre- and post-natal formed dentine and enamel correlate with blood lead levels at the time of their formation [54]. Tooth lead levels have been used as an indicator of exposure in adults as well [55]. Bone and tooth lead levels are more indicative of long term lead exposure levels, relative to blood, as the half-life of lead in bone is orders of magnitude greater and increases with age [46, 56]. The slow rate-of-change in bone lead levels limits their efficacy in describing recent exposures.

Bone is not a homogenous tissue and differences in lead sequestration exist. Due to its slower relative turnover rate, lead in cortical bone may have a longer half-life of retention than trabecular bone. Distribution throughout bone is not uniform, and it appears that trabecular bone has a slightly higher (10%) Pb/Ca ratio compared to cortical bone [57, 58]. Furthermore, long term lead treatment in goats resulted in the highest skeletal lead levels in trabecular bone specifically subchondral bone at the distal and proximal ends of long bones [57]. The tidemark is the area of interface between subchondral bone and articular cartilage. Interestingly, this region is a particularly strong repository for lead, with approximately 13 fold higher concentrations than surrounding bone [59].

To measure bone lead levels in living subjects, k-shell X-ray fluorescence spectrometry (k-XRF) can be used. The latest generation of instruments utilizes a ^{109}Cd source to excite the lead K shell electrons to produce gamma rays (i.e., X-ray fluorescence) and a four-detector array each with its own multi-channel analyzer. This system is more precise than earlier generations and can accurately detect lead levels in the 2-4 micrograms/gram of bone range.

Synovial fluid

Villegas-Navarro *et al.* found that in cattle, lead concentration in synovial fluid is an average of four times higher than blood [60]. Additionally, it has long been known that in the case of periarticular retained lead fragments, the low pH of synovial fluid contributes greatly to the solubilizing of lead and can result in levels a full order of magnitude higher than blood lead levels [61, 62].

Articular cartilage

The above findings show that adjoining tissues (synovial fluid, subchondral bone, and the tidemark) contain accumulated lead, and would purport articular cartilage as a lead repository as well. This was indeed found to be the case, as articular cartilage of exposed individuals had a Pb/Ca ratio 40% greater than even trabecular bone [58].

Skeletal effects of lead toxicity

Decreased motor skills

Lead exposure can disrupt nerve conduction in young and old. Disruptions of fine motor skills were noted in children suffering from lead intoxication [63]. BLLs between 35-60µg/dL were associated with lower scores of "Gross and Fine Motor Composite scores from the Oseretsky scales" [64]. Later in life, exposure results in a curvilinear relationship between lead and reaction time [65]. Lead also reduces hemoproteins such as cytochromes, resulting in impaired cellular energetics and, subsequently, suppressing myelin and nerve conduction [66]. The contribution of lead-induced neuropathy to significant skeletal problems, such as alterations in gait or increased risk of fall remains to be elucidated.

Suppression of 1,25-dihydroxycholecalciferol

Until relatively recently it was held that skeletally sequestered lead was inert with regard to the body's soft tissues; however, a growing body of evidence shows that lead can exert its toxic effects upon the skeletal system directly and indirectly. One of the most important and far-reaching of these effects is reducing vitamin D levels. The effect of lead on vitamin D levels was proposed as an explanation for the observed correlation between elevated lead levels and osteoporosis [26].

This correlation was corroborated by associative findings linking lead exposure to reductions in active vitamin D levels [67, 68]. The ability of EDTA chelation to restore 1,25-vitamin D levels bolstered the hypothesis that lead was the cause of the suppression. The underlying mechanism is most likely due to several non-mutually exclusive factors. It is well established that oral lead exposure can lead to appetite suppression and vomiting [69]. Reduction in overall food intake is a putative contributor to reduced vitamin D intake. Another possibility is that absorbed lead is perceived as calcium by the body and reduces vitamin D production through feedback inhibition. Lead has the ability to bind to the active site of calcium uptake proteins in the intestinal mucosa; however, further study of *in vivo* processes revealed that lead is primarily absorbed in the distal small intestine while vitamin D-dependent calcium absorption occurs in the duodenum [70]. Due to their differing locations of absorption, it is unlikely that lead inhibits vitamin D production by acting as a calcium mimetic in intestinal calcium uptake channels. The ability of EDTA to restore 1,25-vitamin D levels without altering levels of 25 OH vitamin D points to lead impairment of renal biosynthesis of vitamin D. Although lead can suppress the hydroxylase enzyme responsible for the hydroxylation of 25 OH vitamin D even when present at low levels [71], this renal impairment has been shown to be a secondary effect of lead toxicity resulting from a reduction in the heme body pool [72].

Lead induced musculoskeletal pathologies result from direct effects of lead on the cells of this system; however, the possible contribution of vitamin D suppression to these skeletal pathologies should be kept in mind.

Developmental effects

The spatial distribution of lead in the proximity of the growth plate is evident. Growth arrest, arising from lead effects on growth plate chondrocytes, manifests radiographically as lead lines [73, 74]. X-ray microanalysis of growth-plate cartilage matrix sites also denoted localized lead [75].

Exposure to lead from conception to the time of growth plate closure can disrupt normal skeletal development. By disrupting cellular function, lead causes bone malformations, reduces bone formation rates and stature, and precipitates growth plate closure. In rats and mice, lead exposure resulted in fetal bone malformations [76, 77]. A number of parameters of skeletal growth were found to be diminished by lead. In humans, chest and head circumference and overall length or stature are measured. Additionally, in animal models tail length, bone strength, and individual long bone length are compared. Ronis *et al.* found that mice exposed to lead from gestational day 4 through day 55 had significantly "reduced somatic growth, longitudinal bone growth, and bone strength" compared to controls [78]. Maternal lead exposure prior to and throughout pregnancy was likewise shown to reduce early post natal growth, as measured in the offspring by tail length and tail vertebral bone growth [79]. Specifically, in mice dosed with lead, significantly shorter mean growth plate lengths were observed [80, 81]. In humans, elevated lead levels have been associated with decreased height by retrospective study. Analyses of NHANES II data found that blood lead levels of 5-35μg/dL correlate strongly with growth retardation and reduced stature in children aged 1-7 [82, 83]. Analysis of NHANES III data estimated a 1.57cm decrease in stature and 0.52cm decrease in head circumference for every 10μg/dL increase in blood lead concentration [84]. Even in utero, lead may be exerting skeletal effects, as prenatal lead exposure has been linked to shorter birth lengths [85, 86]. Intriguingly, the findings regarding the efficacy of chelation in the amelioration of these effects are ambivalent. Chelation has been shown to return skeletal maturation to normal [74], but larger studies have found no effect or a negative correlation between chelation and growth parameters [87].

Lead has been shown to delay growth plate chondrocyte maturation [88, 89], but the most probable mechanism by which lead affects skeletal maturation is through osteoblast pathology. Historically, there have been a number of reports describing the effect of lead on osteoblasts [90-93]. These studies have been performed in transformed cell lines, freshly isolated normal cells, and *in vivo*. Uniformly, it has been observed that lead is deleterious to the functioning of these cells. This metal ion has been shown to inhibit the

secretion of osteonectin, decrease alkaline phosphatase activity, and to depress type I collagen synthesis [90, 91]. Lead also adversely affects osteoblast cell proliferation and response to regulatory growth factors [92, 94].

In animal experiments directly analogous to what occurs in humans, it has been shown that osteoblast activity is suppressed and stem cell frequency is decreased with lead exposure. In dogs, lead exposure resulted in a suppression of osteoblastic activity that continued after cessation of exposure [95]. In mice that were exposed to lead in their drinking water for 6-12 weeks, osteogenic precursor cells were isolated from bone marrow and cultured under differentiating conditions [96]. Mineralizing nodule area was used as a measure of osteogenic precursor cell frequency. Even a brief exposure of the animals to lead resulted in an approximately 50% decrease in osteoprogenitor cell number. Exposure resulted from imbibing 55ppm lead in the water. This concentration achieved blood lead levels of approximately 20μg/dl in mice. Comparable BLLs may also be observed in similarly exposed humans.

One of the key paracrine factors that mediate the flow of cells from mesenchymal stem cells into osteoprogenitor cells is TGF-β. The current thinking is that the TGF-β pathway stimulates the expansion of osteoblast numbers and maintains a pool of cells that can be primed for new bone formation [97]. Inhibition of this pathway would then favor a depletion of osteoprogenitor cell number. Recent data support this observation and suggest that the mechanism by which lead exerts its effects on bone formation may involve a decrease in the pool of phosphorylated Smads in exposed cells.

Delayed tooth development and increased incidence of dental caries

Lead also affects another of its mineralized repositories, delaying tooth development and increasing the incidence of dental caries. Eruption rate and enamel formation are both susceptible to lead. In several instances lead has been shown to delay tooth eruption rate [74, 98], and in rats this was accomplished via IP injection of lead acetate [99]. Evaluation of enamel mineralization by microhardness testing in rats showed that the lead-exposed animals have decreased enamel mineralization in developing regions but not in mature areas [100]. These findings implicate enamel development as a target of lead toxicity. A potential mechanism by which lead might be exerting these effects is enzyme disruption, a mechanism common to other lead pathologies. Enamel mineralization occurs on a protein scaffold (i.e. amelogenin) which is then removed by matrix metalloproteinases [101]; lead, however, disrupts the activity of these enzymes [102]. Interestingly, these lead-sensitive enzymes also play a role in joint homeostasis, and this will be discussed later at length.

A number of epidemiological studies have found an association between high lead levels and the presence of dental caries. Analysis of the third NHANES study showed an association between elevated blood lead levels and dental caries in both deciduous and permanent teeth [103]. In a survey of children aged 6-10 a positive correlation between lead levels and dental caries was again found, with a stronger association observed in primary teeth than permanent. The study also found higher incidence of caries on occlusal, lingual, and buccal tooth surfaces than mesial or distal surfaces [104]. The ability of lead to exert effects beyond initial exposure was again demonstrated when it was found that toddlers (ages 18-37 months) exposed to lead were more likely to have developed caries on the lingual surface of their primary teeth when again sampled in second or fifth grade [105].

Lead effects on bone and teeth overlap in the area of periodontal bone. Epidemiological analysis of NHANES III data revealed a statistically significant association between periodontal bone loss and elevated blood lead levels [106]. The specific area of periodontal bone affected was the dental furcation, the bone connecting to the tooth between the roots.

Decelerated fracture healing and increased incidence of non-union

Paralleling the inhibitory effect of pre- and peri-natal lead exposure on skeletal development, lead exposure later in life has been shown to delay fracture healing and increase the incidence of fibrous non-unions. In a closed tibial fracture model, bridging cartilage formation and overall

amount of cartilage types II and X were suppressed and delayed, as were maturation and calcification of osseous tissue, in mice that had been treated with lead for 6 weeks [96]. No effects on osteoclasts were observed. Lead effects were therefore attributed to disruption of chondrocyte function, which resulted in inhibition of endochondral ossification. A subsequent study found that lead induced chondrogenesis in progenitor mesenchymal stem cells [107]. Lead was shown to alter chondrocyte populations through the modulation of TGF-β and BMP signaling. Taken in concert, these findings of lead effects on chondrocytes offer a mechanistic explanation of shorter birth lengths and overall achieved stature. Developmentally, lead causes an initial increase in chondrogenesis resulting in increased growth plate activity but with premature closure and overall reduction in bone mineralization and density. In the fracture callus this process is paralleled, cartilaginous callus formation is precipitated but ossification is delayed and reduced. Not only are these findings important for observing lead effects on fracture healing, but in the larger scope of skeletal pathology they show that lead can affect chondrocytes through disruption of TGF-β signaling.

Osteoporosis

While the developing skeleton may be susceptible to lead toxicity at lower doses and shorter exposure lengths, the adult skeleton is nonetheless vulnerable [108]. Along with increased tooth degeneration and disruption of fracture healing, numerous studies have identified a reduction in bone density as a toxic effect of lead exposure. Examination of NHANES II data revealed a positive correlation between BLL and osteoporosis in post-menopausal women [26]. A case study presented by Berlin *et al.* similarly found evidence of elevated skeletal turnover and osteoporotic fracture in a lead poisoned individual [109]. Animal studies have further elucidated lead effects on bone density. After 7 months of low-level lead exposure adult beagles experienced reductions in both appositional rates and bone formation rates [110]. In adult rats exposed to lead, maintaining biologically relevant BLLs of 21μg/dl for one year, a significant decrease in bone density was observed; interestingly, higher BLL expedited the onset of osteopenia [111]. Retardation of bone formation, increased trabecular resorption, and inhibition of osteoid matrix ossification were all observed in lead exposed rabbits [112]. In lead exposed mice bone volume, trabecular number, and trabecular thickness were all decreased and an increased number of trabecular spaces were observed. In lead exposed adults Ronis *et al.* found decreased bone density and less formation of new endosteal bone [78]. Lead-induced disruption of bone density resulted from inhibition of osteoblastogenesis [78], as well as increased osteoclast number, potent inhibition of downstream effects of vitamin D, and (it was speculated) inhibition of collagen or collagen precursor synthesis [112]. Insidiously, lead sequestered in bone may inflate DEXA densities by 4-11% while simultaneously comprising a structural deficit (Puzas unpublished data).

Osteoarthritis

Extensive empirical research into how lead might affect the joint is yet to be conducted; however, there is a large body of observational research and clinical case studies that suggest an association between lead exposure and joint pain and degeneration [113]. In addition, essential molecular components of the joint exhibit vulnerability to lead.

Occupationally exposed individuals present with increased incidence of joint pain that correlates with duration of exposure. Lead smelter workers, construction workers, lead-acid battery manufacturers, and welders with elevated blood lead levels exhibit increased incidence of multi-focal arthralgias and stiffness [114-119]. In addition to systemic exposure, there are also numerous reports in the literature, in both humans and animals, associating the genesis of osteoarthritis with lead exposure of a single joint, due to the presence of a periarticular lead pellet or bullet fragment [61, 120-124]. In controlled experiments periarticular implantation of lead pellets resulted in significantly increased joint degeneration compared to stainless steel pellet controls [125, 126]. In these cases the underlying mechanism of joint degeneration remains unknown; however, no direct mechanical interference by the pellet was found.

The above findings speak to the association of lead with joint degeneration, but osteophyte

formation is also a hallmark of OA. Lead may play a role in this, although the evidence is less conclusive. Similar to the chondrocalcinosis seen in osteophyte formation, lead has been shown to be a potent calcergen, inducer of ectopic calcification in other soft tissues [127, 128].

Although the underlying mechanism remains to be elucidated, *in vitro* studies have identified several molecular targets of lead toxicity. As has been previously discussed, articular chondrocytes rely on TGF-β signaling to maintain production of functional joint components, such as type II collagen, aggrecan, and hyaluronic acid [113]. Interestingly, this signaling pathway is disrupted by lead [129-132]. In numerous other pathologies lead acts via generation of reactive oxygen species (ROS) [133], and the joint has low basal ROS scavenging capacity (ref) and contains a number of ROS-sensitive targets. TGF-β signaling itself is ROS-sensitive [134]. Furthermore, at elevated levels ROS can damage the joint matrix by directly attacking matrix constituents, activating or up-regulating MMPs, altering signaling pathways that maintain normal joint function, and inducing apoptosis [135-139]. Antioxidants are currently being evaluated for OA treatment.

SUMMARY

Due to its continued use, persistence in the environment, and re-release of skeletal repositories, lead exposure remains a risk. The long-held belief that the skeleton is invulnerable to lead toxicity has been disproven. In fact, current research indicates that the musculoskeletal system may be among the most sensitive. This susceptibility stems from 1) bioaccumulation of lead in and around bones and joints, resulting in elevated exposure levels, and 2) prolonged retention ($t_{1/2}$ = 20-30 yrs), resulting in chronic exposure. The observation of growth retardation at blood lead levels as low as 5μg/dL illustrates this susceptibility. What's more, virtually all elements of the musculoskeletal system demonstrate vulnerability to lead. It also appears that the skeleton remains sensitive to lead toxicity throughout all developmental stages. In utero and early developmental lead exposure can reduce stature, lead exposure during midlife can impede fracture healing and increase dental caries, and later in life lead may contribute to osteoporosis and osteoarthritis.

Although significant gains have been made in understanding lead effects on the skeleton, further research is needed. Evaluation of the actual contribution of lead to musculoskeletal diseases and delineation of mechanism in diseases such as osteoarthritis will be essential to the improvement of therapeutic outcomes. The current scope of musculoskeletal diseases is staggering, and in parallel with an increasingly aged population, they are projected to rise drastically. Taken together with the degree to which the skeleton is vulnerable to lead, the importance of continued research in this area cannot be overemphasized.

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