Methodological issues related to studies of lead mobilization during menopause

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Abstract

While there has been a substantial decline in lead exposure in the United States during the past two decades, mobilization of existing lead stored in bone potentially represents an important endogenous source of exposure for menopausal women. It has been hypothesized that lead may be mobilized from skeletal stores during conditions of high bone turnover, such as during menopause. However, such mobilization has not been documented in prospective studies. This discussion is focussed on some of the methodological difficulties to be anticipated in longitudinal studies of lead mobilization specific to menopause and the issues that need to be taken into account when evaluating the results of such studies. To evaluate whether lead mobilization occurs during menopause, a prospective repeated measures design is needed using X-ray fluorescence analysis of lead in bone and serial measurements of blood lead. Potential confounders and effect modifiers also need to be taken into account in the statistical analysis.

Key words: lead; menopause; estrogens

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con estudios sobre movilización de plomo
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Resumen

Mientras que la exposición a plomo ha declinado sustancialmente en Estados Unidos durante las dos décadas pasadas, la mobilización del plomo almacenado en los huesos representa potencialmente una importante fuente de exposición endógena en mujeres menopáusicas. No obstante, tal movilización no ha sido documentada por estudios prospectivos. La presente discusión se enfoca en algunas de las dificultades metodológicas a se anticipadas en estudios longitudinales de mobilización de plomo específicamente en la menopausia, y los aspectos que necesitan ser considerados cuando se evalúan los resultados de tales estudios. Para evaluar si durante la menopausia el plomo es mobilizado, se requiere de un diseño prospectivo con mediciones repetidas utilizando análisis de fluorescencia de rayos-X para determinar el plomo en hueso y una serie de mediciones de plomo en sangre. Los potenciales confusores y modificadores del efecto también deben tomarse en cuenta en el análisis estadístico.

Palabras clave: plomo; menopausia; estrógenos

Despite the substantial decline in lead exposure that has occurred in the United States during the past two decades, certain subgroups, such as the poor, inner-city residents, and minorities, are more likely to have elevated levels of blood lead.[1] Recently, pregnant and lactating women and those undergoing menopause have been identified as additional groups in the population who may be at risk for increased blood levels because of potential lead mobilization during conditions of high bone turnover. To date, no studies have prospectively documented lead mobilization during these conditions. Since increased levels of lead in circulation can potentially adversely affect physical and mental well-being of both the women and their fetuses, such data need to be collected.

In this paper, we discuss some of the difficulties, particularly methodological issues, that can be anticipated in studying lead mobilization specifically

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during menopause. Suggested approaches for overcoming some of these difficulties are also set forth.

Traditionally, the storage of lead in bone was thought to represent a removal of lead from "active sites" in soft tissue, in essence, a form of sequestration that effectively immobilized the body lead burden. However, there is now limited evidence that skeletal lead may be mobilized during conditions of high bone turnover, such as occurs during aging as well as pregnancy, lactation, and particularly menopause.[2] Most of the evidence is based on animal studies[3] and case reports of lead toxicity during pregnancy in women who had not had recent lead exposure.[4,5] A recent prospective study of women residing near a lead smelter in Sweden observed a statistically significant increase in blood lead concentrations during pregnancy in both the exposed group and an unexposed control population.[6] Similarly, serial measurements in a group of pregnant women in Mexico showed a significant rise in blood lead during the second half of the pregnancy.[7] There is also a recent case report of a woman who developed lead poisoning from mobilization of bone stores during thyrotoxicosis,[8] an acute condition in which there is significant bone turnover.

The rate of accumulation of lead within the skeleton is a function of age, bone type (cortical or trabecular) and skeletal site.[9] Lead has a very long half-life in cortical bone (approximately 10,000 days).[10] As a result, lead levels in bone accumulate progressively with age until middle or later life, when some decline occurs. In addition to age-related loss that occurs slowly and in both sexes, bone turnover is accelerated during menopause so that more bone is resorbed than formed. More specifically, any increase in blood lead level would be expected to be highest during early menopause, as bone turnover increases dramatically during the first three years after menopause, but then slows to a rate only slightly above the premenopausal bone loss rate in both Caucasian and African-American women.[11-13] Bone turnover is also influenced by maternal diet.[14,15]

The magnitude of lead mobilization from bone and its significance for overall lead exposure in menopausal women has not been empirically measured. It has been estimated that trabecular bone loss is approximately 2-6 percent per year during the first 3 to 4 years after menopause.[12,13] After that it decreases for 5-8 years and finally levels off at less than 1 percent per year. Cortical bone loss has been shown to follow a similar pattern, but the loss is generally less rapid. Thus, the reported increase in blood lead levels among recently menopausal women suggests that lead may be mobilized at rates consistent with the increase in bone loss.

During menopause, calcium and other minerals are mobilized from the bone. In the skeleton, lead is contained in the mineral matrix, apparently in close chemical association with calcium and phosphate.[16] It has been hypothesized that the accelerated bone mineral turnover that occurs during pregnancy, lactation, and menopause may be coupled with mobilization of lead from the bone.[2,17] Such lead, when mobilized, could move into the blood compartment and exert toxic effects. There is rapid exchange at all bone surfaces in close contact with the blood, slow exchange by diffusion through the crystalline matrix, incorporation into mineralizing bone and resorption of bone mineral into the blood.[18,19] Lead is believed to compete with calcium for transport and binding sites and is released along with calcium when bone is resorbed.[20,21] Calcium and lead enter and leave the bone in a similar manner although the rate of bone uptake is more rapid for calcium than for lead.[22,23]

Cross-sectional data also support the hypothesis that blood lead levels may increase during menopause. The Second National Health and Nutrition Examination Survey (NHANES II) showed higher blood and plasma lead levels in post-menopausal women. The NHANES II mean blood lead levels were 14.19 μg/dl in nulliparous postmenopausal women and 12.97 μg/dl in parous postmenopausal women as compared to 11.66 μg/dl in premenopausal women. Since lead may be mobilized during pregnancy, the lower level among the parous women is thought to reflect prior mobilization of lead stores. The differences between the pre and postmenopausal women are not large but they were statistically significant. It should be noted, however, that these means are based on the evaluation of cross-sectional data rather than longitudinal data from the same individuals. The study design could therefore have obscured the magnitude of the changes within women pre and post menopause. On the other hand, cohort effects are likely to have resulted in lower lead levels among the younger as opposed to the older women. In addition, pooling data from women at all stages of menopause could have lead to an underestimate of the effect of menopause on circulating blood levels as lead mobilization would be expected to be highest during early menopause. Indeed, there was an inverse correlation between blood levels and years since menopause in the data from the NHANES II data.[17]

A study of Mexican-American women who participated in the Hispanic Health and Nutritional Examination Survey (HHANES 1982-1984) also has shown that postmenopausal women had higher blood lead levels than premenopausal women.[24] Among the

post-menopausal women, those who recently (4 years or less) had reached menopause had higher blood lead concentrations than those who had undergone menopause earlier. This study took into account potential risk factors of elevated blood lead levels such as age, pregnancy history, body mass index and cigarette smoking. However, no data were available on estrogen replacement therapy. Grandjean *et al*.[25] in a study from Scandinavia also detected significantly higher blood lead concentrations in postmenopausal as compared to premenopausal women.

Analysis of Current *versus* Cumulative Lead Exposure

Evaluation of the occurrence of lead mobilization requires a sensitive and reliable biological indicator of chronic lead exposure. Previous studies of chronic lead exposure have been forced to rely either on determinations of current blood level, historical reconstructions of exposures from past blood levels or previous air lead measurements.

Current blood lead determination is not an adequate index of chronic lead exposure,[26] because the half life in blood is only approximately 36±5 days.[10] Moreover, the concentration of lead in blood is a composite index that reflects the equilibrium between current exposure, excretory loss and the movement of lead from bone and other deep compartments to blood. The relative contribution to the blood level from each of these sources varies with current exposure and the magnitude of the total body burden. In one group of currently employed lead workers with varying durations of exposure, the mean endogenous contribution was estimated as no more than 10-20%,[27] whereas in workers retired after decades of exposure, virtually all lead reflected past exposure.[28,29] Current blood levels however, are useful in assessing possible toxic effects that are of relatively acute onset, such as might occur during conditions of accelerated bone turnover following surgical menopause or pregnancy.

Historical reconstructions of past exposure (e.g., area under the curve of periodic blood lead measurements) are inherently less accurate than direct biological measurement of body lead burden. Moreover, the data necessary to permit such reconstruction of past exposure are available only in certain industrial populations in whom repeated blood monitoring is legally mandated.[30]

X-ray fluorescence analysis (XRF) of lead in bone provides a non-invasive, accurate and a relatively rapid technique for estimating past cumulative lead absorption.[31,32] The technique takes advantage of the fact that approximately 90% to 95% of the body burden of lead is retained in bone[33] and that lead in cortical bone has a half-life of approximately 10,000 days.[10,30]

Serial bone densitometry assessments are also important in lead mobilization studies to document bone turnover. In addition biochemical markers, such as N-telopeptides, pyridinolines and bone alkaline phosphatase may be useful to monitor bone loss[34] but some of these markers are subject to diurnal changes and high assay variation.[35]

Natural *versus* Surgical Menopause

To avoid the limitations of cross-sectional studies comparing blood lead levels of premenopausal to postmenopausal women, a prospective repeated-measures design is needed. Specifically, baseline XRF analysis of lead in bone and blood lead levels should be performed before menopause and at selected intervals after menopause (e.g., at 6 and 18 months after menopause). Bone densitometry should ideally be performed at the same intervals to determine bone turnover. The ideal population for such a study, however, is not as clear-cut. This design would be inefficient for studying women undergoing natural menopause, as the typical range for age at onset is from 45-55 years although some women may not become menopausal until closer to the age of 60 years. Even when a woman begins to experience perimenopausal symptoms such as irregular or heavy menstrual periods, it is not possible to predict when menopause actually occurs as perimenopausal symptoms can persist for years. Also, bone loss appears to accelerate as ovarian hormone production declines. An alternative to following women undergoing a natural menopause is to identify women scheduled to have a surgically induced menopause, that is, a bilateral salpingo-oophorectomy. It should be noted that women who undergo a hysterectomy without a bilateral oophorectomy should not be considered postmenopausal simply because of the cessation of menses. Since the critical factor is the loss of ovarian function and consequently estrogen deficiency, both ovaries must be removed for the woman to experience menopause. Surgically menopausal women represent a unique and efficient model for studying lead mobilization as these women will undergo "instant" menopause at a predetermined time. Riggs et al.[11] noted in a review of osteoporosis that it is more difficult to demonstrate the accelerated postmenopausal phase of bone loss after natural menopause than after oophorectomy because the onset of estrogen deficiency is more gradual and variable in natural menopause.

Nevertheless, the choice of surgically menopausal women has some drawbacks. Presumably, because the withdrawal of estrogen is immediate rather than gradual, surgically menopausal women are more likely to take hormone replacement therapy. Such therapy, in turn, prevents bone loss and, consequently, lead mobilization.[36] If a very high proportion of surgically menopausal women takes hormone therapy, it may be difficult to detect any evidence of bone turnover and lead mobilization. Furthermore, surgically menopausal women may not be representative of women undergoing a natural menopause. Obviously, women who are having a bilateral oophorectomy for a malignant condition should not be included in any study of lead mobilization as the cancer itself and/or the therapy could affect bone homeostasis. With regard to bilateral oophorectomies for benign conditions, the most common indications are an accompanying hysterectomy for fibroids with or without bleeding, benign pelvic mass, ovarian cysts, endometriosis, and a family history of ovarian cancer. Fibroids are by far the most common indication. Although fibroids are prevalent among perimenopausal women, women who need a hysterectomy may differ from postmenopausal women who have an intact uterus. It should be noted that an increasing proportion of women requests a unilateral oophorectomy whenever possible, as it is now recognized that the ovaries produce hormones other than estrogens (e.g., androgens) that are important for sexual and general well-being.[37] Thus, it is likely that rates of bilateral oophorectomies will decline.

Consideration of Potential Confounders and Effect Modifiers

In order to determine whether skeletal lead is mobilized during menopause, potential confounders and effect modifiers need to be taken into account. Probably the most important confounder is use of estrogen replacement therapy (ERT) as it has been shown to prevent or slow down bone loss. It is nevertheless difficult to anticipate the frequency of ERT in a given cohort of women. A review reported that among women with bilateral oophorectomies, 31-89% of women started to take ERT and 13-71% continued to use ERT for 5 years.[38] Data from the Epidemiologic Follow-up Study to the First National Health and Nutrition Examination Survey showed that among those who had reached menopause from the 1945-1954 birth cohort, 63% had ever used ERT.[39] Furthermore, 43% of the ever users had used ERT for at least 5 years. The proportion of ERT use was higher among women who were white, who were more highly educated or who had experienced a surgical menopause. Other studies have also shown wide geographic differences that ranged from a low of 25.2% for use of menopausal estrogens in the Northeast to a high of 52.5% in the West based on 1987 national survey data.[40]

In NHANES II, age, black race, postmenopausal status, number of cigarettes smoked and alcohol use were positively associated with blood lead and plasma lead. Income and years since menopause were negatively associated with blood and plasma lead.[17] Having had any pregnancy decreased the postmenopausal increase significantly for plasma lead but the association was of borderline significance for whole blood lead. Similarly, in the HHANES,[24] increasing age, being postmenopausal, recency to menopause, lower income level, less education, increasing urbanization, and lower body mass and cigarette smoking were related to higher blood lead levels. 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. 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.

These data underscore the importance of controlling for factors that affect lead levels as well as other risk factors for lead mobilization (e.g., menopausal status, parity, and insufficient calcium intake). As shown in the HHANES data, effect modifiers may also be important for determining relationships among risk factors.

Conclusion

While acute lead exposure has declined substantially in the United States, high bone turnover which commonly occurs among menopausal women who are not on ERT, represents a potential source of endogenous lead. Other conditions which were not considered in this paper that could potentially increase skeletal lead mobilization include certain illnesses or medications, immobility due to spinal cord injury, prolonged bed rest, and long-duration spaceflight. Quantitative assessment of potential efflux of bone lead has only been recently made possible with the introduction of XRF analysis. This technique, along with serial measurements of blood lead and bone mineral density can be used to estimate the degree of transfer of lead from the bone into the blood compartment. These measurements are also important during menopause, a transi-

tional stage when women appear to be subject to signs of neuropsychological dysfunction that may be related, in part, to endogenous lead exposure.

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