Lead Exposure at an Early Age Substantially Increases Lead Retention in the Rat

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It has been hypothesized that the high rate of bone remodeling during childhood and the consequent high calcium and lead turnover result in a substantial reduction in bone lead stores so that much of the lead incorporated in bone during childhood does not persist into adulthood. We studied the effect of age at lead exposure on blood and organ concentrations of lead, calcium, and zinc 1–5 months after termination of lead ingestion. Blood and organ lead concentrations and contents 4 weeks after lead exposure ceased were significantly higher in the rats exposed beginning at 5 weeks of age than in those exposed beginning at 10 or 15 weeks old. Bone lead declined as the time since exposure increased. Despite this trend, the rats exposed when youngest had bone lead concentrations at 20 weeks after the termination of lead exposure that were higher than those of the other rats only 4 weeks after cessation of lead ingestion. Multiple regression analysis demonstrated that age at lead exposure remained a significant predictor of blood and organ lead concentrations and contents even after the inclusion of total lead consumed, body weight, and age at organ harvesting in the regression analysis. There were only small differences in organ calcium and zinc concentrations among treatment groups except for kidney calcium. The results do not support the hypothesis of rapid depletion of bone lead stores in young animals, but rather suggest that younger age at lead exposure is associated with greater lead retention and toxicity even in the absence of continued lead exposure. Key words: age, bone, calcium, lead, rats, retention, zinc. Environ Health Perspect 105:412–417 (1997)

More than 90% of the body lead burden in rats and humans is in the skeleton (1). The half-life of bone lead is long—5–20 years or more in humans—with lead in cortical bone having a longer biological residence time than lead in trabecular bone (1–3). The long half-life suggests that environmental exposure to lead can result in bone lead accumulation that may persist for many years. Unfortunately, the skeleton cannot be considered as merely an inert repository for lead, nor can it be assumed that this lead will remain indefinitely sequestered in bone, since several factors may influence its release from bone into the bloodstream. In a previously lead-exposed individual, these factors include age and sex, nutritional status, pathological conditions such as osteoporosis and kidney disease, the skeletal demineralization that occurs with aging, and, in particular, menopause and pregnancy (1). In addition, remodeling is a continuous cycle of bone destruction and renewal that occurs throughout life in mammals. Thus, lead accumulated in bone is a potential internal source of lead that may be mobilized and redistributed via the bloodstream to soft tissues, resulting in toxic manifestations.

Lead and calcium bind to the same sites in bone, and turnover of bone calcium occurs as often as several times per year in infants and children up to and through the adolescent growth spurt (3). It has been hypothesized that the high rate of bone remodeling during childhood and the related high turnover of calcium and lead result in a substantial reduction in bone lead stores, suggesting that much of the lead incorporated into bone during childhood does not persist into adulthood (3–5). It would follow that younger age at lead exposure results in a lower future body lead burden. If this were true, then the skeletal lead burden of females excessively exposed to lead at a young age would not persist long enough to be a problem during pregnancy, lactation, or menopause.

The rat is considered to be a good model for the study of lead toxicity in humans (4). Using rats, we tested the alternative hypothesis that younger age at lead exposure results in a greater accumulation of lead stored in the skeleton as well as in soft tissues. The objective of this study was to determine the effect of age during lead exposure on blood and organ lead concentrations 1–5 months later. Organ concentrations of the essential divalent metals calcium and zinc were also measured for comparison to lead.

Materials and Methods

Animal care and treatment. Weanling female Sprague-Dawley rats (Taconic Farms, Germantown, NY) (n = 40, 4 weeks old) were allowed to acclimate to the vivarium environment for a 1-week period. They were housed individually in plastic cages and weighed weekly during the study. Twelve-hour light:dark cycles and constant temperature and humidity were maintained throughout the study. After 1 week, rats were fed a modified AIN–76 diet (Research Diets, Inc., New Brunswick, NJ) containing 0.5% calcium and were maintained on this diet for the remainder of the study. The composition of this diet has been previously described (6). The rats were then randomly assigned to one of three treatment groups. The largest group (n = 24 with three subgroups of eight rats each) was given drinking water containing 250 mg/l of lead as the acetate (Fisher Scientific, Fair Lawn, NJ) beginning at 5 weeks of age. Lead exposure of the other two groups (n = 8 per group) via drinking water with the same lead concentration began at 10 (midadolescence) and 15 (young adulthood) weeks of age. Glacial acetic acid was added to the drinking water solutions at a concentration of 12.5 μl/l to prevent the precipitation of lead carbonate. Drinking water consumption was monitored. The objective was to achieve similar total lead ingestion in the three treatment groups.

Administration of drinking water containing lead to eight rats in each group for 5 weeks was followed by a 4-week period without lead exposure. The 16 additional rats exposed to lead beginning at 5 weeks of age were studied 8 or 20 weeks after the cessation of lead exposure. After the above intervals, blood was withdrawn by cardiac puncture from rats anesthetized with 25 mg/kg sodium pentobarbital (Steris Laboratories, Phoenix, AZ). Rats were killed by decapitation while under anesthesia. The organs harvested from each rat were the liver, kidneys, left femur, brain, and the bones of the lower vertebral column. Organs were briefly immersed in deionized/distilled water to remove surface blood contamination, and were then air-dried and stored at -70° in presired, weighed polyethylene or polypropylene containers.

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A protocol describing the above procedures was approved by the Institutional Animal Care and Use Committee at the New Jersey Medical School (Newark, NJ).

**Laboratory analyses.** Whole blood lead concentrations were determined by electrothermal atomic absorption spectrophotometry (6, 7). A quality control sample, Bio Rad whole blood control level 3 (Bio Rad, Anaheim, CA), was used for evaluation of the accuracy of these analyses. Concentrations determined for this sample were within 8% of the certified value.

Organ concentrations of calcium and zinc were determined by previously described techniques using flame atomic absorption spectrophotometry (7). Briefly, organs were ashed with a 3:1 mixture of double distilled nitric and perchloric acids (GFS Chemicals, Columbus, OH), and the residue was quantitatively transferred to a 10 or 25 ml volumetric flask and diluted with distilled, deionized water. Further dilutions were necessary for some analyses, which were conducted by using flame atomic absorption spectrophotometry. National Institute of Standards and Technology bovine liver (SRM 1577b, Gaithersburg, MD) was used as a quality control sample for all analyses. Assays of this sample in our laboratory gave results within 5% of certified values. Lead concentrations of the same ashed samples were determined by electrothermal atomic absorption spectrophotometry. The precision (coefficient of variation; CV) of the assays, based on analysis of standard reference materials and/or quality control samples, was 0.01 to 0.07. Calculations of concentrations were based on wet tissue weight. In addition to concentrations, the total organ content of lead was also calculated to correct for possible differences in organ size among individual animals and treatment groups.

**Statistics.** Data reduction and analysis were performed using dBase III+ (Ashton-Tate, Torrance, CA) and the Statistical Analysis System (SAS Institute, Cary, NC). ANOVA (SAS General Linear Models, SAS Institute, was used to evaluate the effects of treatment group and interval since the end of exposure on blood and organ metal concentrations and contents. If ANOVA indicated that there were statistically significant (p<0.05) differences among groups for a specific measurement, then pair-wise comparisons were made by Duncan’s multiple range test at α = 0.05. Kidney lead and calcium concentrations are known to vary considerably among individual animals, even for rats in the same treatment group (6–9). Therefore, they were log transformed before ANOVA evaluation.

Because other factors (age at organ harvesting, body weight, total lead ingested) besides age during lead exposure might influence blood and organ lead concentrations and content, stepwise multiple regression analyses were performed to assess this possibility. In these analyses, the blood or organ (brain, femur, kidney, liver, spinal column bone) lead concentration was the dependent variable. The independent variables were age during lead exposure, age at organ harvesting, body weight at the midpoint or end of lead exposure, and total lead consumed. The same approach was used to evaluate organ lead content of the five organs studied as a dependent variable. Thus, a total of 22 separate multiple regression analyses were done (11 using body weight at the midpoint of lead exposure and 11 using body weight at the end of lead exposure).

**Results**

Figure 1 depicts growth curves for the rats studied. Age at the initiation of lead exposure did not influence growth because weight gain was comparable for rats in the three treatment groups (ANOVA, p>0.05).

Daily drinking water consumption during the 5-week period of lead exposure was 16.4 ± 2.3, 19.6 ± 3.0, and 15.1 ± 0.1 ml/day for the rats given lead starting at 5, 10, or 15 weeks old, respectively. These values did not differ significantly (ANOVA, p>0.05), indicating that lead ingestion via the drinking water was comparable for the three treatment groups. Mean daily lead doses based on body weight at the midpoint of lead exposure were 23 ± 1, 21 ± 2, and 16 ± 1 mg/kg/day for rats given lead starting at 5, 10, or 15 weeks old, respectively.

Figure 2 contains the blood and organ lead concentrations of the rats studied. Both greater age at the start of lead exposure and a longer interval since the end of exposure were associated with significantly (ANOVA, p<0.01) lower blood and organ lead concentrations. However, the relative differences for different age groups and with increasing interval since cessation of lead exposure varied among the organs studied. For example, the ratios of the lead concentrations (measured 4 weeks after the end of lead exposure) for the group exposed beginning at 5 weeks of age to that exposed at 15 weeks were 6.83 for kidney, 4.31 for femur, 3.02 for spinal column bone, 2.46 for brain, 1.88 for liver, and 1.70 for blood. For the rats exposed beginning at 5 weeks of age, the ratios of the concentrations 4 weeks after the end of exposure to those at 20 weeks were 6.61 for brain, 3.02 for liver, 2.34 for kidney, 1.82 for femur, 1.80 for blood, and 1.62 for spinal column bone.

Figure 3 depicts the total lead content of the five organs analyzed. The pattern of values is similar to that for the lead concentrations of these organs.

Stepwise multiple regression analyses demonstrated that the independent variables—body weight at the midpoint or end of lead exposure, age at organ harvesting, and total lead consumed—significantly
Figure 2. Blood and organ lead concentrations of rats that ingested 250 mg/l of lead in their drinking water for 5 weeks beginning at 5 (red), 10 (blue), or 15 (green) weeks of age. Blood and organs were excised 4, 8, and 20 weeks after the end of lead exposure. Bars represent the mean, n = 8 per group except the brain, for which n = 6–8. Concentrations not marked with the same letter (A, B, C, D) are significantly different (Duncan's test, p<0.05). Means ± standard error are indicated on each bar.
Figure 3. Total organ lead content of rats that ingested 250 mg/l of lead in their drinking water beginning at 5 (red), 10 (blue), or 15 (green) weeks of age. Organs were excised 4, 8, and 20 weeks after the end of lead exposure. Bars represent the mean, $n = 8$ per group except the brain, for which $n = 6–8$. Values not marked with the same letter (A,B,C,D) are significantly different (Duncan’s test, $p<0.05$). Means ± standard error are indicated on each bar.
influenced blood and most organ lead concentrations and contents (p<0.05). However, even after stepwise inclusion of the above independent variables in the regression analysis, age during lead exposure remained significantly associated with lead concentrations or contents for blood and all organs except the brain.

Table 1 contains the results of the determinations of calcium and zinc concentrations in the five organs studied. Lead exposure had only modest effects on organ calcium and zinc, with the exception of kidney calcium concentrations (Table 1).

**Discussion**

The results for the blood and organ lead concentrations do not support the hypothesis of more rapid depletion of bone lead stores in younger versus older animals; instead, they suggest that younger age at lead exposure is associated with greater lead retention and toxicity subsequent to the cessation of lead exposure. In fact, the differences in organ lead concentrations among treatment groups were substantial, as reflected in the ratios calculated, which indicate that blood and organ lead concentrations 4 weeks after the end of lead exposure were 1.70- to 6.83-fold higher in the rats exposed beginning at 5 weeks of age than in the rats exposed beginning at 15 weeks of age. Lead retention was particularly high for calcified tissue and the kidney; this is consistent with the results of other studies (4-6-9).

The cause of the consistently higher blood and organ lead concentrations in the rats youngest during exposure was not determined in this investigation, but is not due to differences in the quantities of lead ingested, which were comparable in the 3 treatment groups. In addition, the multiple regression analyses demonstrated that consideration of body weight during lead exposure, age at organ harvesting, and total lead consumed did not alter the significant effects of age during lead exposure on blood and organ lead concentrations and contents. One explanation may be the greater gastrointestinal absorption of lead in young versus older rats (10), though other differences in lead metabolism and in excretion may also contribute to the higher blood and organ concentrations in the rats that were youngest during lead exposure. Kostial et al. (11) reported that a large fractional absorption of lead in suckling rats falls rapidly (toward adult levels) during a period of days to at most a few weeks after weaning. Weaning of rats is usually completed by 3-4 weeks of age. Rats in the current study were exposed to lead for 5 weeks beginning at 5, 10, or 15 weeks of age; they exhibited decreasing body lead retention with increasing age at the onset of lead exposure. These results are consistent with the observations of Kostial, but suggest that increased lead absorption may persist well beyond weaning and into adolescence in the rat.

Another reason for the higher bone lead concentrations in the rats exposed when youngest may be the observation that skeletal uptake of absorbed lead is much greater in rapidly growing mammals than in mature animals (5). Thus, the combination of increased gastrointestinal absorption and increased skeletal uptake in young animals may interact to enhance lead deposition in calcified tissue.

The consistent pattern of decreasing organ lead concentrations with increasing rat age at exposure differed from that of calcium and zinc. This may reflect reduced lead absorption with increasing age and the cessation of lead exposure 4-20 weeks prior to blood and organ harvesting; in contrast, ingestion of the essential divalent metals from food occurred throughout the study. However, differences in the metabolism of lead and the other metals may also play a role.

The markedly increased kidney calcium concentrations in the two groups that were older during lead ingestion than in the rats that were youngest during lead ingestion may reflect the well-known development of nephrocalcinosis that occurs in rats. Although lead and calcium share a number of metabolic similarities (12), the trend in kidney calcium concentrations in the rats studied is the opposite of lead, which decreased with increasing age during lead ingestion. It is possible that lead exposure at an early age may retard the later development of nephrocalcinosis.

It is well known that bone lead concentrations increase with age (2,5,12). The bone lead concentrations and content of the youngest rats studied declined with increasing time (from 4 to 8 to 20 weeks) since the end of lead exposure. However, children who accumulate considerable lead stores are likely to experience further increases in bone lead concentrations and content with age because it is not possible to avoid additional lead exposure. Nevertheless, for toxins with long biological residence times, such as lead, the body burden is determined primarily by the exposure history rather than current ingestion or inhalation (4).

The substantial turnover of calcium and lead in bone during early life is the result of the extensive modeling necessary to maintain the proper shape of growing bones as they increase in mass and size. O'Flaherty (3) has suggested that not all bone participates equally in this process because some regions of bone turn over

| Organ          | Age at start of lead exposure (weeks) | Time since end of lead exposure (weeks) | Calcium μmol/g | Zinc nmol/g |
|----------------|--------------------------------------|----------------------------------------|----------------|-------------|
| Brain          | 5                                    | 4                                      | 1.05 ± 0.10    | 195 ± 2A    |
|                | 5                                    | 8                                      | 0.94 ± 0.11    | 183 ± 2B    |
|                | 5                                    | 20                                     | 1.04 ± 0.13    | 191 ± 2A    |
|                | 10                                   | 4                                      | 0.90 ± 0.04    | 186 ± 3B    |
|                | 15                                   | 4                                      | 0.91 ± 0.03    | 188 ± 2AB   |
| Kidney         | 5                                    | 4                                      | 27 ± 14C       | 457 ± 18    |
|                | 5                                    | 8                                      | 51 ± 27BC      | 395 ± 18    |
|                | 5                                    | 20                                     | 43 ± 19C       | 437 ± 17    |
|                | 10                                   | 4                                      | 197 ± 49AB     | 447 ± 25    |
|                | 15                                   | 4                                      | 142 ± 42AB     | 438 ± 24    |
| Liver          | 5                                    | 4                                      | 0.78 ± 0.03A   | 455 ± 9A    |
|                | 5                                    | 8                                      | 0.71 ± 0.02AB  | 445 ± 7AB   |
|                | 5                                    | 20                                     | 0.75 ± 0.03AB  | 424 ± 10B   |
|                | 10                                   | 4                                      | 0.70 ± 0.02B   | 432 ± 10AB  |
|                | 15                                   | 4                                      | 0.67 ± 0.02B   | 442 ± 12AB  |
| Femur          | 5                                    | 4                                      | 4503 ± 230     | 2439 ± 105C |
|                | 5                                    | 8                                      | 4507 ± 63      | 2628 ± 38BC |
|                | 5                                    | 20                                     | 4436 ± 76      | 2831 ± 48AB |
|                | 10                                   | 4                                      | 4469 ± 132     | 2551 ± 91C  |
|                | 15                                   | 4                                      | 4841 ± 83      | 2866 ± 62A  |
| Spinal column  | 5                                    | 4                                      | 3420 ± 76B     | 2269 ± 86B  |
| bone           | 5                                    | 8                                      | 3148 ± 77C     | 2144 ± 60B  |
|                | 5                                    | 20                                     | 3731 ± 49A     | 2672 ± 43A  |
|                | 10                                   | 4                                      | 3670 ± 43A     | 2515 ± 53A  |
|                | 15                                   | 4                                      | 3667 ± 78A     | 2659 ± 57A  |

Data are mean ± standard error. n = 8 for all data except brain calcium, for which n = 6-8. Concentrations not marked with the same superscript letter (A,B,C) are significantly different (Duncan’s test, p<0.05).

*Rats were given 250 mg/l of lead in their drinking water for 5 weeks.
more rapidly than others, whereas pockets of old (neonatal) bone persist throughout life. Greater gastrointestinal lead absorption and/or skeletal lead uptake in young animals in combination with persisting pockets of neonatal bone may explain the relatively high lead concentrations found 4–20 weeks after cessation of lead exposure of the youngest rats in the current study.

The data demonstrate that exposure at a young age can lead to substantial skeletal lead accumulation and retention despite a high rate of bone remodeling, and suggest that extensive turnover in the skeleton cannot overcome the greater absorption of lead that occurs in young animals. Kosnett et al. (13) have noted that even by the end of the sixth decade of life, more than one third of the skeletal mass is composed of unremodeled bone acquired during childhood and adolescence, and they suggest that a substantial fraction of the lead incorporated into bone at a young age will remain there for many years. The results of the current study support this suggestion.

It is well known that the short-term toxic effects of lead, especially on the central nervous system, are greater for young children than for adults (14). The present study suggests that retention of lead in the skeleton by children exposed at a young age may also pose long-term health problems. A particular concern is the release of lead from the skeleton in women during pregnancy and lactation, which can then be transferred to the fetus or neonate (7,15). In addition, recent studies demonstrate that increased skeletal lead stores are associated with aggressive behavior in young boys (16), reduced hematocrits and hemoglobin concentrations in men (17), and development of hypertension in males aged 48–92 (18).

If the results of this study are applicable to humans, excessive lead exposure or lead poisoning as a child may result in the retention of substantially more lead in the skeleton than would exposure at a later age. Since lead is a ubiquitous and widespread contaminant, it will not be possible to eliminate additional environmental exposure of people of all ages. This inevitable exogenous exposure will be augmented by endogenous exposure as a result of past and ongoing bone lead accumulation.

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