

Hormone Replacement Therapy May Reduce the Return of Endogenous Lead from Bone to the Circulation

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Hormone replacement therapy (HRT) in postmenopausal women suppresses the increase in bone resorption expected as circulating levels of endogenous estrogen decline. We tested the hypothesis that bone lead content might remain elevated in women on HRT. Fifty-six women who at recruitment were on average 3.5 years postmenopausal were placed on calcium supplementation. Six months later, 33 of these women were prescribed either low dose or moderate dose hormone replacement in addition to the calcium supplementation. After approximately 4 years of hormone replacement, lead content was measured at the tibia and calcaneus by *in vivo* fluorescence excitation, and lead concentrations were measured in serum, whole blood, and urine. Women not taking hormones had significantly lower lead concentrations in cortical bone compared to all women on HRT ($p = 0.007$). Tibia lead content (mean \pm SD) for women on calcium only was 11.13 ± 6.22 $\mu\text{g/g}$ bone mineral. For women on HRT, tibia bone lead was 19.37 ± 8.62 $\mu\text{g/g}$ bone mineral on low-dose HRT and 16.87 ± 11.68 $\mu\text{g/g}$ bone mineral on moderate-dose HRT. There were no differences between groups for lead concentrations measured in trabecular bone, whole blood, serum, or urine. Hormone replacement maintains cortical bone lead content. In women not on HRT, there will be a perimenopausal release of lead from bone. **Key words:** bone resorption, endogenous lead, estrogen, hormone replacement, menopause. *Environ Health Perspect* 103:1150–1153 (1995)

The moderate-dose, cyclical regime was 0.625 mg/day Premarin for days 1–25 and 5 mg/day Provera for days 16–25 of a monthly cycle. After 2 years, the moderate-dose, cyclical regime was changed to a moderate-dose, continuous regime which consisted of 0.625 mg/day Premarin and 2.5 mg/day Provera.

Longitudinal measurements of bone mass were made at the one-third radius and lumbar spine using photon absorptiometry (3,4). These sites were selected as representative of cortical and trabecular bone. There were no statistically significant differences in age, weight, height, years since menopause, or bone-mass variables between the three groups at entry into the study (4). There were also no differences between groups for variables such as smoking, drinking, diet, or parity (5). A questionnaire designed to identify occupational, recreational, and environmental exposure to lead was administered to each subject at the time of bone lead measurement. No differences in lead exposure could be detected between groups.

The calcium-only group consisted of 23 women, 2 of whom originally chose HRT but declined their assigned therapy and continued on calcium only. One additional subject was transferred to the calcium-only group after less than 3 months on low-dose HRT. There were 33 women who chose calcium supplementation plus HRT. Sixteen of these were on low-dose, continuous HRT and 11 were on moderate-dose HRT. The mean duration of HRT for subjects on the low-dose regime was 4.10 years (SD = 0.22 years). For the subjects on moderate-dose HRT, the mean duration was 3.90 years (SD = 0.37 years). A fourth group of six women did not comply with their assigned HRT regime between the times of enrollment and the measurement of lead concentrations. Their mean duration of exposure to hormones was 2.15 years (SD = 0.49 years).

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The concentration of lead in bone apparently increases steadily throughout life. By the time a woman reaches the age of menopause she can expect to have a bone lead content of about 12 $\mu\text{g/g}$ mineral in cortical bone, with somewhat higher levels in trabecular bone (1). Because about 95% of body lead resides in the skeleton, a typical endogenous lead burden for a menopausal woman will be 30 mg.

Endogenous lead has access to the circulation through the normal processes of mineral exchange and bone turnover. The concentration of lead in bone can decrease if the rate of lead transfer from bone to the circulation during mineral exchange and bone turnover is greater than the rate of lead transfer from the circulation to bone. Such a situation can best be achieved by reducing the rate of ingestion of lead such that the concentration of lead in newly formed bone will fall and the amount of lead available for exchange from the circulation is reduced.

Recent declines in blood lead levels achieved by reducing environmental lead exposures (2) should also produce reductions in bone lead content by decreasing uptake of lead into bone. Superimposed on this effect, the increase in bone resorption associated with menopause should increase the release of lead from bone. Since hormone replacement therapy (HRT) opposes the increase in both bone resorption and

mineral exchange, it would be expected that HRT would maintain bone lead concentration and suppress the transfer of endogenous lead to the circulation. Women on HRT should have higher concentrations of bone lead and lower concentrations of plasma lead. To test this possibility, we compared the concentrations of lead in bone, serum, urine, and whole blood for subjects who were either on calcium supplementation or on HRT plus calcium supplementation.

Materials and Methods

The subjects who volunteered for these measurements were participants in a study of the impact of HRT on bone mass. The protocol for that study, which has been described previously (3), was approved by the Research Advisory Committee of the Faculty of Health Sciences, McMaster University. White women, who were typically between 1 and 5 years postmenopause, were recruited from the local community and placed on calcium supplementation (500 mg/day). Six months later each woman chose either to add HRT to the calcium supplementation or to remain on calcium alone. Those given HRT received either a low-dose, continuous or a moderate-dose, cyclical regime. The low-dose regime consisted of 0.3 mg/day equine estrogen (Premarin) and 2.5 mg/day medroxyprogesterone (Provera).

The lead concentrations of bone, blood, serum, and urine were measured during the fourth or fifth year of HRT. Bone lead was measured at the mid-tibia and the right calcaneus in all subjects using the instrument described by Gordon et al. (6). These sites are considered to be representative of cortical and trabecular bone. Briefly, the bone was exposed to a beam of 88-keV photons emitted from a ^{109}Cd source. Characteristic X-rays emitted from any lead present in the bone are detected by a high-purity germanium detector. At the same time, 88-keV photons coherently scattered by bone mineral into the detector are monitored. With appropriate calibration, the system yields a measurement of bone lead content in units of micrograms of lead per gram of bone mineral. The standard deviation of repeated *in vivo* measurements is typically between 2 and 5 $\mu\text{g Pb/g mineral}$ at the tibia (7). For calcaneal measurements the uncertainty is greater (typically 8 $\mu\text{g Pb/g mineral}$) because a smaller mass of bone is measured and because the overlying tissue is thicker at the calcaneal site compared to the tibial site.

Serum and urine lead was measured using a technique developed by Bowins and McNutt (8). A tracer quantity of enriched ^{204}Pb was added to each serum or urine sample, which was acidified with nitric acid. Aliquots were then volatilized in a graphite furnace and introduced into an inductively coupled plasma mass spectrometer. The technique yields a standard deviation of 0.006 $\mu\text{g/dl}$ at a lead concentration of 0.03 $\mu\text{g/dl}$.

Blood lead was measured by flameless atomic absorption spectrophotometry. The coefficient of variation from measurements repeated over a 3-month period for a control sample with a lead concentration of about 60 $\mu\text{g/dl}$ was 4%.

We used one-way analysis of variance to search for differences between groups in the concentrations of lead in blood, serum, and urine as well as for the bone lead content of the tibia and calcaneus. Two-sample *t*-tests were used to test for significant dif-

ferences between subjects who had never taken hormones and those who had complied fully with their regime. Only those subjects with complete data sets were included in the analysis, and differences associated with a *p*-value <0.05 were considered significant.

Results

As reported elsewhere (3), moderate-dose HRT increased bone mineral mass at the spine, while low-dose HRT eliminated a rate of loss of 0.3 g mineral/year observed on calcium alone. HRT produced no differences in rates of change of mineral mass at the radius.

Complete results for whole blood and serum lead concentrations, 24-hr urine excretion of lead, and the lead content of the tibia and calcaneus were obtained in 22 subjects in the calcium-only group, 15 in the low-dose HRT group, 11 in the moderate-dose HRT group, and 6 subjects in the partial HRT group. Two serum samples, one from the calcium-only group and one from the low-dose HRT group, were lost to analysis due to a technical failure of the mass spectrometer. The mean values and standard deviations for the measured variables are given in Table 1 for each group of subjects. One-way analysis of variance showed that tibia lead content was significantly lower in the group of subjects not taking hormones (*p* = 0.049). No other statistically significant difference existed between groups. The mean tibia lead content is shown for each group in Figure 1.

If the subjects are assigned to two groups, one of which is composed of subjects who had never taken hormones (*N* = 22), the other of those subjects who fully complied with their hormone replacement regime (*N* = 26), a two-sample *t*-test shows a significant difference in tibia bone lead (*p* = 0.007) but no difference in calcaneal bone lead (*p* = 0.47).

Discussion

These results suggest that postmenopausal women who are on HRT will have a

greater skeletal lead burden than women not on hormones. The excess lead is located within cortical bone, rather than trabecular bone, probably because the retention time in cortical bone is at least twice that of trabecular bone (9). This is consistent with measures of blood lead made during the second National Health and Nutrition Examination Survey (NHANES II) (10). In 849 women between 40 and 60 years of age, blood lead concentration postmenopause was 13.0 $\mu\text{g/dl}$, whereas in premenopausal women it averaged 11.9 $\mu\text{g/dl}$. The increased blood lead is thought to be due to release of lead from bone as a consequence of menopause-related increases in bone turnover. In our study, hormone replacement did not produce a significant difference in blood lead concentration. The average concentrations in our subjects were approximately one-third those observed in NHANES II but were similar to the values reported in NHANES III (11). Hormone replacement prevents the menopause-associated increase in bone turnover, and lead would be expected to remain in the skeleton. The menopause-related increase in blood lead is greater for white than for black women because the increase in bone turnover is greater for white women (12). Especially at risk for increased blood lead after menopause are white women who have had no children. This is thought to be because skeletal lead burdens would have been reduced by a postpartum increase in bone turnover in lactating women (13,14), resulting in less lead being available for mobilization after menopause (15).

The transfer of calcium between bone and the circulation takes place through the processes of mineral exchange and bone turnover. The purpose of exchange and turnover is to release minerals to the circulation, repair damaged bone, replace effete

Table 1. Mean values (SDs) for the measured variables

Variable	Hormone replacement therapy ^a			
	None (<i>N</i> = 22)	Low dose (<i>N</i> = 15)	Moderate dose (<i>N</i> = 11)	Partial HRT (<i>N</i> = 6)
Blood lead ($\mu\text{g/dl}$)	4.60 (1.59)	4.08 (1.60)	5.22(3.36)	3.35 (1.32)
Serum lead ($\mu\text{g/dl}$)	0.074 (0.048)	0.106 (0.065)	0.061 (0.025)	0.086 (0.057)
Urine lead ($\mu\text{g/day}$)	2.77 (1.70)	2.23 (0.90)	2.93 (2.20)	2.10 (1.78)
Tibia lead ($\mu\text{g/g}$)	11.13 (6.22)	19.37 (8.62)	16.80 (11.68)	14.66 (8.41)
Calcaneus lead ($\mu\text{g/g}$)	21.12 (13.55)	24.02 (10.88)	23.83 (14.18)	17.63 (16.09)

^aLow dose consisted of 0.3 mg/day estrogen and 2.5 mg/day medroxyprogesterone; moderate dose consisted of 0.625 mg/day estrogen on days 1–25 and 5 mg/day progesterone on days 16–25 for 2 years, then 0.625 mg/day estrogen and 2.5 mg/day progesterone for 2 years; partial therapy included women who did not comply with their assigned regime. See Materials and Methods for details.

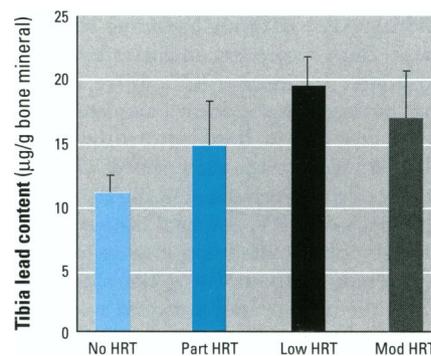


Figure 1. Mean tibia lead content and standard error of the mean for postmenopausal women who have never used hormone replacement therapy (No HRT) and those who partially complied (Part HRT) or fully complied with a low dose (Low HRT) or moderate dose (Mod HRT) hormone replacement regime.

bone and reorganize bone in response to altered mechanical environments. It has been estimated that each day the mass of calcium involved in exchange is about 10 times the mass of calcium involved in turnover (16). In recently proposed compartmental models of lead metabolism, the assumption is made that these same processes are responsible for the movement of lead between the circulation and bone (17,18). Lead on bone surfaces is considered to be rapidly exchangeable, whereas lead distributed throughout the bone volume is in exchange with bone surface lead. In reality, not all bone lead will be equally exchangeable with circulating lead, but there is likely to be a spectrum of accessibility ranging from freely exchangeable to non-exchangeable. The latter will correspond to lead fixed within bone crystals and available only after osteoclastic resorption of bone (19).

The exchange of calcium between bone and the circulation is under the influence of parathyroid hormone (20). The sensitivity of postmenopausal bone to the effects of parathyroid hormone is reduced by estrogen replacement. That is, for the same parathyroid hormone concentration, the estrogen replete woman will have lower serum calcium (21,22). The rate of resorption of bone is decreased by estrogen through a reduction in the activation frequency of new remodeling cycles with an increase in the mean age of the bone (23–25). It is known that elements that are bone-volume seekers such as lead and the alkaline earth elements are retained to a greater extent in older bone than in newly formed bone (26).

The relative contributions of exchange and turnover to the reduction in bone lead content after menopause can be estimated from our data. In premenopausal women the rate of bone turnover is about 150 mg Ca/day, and, without hormone replacement, bone resorption increases by about 20% after menopause (27). If we assume that hormone replacement completely prevents any rise in bone resorption, then HRT should prevent the turnover of about 30 mg calcium each day. During the course of 4 years of HRT, the total mass of calcium protected could amount to about 44 g of calcium or about 100 g bone mineral. Thus, the mass of lead protected by a hormone-induced reduction of bone turnover could amount to almost 2 mg. The observed difference in concentration at the tibia between the calcium-only group and the HRT groups is about 6 µg/g bone mineral or, for a bone mass of 2000 g, a total mass of lead of 12 mg. Thus, about 10 mg of lead remains in bone because of a hor-

mone-induced reduction in exchange. It should be noted that this estimate neglects the lead present in trabecular bone and also any differences in bone mass produced by HRT.

This study is an observational report on a convenience sample of subjects. It is limited by its cross-sectional nature, and it is conceivable that differences in bone lead may have existed before HRT was started. The fraction of bone lead released after menopause may be greater in some women than indicated by this work. All women in this study took calcium supplementation, and it is possible that in subjects with calcium deficiency, the mass of bone involved in exchange and turnover processes may be even greater. In addition, hormone replacement did not start immediately after endogenous estrogen levels fell. The mean time after menopause that women started HRT was 3.5 years (4). Consequently, all subjects in this study suffered a period without HRT and therefore will have lost some lead from the bone. The woman who is likely to release the most lead from her skeleton is the woman who has accumulated considerable lead from her environment, who suffers a significant perimenopause increase in bone turnover and mineral exchange, and who may have a poor calcium intake.

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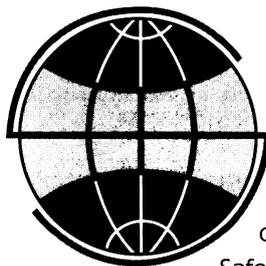
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XIVth World Congress on Occupational Safety and Health

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The XIVth World Congress on Occupational Safety and Health will be held in Madrid from April 22 to April 26, 1996. The organizers are the Spanish Ministry of Labour and Social Security, through the National Institute for Occupational Safety and Health (INSHT), the International Labour Office (ILO), Geneva, and the International Social Security Association (ISSA), Geneva.

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The main focus of this Congress will be on the consequences for occupational safety and health of processes of international and regional integration (e.g. EU, NAFTA) and of the globalization of economic relations, on an in-depth analysis of chemical risks and on new proposals for cooperation and participation within enterprises. Other specific issues will also be dealt with, such as training and information, control of working conditions or new responsibilities. Special emphasis will be placed on small and medium-sized enterprises and sectors facing specific problems with regard to safety and health at work, such as the construction sector and agriculture.

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