The deleterious biological effects of lead ingestion are well established. There has been a growing concern that ingestion of certain oyster shell-containing calcium supplements and antacids might contribute to an increased risk of lead poisoning in individuals who consume these products [1]. Consumers of calcium supplements and calcium antacids, especially pregnant and lactating women, their unborn fetuses, infants, and small children would thus be exposed to a serious and documented risk of reproductive and developmental harm. Because calcium supplements are invariably prescribed in the prevention and treatment of osteoporosis, elderly osteoporotic individuals may also be at increased risk for lead toxicity. Because the skeleton represents the major repository of lead in the human body, containing 90% or more of total lead content [2], we reasoned that measurement of skeletal lead concentration in osteoporotic individuals before and after calcium supplementation would disclose whether there was significant lead accumulation from administration of calcium supplements.

The patient population was composed of 11 women and three men with a mean age of 60 years (range 38–76). All patients had osteoporosis as evidenced by reduced vertebral bone mass, histologic evidence of osteoporosis, and at least one vertebral fracture. All patients and controls were involved in research protocols approved by the Institutional Review Board on the Use of Human Subjects. A transcortical iliac crest bone biopsy was obtained from each patient after obtaining informed consent and before beginning calcium citrate therapy (400 mg of calcium twice daily as Citracal®, Mission Pharmacal). A second bone biopsy was procured on the opposite side after completing, on average, 4.75 years of calcium therapy (range 1–9 years). Four healthy men (mean age 35 years, range 20–53) who participated in a different protocol without calcium supplementation and who also underwent transcortical iliac crest bone biopsy 12 weeks apart served as methodology controls.

Bone biopsies were dehydrated and embedded in methylmethacrylate for sectioning and histologic analysis. Upon completion of bone histology, a 100-μm longitudinal section was cut from each biopsy with a low-speed wafering saw. These sections were then ashed overnight in tared platinum crucibles at 600 °C and the ash weight of bone (representing the mineral phase) then determined. Ashed bone specimens (5–20 mg ash weight) were dissolved in 1 mL of 5% nitric acid and diluted to 2 mL with water from a MilliQ (Millipore) water purification system. In addition, sections of methylmethacrylate that contained no bone specimen were also removed and subjected to the same procedure to ensure that the embedding material contained no significant amounts of lead. Bone lead was quantified via electrothermal atomic absorption spectrometry. The analytical equipment was a Varian SpectrAA 20 equipped with a graphite tube assembly (GTA 96) electrothermal oven (Varian Instruments). A calibration curve was prepared over the range of 0.2–1 ng of elemental lead in 2.5% nitric acid with a total injection volume of 25 μL. The sample was dried at 100 °C (ramp 5 s, hold 10 s), ashed at 400 °C (ramp 10 s, hold 8 s), and atomized at 2000 °C (5 s). The absorbance was measured at 283.3 nm. A deuterium background correction was used because the above-mentioned spectrophotometer was not equipped with Zeeman correction capabilities. Samples (25 μL) were assayed directly without further additions. This methodology gave a detection limit of 0.05 ng of lead when processed and assayed as described above. The intraassay CV was 3.9% when determined on three specimens assayed 15 times each (range 1.6–6.5%). The interassay CV was 4.5% when determined for three different concentrations on five different days (range 2.5–5.6%). Assessment of method accuracy yielded an average recovery of 107% (range 97–116%). Preliminary studies in a few specimens demonstrated comparable results for either direct addition or by calibrator additions. Thus, all results were obtained by the method of direct analysis.

Table 1 summarizes the results. For all 14 patients, mean bone lead increased nonsignificantly (P = 0.203) from 3.3 ± 1.8 to 4.2 ± 1.8 ng/mg bone ash. All mean values were within reported normal values of 1–8 ng/mg bone ash when performed by electrothermal atomic absorption spectrometry [3–5]. Variability of lead in the iliac crest was observed to be minimal on the basis of sampling from contralateral sites 12 weeks apart in the four healthy subjects. It is interesting to note that pre- and postmean

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Bone lead (ng/mg bone ash)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>35 ± 14</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>Patients (n = 14)</td>
<td>60 ± 12</td>
<td>3.3 ± 1.8</td>
</tr>
</tbody>
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*a* Calcium treatment was 400 mg of calcium twice a day as calcium citrate for a mean treatment period of 4.75 years in patients only.

b Values expressed as mean ± SD.
values in the patients were significantly higher than those in the healthy subjects. This difference in basal bone lead concentration might be the result of previous long-term calcium supplementation in the older patient population. To address this issue, we reviewed the 14 patients’ charts and determined that three patients disclosed prior calcium supplementation. Two men took calcium carbonate, one at 800 mg/day for 12 years and the other at 2 g/day for 2 years. The remaining woman took calcium citrate at 200 mg/day for 2 years. The mean bone lead content in these three patients (2.8 ± 1.0 ng/mg bone ash) was not significantly different and actually less than that for the remaining 11 patients (3.4 ± 2.0, P = 0.0646). The value was not significantly different from the four younger subjects (P = 0.227). This suggests that prior calcium supplementation may not totally explain the differences in basal bone lead concentration between the young controls and older patients. However, because the number of patients for which this comparison was made is small, this contention cannot be dismissed. Alternatively, a longer lifetime exposure to environmental lead in the older patient population may also explain this difference. In addition to the above findings, there was no correlation between the duration of calcium administration and the change in bone lead concentrations (Fig. 1). This is not surprising as the lead concentration in the Citracal tablet was 100 ng/tablet as determined by inductively coupled plasma mass spectrometry (personal communication C. Wabner, Mission Pharmacal). Thus, at 800 mg of calcium per day (4 tablets), a total lead ingestion of 400 ng/day from calcium citrate would be expected. Over 5 years, this would represent 22% of the total bone lead increase observed during the treatment period in the present study, assuming all ingested lead was absorbed and deposited in the skeleton of a 70-kg person with a total bone mineral phase of 3500 g.

Another consideration is the effect that a skeletally active drug such as estrogen, diphosphonate, or sodium fluoride might have on bone lead concentration when taken concurrently with a calcium supplement. Indeed, it would be expected that such drugs, which increase bone mass, would raise bone lead concentration as the newly formed bone becomes mineralized with calcium from the oral calcium supplement. In the current patient population, 11 patients took Neosten® (sustained-release sodium fluoride, 25 mg twice a day) concurrently with Citracal. When compared with the three patients who took Citracal alone, there was no significant difference between the change in bone lead content between the two groups (0.7 ± 2.7 for fluoride-treated vs 1.6 ± 2.4 ng/mg bone ash for calcium alone, P = 0.639). At present, we do not know if concurrent use of estrogen or bisphosphonates with calcium supplementation would yield similar findings.

Thus, on the basis of this small number of patients, we conclude that calcium supplementation in the form of calcium citrate (Citracal) does not present a potential risk for lead intoxication.

References


Effect of Hemolysis on the Concentration of Insulin in Serum Determined by RIA and IRMA, Didier Chevenne,1,2*, Annick Letailleur,1 François Trivin,2 and Dominique Porquet1 [1Hoˆp. Robert Debré, Lab. de Biochim.-Hormonol., 48 Blvd. Séruier, 75019-Paris, France; 2Hoˆp. Saint-Joseph, Lab. de Biochim., 185 rue Raymond Losserand, 75674-Paris Cédex 14, France; *author for correspondence: fax (33) 1 40 03 47 90]

Insulin-degrading enzyme (IDE; EC 3.4.99.45) was first described 40 years ago [1]. It is widely distributed in various tissues, including red blood cells (RBC) [2–4]. IDE may not play a key role in insulin metabolism, and fundamental questions on the biological role of IDE remain [2, 3]. Recently, IDE was characterized as a peroxisomal protease [3]. The specificity of IDE is selective: Only insulin and transforming growth factor-α (K_m = 0.1 μmol/L) are good substrates; insulin-like growth factor 1 and proinsulin are poor substrates [2–4]. On denaturing