Oral supplementation with NaFeEDTA reduces blood lead in postmenopausal but not premenopausal Korean women with anemia

Hee-Seon Kim, Ph.D., M.P.H., Min-Kyoung Kim, M.S., and Byung-Kook Lee, M.D., Dr. Med. Sc., M.Sc.O.He.

Abstract

Objective: The objective was to evaluate the effect of iron supplementation for 6 mo on blood lead (PbB) concentration in populations of premenopausal (PRE-M) and postmenopausal (POST-M) Korean women.

Methods: We conducted a community intervention trial in Asan, Republic of Korea. Of the 137 enrolled women with hemoglobin (Hb) levels lower than 12 g/dL, 37 were PRE-M and 100 were POST-M. Each woman received the equivalent of 9 mg of supplemental iron per day in the form of sodium-iron ethylene-diaminetetra-acetic acid (NaFeEDTA) syrup for a period of 6 mo.

Results: The initial PbB concentrations were 2.56 ± 0.99 µg/dL (mean ± SD) for the PRE-M women and 3.27 ± 1.24 µg/dL for the POST-M women. The differences were statistically significant (P < 0.01). After 6 mo of NaFeEDTA supplementation, the PbB concentration decreased in the POST-M group but no change was observed in the PRE-M group. When age and baseline Hb levels were adjusted for, PbB concentrations decreased by 0.10 and 0.31 µg/dL in the PRE-M and POST-M groups, respectively, and the results were significant using a multivariate model (P = 0.016). Iron status determined by zinc protoporphyrin, transferrin saturation, and Hb improved significantly in both groups, but serum ferritin decreased significantly in the POST-M women (P < 0.001), with no change in the PRE-M group.

Conclusion: After 6 mo of supplementation with the equivalent of 9 mg of iron/d in the form of NaFeEDTA, the PbB concentrations in Korean women with anemia appeared to depend on their menopausal status. © 2009 Published by Elsevier Inc.

Keywords: Sodium-iron ethylene-diaminetetra-acetic acid; Iron supplementation; Blood lead; Iron status; Zinc protoporphyrin

Introduction

Due to increased concerns about environmental pollution, nutritional intervention has become an important means of mitigating the toxicity of environmental pollutants and modulating health and disease associated with chemical insults [1]. Adequate dietary iron intake is essential for the prevention of lead toxicity for workers with high blood lead (PbB) concentrations [2], and improved iron nutrition is important for the reduction of PbB toxicity [3]. These results suggest that nutritional intervention with iron might be an efficient secondary preventive intervention for controlling high PbB.

Nutritional iron supplied in the form of sodium-iron ethylene-diaminetetra-acetic acid (NaFeEDTA) has high bioavailability because its uptake is not generally affected by inhibitors of iron absorption [4,5]. In the past, the use of NaFeEDTA was restricted to experimental trials because of concerns over its potential chelation of important metabolic minerals and toxic metals such as aluminum, cadmium, and Pb [6]. However, analyses thus far on the safety and the
toxicology of NaFeEDTA [7,8] have suggested no adverse health effects of its use.

The binding of EDTA to minerals can be evaluated from data on the stability constants (log K) at the pH optimum of maximum binding [9]. These constants are relatively high for potentially toxic elements such as aluminum (15.5), cadmium (15.0–16.1), and Pb (16.8–17.7) [10]. Very little is known about the effect of NaFeEDTA on the absorption and retention of these potentially toxic elements. A link between iron status and PbB is supported by animal studies [3]. Iron and Pb are speculated to compete for an absorptive pathway in the small intestine [11,12] and children consuming inadequate amounts of iron show increased absorption of Pb [13]. Anemia is a common condition in women of child-bearing age and in the older population [14]. In Korea, the relation between anemia and PbB is of key interest because of the relatively high prevalence of anemia in older Korean women [15,16] and increased exposure to Pb from environmental pollution.

The objective of this study was to evaluate the effects of a 6-mo regimen of iron supplementation with NaFeEDTA on the PbB levels and iron status of community-dwelling premenopausal (PRE-M) and postmenopausal (POST-M) Korean women.

Materials and methods

Subjects and experimental design

This was a community intervention study of iron supplementation, conducted in the city of Asan, Republic of Korea. During January 2004, the community-based health and nutrition survey was conducted with 1200 residents of six communities in Asan. In total, 849 potential women participants were screened for anemia by measuring their blood hemoglobin (Hb) levels in accordance with World Health Organization (WHO) criteria [17]. Menopausal status of the women was determined based on their answers to the following questions: 1) Do you still menstruate? 2) Have you stopped menstruating because you underwent surgery? Among the women satisfying the WHO selection criteria for anemia (Hb <12 g/dL), 37 were PRE-M and 100 were POST-M. The 137 women selected as participants were healthy except for 47 with hypertension and 8 with diabetes. Participants consulted with a physician on the project team about their current medications and conditions and they were provided with the equivalent dose of 9 mg of iron per day in the form of bottled NaFeEDTA syrup from January to June 2005. Because only 4 of the 100 POST-M women reported menopause due to hysterectomy (answered yes to question 2), we did not differentiate our results from the cause of menopause. No women were in amenorrhea due to disease, pregnancy, or lactation and none of postmenopausal women reported the use of hormone replacement therapy. The subjects took their NaFeEDTA syrup supplement once a week as a single scoop containing 63 mg of iron or as a daily dose of 35 drops with 9 mg of iron. Every 2 wk, the appropriate supplements were distributed to the participants at their local public health centers and the nursing staffs at each center monitored the participants. Although only 130 of the original 137 participants fully completed the 6-mo regimen of nutritional intervention with NaFeEDTA (two refused supplementation, and five dropped out due to stomach discomfort), all 137 participants were included in the final data analysis because this was designed as an intention-to-treat study. The participants provided their written informed consent and received a detailed explanation of the study that was approved by the institute research board of Soochunhyang University, Asan, Korea.

Data collection and analysis

At the start of the study (T1), demographic information was obtained by participant surveys, and anthropometric measurements including height, weight, and percentage of body fat were made using Inbody 3.0 (Biospace, Seoul, Korea) with the participants wearing no sweaters, socks, or shoes. All participants were evaluated at T1 for PbB, zinc protoporphyrin (ZPP), and biochemical indicators of iron including Hb, transferrin saturation (TS), and serum ferritin (SF). At the end of the study (T2), after 6 mo of nutritional intervention with NaFeEDTA, the participants were re-evaluated for PbB, ZPP, Hb, TS, and SF.

Approximately 10 mL of venous blood was collected from each participant after an overnight fast at T1 and T2 and divided into two tubes: 4 mL for PbB, ZPP, and Hb analyses, and 6 mL of blood was immediately separated for analyses of serum iron, total iron-binding capacity, and SF. PbB was measured in duplicate with a Zeeman background-corrected atomic absorption spectrophotometer (Z-8100; Hitachi, Tokyo, Japan) using the standard addition method of the National Institute of Occupational Safety and Health [18] at the Institute of Environmental and Occupational Medicine, Soochunhyang University, which is a certified reference laboratory for Pb measurement in Korea. ZPP levels were measured by a portable hematofluorimeter (Aviv-206; Aviv, Lakewood, NJ, USA) at the study sites [19]. Hb was assayed by the cyanmethemoglobin method (model Ac-T; Beckman Coulter, Fullerton, CA, USA). Serum iron and total iron-binding capacity levels were measured by spectrophotometry (TBA-40FR biochemical analyzer; Hitachi) and TS was calculated by dividing the serum iron by total iron-binding capacity and multiplying by 100. SF concentration was determined in duplicate by the immunoaffimetric assay at Samkwang Reference Laboratories, Seoul, Korea (College of American Pathologists Accredited Laboratory 69944-01).
Body mass index (kg/m²) 23.8
Height (cm)† 159.1
Weight (kg)† 60.0
Age (y)† 44.2

* Values are means ± SDs or percentages of subjects. Significance levels were determined by Student’s t test for mean differences and chi-square test.
† P < 0.001.

### Results

The group of PRE-M women constituted 27% of the total participants and were significantly younger, heavier, and taller (P < 0.001) than the women in the POST-M group. Body mass index and percentage of body fat were not significantly different between the two groups. Each group displayed significantly different (P < 0.001) demographic characteristics such as education level and family type (Table 1). All participants were anemic at the beginning of the study (T1), but after 6 mo of NaFeEDTA supplementation (T2), the prevalence of anemia in the PRE-M group was only 30% and that in the POST-M group was 39% (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Characteristics of subjects at baseline*</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>Age (y)†</td>
<td>44.2 ± 8.9</td>
<td>72.3 ± 7.7</td>
</tr>
<tr>
<td>Age at menopause (y)</td>
<td>—</td>
<td>48.7 ± 6.9</td>
</tr>
<tr>
<td>Weight (kg)†</td>
<td>60.0 ± 9.0</td>
<td>50.4 ± 9.0</td>
</tr>
<tr>
<td>Height (cm)†</td>
<td>159.1 ± 6.0</td>
<td>148.8 ± 7.0</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.8 ± 3.7</td>
<td>23.0 ± 3.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>29.1 ± 5.7</td>
<td>30.6 ± 6.9</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Prevalence of anemia†</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>T2</td>
<td>30</td>
<td>39</td>
</tr>
</tbody>
</table>

### Statistics

All statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as P < 0.05 based on Student’s t test and chi-square test to detect differences between the PRE-M and POST-M groups. Paired t test was performed to detect significant differences between baseline and postsupplementation values (T2 – T1). Multivariate analysis of variance was performed to detect any significant differences in PbB levels between T1 and T2 values (within group) by menopausal status (between groups), after age and baseline Hb were adjusted as covariates. Two participants who rejected supplementation and five dropouts, all belonging to the POST-M group, were included in the data analyses because of the intention-to-treat study design.

However, when anemia was limited only to iron-deficiency anemia (IDA), as defined by TS <16% or SF <12 μg/L and Hb <12 g/dL, the initial anemic prevalences at T1 were 92% for the PRE-M and 29% for POST-M groups; after 6 mo of nutritional intervention with NaFeEDTA at T2, these prevalences were decreased to just 24% for PRE-M women and 9% for POST-M women. Baseline (T1) PbB levels ranged between a minimum value of 0.65 μg/dL and a maximum of 6.94 μg/dL, with a mean ± standard deviation of 3.07 ± 1.22 μg/dL for all total participants. Although T1 PbB levels were lower in the PRE-M group than in the POST-M group (P < 0.01, 2.56 versus 3.27 μg/dL, respectively), T1 ZPP was higher in the PRE-M than in the POST-M group (P < 0.01, 88.4 versus 61.1 μg/dL, respectively). Baseline (T1) biochemical indices of iron status were significantly lower in the PRE-M group but this dif-

Hb, hemoglobin; IDA, iron deficiency anemia; NaFeEDTA, sodium-iron ethylene-diaminetetra-acetic acid; PbB, blood lead; SF, serum ferritin; T1, baseline; T2, after 6 mo of supplementation; T2 – T1, difference between baseline and postsupplementation values; TS, transferrin saturation; ZPP, zinc protoporphyrin

* Values are means ± SDs or percentages of subjects.
† Hb levels <12 g/dL.
‡ Defined by anemia with TS <16% or SF <12 μg/L.
§ Significantly different between groups by Student’s t test.
¶ Significantly different within a group by paired t test.
** P < 0.001.

** P < 0.01.
ference became insignificant after 6 mo of supplementation (T2) with the exception of SF. After 6 mo (T2), PbB levels decreased significantly in the POST-M group ($P < 0.01$) but PbB levels in the PRE-M group were not significantly so by paired $t$ tests (Table 2). In the multivariate model that adjusted for age and baseline (T1) Hb levels, the NaFeEDTA nutritional supplementation significantly lowered PbB levels according to menopausal status ($P = 0.016$; Fig. 1).

Iron status indices were not consistent after 6 mo of iron supplementation. ZPP decreased significantly and Hb and TS increased significantly in both groups. SF concentration, however, showed a significant decrease in the POST-M group, whereas no difference was observed in the PRE-M group. When age and baseline Hb were adjusted in multivariate models, mean estimates showed a similar tendency as the raw values presented in Table 2 except for post-supplementation (T2) Hb and SF (data not shown). Post-supplementation mean estimates of Hb and SF showed significantly higher values in the PRE-M group than in the POST-M group (12.22 versus 12.18 g/dL for Hb and 46.4 versus 41.7 $\mu$g/L for SF).

**Discussion**

This study was conducted as part of a nutritional intervention project to improve the general health of a rural community. Consequently, all women with anemia in the selected communities were treated with iron supplementation without a placebo-controlled group as per our intention-to-treat study design. Similar to most rural communities in the Republic of Korea, rural Asan has a relatively larger percentage of older individuals than younger individuals. Therefore, our selecting a disproportionate number of PRE-M (37) and POST-M (100) participants for our study was not unexpected. However, changes in PbB levels after the 6-mo supplementation were different between the two groups, after age and baseline iron status were adjusted. These findings support the hypothesis that iron therapy can lower Pb levels, but the magnitude of the change could be dependent on the physiologic status of individual subjects.

Several studies investigating the effects of iron supplementation on PbB levels in children have yielded controversial results. A randomized placebo-controlled trial in a smelter complex in Mexico demonstrated a decrease in PbB levels due to a general decrease in Pb pollution in the area, but no additional reduction in PbB levels due to iron supplementation (30 mg/d of elemental iron given as ferrous fumarate for 6 mo) was observed in the first-grade children in this study [20]. In another study, children 2–5 y of age who were iron deficient received 200 mg/d of ferrous sulfate and no differences in PbB levels were observed after 4 mo between children with low and high PbB levels [21]. In this particular study, the children with high PbB levels who received iron therapy showed a significant increase in PbB levels over time, when compared with the PbB and Hb levels of the children given a placebo. In contrast, when Indian school children were fed iron-fortified rice meal at a dose of 15 mg/d for 16 wk, chronic Pb intoxication decreased [22]. In addition, Wolf et al. [23] found the greatest decrease in Pb levels among non-anemic Costa Rican children with depleted iron stores, followed by children with ID, suggesting that the beneficial effect of iron supplementation on PbB levels was dependent on iron status. Some of the discrepancies found in these children’s trials could be attributable to physiologic differences of the subjects, which resulted in differential abilities to metabolize iron and Pb burdens in vivo, similar to the differences we observed with respect to menopausal status.

In our study, an unexpected decrease of SF after iron supplementation was seen among the POST-M women, whereas the other iron indices, Hb and TS, increased and PbB levels decreased. In contrast, the PRE-M women showed significantly improved iron status by ZPP, Hb, and TS, but the changes in PbB and SF levels were not significant. The mean baseline (T1) SF concentration in the POST-M women was unusually high for women with anemia. SF levels can be increased by the presence of inflammation, infection, trauma, certain chronic diseases, iron overload (excessive iron stores), viral hepatitis, and certain cancers such as Hodgkin’s disease [24]. Anemia in older populations is frequently considered a type of pathologic condition caused by underlying diseases. Thus, causes of anemia include iron, folate, and vit $B_{12}$ deficiencies; renal insufficiency; anemia of chronic inflammation, formerly termed anemia of chronic disease; and unexplained anemia.

![Fig. 1. Changes in the estimated mean concentrations of PbB before and after 6 mo of sodium-iron ethylene-diaminetetra-acetic acid supplementation after corrections for age and baseline hemoglobin levels. PbB, blood lead; POST-M, postmenopausal; PRE-M, premenopausal.](image-url)
When the cause of anemia was distinguished from other common causes as specifically IDA, the POST-M women in this study had a prevalence of IDA of only 29% at baseline (T1). Therefore, some of our older participants may have had certain pathologic conditions that were not recognized at the beginning (T1) of our study, aside from the aforementioned self-reported 47 subjects with hypertension and 8 with diabetes.

Despite the unknown etiology of the anemias in 71% of the POST-M and 8% of the PRE-M women, iron supplementation clearly increased Hb and TS indices and decreased ZPP in both groups. The decreased PbB levels and ZPP observed in the POST-M group might be attributable to a change in iron utilization in the blood because NaFeEDTA could effectively mobilize the stored endogenous iron from SF, probably due to absorbed EDTA [25], and provide exogenous iron. An animal study demonstrated that the distribution of iron in the body was markedly different for rats exposed to iron in the form of NaFeEDTA than for rats exposed to other forms of iron such as FeSO4 or FeCl3. NaFeEDTA was less effective than FeSO4 in elevating levels of stored iron [26], most likely due to the different postabsorptive distribution patterns for iron chelated with EDTA [27]. However, the concentration of kidney non-heme iron was markedly higher in rats fed NaFeEDTA and ZPP observed in the POST-M group might be attributable to a change of iron utilization in the blood because NaFeEDTA could effectively mobilize the stored endogenous iron from SF, probably due to absorbed EDTA [25], and provide exogenous iron.

Iron status is the most important factor affecting iron absorption [29] and the iron absorption rate is inversely related to the concentration of SF, an index of body iron stores [30]. With iron repleted, however, the anemic POST-M women (those who did not have IDA) may have had downregulated iron absorption due to their high SF levels [31] and, consequently, downregulated lead absorption because iron and Pb share the common intestinal divalent metal transporter-1 [32]. In fact, the significant decrease in PbB levels in the POST-M group could be explained by this downregulation of Pb absorption. One resultant possibility is that the PbB mobilized to the kidneys and was excreted by the same mechanism as for increased iron excretion by EDTA released from NaFeEDTA [28]. In contrast, for the PRE-M women who were anemic, exogenous iron could have been effectively absorbed to increase Hb and serum iron, but exogenous iron might not have been enough to change the levels of stored iron or PbB. Although EDTA certainly binds to the PbB with the same affinity in the PRE-M and POST-M groups, the impaired iron status of the PRE-M group could influence the rate of reabsorption in the kidneys, and the PbB mobilized to the kidneys could be reabsorbed via divalent metal transporter-1 with iron in the loop of Henle and distal convoluted tubules rather than being excreted [25]. In this albeit rather convoluted way, a possible explanation arises for our nutritional supplementation with iron in the form of NaFeEDTA that differentially affected the iron status and PbB levels according to menopausal status. However, note that we cannot provide definite reasons for the differences between the two groups in the present study because we cannot determine if these differences were due to EDTA, physiologic conditions of menopausal status, or some combination of the two.

Our study has a few potential limitations that may affect any inferences derived from our findings. The participants were primarily from the low and middle classes who voluntarily attended the community health-promoting project and were not a random sample of the general population. Thus, our findings cannot be generalized to all women. In addition, only 27% of our study group (n = 37) were PRE-M women, limiting our ability to conduct a more in-depth analysis. The PbB levels of our participants and the supplemental iron doses were generally lower compared with other iron intervention studies seeking to reduce PbB. We used lower iron supplementation levels primarily due to our concerns about the levels of free EDTA released from NaFeEDTA for oral delivery, and we tried to follow the safe level proposed by the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives [33]. Relatively low baseline (T1) PbB and iron supplementation levels in our study would have limited the results to be detected into a further statistically significant level and possibly lead to the underestimation of the efficacy of iron intervention. Despite these possible limitations, iron supplementation in the form of NaFeEDTA significantly lowered PbB levels in POST-M women.

Although primary prevention of Pb exposure is optimal, it may not be feasible when exposure is ubiquitous or when it involves sources not under the control of the community as the case of the present study. Under such circumstances, nutrient supplements will play a mitigating role and our study has provided evidence. The fraction of disease attributable to low-level environmental Pb exposures may be small, but if a large population in a community is exposed, even a relatively small exposure makes a notable contribution to disease. Furthermore, when Pb exposure is combined with poor nutrition, the negative health effects of both are greatly compounded.

Our study demonstrated the appropriate nutritional intervention with iron in the form of NaFeEDTA and significantly lowered the PbB levels of POST-M women with anemia living in communal dwellings in rural Asan, Republic of Korea.

References


[28] Zhu L, Miller DD. Tissue iron distribution and urinary mineral excretion vary depending on the form of iron (FeSO4 or NaFeEDTA) and the route of administration (oral or subcutaneous) in rats given high doses of iron. J Agric Food Chem 2007;55:8793–9.


