0163–4984/96/5202–0155 $06.75

An Improved Method for Estimating Original Mineral Contents in Excavated Bone Using Sulfur

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Received January 13, 1995; Accepted February 28, 1995

ABSTRACT

Trace element analysis in excavated bones is complicated by the lack of a reliable index for estimating the original amount of bone material. In this study, we subjected modern human bones to alkali treatment to simulate aging. Alkali treatment of vertebrae with attached muscle did not affect sulfur (S) content; it increased the magnesium (Mg), phosphorus (P), and zinc (Zn) contents, and tended to decrease iron (Fe) content of the bones. When vertebrae cleaned of muscle were used, alkali treatment did not affect S and Fe contents but increased Mg, P, Ca, and Zn contents. Ca and S contents were higher in excavated bones (200–1300 yr old) than in their surrounding soils. In contrast, S, Mg, and Ca contents per dry weight did not differ between the excavated bones and the alkali-treated modern bones. These results indicate that S can provide a more accurate index of excavated bones than the often-used Ca content or dry wt measures, especially for bones excavated from calcium-rich soils.

Index Entries: Bone elements; bone excavation; paleosteology; anthropology.

INTRODUCTION

Bones obtained from excavations at historical or ancient sites can provide information about the histories of these sites *(1)*. Measurements

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of various trace elements in excavated biomaterial can provide information on subjects such as the mode of living and foods *(2–9)*, and on changes in environmental pollution *(10–12)*. The concentrations of trace elements in excavated bones are usually reported in terms of dry wt and/or calcium content. However, a problem with these types of analyses is that the constituents of bones that have been underground for long periods are generally modified by decomposition and diffusion, both into and out of the bones. The present study was undertaken to determine whether one of the common elements found in bone could be used as an index of the original amount of bone, and thus could be used to determine original concentrations of trace elements.

MATERIALS AND METHODS

Bone Sampling

Recent human vertebrae were resected from cadavers that had been used for teaching medical students. Excavated human bones were collected from graves of the 6–17th centuries in Tokushima and Matsuyama, Japan. Soil samples were collected by shaving off soils that had adhered to the bones.

Reagents

Pure $CaCl_2$, $MgCl_2$, and $Zn(NO_3)_2$ for use as atomic absorption spectrometry standards were obtained from Wako Pure Chemicals (Osaka, Japan). Volumetric standard solution of H_2SO_4 and ion chromatographic standard solution of NaH_2PO_4 for use as atomic absorption spectrometry standards were also purchased from Wako Pure Chemicals. Distilled water was filtered through Milli-Q (Nippon Millipore, Tokyo, Japan) to make extra-purified water.

Alkali Treatment

Vertebrae with attached muscle (muscle-attached vertebrae) that had been resected from a single vertebral column were cut into two halves. One half was heated in 5% NaOH solution for 3 h, washed in running tap water for 6 h, soaked in distilled water for 6 h, and then dried by heating at 80°C for 16 h. After weighing, 5 mL of HNO_3 were added, and each vertebra was heated for 2 h at 100°C in a 50-mL volumetric flask. After addition of 2.5 mL of perchloric acid, the sample was heated for 2 h and then diluted to a volume of 50 mL with distilled water. The other half of the vertebra was not treated with alkali, but was otherwise prepared by the same procedure as above. Vertebrae from the other vertebral columns were cleaned of muscle (muscle-removed vertebrae) and cut into two halves. one half was heated in 5% NaOH solution for 1.5 h, washed in running tap water for 24 h and then soaked in distilled water for another 24 h. After drying for 16 h at 80°C, the vertebrae were treated with HNO_3 and $HClO_4$ for wet combustion. The other half was not treated with 5% NaOH solution. For dissolution, excavated bones and their surrounding soils were also treated with HNO_3 and $HClO_4$.

Measurements

Sample solutions were filtered through no. 7 filter paper (Toyo Roshi, Tokyo, Japan) and the filtrate applied to an inductively coupled plasma atomic emission spectrophotometer (ICP-AES, ICPS-1000III, Shimadzu, Kyoto, Japan). The operating conditions were: RF generator, 1.2 kW; plasma argon flowrate, 1.2 L/min.; cooling gas flowrate, 14 L/min.; carrier gas flowrate, 1.0 L/min.; entrance slit, 20 μm; exit slit, 30 μm; observation height, 15 mm; integration time lapse, 5 s. Ca emission was measured at 393.366 nm, Mg at 279.553 nm, Zn at 213.856 nm, S at 180.731 nm, and P at 178.287 nm.

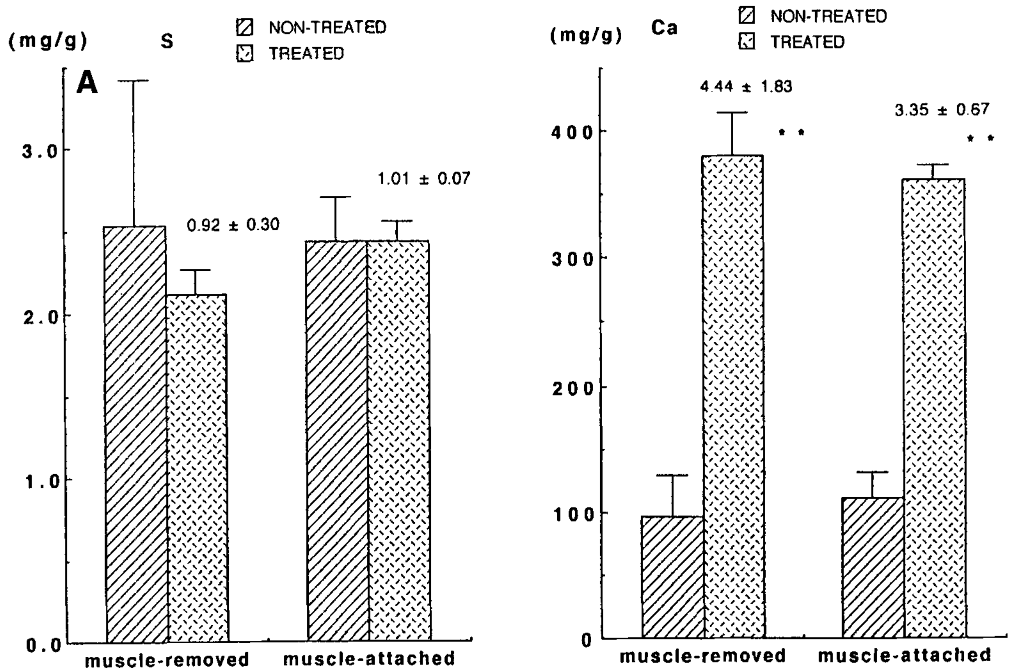
Statistical Analysis

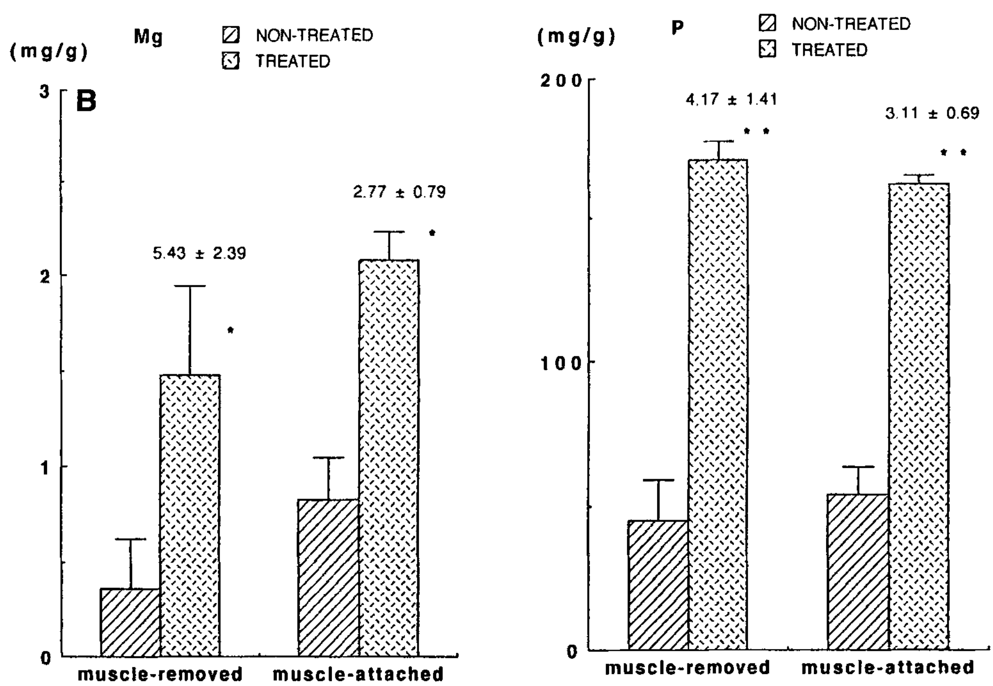
All results are expressed as mean ± SD, and the differences between groups were analyzed by a one-way analysis of variance (ANOVA). In addition, group means were compared using the Dunnett method.

RESULTS

Figure 1 shows the effects of alkali treatment on muscle-removed and muscle-attached modern human vertebrae. The alkali treatment was used to remove protein and some minerals to simulate the effects of aging. Alkali treatment did not affect the structure of muscle-attached bones but it removed a significant amount of mineral and severely weakened the muscle-removed bones. Sulfur (S) content in the bone did not differ in the alkali-treated and nontreated groups or in the muscle-attached and muscle-removed groups. Magnesium (Mg), phosphorus (P), and calcium (Ca) contents were increased by alkali treatment, but did not differ in the muscle-attached and muscle-removed groups. Zinc (Zn) content also increased with alkali treatment, but more so in the muscle-removed bones than in the muscle-attached bones. Iron (Fe) content in alkali-treated bones was higher in the muscle-removed group than in the muscle-attached group, but did not differ in the alkali-treated and nontreated groups.

Figure 2 shows mineral contents in the excavated bones and their surrounding soils. Ca, P, and S contents in the bone samples were higher than the contents in the soil, but Mg and Zn contents in the bone did not differ from those in the soil. Fe content was significantly lower in the bone than in the soil.


(mg/g)
S
NON-TREATED
TREATED
A
3.0
2.0
1.0
0.0
0.92 ± 0.30
1.01 ± 0.07
muscle-removed
muscle-attached
(mg/g)
Ca
NON-TREATED
TREATED
400
300
200
100
0
4.44 ± 1.83
3.35 ± 0.67
* *
* *
muscle-removed
muscle-attached


(mg/g)
Mg
NON-TREATED
TREATED
B
3
2
1
0
5.43 ± 2.39
2.77 ± 0.79
*
*
muscle-removed
muscle-attached
(mg/g)
P
NON-TREATED
TREATED
200
100
0
4.17 ± 1.41
3.11 ± 0.69
* *
* *
muscle-removed
muscle-attached

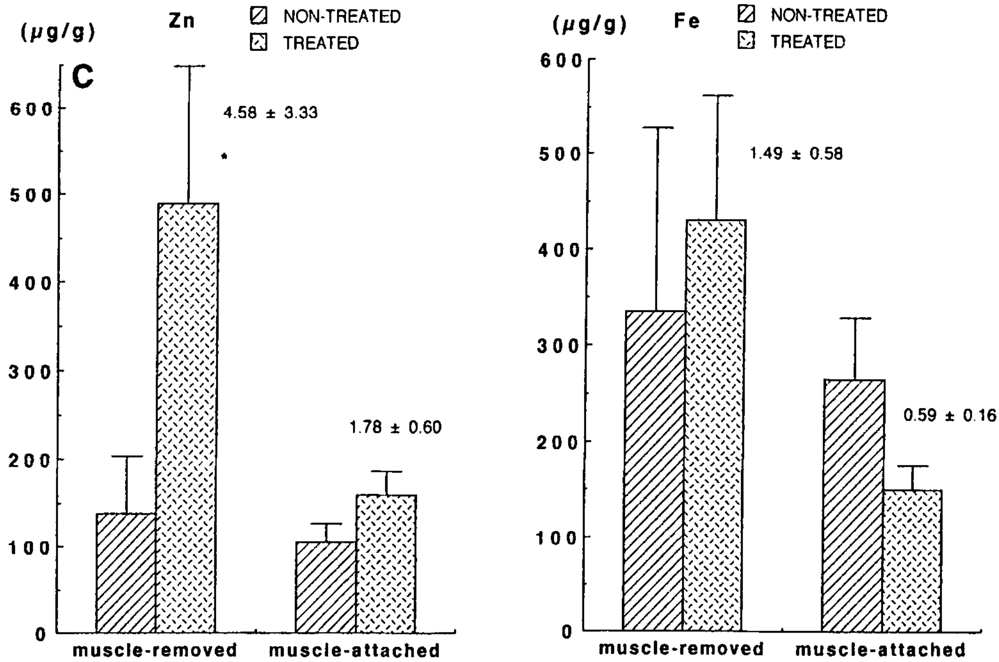


Fig. 1. Effects of alkali treatment and attached muscle on the concentrations of selected elements in modern human bones. Hatched, nonalkali-treated; Cross-hatched, alkali-treated. Numbers above alkali-treated bars are the ratio of concentrations for treated to nontreated bone. Bars show mean ± SD $^*p < 0.05$, $^{**}p < 0.01$ vs nontreated group.

DISCUSSION

In order to analyze mineral contents in excavated bones, a relative standard index of the original bone is needed. Dry wt has been used as a standard index by many studies *(9,10)*, but this index is subject to error because of changes in weight owing to aging and variations in soil moisture. Ca has also been used as a standard *(12)*, because it is a major component of bone, and because hydroxyapatite, the form in which Ca is found, is water insoluble. However, Ca is not suitable as a standard when the bone is excavated from Ca-rich soil.

Alkali treatment was used in an attempt to remove protein and some minerals from the bone to simulate the losses of protein and minerals from excavated bone that occur during aging. As shown in Fig. 1, alkali treatment did not remove P, Mg, or Ca from either muscle-removed or muscle-attached bones, but it more easily removed Fe and Zn from muscle-removed bones than from muscle-attached bones. Furthermore, alkali treatment did not affect the S content/dry wt. Although S is a constituent of cartilage, the data show that S is difficult to remove from the bone. S, Mg, and Ca contents/dry wt did not differ between the excavated bones

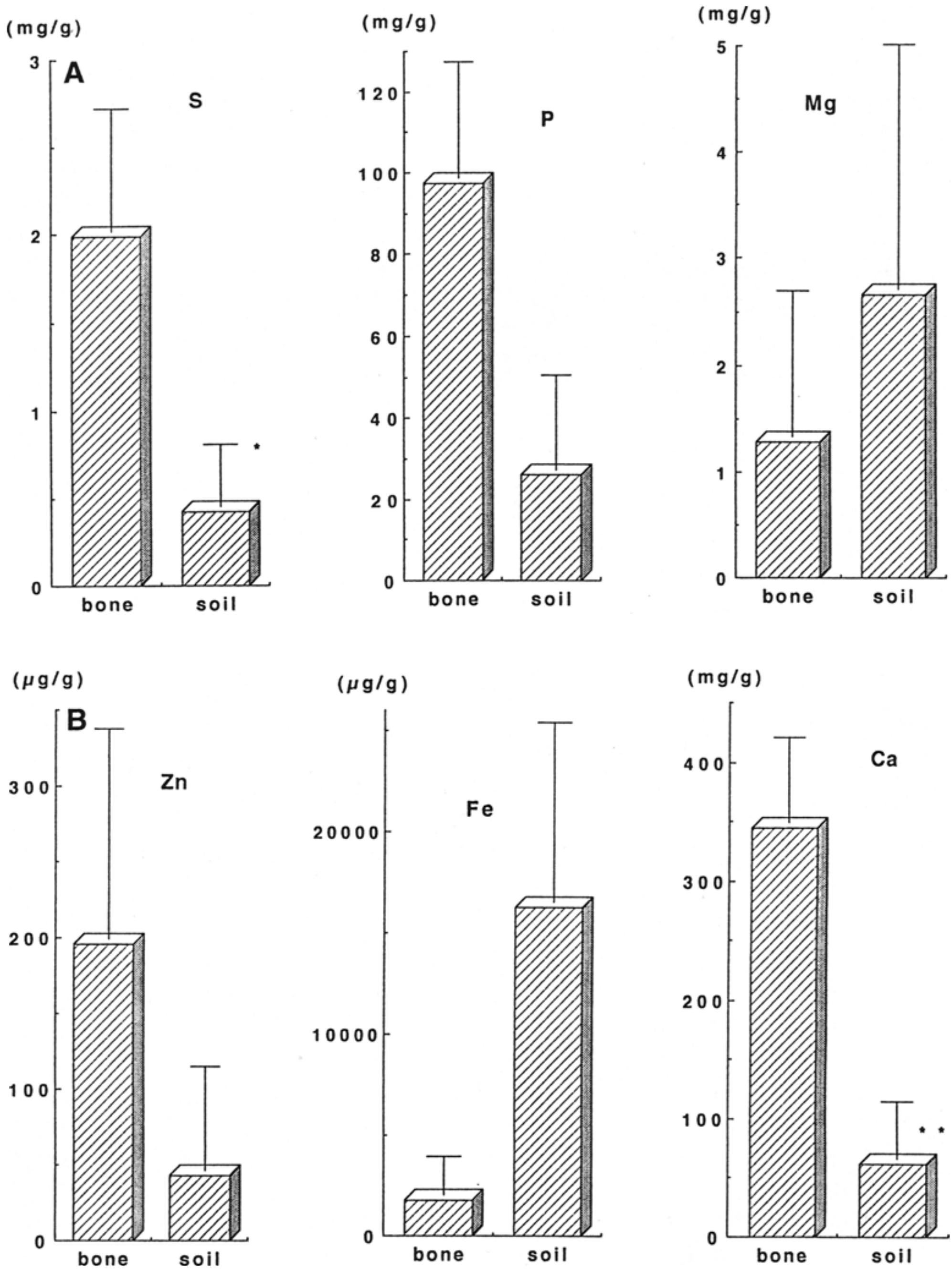


Fig. 2. Concentrations of selected elements in the excavated bones and their surrounding soils. Mean ± SD $*p < 0.05$, $**p < 0.01$ vs bone group.

and alkali-treated modern bones. Moreover, the Ca and S contents were higher in the excavated bone than in their surrounding soils, and so only negligible quantities of these elements would be expected to transfer from soils to the bones.

Based on these results, it appears that the original concentrations of the constituent elements of excavated bone can be more accurately determined from their ratio with respect to S than from their ratios with respect to Ca content or to dry wt. It thus would appear to be useful to include assays of S as well as Ca content and dry wt for analyses of trace elements in excavated bones and their surrounding soils.

ACKNOWLEDGMENTS

We express our gratitude to Yukinori Nishio and his coworkers at the City Museum of Archeology of Matsuyama and to Akira Takakusu and Fumio Nishiwaki at the Department of Anatomy, Nara Medical University, for their help in collecting and preparing both the old and the modern bones.

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