Determination of Fourteen Elements in Bone Samples Using Inductively Coupled Plasma (ICP) Analysis

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Objective. There have been many studies of the concentrations of elements in blood and urine, but few studies have focused on the concentrations of elements in bone. The elements in bone play important roles in a variety of physiological processes. The objective of this study was to assess the optimum conditions for measuring the concentrations of 14 elements in human bone samples.

Methods. Seventy-seven bone samples were collected from bone surgery patients and pretreated using a microwave digest. Inductively couple plasma-atomic emission spectroscopy (ICP-AES) analysis was used to determine the optimal conditions for three variables: sample uptake rate, flow rate of Argon gas (Ar) into the nebulizer and concentrations of nitric acid.

Results. The detection limit of elements ranged from 1 μg/L (Co, Mn and Mg) to 55 μg/L (Pb). The optimal sample uptake rate and the optimal flow rate of Ar into the nebulizer were found to be 1.33 ml/min and 0.35 l/min, respectively. The optimum nitric acid concentration was 1M. Mn (0.7 μg/g) and Cd (1.2 μg/g) in bone samples were present in the lowest concentrations, and Ca (82007.9 μg/g) and Mg (3005.2 μg/g) had the highest concentrations. Except for Cu, there were no significant differences in element concentrations between males and females. There was a high variation in element concentrations among the bone samples.

Conclusions. Further research is needed to investigate the factors that influence the concentrations of elements in bone. The optimal conditions for bone-element analysis using ICP-AES as recommended by the authors was confirmed in this study. **(Mid Taiwan J Med 2001;6:125-32)**

Key words

bone, inductively couple plasma analysis (ICP), multi-elements

INTRODUCTION

Data on trace metals in human organs are useful for evaluating nutrition and for preventing and controlling a variety of diseases caused by mineral or trace element imbalance. Recent advances in analytical technology, such as inductively coupled plasma-atomic emission spectroscopy (ICP-AES), inductively coupled plasma-mass (ICP-MS), atomic absorption spectroscopy (AAS), neutron activation analysis (NAA), X-ray fluorescence spectroscopy (XRF) and protein induced X-ray and gamma-ray (PIXE and PIGE) [1-5], make it possible for very low concentrations of elements in human tissues to be detected. Numerous studies [6-9] have determined the levels of a wide variety of

Received : April 23, 2001. Revised : June 18, 2001.
Accepted : July 13, 2001.
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elements present in human hair, urine, blood, serum, saliva and bone. However, no consensus regarding the precise levels of elements in human biospecimens has been reached, due to the lack of a standardized analytical methodology, problems with contamination, and fluctuating physiological and nutritional status. It is necessary, therefore, to establish a national database of elements in the human body. This database could be used for studies of nutrition and diseases, allowing for a better understanding of the health risks posed by exposure to certain trace elements. This information could also help researchers further understand the role that elements play in human physiology.

In Taiwan, few studies have evaluated the levels of elements in human bone. Most previous studies were primarily concerned with assessing the concentrations of specific elements in blood and urine. ICP-AES can simultaneously determine the concentrations of elements in different kinds of biological specimens. ICP-MS is much more sensitive and more specific than ICP-AES, but it is not suitable for general use. Also, ICP-MS has not been widely used in previous studies because it is expensive. As such, prior to the commencement of this study, the optimal conditions for the analysis of 14 elements in bone were established using ICP-AES. Tzeng et al [10] used ICP-MS to measure the concentrations of elements in biological samples (rice and oysters) and tested different variables to determine the optimal conditions. They concluded that the sample uptake rate into the ICP-Mass, the flow rate of Argon gas into the nebulizer, and the concentration of nitric acid were the most significant factors, and that the radio frequency and width of the slit in the ICP-Mass were not significant factors. The objective of this study was to measure the concentrations of 14 elements in human bone samples and to evaluate the optimum conditions for analysis. Based on the findings of the current study, the authors intend to conduct further research into bone-elements in the Taiwanese population.

MATERIALS AND METHODS

Sample Collection

Seventy-seven compact bone samples were taken from 70 bone surgery patients (femoral neck fracture (N=13), ischemic necrosis of femoral head (N=18), osteoarthritis (N=24) and others (N=17). None of the subjects regularly took mineral supplements and no one had previously been exposed to metals. Of the 70 subjects, 53 were male (66.3%). Forty-one subjects (51.3%) were aged 61–80 years, and 22 subjects (27.5%) were aged 41–60 years. Eight subjects (10%) were aged below 40 years and 9 subjects (11%) were aged over 80 years. Except for Cd and Pb, there were no significant differences between the four age groups. There was a positive correlation between age and Cd and Pb concentrations (Kuo et al, 2000). During surgery, 77 bone samples were taken and immediately stored at −70 ℃. Bone samples for chemical analysis were obtained by cutting a 10 mm thick slice from each of the damaged bones' central cavities. Subsequently, 0.5–1.0 mm segments of each slice were cut concentrically, using a stainless steel wire saw, to provide a sequence of samples from the outer to the inner bone cortex. The mass of each sample was approximately 200 mg. Although the steel saw used was electroplated with chromium, accuracy testing found that samples were not contaminated, and there were no samples with high concentrations of this element. Three bone sample digestion methods were tested: dry ash (8 h, 550 ℃); wet digest (nitric acid, 85 ℃); and microwave digest (CEM standard methods). Microwave digest conditions were as follows: pressure, from 50–150 PSI; temperature, from 0–180 ℃; total reaction time, 50 minutes; power, 630W. Before microwaving, 0.5 g of sample and 10 mL of 70% nitric acid were added to each lined digestion vessel (LDV). Twenty-five milliliters of digested sample was then transferred to a beaker and heated at 85 ℃ until dry. A 25 mL sample was achieved by adding distilled water before analysis by ICP. Detailed information is

Table 1. The optimum conditions of ICP

Instrument	JY74 ICP-AES Monochromator HR1000 /polychromator
Ar gas	99.99%
Power output	1000W
RF	40.68MHZ
Plasma gas flow rate	12 l/min
Sheath gas flow rate	0.15 l/min
Auxiliary gas	0.1 l/min
Nebulizer flow rate	0.35 l/min
Sample uptake rate	1.13 ml/min

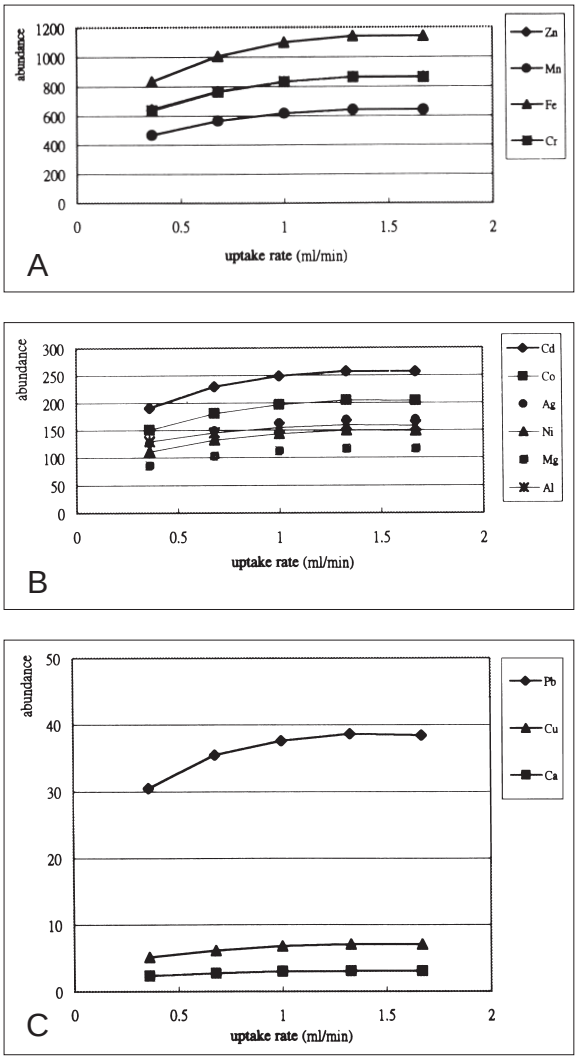

Fig. 1 Relationship between sample uptake rate and signal intensity of 14 elements.

described by Kuo et al [11]. Bone samples were analyzed for 14 elements (As, Zn, Cd, Pb, Ni, Cu, Mn, Fe, Cr, Mg, Al, Cu, Ag and Ca).

Experiment

Bone samples were pretreated by microwave digest (CEM standard method). Using standard reference materials (SRM), it was found that the accuracies for the following elements were: Zn (85%), Fe (79%), Mg (90%), Ca (105%). Precision of the samples was assessed by testing each of the 14 elements four times. The average difference, ranged from 2.9% to 9.4% [11]. The optimal conditions for analysis using ICP-AES were determined. The conditions of the apparatus are shown in Table 1. Diluted samples were nebulized and delivered into the plasma torch section with argon gas. Three conditions were tested. 1) The sample uptake rates (0.36, 0.68, 1.0, 1.33, 1.67 ml/min) into the ICP-AES. The uptake rate was tested by pumping 10 ppm of the multi-elements into the ICP-AES and then measuring the signal intensity. 2) The flow rates of argon into the nebulizer (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 l/min) at a sample flow rate of 1.33 ml/min. The signal intensity at each of the eight flow rates was measured. 3) The nitric acid concentrations (0.00, 0.01, 0.5, 1.0, 2.5 and 5.0 M). Five different concentrations of nitric acid were added to a standard (10 ppm) of the multi-elements and the signal intensity at each of the 5 acid concentrations was measured. For a detailed description of the quality control procedure, please refer to Kuo et al [11]. The SAS/PC+ 6.12 software package [12] was used for statistical analysis. The flow rate of the nebulizer, the uptake rate and the concentrations of nitric acid were plotted against the intensity for the 14 elements. The mean, standard deviation, and percentage were used to describe the results. The Student's *t*-test was used to compare the concentrations of the 14 elements in males and females.

RESULTS

The sample uptake rates of elements at different intensities are shown in Fig. 1. Three graphs were made due to the wide variation of ICP intensity (Figs. 1 A-C). Fig. 1A shows that the abundance of Zn, Mn, Fe and Cr

exceeded 400 and increased slightly when the sample uptake rate increased. At a flow rate of 1.33 ml/min of Ar into the nebulizer, the sample uptake rate did not result in any further increase in intensity. Figs. 1 B&C revealed a similar trend. Because the pressure caused by Ar flow rates above 1.33 ml/min could damage the machine, we did not increase the intensity above 1.33 m/min, even though this flow rate may be lower than the optimum level. Also, if the sample uptake rate is too high, the droplet diameter increases, resulting in obstruction of the nebulizer and a lower sensitivity. It has been found that radiofrequency of power and the nebulizer flow rate both affect ICP/MS [14]. Also increased power could reduce the life span of the machine and thus ran the machine at about 1000W [13,14]. As such, the current study used 1000W power and RF 40.68MHz. Large emission intensity fluctuations are observed from analyte species in inductively coupled plasma. Low plasma response and peaks in emission intensity of atoms are accompanied by depressions in ion emission. This phenomenon appears to be due to localized cooling by aerosol droplets. These emission spikes are caused by atomization and ionization of analyte from vaporizing particles [15,16].

Fig. 2 shows the nebulizer flow rate plotted against intensity for different elements. There are similar trends for Figs. 2 A-C. There was an increase in intensity with increased flow rate, peaking at 0.35 l/min. Pb was the exception, peaking at 0.3 l/min. Overall, the optimum flow rate was 0.35 l/min. The authors considered flow rates above 0.35 to be damaging to the nebulizer. These results are inconsistent with Tsai's finding [17] that the optimum flow rate of Ar was around 0.7 l/min, possibly because Tsai used an ICP/MS machine (Perkin-Elmer Sciex Elan 5000). However, the overall trends were very similar in both studies. The nebulizer flow and the sample uptake rates are two of the factors that can cause non-spectroscopic interference in the ICP machine, making it necessary to

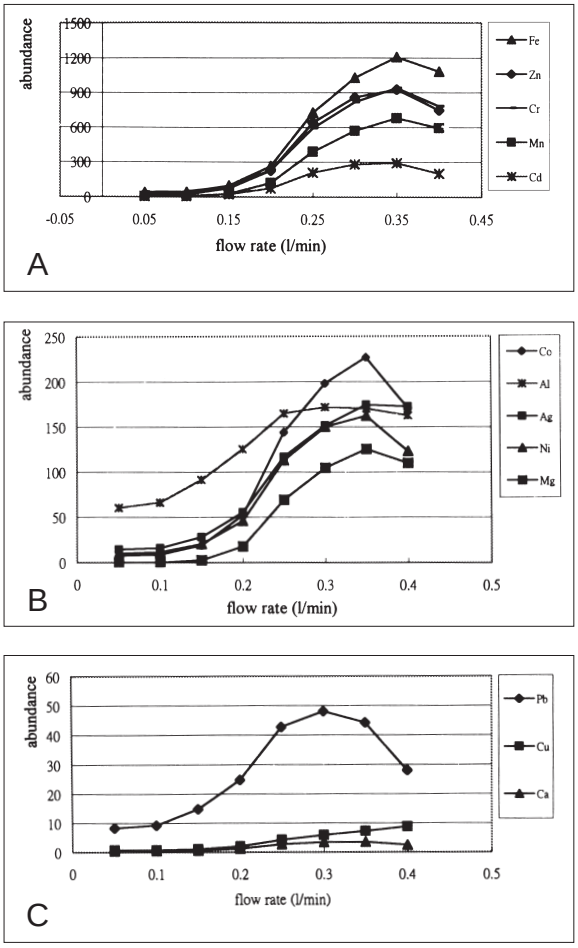

Fig. 2 Relationship between flow rate of nebulizer signal intensity of 14 elements.

establish optimum conditions for the measurement of element concentrations. In previous studies [18,19], dilution method, standard addition, internal standard, and isotope dilution methods were employed in order to overcome the problem of non-spectroscopic interference common to ICP/MS machines. There are few data available with regard to ICP/AES and non-spectroscopic interference. When there is a large difference in the concentration of different elements in the specimen, there is a greater probability of non-spectroscopic interference. The current study compared SRM samples with the results from ICP/AES and found that there was a high degree of consistency. Concentrations of As, Cd, Pb, Mn, Al and Cu were so low that they were not detected.

The intensities of ICP-AES plotted

against nitric acid concentrations for the 14 elements are shown in Figs. 3 A-C. Nitric acid concentrations were 0.00, 0.01, 0.5, 1.0, 2.5 and 5.0M. Overall, intensity was lowest at 0.5M and the optimal level was found to be 1.0M. Intensity decreased slightly when concentrations exceeded 1.0M. The authors speculate that this may be because the viscosity of nitric acid increases with increased acidity, thereby diminishing the efficiency of the nebulizer. Tsai [17] reported that (using ICP-MS) different elements produced varying responses to different nitric acid concentrations. For example, when 0.5M nitric acid was used, Al and Mo both had a low intensity and there was interference. For Zn and Cd, interference occurred when the acidity exceeded 0.5M. This may be related to the increased free energy (eV) which results in greater suppression. Tsai suggested that the optimum nitric acid concentration was 0.1M to 0.2M. Comparing nebulizer flow rates and sample flow rates, in our study, nitric acid concentrations did not significantly affect the intensities of the 14 elements.

Based on the optimum conditions described above, the concentrations of the 14 elements were determined. Table 2 shows the mean, median and standard deviation, wavelength and detection limit for the 14 elements.

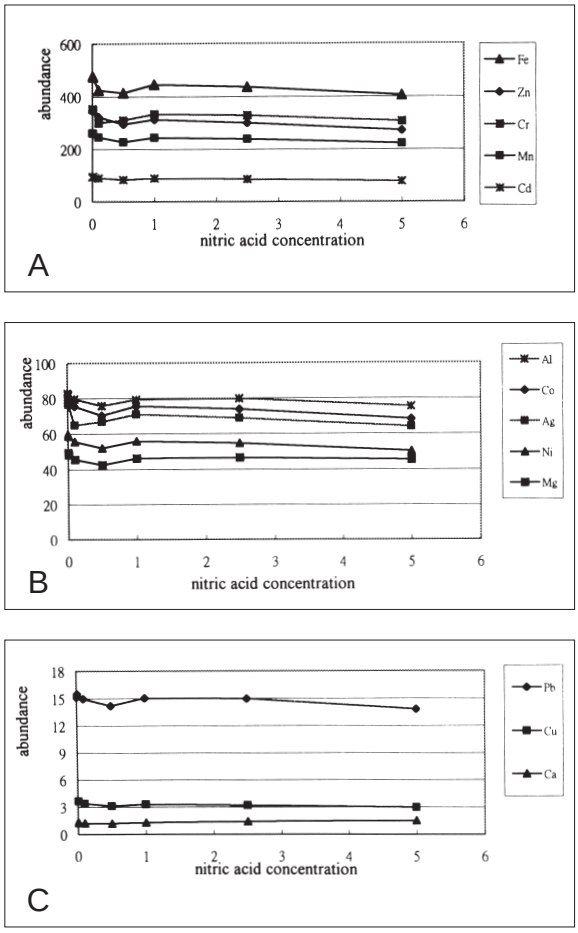

Fig. 3 Relationship between nitric acid concentration and signal intensity of 14 elements.

DISCUSSION

Since bone is comprised largely of Ca and Mg, these elements, as well as Zn, were present in the highest concentrations. Bone tissue is primarily composed of the calcium compound hydroxyapatile [$Ca_{10}(PO_4)_6(OH)_2$] which deposits and matures in the interstices of the collagen molecules. The subjects in this study had not been exposed to occupational elements and so certain elements such as Pb, Mn, Cd and As are present in very low concentrations. Pb concentrations were low (7.1 μg/g), ranging from 3.73 μg/g to 19.6 μg/g and increased with age. These levels are lower than those found in Stack's [20] study of workers in a lead battery manufacturing plant (mean = 18.2 μg/g). One of the physiological properties of bone is that it can store certain elements for a long period of time. Therefore, compared with other biospecimens like blood, saliva and urine, concentrations of elements such as Pb, Co and Cr can be much higher in bone. However, there is often a great deal of variation in concentrations between samples. This may be due to differences in the subjects' dietary intake and in individual variations, such as age, gender, health status and residential location. Large quantities of toxic elements such as lead, cadmium and arsenic have been released from industrial plants in past decades and exposure to these elements may pose a health risk to populations living in or near industrially polluted regions in Taiwan. In order to better understand the role of elements in human physiology, it is vitally important to conduct further research of

Table 2. Concentrations, wave-lengths and detection limits of 14 elements in bone samples

Element	Mean ± SD (μg/g)	Median (μg/g)	Wave-length (nm)	Detection limit (μg/L)	
				Instrument	Method
As	3.6 ± 3.5	2.9	193.699	6	9
Zn	115.0 ± 43.7	99.9	213.856	2	3
Cd	1.2 ± 0.7	1.0	220.353	5	6
Pb	7.1 ± 3.4	5.9	214.438	55	57
Ni	7.0 ± 10.5	3.7	238.892	10	13
Co	2.2 ± 3.2	1.11	231.604	1	2
Mn	0.7 ± 1.2	0.38	257.610	1	2
Fe	20.3 ± 24.0	8.9	259.940	3	6
Cr	11.9 ± 37.6	5.1	267.716	4	6
Mg	3005.2 ± 1687.7	2673.0	279.553	1	1
Al	52.1 ± 116.2	18.6	309.271	35	50
Cu	3.6 ± 0.35	2.18	324.754	3	4
Ag	2.8 ± 3.5	1.7	329.068	5	7
Ca	82007.9 ± 96620.5	62492.6	393.366	15	17

Table 3. Concentrations of 14 elements in bone based on sex

Element	Male	Female	*p* value*
As	3.86 ± 4.11*	3.13 ± 0.80	0.23
Zn	109.27 ± 40.4	126.55 ± 47.88	0.12
Cd	1.16 ± 0.47	1.26 ± 0.98	0.62
Pb	6.77 ± 3.16	6.76 ± 9.82	0.32
Ni	7.63 ± 3.78	7.56 ± 11.72	0.76
Co	2.33 ± 3.91	1.88 ± 1.28	0.45
Mn	0.49 ± 0.72	0.97 ± 1.80	0.21
Fe	21.31 ± 24.35	18.55 ± 23.69	0.63
Cr	6.48 ± 6.50	22.01 ± 62.29	0.21
Mg	2843 ± 1634	3304 ± 1773	0.27
Al	57.08 ± 140.33	42.79 ± 47.21	0.51
Cu	2.64 ± 1.67	5.52 ± 7.38	0.07
Ag	2.93 ± 4.19	2.44 ± 1.32	0.45
Ca	84649 ± 112375	77115 ± 58772	0.70

*Data are expressed as mean ± SD.

elements in bone.

The sample size (N=77) of this study may not be large enough for any firm conclusions to be made. Kuo et al [11] reported that there were no significant differences in bone-element concentrations for four bone disease etiologies. In our study, it was assumed that these bone-element concentrations represented those found in the Taiwanese population. However, the authors recommend that further studies include the following: a large sample size and a wider variety of bone types.

Table 3 compares the concentrations of the 14 elements in males and females. Except for Cu, there were no significant differences between the two genders. Cu concentrations among females were twice as high as in males (p = 0.07). Mg concentrations were also higher among females than males, but this was non-significant. Ca concentrations were higher among men (84,649 μg/g) than women (77,115 μg/g), but this was not significant due to a high degree of variation. The factors affecting variations in bone-element concentrations are many and difficult to understand. It is clear that there are two main factors affecting concentrations of bone-elements: genetic (individual metabolism of elements) and environmental (dietary intake of elements). However, the precise etiologies of elements in bone have yet to be clarified.

In conclusion, the optimal sample uptake rate and the optimal flow rate of Ar into the nebulizer were found to be 1.33 ml/min and

0.35 l/mm, respectively. Flow rates exceeding these values could damage the machine. The optimum nitric acid concentration was 1M. Mn and Cd were present in the lowest concentrations, and Ca and Mg were highest. There was high variation among the bone samples. Except for Cu, there were no significant differences between males and females for the 14 elements. Further studies need to be conducted to confirm the optimal conditions for bone-element analysis using ICP-AES and to investigate the factors that influence the concentrations of elements in bone.

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以感應耦合電漿儀測定台灣人體骨中十四種元素之濃度

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目的　有關人體元素濃度之相關研究較多如血液及尿液，但人體骨中元素濃度之研究則較少，且骨中元素濃度與生理作用有關。因此，本研究之目的在評估以感應耦合電漿儀(ICP-AES)分析國人骨中之14種元素之適當分析條件。

方法　77件骨頭樣本來自中部某醫學中心經微波消化之前處理，再以感應耦合電漿原子發射儀分析，測試三個適當儀器分析條件包括樣本吸收率，氬氣之流速及硝酸之濃度。

結果　顯示元素偵測極限範圍從1 μg/L(鈷、錳及鎳)至55 μg/L(鉛)，而樣本吸收率及氬氣最適流速分別爲1.33 mL/min及0.35 l/min，硝酸最適之濃度則爲1M。在人體骨中元素的量以錳(0.7 μg/g)及鎘(1.2 μg/g)濃度最低，鈣(82007.9 μg/g)與鎂(3005.2 μg/g)濃度最高，不過本研究骨中所含元素濃度變異性較大，除銅原元素外，人體骨中所含元素濃度在男女性並無統計性之差異。

結論　應繼續探討影響人體骨中元素濃度之相關因素，並進一步確定以感應耦合電漿儀分析骨中元素之最適條件。（中台灣醫誌 **2001;6:125-32**）

關鍵詞

骨頭，感應耦合電漿(ICP)分析，多種元素

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收文日期：4/23/2001　　修改日期：6/18/2001

接受日期：7/13/2001