Calcif Tissue Int (1994) 54:34–37

Calcified
Tissue
International

Magnesium Distribution in Human Bone

S. Tsuboi[1], H. Nakagaki[1], K. Ishiguro[1], K. Kondo[1], M. Mukai[2], C. Robinson[3], J. A. Weatherell[3]

Departments of [1]Preventive Dentistry and Dental Public Health and [2]Operative Dentistry, School of Dentistry, Aichi-Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464, Japan
[3]Division of Oral Biology, Leeds Dental Institute, University of Leeds, Claredon way, Leeds LS2 9LU, UK

Received: 13 May 1993 / Accepted: 10 August 1993

Abstract. The present study was undertaken to reveal the magnesium distribution in human bone. Sixty human ribs, obtained from subjects aged 10–80 years of age, were used. Transverse sections were prepared from the middle region of the human ribs. Adjacent sections were ground to a thickness of about 1000 μm. One section was used for magnesium determination by atomic absorption spectrophotometry, and the other was used for analysis with X-ray microanalysis. Thirty micron thick samples were abraded continuously from the periosteal and the endosteal surfaces by abrasive microsampling, as previously described by Weatherell et al. [3]. Results showed that magnesium concentrations were higher in both the periosteal and endosteal surfaces and did not change with age in general, although it tended to be higher among teenagers and lower over 80 years old.

Key words: Magnesium — Bone — Aging — Human

Magnesium (Mg) is one of the more important minor cations in the skeletal tissues. It can be incorporated into the mineral phase and can markedly affect its behavior, particularly its solubility [1]. Mg concentration often mirrors that of fluoride, especially when elevated fluoride concentrations are evident [2–4].

Though there is considerable information concerning bulk Mg concentrations in bone, there is little data concerning its topographical distribution, especially in relation to age and sex. With this in mind, a study of Mg distribution across the cortex of human ribs has been carried out.

Materials and Methods

Bone Materials

Whole ribs were from hospital patients in Nagoya, Japan, and stored in a fluoride-free, 10% neutral formalin solution (pH 7.4). The patients consisted of 30 male and 30 female subjects with age ranging from 17 to 93 years. The tissue obtained was removed from patients undergoing surgery for removal of neoplastic tissue. They had been life-long residents in a region where the fluoride concentration in the drinking water was about 0.1 ppm.

Human ribs were defatted in ethanol and diethylether. Soft tissues were then carefully removed using a scalpel under a microscope. The ribs were cut transversely across the middle region using a Maruto Slicer (Maruto, Japan). Two adjacent transverse sections were prepared and ground to a thickness of about 1000 μm using a whetstone (#1200 King Deluxe Stone, Japan). One of the ground sections was used for the determination of Mg distribution and the other was used for the X-ray microanalysis (XMA).

Sampling

The transverse cut sections of bone intended for Mg determination were attached to a glass slide using double-sided adhesive tape. With a scalpel, a line was marked on each specimen midway between the periosteal and endosteal surface. Each was cut into paired blocks about 1000 μm wide extending across the cortical bone from the periosteal to endosteal surface using a thin diamond disk (86XXSI Horiko, Germany). These specimens were then mounted vertically on brass rods with an adhesive (Quick 5, Konishi, Japan). One of these paired specimens was attached at the periosteal surface and the other at the endosteal surface. The brass rods were then mounted on a Mikrokator (C. E. Johanson, Sweden). Successive layers 30-μm thick were then abraded from the specimens using 15 μm grade silicon-carbide Imperial Lapping Film (3M, USA) [3]. The abrasion progressed from the surface and proceeded as far as the 'half-way' line marked on each specimen as previously described by Narita et al. [5].

Determination of Mg and Calcium

Four microliters of 1 M $HClO_4$ were placed on the silicon-carbide film to extract the mineral from each powdered sample of cortical bone. Using suction, the acid was transferred from the film into a solution containing 3000 ppm $La(La(NO_3)_3 \cdot 6H_2O)$. The extraction procedure was repeated two more times to produce a total solution of 2.5 ml. Two 1-ml samples were removed for Mg determination and the remaining solution was used for calcium (Ca) determination. From the analysis, the concentration of Mg was calculated using the Mg/Ca ratio obtained. This was based on the assumption that the Ca concentration of the bone was about 22.5% in the tissue (see table, Zipkin [6]) and 39.8% in the mineral hydroxyapatite [7]. The results are expressed as percent per weight of tissue and mineral. The distribution of Ca across the bone was also determined by X-ray microanalysis (JCMA-733, JEOL, Japan). This method was utilized to ensure that the changing values of Mg concentration, as represented by the Mg/Ca ratios, did not depend to any significant extent upon change in Ca concentration across the bone. Student's *t*-test and analysis of variance (ANOVA) were used to measure significant differences in samples [8].

Three regions—periosteal, middle, and endosteal—were distinguished, with periosteal and endosteal regions defined as the outermost, middle, and innermost 90 μm-thick layers of the bone cortex.

Correspondence to: S. Tsuboi

Table 1. Average Mg concentrations in periosteal, middle, and endosteal regions of the cortex. Both periosteal and endosteal regions were higher than the middle region

Age (yrs)	Male				Female			
	(n)	Periosteal	Middle	Endosteal	(n)	Periosteal	Middle	Endosteal
10–19	(2)	0.285 ± 0.005	0.245 ± 0.005	0.247 ± 0.020	(0)			
20–29	(3)	0.218 ± 0.036	0.198 ± 0.020	0.258 ± 0.031	(1)	0.283	0.217	0.247
30–39	(4)	0.219 ± 0.018	0.189 ± 0.011	0.218 ± 0.012	(1)	0.200	0.170	0.190
40–49	(2)	0.262 ± 0.051	0.215 ± 0.012	0.227 ± 0.034	(5)	0.223 ± 0.016	0.189 ± 0.005	0.213 ± 0.005
50–59	(6)	0.247 ± 0.018	0.211 ± 0.013	0.243 ± 0.019	(6)	0.230 ± 0.013	0.198 ± 0.011	0.234 ± 0.009
60–69	(4)	0.242 ± 0.027	0.206 ± 0.022	0.231 ± 0.027	(8)	0.217 ± 0.010	0.209 ± 0.012	0.230 ± 0.014
70–79	(4)	0.240 ± 0.014	0.199 ± 0.009	0.222 ± 0.023	(5)	0.258 ± 0.016	0.216 ± 0.012	0.256 ± 0.022
80–89	(3)	0.235 ± 0.023	0.173 ± 0.026	0.214 ± 0.027	(3)	0.222 ± 0.016	0.177 ± 0.003	0.203 ± 0.013
90–	(1)	0.200	0.187	0.190	(0)			

Mean ± SE (%)

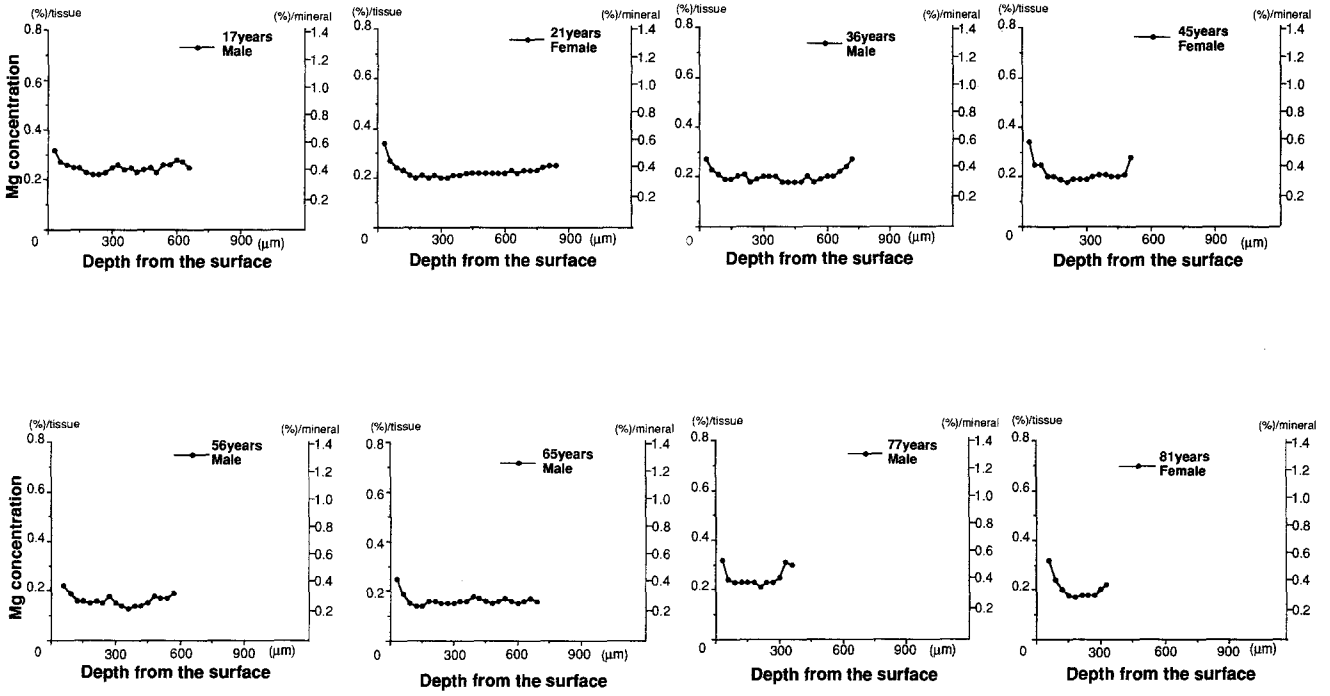

Fig. 1. Profiles of Mg concentrations with age in the cortical bone of human ribs, becoming gradually thinner.

Results

Table 1 shows average Mg concentrations in periosteal, middle, and endosteal regions of the cortex. Typical Mg profiles of the cortical bones in 10 ~80-year-old subjects are shown in Figure 1. Mg concentrations in the three regions of the cortex related to age are shown in Figure 2. Mg concentrations were significantly higher in the periosteal and endosteal regions and lowest in the middle of the cortex. No significant differences in age and sex were observed, although it tended to be higher among teenagers and lower over 80 years old. Table 2 shows ANOVA for the data. The coefficients (rate) of contribution were 56.3% in the region factor, 18.8% in the age factor, and 12.5% in the interaction factor of age and sex. Mg profiles of the periosteal, middle, and endosteal regions observed by XMA are shown in Figure 3. Mg distribution was similar to the distribution determined by chemical analysis. The Mg concentration showed a tendency to increase near or in the region of the haversian canal.

Discussion

Bone has a complex hierarchical structure, which, despite much investigation, is still not well understood [9]. The main inorganic phase of bone, as well as pathologically calcified collagenous tissues, is a basic Ca phosphate which is idealized as hydroxyapatite. Biological apatites are characterized by poor crystallinity and nonstoichiometry due to the presence of significant amounts of foreign ions [9]. Mg is one of the important cations in the bone. Fluoride increases the metabolic requirement for Mg [4].

Concentrations tended to be higher in the periosteal region, falling in the midcortex, and sometimes showing a rise towards the endosteal surface. This might reflect the fact

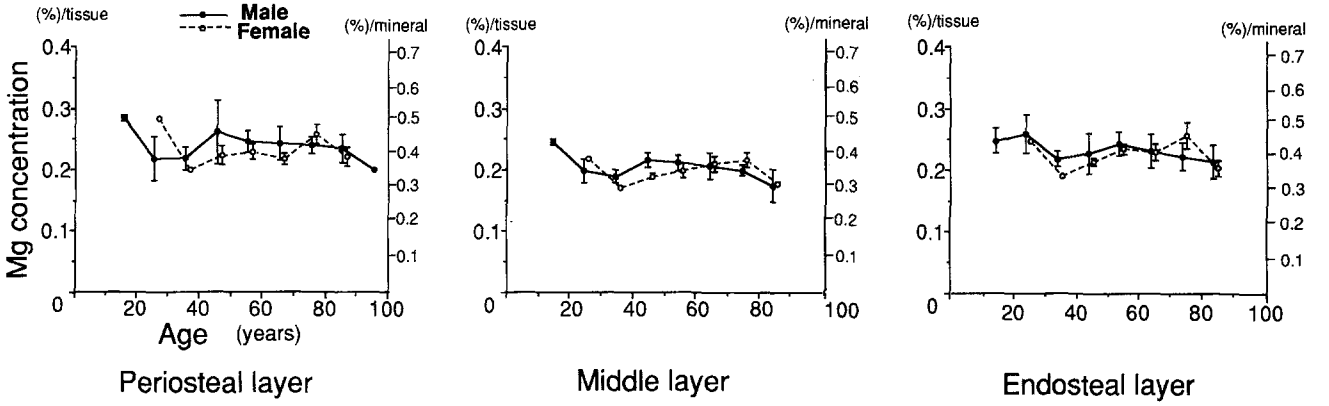

Fig. 2. Average Mg concentrations with age and sex in the cortical bone of human ribs. No obvious differences were observed in age and sex.

Table 2. ANOVA for the data of the three regions of cortex (A, age; B, sex; C, region). The coefficient (rate) of region (C) was the biggest factor

Source	Degree of freedom	Sum of square	Mean square	F-value	*P*-value	Contribution (%)
(A)	5	0.003	0.001	7.964	0.003	18.75
(B)	1	0.000	0.000	5.183	0.046	0.00
(C)	2	0.009	0.005	70.482	0.000	56.25
(AB)	5	0.002	0.000	5.832	0.009	12.50
(AC)	10	0.001	0.000	1.204	0.388	6.25
(BC)	2	0.000	0.000	0.826	0.466	0.00
Error	10	0.001	0.000			6.25
Total	35	0.016	0.006			100.00

A, age; B, sex; C, region

(−) $p > 0.05$
* $p < 0.05$
** $p < 0.01$

* $p < 0.05$
*** $p < 0.001$

	−39	40–49	50–59	60–69	70–79	80–
−39						
40–49	(−)					
50–59	(−)	(−)				
60–69	(−)	(−)	(−)			
70–79	(−)	(−)	(−)	(−)		
80–	(−)	(−)	*	(−)	**	

	Periosteal	Middle	Endosteal
Periosteal		***	*
Middle	***		
Endosteal	*		

that bone surfaces tend to be more prone to remodeling, compared with interior tissue. Newly formed bone may therefore contain smaller, less mature apatite crystals. These would present a greater surface to mass ratio and could more easily accommodate Mg in their surfaces. In the present study, this pattern of fluoride distribution was maintained despite aging. This suggests that even with cortical thinning, Mg concentrations were maintained in both inner and outer surfaces. One could conclude from this that access to the circulation and tissue fluid is important. This also supports the view that a substantial part of the Mg is in rapid equilibration with the bone/tissue fluid. This is supported by overall Mg deficiency in menopausal osteoporosis which is reflected in lower bone Mg [10]. The differences in remodeling patterns would need to be known in order to draw strict parallels. The Mg concentrations at the periosteal, middle, and endosteal regions showed no significant correlation with age and sex, except a hint of a fall in the youngest age groups, at the periosteal surface. This data may relate to the result of Burny and Wollast [11] which showed a higher Mg concentration in younger cortical bones. Recently, Ishiguro et al. [12] reported that fluoride concentrations of the cortical human bone increased with age, differing from the present Mg result which did not increase with age. When a hydroxyl ion of hydroxyapatite changes to the fluoride ion, it increases chemical stability and is the main reason for fluoride accumulation. Mg, on the other hand, does not replace Ca^{2+} in a simple manner. Precise locations of additional Mg^{2+} are not clear. Mg tends to decrease apatite stability which might explain why Mg^{2+} did not increase directly with age. The ease of access of bone mineral to extracellular fluid and the circulation as a means of facilitating Mg^{2+} uptake was supported by the Mg^{2+} distribution around haversian canals. Compared with adjacent bone samples, Mg concentrations tended to increase around the haversian canal. It is unfortunate that the bone was not labeled with fluorochrome (i.e., tetracycline) prior to biopsy to localize sites of active bone formation (versus resorption). More detailed studies concerning bone metabolism are clearly required.

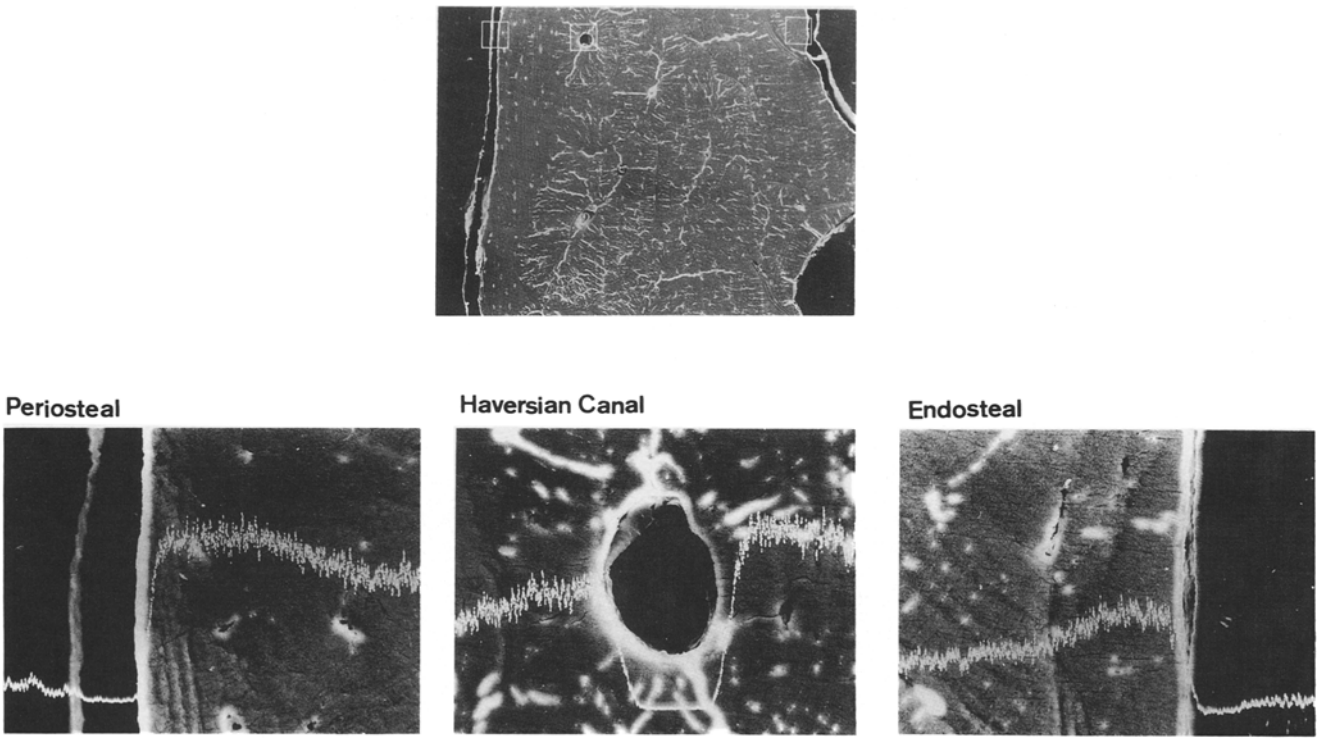

Fig. 3. Mg distribution in the cortical bone of human ribs observed by XMA, showing high concentrations in the regions of periosteal, endosteal, and haversian canal.

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