

Zn AND Cu CONTENT IN HUMAN CANCELLOUS BONE

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Duplicate cancellous bone samples from the iliac crest were obtained from autopsies of 88 persons with a normal mineral status and 50 having osteoporotic changes due to chronic immobilizing diseases. The following parameters were determined: the mineral density with gamma-ray attenuation, the compressive strength with a strain transducer, the concentrations of Ca, Mg, Cd, Co, Cu, Mn, Ni, Li, Pb, Sr and Zn with an atomic absorption spectrophotometer and the concentration of F with an ion-selective electrode. The data were analyzed with linear multiple regression analysis.

Altogether 34.8 per cent of the Zn concentration was accounted for in all the variables. The selected variables, after the nonsignificant and those with very low explanatory power were removed, explained 38.3 per cent. The two most important variables were the F concentration (16.1 per cent) and Cu concentration (13.7 per cent). The third variable was Mn concentration (8.5 per cent). All variables explained 41.7 per cent of the Cu concentration and five selected variables 40.7 per cent, respectively. The concentration of Zn explained itself as much (20.1 per cent) as four other variables (Ca 7.7 per cent, Mn 5.9 per cent, Sr 3.9 per cent and Co 3.2 per cent) together. Age and sex did not significantly affect the concentration of Zn and Cu. No remarkable difference was found between the bone samples representing a normal mineral status and osteoporosis.

Key words: bone; Zn; Cu

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Deficiency of Zn has in many animal experiments been found to lead to disturbances in bone mineralization (Brown et al. 1978, Calhoun et al. 1974, Hurley et al. 1969, Vincent 1963). There have been corresponding findings with regard to Cu (Baxter 1951, Teague & Carpenter 1951, Underwood 1977). Zn is evidently of importance for organic structure (Belanger et al. 1977) as well as crystallization (Lappalainen & Knuuttila 1981). Cu is of importance in the formation of collagen cross-linking (Rucker et al. 1969). In spite of the role of Zn and Cu in mineralization, the deficiency of these trace elements, in animal experiments, has not been found to affect the Ca content of bone per the weight of bone or ash content (Brown et al. 1978, Rucker et al. 1969).

Zn increases, however, concomitantly with Ca during bone formation (Calhoun et al. 1974).

Instead an inverse interrelationship between Zn and Cu contents has been observed as regards concentrations in bone both in rats (Schwarz & Kirchgessner 1979) and in pigs (Pond et al. 1978). The Cu depletion slightly increased the Zn content of bone. The antagonism between Zn and Cu has been widely reviewed by Bremner (1979). On the other hand, when the Zn and Cu status of the elderly was studied, no significant relationship was obtained in either plasma or hair (Vir & Love 1979).

The purpose of this study was to investigate in bone specimens of the iliac crest of humans: 1) The relation between Zn and Cu concentrations

and 2) how age, sex, bone strength, mineral density, Ca, Mg, Cd, Co, Fe, F, Li, Mn, Ni, Li, Pb, Sr contents affect Zn and Cu concentrations.

MATERIAL AND METHODS

Bone material

Duplicate cancellous bone samples from the anterior iliac crest were obtained from autopsies of 25 women and 63 men who had died suddenly due to acute coronary artery disease or accidents. The samples representing osteoporotic bone were taken from 26 women and 24 men, whose cause of death was chronic immobilizing diseases. Previous studies (Alhava et al. 1977, Karjalainen & Alhava 1977) have indicated that this division separates osteoporotics and those with a normal mineral status. The age distribution of the total material was as follows: 17–20 year-olds, 4, 20–30 year-olds, 22, 40–59 year-olds, 44 and 60–88 year-olds, 68.

One sample was fixed in 40 per cent ethyl alcohol and the other sample was treated with sodium hypochlorite, hydrogen peroxide and xylene to remove bone marrow and fat as described in detail by Alhava et al. (1977).

Assays

The bone mineral density according to a modification of the gamma-ray attenuation as described by Alhava et al. (1980), as well as the compressive strength with a strain transducer as presented by Nokso-Koivisto et al. (1976), were determined from the samples fixed in alcohol. From the macerated bone samples ashed in a solution of conc. HCl and HNO₃ (2:1) the concentrations of Ca, Mg, Cd, Co, Cu, Mn, Ni, Li, Pb, Sr and Zn were determined with an air-acetylene burner and an atomic absorption spectrophotometer (Perkin Elmer Model 372, Perkin Elmer Corporation, Norwalk, Connecticut, USA). The detailed operating parameters were as described in the Perkin Elmer operation manual (1976) and in a previous study of Knuutila et al. (1980). The reproducibility of repeated assays of different metals was within 5 per cent. Fluoride was de-

termined with an ion selective electrode (Orion 94–09) as earlier presented by Alhava et al. (1980).

Statistics

Variables related to Zn and Cu concentrations were studied with multiple linear regression analysis. First all variables were introduced into the model and then the model was gradually reduced to contain variables that most increased the value of the multiple correlation squared (MCS).

RESULTS

When all variables were introduced into the multiple regression, 34.8 per cent of the Zn concentration was explained. The model was gradually reduced and 12 subsequent models were constructed. The following variables, which had a very low explanatory power or a nonsignificant F-value, were removed: Pb, mineral density, Co, Mg, bone strength, Cd, Li, Ca, Ni, Sr, age and sex. Osteoporosis was a dummy variable in the model and it was also nonsignificant thus indicating no remarkable difference between the persons with normal mineral status and those with reduced Ca level. Three variables in the last model explained altogether 38.3 per cent of the Zn concentration (Table 1). The two most important variables were the F concentration (16.1 per cent) and the Cu concentration (13.7 per cent). The overall significance of the model tested with the F-test showed a statistically significant F-value ($P < 0.001$).

The multiple regression model was also statistically significant in the case of Cu. Removing Mg, bone strength, F, mineral density, Li, Ni, Pb, Cd, age, sex, a dummy variable (= osteoporosis) from the Cu-model changed the explanation percen-

Table 1. Selected factors related to the Zn concentration of bone based upon the multiple linear regression analysis

Variable	Regression coefficient	Standard error of coefficient	Significance of coefficient	Multiple correlation squared
F	0.023	0.007	$P < 0.01$	0.161
Cu	8.162	2.797	$P < 0.01$	0.137
Mn	10.91	2.548	$P < 0.001$	0.085
Constant term	-0.00344			

Table 2. Selected factors related to the Cu concentration of bone based upon the multiple linear regression analysis

Variable	Regression coefficient	Standard error of coefficient	Significance of coefficient	Multiple correlation squared
Zn	0.0054	0.002	$P < 0.05$	0.210
Ca	0.00002	0.0000	$P < 0.001$	0.077
Mn	0.205	0.076	$P < 0.01$	0.059
Sr	0.034	0.012	$P < 0.01$	0.038
Co	0.088	0.038	$P < 0.05$	0.032
Constant term -0.00288				

tage from 41.7 to 40.7. The most important single variable was the Zn concentration, which explained 20.1 per cent (Table 2). The regression coefficient indicates measurable changes of the Cu concentration when the Zn concentration is, for example, changed about 50 µg/g. The explanatory power of the other variables is as follows: Ca (7.7 per cent), Mn (5.9 per cent), Sr (3.8 per cent) and Co (3.2 per cent). The mean concentration \pm S.D. of Cu in the whole material was 1.3 ± 0.5 µg/g and of Zn 113.9 ± 40.7 µg/g, respectively.

DISCUSSION

The average Zn content in the iliac crest samples was similar to that measured in long bones

(Strehlow & Kneip 1969). When the dry weight concentrations are compared with ash weights the results have been multiplied by 1.4 (mean Ca concentration 2.95 mg/g). Methodological differences could explain the variance in Zn concentrations obtained from bone in different studies (Alhava et al. 1977, Spadaro & Becker 1970). The majority of Zn in bone has been found to exist in the mineral phase (Brätter et al. 1977, Spadaro & Becker 1970). Of the measured variables Ca did not, however, prove to be a significant factor explaining Zn concentration. This might indicate that Zn as a structural part of the apatite crystal is not related to its growth but to the nucleation. Thus its effect could cause a gain in the critical size of the lattice in the initial phase of mineralization. Some evidence for the possible nucleator role of Zn has been previously pre-

Table 3. Correlation matrix of selected factors

	Zn	Cu	F	Ca	Mn	Sr	Co
Zn	-	0.479 $P=0.000$	0.381 $P=0.000$	-0.018 $P=0.418$	0.527 $P=0.000$	0.286 $P=0.000$	0.253 $P=0.001$
Cu	0.479 $P=0.000$	-	0.218 $P=0.005$	0.273 $P=0.001$	0.478 $P=0.000$	0.337 $P=0.000$	0.328 $P=0.000$
F	0.381 $P=0.000$	0.218 $P=0.005$	-	-0.151 $P=0.039$	0.341 $P=0.000$	0.219 $P=0.004$	0.102 $P=0.112$
Ca	-0.018 $P=0.418$	0.273 $P=0.001$	-0.151 $P=0.039$	-	0.060 $P=0.242$	0.131 $P=0.063$	-0.097 $P=0.125$
Mn	0.527 $P=0.000$	0.478 $P=0.000$	0.341 $P=0.000$	0.060 $P=0.242$	-	0.183 $P=0.014$	0.285 $P=0.000$
Sr	0.286 $P=0.000$	0.337 $P=0.000$	0.219 $P=0.004$	0.131 $P=0.063$	0.183 $P=0.014$	-	0.047 $P=0.288$
Co	0.253 $P=0.001$	0.328 $P=0.000$	0.102 $P=0.112$	-0.097 $P=0.125$	0.285 $P=0.000$	0.047 $P=0.288$	-

sented by Knuutila et al. (1980), Lappalainen & Knuutila (1981).

The most important variable explaining the Zn concentration was F, the location and role of which is to a great extent in the mineral phase. One explanation for the interrelation between Zn and F could be in fact the change from Zn-OH coordination to Zn-F coordination in the central core of the lattice. It is well known that the Zn-F affinity is greater than the Zn-OH affinity. Although it is evident that there is a relationship between Zn and F, the regression coefficient is not very high, allowing also other interrelations concerning F. In the reduced model of Zn as well as of Cu, Mn was a significant explanatory factor. The role of Mn has been clearly established as essential for normal skeletal development (Caskey et al. 1939, Hurley et al. 1961). Furthermore Mn is the important ion in the metabolism of mucopolysaccharides (Leach 1971) affecting the formation of the organic matrix of bone.

The significance of Cu for the organic matrix of bone has been emphasized by Pinnell & Martin (1968) and Rucker et al. (1969). In the present results the factor which most increased the MCS-value after Zn was Ca, which indicates that a great part of the Cu is bound to the mineral phase. It was also found that the Cu concentration in dental enamel was greater than that in dentine (Lappalainen & Knuutila 1981), although the amount of organic matrix in enamel is about 5 per cent and that in dentine about 35 per cent. In chicken bone about 62 per cent of Cu is situated in the mineral phase (Rucker et al. 1969). On the other hand quite a small amount of Cu is not likely to make it a significant cation in the crystals. The Cu concentration in this study was lower than that presented earlier by Adelstein & Vallee (1964).

The antagonistic interrelation between Zn and Cu has been established especially in non-mineralized tissues in several studies, as reviewed by Bremner (1979). Furthermore this type of interrelation has also been found in the serum of rats (Murthy et al. 1974). A controversial result, compared to animal studies (Pond et al. 1978, Schwarz & Kirchgessner 1979), was observed in human bone (regression coefficient between Zn and Cu was positive) when the concentrations of

Zn and Cu varied within normal limits. This situation in bone could be due to the location of both cations primarily in the mineralized phase. The diadocia between Zn and Cu is not common in the minerals existing in nature. This means that the competition of Zn and Cu from the same positions is not evident. On the other hand more information is needed about the relation between Zn and Cu in bone; for example, by comparing the organic and mineral phases separately.

In conclusion, it can be established that the results provided evidence for a positive correlation between Zn and Cu in bone when several other factors were also taken into consideration. In addition the connection between Zn and F proved to be significant.

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