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SERUM CALCITONIN AND BONE MINERAL CONTENT IN PATIENTS WITH OSTEOGENESIS IMPERFECTA

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Serum calcitonin and bone mineral content in the forearm, measured by photon absorptiometry, were investigated in 21 patients with osteogenesis imperfecta tarda. The bone mineral content was significantly reduced as compared with normal controls, whereas the bone mineral content corrected for bone width was normal in adult patients but subnormal in children and young adults. Serum calcitonin did not differ significantly from that in normal individuals and no relation was found between serum calcitonin and bone mineral content.

Key words: bone mineral content; osteogenesis imperfecta tarda; photon absorptiometry; serum calcitonin

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Osteogenesis imperfecta is an infrequent generalized disease in bone and connective tissue characterized by increased bone resorption, osteopenia and spontaneous fractures (Rosenberg et al. 1977). Both short-term and long-term studies of calcitonin treatment have suggested an improvement in bone changes in patients with osteogenesis imperfecta tarda (Rosenberg et al. 1977, Castells et al. 1972).

Measurements of serum calcitonin (s-CT) in patients with osteogenesis imperfecta have not been reported before and bone mineral content, measured by photon absorptiometry, has only been investigated in a few patients (Rosenberg et al. 1977). The present study was carried out to describe the bone mineral content and biochemical values of calcium-phosphorus metabolism, including s-CT, in patients with osteogenesis imperfecta and to evaluate whether a lack of s-CT could explain the decrease in bone mass in these patients.

PATIENTS AND METHODS

Patients. The study comprised 21 patients with osteogenesis imperfecta tarda (13 females and 8 males) 14 – 78 years of age (mean 33.1 years) (Table 1). All patients had previously sustained spontaneous fractures; five had had 10 – 15 fractures, the others more than 15. All patients appeared with blue sclerae. No patients had symptoms of gastrointestinal disease and all had normal kidney function.

Methods. Serum concentrations of calcium, phosphorus, alkaline phosphatase and calcitonin were measured after an overnight fast. Serum calcium was corrected for individual variation in serum protein concentration (Pedersen 1973) and calculated as the calcium concentration corresponding to a protein level of 70 g/l (s-calcium corr.).

Calcitonin. Calcitonin was determined by a radioimmunoassay using a commercial antibody to human calcitonin (Calbiochem, USA) and synthetic human M-calcitonin (Ciba, Switzerland)

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Table 1. Clinical and biochemical data in patients with osteogenesis imperfecta

Pat. no.	Age/sex (years)	s-calcitonin pg/ml	s-calcium mg/100 ml	s-phosphorus mg/100 ml	alk. phosphatase units
1	15/m	20	9.5	4.8	641
2	21/m	45	10.3	3.5	175
3	24/m	35	—	—	—
4	28/m	35	9.9	2.8	188
5	28/m	25	9.8	3.2	170
6	29/m	10	—	—	—
7	46/m	40	9.5	2.6	120
8	77/m	35	9.9	2.7	148
9	14/f	10	9.8	4.2	500
10	15/f	30	9.7	3.6	316
11	16/f	10	9.8	3.4	215
12	17/f	40	9.5	4.1	177
13	20/f	70	9.2	4.3	204
14	32/f	220	9.3	2.8	131
15	36/f	10	9.5	3.3	134
16	37/f	35	9.4	3.6	149
17	39/f	20	9.4	3.2	229
18	43/f	10	9.8	3.7	134
19	43/f	30	9.6	3.2	177
20	48/f	10	9.9	3.1	160
21	64/f	70	9.6	2.5	225
Mean	33.0	39	9.65	3.4	221
Normal range		10–120	9.2–10.5	2.7–4.7	A: 80–220 C:200–800

A = adults
C = children

for standards and iodination. ^{125}I-calcitonin was prepared by the chloramin-T method (Tashjian 1969). The labelled antigen was purified by absorption to and elution from Quso G32 (Philadelphia Quartz Co., Philadelphia, USA) (Tashjian 1969). More than 90 per cent of the labelled, purified antigen could be bound by the antibody. Assay conditions were modified from Dietrich et al. (1975). 200 µl serum and 250 µl antibody dilution in 0.1 M tris –0.25 per cent HSA (pH = 7.3) was preincubated for 2 days at 4°C. 50 µl ^{125}I-calcitonin dilution giving about 5000 cpm was added, followed by 2 days incubation at 4°C. Antibody-bound calcitonin was precipitated by polyethylene glycol (PEG 6000). Standards were prepared in serum from totally thyroidectomized patients. Each serum was made in duplicate together with a tube without antibody to correct for incubation damage and non-specific binding of the tracer. The lowest detectable concentration was 20 pg/ml. The precision of double determinations was 10 pg/ml. Reproducibility was determined using a control serum with a calcitonin level of 140 pg/ml. In nine assays the coefficient of variation was 14 per cent. The normal material comprised 40 healthy subjects with a mean age of 34 years (range: 20 – 56).

Bone mineral content (BMC) in the forearm was measured using a GAMMATEC osteodensitometer model GT 30 with a 50 mCi ^{125}I source (Christiansen et al. 1975). The scan site was selected automatically by the instrument as the most distal position where the separation of radius and ulna exceeds 8 mm, and six scans with a separation of 4 mm between each were carried out and the mean values displayed. The combined width of radius and ulna (BW) was measured on the photon absorption curves and the mean of the two arms calculated.

The results were expressed as absolute values (g/cm) and in per cent of the mean values for normal controls of the same age and sex.

The reproducibility of the BMC measurement

Table 2. Bone mineral content in patients with osteogenesis imperfecta

Pat. no.	Age/sex (years)	BMC g/cm	BMC %	BW cm	BMC/BW g/cm²	BMC/BW %
1	15/m	21.5	50	2.6	8.3	85
2	21/m	45.4	80	2.5	18.5	102
3	24/m	37.5	66	2.4	15.6	87
4	28/m	43.7	77	2.6	17.1	94
5	28/m	45.4	80	2.7	17.1	94
6	29/m	43.0	75	2.6	16.4	91
7	46/m	48.3	82	2.5	19.2	108
8	77/m	44.2	102	2.9	15.4	117
9	14/f	20.9	65	2.3	9.1	87
10	15/f	18.0	51	2.6	7.0	54
11	16/f	22.8	65	2.2	10.4	70
12	17/f	26.3	67	3.0	8.8	72
13	20/f	33.7	81	2.0	17.3	111
14	32/f	38.2	92	2.5	15.3	105
15	36/f	38.4	92	2.4	15.9	109
16	37/f	30.9	74	2.6	11.9	82
17	39/f	31.9	78	2.6	12.5	86
18	43/f	29.7	71	2.3	12.7	88
19	43/f	36.2	86	2.9	12.7	88
20	48/f	33.1	79	3.0	11.2	77
21	64/f	29.4	107	2.4	12.2	133
Mean			77.1			92.4

BMC = bone mineral content
BW = bone width

expressed as the variation coefficient was about 1 per cent in normal persons (Christiansen & Rødbro 1977).

Statistical evaluation. For comparisons of group means the Mann-Whitney U-test was used and for correlation analysis Spearman's rank correlation test.

RESULTS

Table 2 shows the bone mineral content in patients with osteogenesis imperfecta expressed in arbitrary units (dimension g/cm) and as per cent of normal sex and age matched controls (BMC per cent). A reduced bone mineral content ($P < 0.01$) and a reduced bone width ($P < 0.01$) were found in the patients as compared with the controls. No significant relation between the total number of fractures and the BMC could be demonstrated. After correction of the BMC for bone width (BW) no difference was found in the bone mass between adult patients and normal controls, whereas the children and young adults showed subnormal values. Thus BMC per cent ($R = 0.72$, $P < 0.01$) and to a lesser degree BMC/BW per cent ($R = 0.46$, $P < 0.05$) increased with age.

The biochemical values of calcium-phosphorus metabolism including s-CT are given in Table 1. The mean s-CT concentration of 39 pg/ml (range 10 – 220) did not differ from the normal mean value of 38 pg/ml (range 10 – 120). Serum alkaline phosphatase was moderately increased in three patients and normal in the others. All patients revealed normal serum concentrations of calcium corr. and phosphorus.

DISCUSSION

The present study shows a markedly reduced bone mineral content, measured by photon

absorptiometry, in patients with osteogenesis imperfecta as compared with age and sex matched normal controls. Some interference with the BMC measurements caused by post-traumatic osteoporosis following forearm fractures could not be excluded, as most patients previously had had one or more fractures of the forearm. The decrease in bone mineral content could mainly be explained by a similar reduction in bone width as the bone mineral content corrected for bone width was normal.

These findings are supported by a previous radiological study of the width of the second right metacarpal bone in adult patients with osteogenesis imperfecta (Paterson 1978) showing a significant reduction in total bone width, whereas the combined cortical width was normal. Examination of bone biopsies from the iliac crest of children and young adults by bone histomorphometry (Riley et al. 1973) has shown a reduced cortical width, a reduced osteoid width and a decreased amount of osteons. The bone formation rate was reduced in children less than 10 years old and normal in children more than 10 years of age. The difference in cortical width between the two investigations (Paterson 1978, Riley et al. 1973) may be due to the difference in age between the patient groups. The more pronounced reduction in bone mineral content in children found in the present study is in agreement with these observations and indicates that a more severe degree of disease is present in children as compared with adults.

In both short-term and long-term studies of calcitonin treatment of patients with osteogenesis imperfecta favourable responses have been suggested (Rosenberg et al. 1977, Castells et al. 1972). The study of Rosenberg et al. (1977) showed a rate of increase in forearm bone mass which was greater than that observed in an untreated group of patients with osteogenesis imperfecta tarda. As calcitonin inhibits bone resorption both in animals (Wallach et al. 1967) and in man (Haddad & Caldwell 1972), a lack of calcitonin in patients with osteogenesis imperfecta might be a contributory pathogenetic factor. The present study shows, however, normal s-CT in all but one of the patients included in the study, and there was no relation between bone mineral content and s-CT.

The finding of normal serum calcitonin concentrations in patients with osteogenesis imperfecta is similar to the finding in patients with Paget's disease, in whom calcitonin treatment may induce normalizing bone changes (Kanis et al. 1975), whereas s-CT is normal (Kanis et al. 1977) or even increased (Franchimont & Heynen 1976).

A decreased sensitivity to calcitonin in bone causing a relative lack of calcitonin has been proposed to explain the findings in Paget's disease (Woodhouse et al. 1971) and an acquired tissue resistance to calcitonin secondary to a viral infection has been suggested (Rebel et al. 1975), as nuclear inclusion bodies have been demonstrated in the osteoclasts (Rebel et al. 1975).

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REFERENCES

Castells, S., Inamdar, S., Baker, R. K. & Wallach, S. (1972) Effect of porcine calcitonin in osteogenesis imperfecta tarda. *Pediatrics* **80,** 757–762.

Christiansen, C. & Rødbro, P. (1977) Long-term reproducibility of bone mineral content measurements. *Scand. J. clin. Lab. Invest.* **37,** 321–323.

Christiansen, C., Rødbro, P. & Jensen, H. (1975) Bone mineral content in the forearm measured by photon absorptiometry. *Scand. J. clin. Lab. Invest.* **35,** 323–330.

Dietrich, F. M., Hunziker, W. H. & Fischer, J. A. (1975) Synthetic human calcitonin: Analysis of antibodies obtained from various animal species and determination of immunoreactive

hormone in human sera. *Acta endocr. (Kbh.)* **80,** 465–486.

Franchimont, P. & Heynen, G. (1976) *Parathormone and calcitonin radioimmunoassay in various medical and osteo articular disorders.* Masson Inc., New York.

Haddad, J. G., Jr. & Caldwell, J. G. (1972) Calcitonin resistance, clinical and immunological studies in subjects with Paget's disease of bone. *J. clin. Invest.* **51,** 3133–3141.

Kanis, J. A., Fitzpatrick, K. & Strong, J. A. (1975) Treatment of Paget's disease of bone with porcine calcitonin: clinical and metabolic responses. *Quart. J. Med.* **44,** 399–413.

Kanis, J. A., Heynen, G. & Walton, R. J. (1977) Plasma calcitonin in Paget's disease of bone. *Clin. Sci.* **52,** 329–332.

Paterson, C. R. (1978) Metacarpal morphometry in adults with osteogenesis imperfecta. *Brit. med. J.* **1,** 213–214.

Pedersen, K. O. (1973) Calcium in human serum. Biochemical and clinical aspects. Thesis, University of Aarhus.

Rebel, A., Malkani, K., Baslé, M. & Bregon, C. (1975) *Calcified tissues,* p. 114, FADL's Forlag, Copenhagen.

Riley, F. C., Jowsey, J. & Brown, D. M. (1973) Osteogenesis imperfecta: Morphologic and biochemical studies of connective tissue. *Pediat. Res.* **9,** 757–768.

Rosenberg, E., Lang, R., Boisseau, V., Rojanasathit, S. & Avioli, L. V. (1977) Effect of long-term calcitonin therapy on the clinical course of osteogenesis imperfecta. *J. clin. Endocr.* **44,** 346–355.

Tashjian, A. H., Jr. (1969) Immunoassay of thyrocalcitonin. I. The method and its serological specificity. *Endocrinology* **84,** 140–148.

Wallach, S., Chausmer, A. & Mittleman, R. (1967) In vivo inhibition of bone resorption by thyrocalcitonin. *Endocrinology* **80,** 61–64.

Woodhouse, N. J. Y., Reiner, M., Bordier, P., Kalu, D. N., Fisher, M., Foster, G. V., Joplin, G. F. & MacIntyre, I. (1971) Human calcitonin in the treatment of Paget's bone disease. *Lancet* **i,** 1139–1143.

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