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Noncollagenous Proteins in Normal and Pathological Human Bone

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Summary. The concentrations of α_2HS-glycoprotein, albumin, and sialic acid were measured in the bone of 28 normal individuals and 6 patients with osteogenesis imperfecta, 3 patients with Paget's disease, and 4 patients with either renal osteodystrophy, osteoporosis, osteomalacia, or osteopetrosis. The concentration of α_2HS-glycoprotein in neonatal bone was 3 × higher than in bone from children and 7 × higher than in adult bone. The concentrations of albumin and sialic acid in neonatal bone were 1.5 × higher that in bone from children and twice the levels in adult bone. The concentrations of α_2HS-glycoprotein, albumin and sialic acid were above the normal mean values in the patients with osteogenesis imperfecta, and 4 patients had significantly raised levels of one or more of these proteins. The concentrations of these noncollagenous proteins were also significantly raised in Paget's disease, and the α_2HS-glycoprotein was significantly raised in renal osteodystrophy. The lowest level of α_2HS-glycoprotein was in osteopetrotic bone and the lowest levels of albumin and sialic acid were in osteoporotic bone. The results of this study suggest that the concentrations of α_2HS-glycoprotein, albumin, and sialic acid in bone are related to the rate of bone turnover.

Key words: α_2HS-glycoprotein — Albumin — Bone sialic acid — Osteogenesis imperfecta — Paget's disease.

The noncollagenous matrix of bone contains proteins that are derived from plasma as well as proteins that are synthesized locally [1, 2]. Plasma α_2HS-glycoprotein and albumin are concentrated in the bone matrix whereas other plasma proteins such as immunoglobulin G appear to be associated with the blood vessels of bone [2]. Several classes of noncollagenous proteins are synthesized in bone including sialoprotein [1], osteocalcin [3], and osteonectin [4]. Osteocalcin and osteonectin may have a role in mineralization but the functions of the sialoprotein, α_2HS-glycoprotein, and albumin in bone are unclear [1, 2].

We have determined the concentrations of α_2HS-glycoprotein, albumin, and sialic acid in normal and pathological human bone with a view to gaining an insight into the possible functions of these noncollagenous proteins. We found that α_2HS-glycoprotein, albumin, and sialic acid were present in high concentrations in neonatal bone and that the levels were lower in bone from children and adults. The α_2HS-glycoprotein was highly concentrated relative to albumin. Abnormally high levels of these proteins were also observed in some patients with osteogenesis imperfecta, Paget's disease, and other bone diseases.

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Materials and Methods

Normal tibial and femoral cortical bone was obtained at autopsy from 28 patients who had died from accidental causes or from diseases that were not likely to have affected bone. The samples were classified as neonatal, child (4–13 years) or adult (19–82 years). Tibial cortex was obtained at operation from 5 patients with the type III form of osteogenesis imperfecta and from a patient with the type IA form of this disease [5, 6]. Samples of bone were also obtained at operation or at autopsy from 2 adults and 1 adolescent with Paget's disease and from 4 adults with either renal osteodystrophy (features mainly of osteitis fibrosa), osteomalacia, osteoporosis, or osteopetrosis.

The samples of bone were cleaned, washed with water, and powdered in a freezer mill. The milled bone was decalcified with EDTA and both the supernatant and decalcified matrix were digested with bacterial collagenase [1]. Proteinase inhibitors were not used but we showed, using two labeled substrates, that the collagenase (type VI) obtained from Sigma Chemical Co. St Louis, Mo., U.S.A. was free of proteinase activity [8]. The digest was dialyzed and the retained soluble noncollagenous pro-

Table 1. Changes with age in the composition of normal human bone

Age group	α_2HS-glycoprotein mg/g wet bone	Albumin	Sialic acid μmol/g wet bone	Water %
Neonate	2.03 ± 0.20 (8)	0.81 ± 0.29 (8)	2.82 ± 0.16 (6)	10.31 ± 0.89 (6)
Child [4–13]	0.66 ± 0.08 (5)	0.55 ± 0.15 (5)	1.98 ± 0.20 (5)	8.25 ± 1.45 (5)
Adult [19–82]	0.28 ± 0.03 (15)	0.36 ± 0.05 (15)	1.39 ± 0.14 (14)	8.44 ± 0.32 (14)

Values shown are the mean ± SE, with the number of patients in parentheses

teins were lyophilized and analyzed for their contents of α_2HS-glycoprotein, albumin, sialic acid, and total protein. Alpha$_2$HS-glycoprotein and albumin were measured by radial immunodiffusion using Partigen plates (Behringwerke, Marburg-Lahn, West Germany) containing antiserum to human α_2HS-glycoprotein or to albumin [7]. Sialic acid and water content were measured by previously described methods [8]. The total protein content of the noncollagenous matrix was determined by the method of Lowry et al. [9]. All results were expressed as mg/g or μmol/g of wet undecalcified bone and they were compared using Student's *t* test.

Results

The normal concentrations of the noncollagenous proteins were determined by combining the results from the normal tibial and femoral specimens (Table 1). Significant differences were not observed between the results obtained from these sites except in the adult group. The concentration of sialic acid in the 10 adult tibial specimens was 1.21 ± 0.16 (SE) μmol/g of wet bone which was significantly less than the concentration of 1.82 ± 0.07 μmol/g of wet bone in 26 femoral samples which included the 4 samples in the present study and a further 22 samples in which only sialic acid was measured ($P < 0.001$). The sialic acid results from the 10 adult tibial and 4 adult femoral specimens used in the present study were combined in Table 1 but the sialic acid values in samples of abnormal adult tibia and femur were compared with their respective control values.

The concentrations of α_2HS-glycoprotein, albumin, and sialic acid were highest in neonatal bone (Table 1). The noncollagenous proteins of neonatal bone consisted of 9.1% (w/w) α_2HS-glycoprotein, 2.3% albumin, and 24.6% sialoprotein, using a conversion factor of 13.8% sialic acid content of human bone sialoprotein [10]. The concentration of α_2HS-glycoprotein in neonatal bone was 3 × higher than in bone from children and 7 × higher than in adult bone. The results in the three age groups were significantly different from each other ($P < 0.001$). The α_2HS-glycoprotein in neonatal bone was concentrated approximately 94 × relative to albumin. This concentration factor was calculated using the bone and plasma concentrations of α_2HS-glycoprotein and albumin according to the formula described by Ashton et al. [2]. A mean neonatal plasma albumin concentration of 34 g/l, which was established at the Royal Children's Hospital in Melbourne, was used with a neonatal plasma α_2HS-glycoprotein concentration of 90 mg/100 ml that was obtained from Ashton et al. [2].

The concentrations of albumin and sialic acid in neonatal bone were 1.5 × higher than in bone from children and twice the levels in adult bone. Significant differences in the concentrations of albumin and sialic acid were observed when the neonatal and adult results were compared ($P < 0.01$ and $P < 0.001$ respectively). Significant differences were also observed between the sialic acid concentrations in neonatal and children's bones ($P < 0.01$).

The concentrations of α_2HS-glycoprotein, albumin, and sialic acid exceeded the mean control values in all the patients with osteogenesis imperfecta except for a lower level of albumin in the 16-year-old patient (Table 2). A 23-year-old male had significant elevations of each of these proteins while 1 patient had an elevated α_2HS-glycoprotein and 2 patients had elevated levels of sialic acid. The 17-year-old male also had significantly raised levels of the three proteins when they were compared with adult control values and the 16-year-old male also had a significant elevation of the α_2HS-glycoprotein when it was compared with adult controls. Overall the concentrations of the three noncollagenous proteins in the patients with osteogenesis imperfecta did not show any tendency to decrease between the ages of 6 and 23 years.

Significantly increased levels of two or three of the noncollagenous proteins were observed in the 3 patients with Paget's disease (Table 3). The adolescent patient had an elevated serum concentration of alkaline phosphatase and her mandibular bone showed histological abnormalities resembling Paget's disease. The sialic acid levels were also determined in a further five patients with Paget's dis-

Table 2. Composition of tibial bone from patients with osteogenesis imperfecta

Clinical type	Sex & age	α_2HS-glycoprotein mg/g wet bone	Albumin	Sialic Acid μmol/g wet bone
IA	M (6)	1.05[a]	1.17	2.58
III	F (6)	0.88	0.96	2.85[a]
	M (11)	0.72	0.90	1.98
	M (16)	0.97	0.49	3.59[a]
	M (17)	0.75	0.90	2.67
	M (23)	1.03[a]	0.80[a]	2.52[a]

Values shown are the mean of 2 or more determinations
[a] Results more than 2 SD above the mean control values shown in Table 1

Table 3. Composition of bone from patients with various bone diseases

Disease	Sex & age	Bone	α_2HS-glycoprotein mg/g wet bone	Albumin	Sialic Acid μmol/g wet bone	Water %
Paget's	F (13)	Mandible	0.34	1.54[b]	2.7[b]	ND
Paget's	M (63)	Vertebra	1.82[b]	1.47[b]	4.3[b]	14.1
Paget's	F (65)	Femur	0.69[b]	0.55	3.5[b]	10.2
Osteitis fibrosa[a]	F (37)	Femur	0.58[b]	0.51	2.1	4.8
Osteomalacia	F (60)	Femur	0.29	0.29	2.4	9.2
Osteoporosis	F (56)	Vertebra	0.3	0.21	1.2	9.9
Osteopetrosis	M (23)	Femur	0.19	0.75[b]	3.1	ND
		Ilium	0.15	0.62	1.7	ND

Values shown are the mean of 2 or more determinations
[a] Due to renal failure
[b] Results more than 2 SD above the mean control values shown in Table 1
ND—not done

ease and the concentrations obtained of 4.0 ± 0.15 (SE) μmol/g wet weight of bone were also significantly increased ($P < 0.001$). The concentration of α_2HS-glycoprotein was also significantly increased in a patient with renal osteodystrophy. The lowest value of α_2HS-glycoprotein was observed in a patient with osteopetrosis while the lowest levels of albumin and sialic acid were in a patient with osteoporosis.

Overall, the samples of pathological bone were shown to have significantly increased concentrations of α_2HS-glycoprotein in five, albumin in four, and sialic acid in six specimens. While significant elevations of the three proteins were observed in 2 patients, a further 7 patients had elevations of only one or two of them.

Proteinase inhibitors were not used during decalcification and the collagenase used was shown to be free of nonspecific proteinase activity. The contents of the noncollagenous proteins reported here are higher than those found by Dickson and Bagga [11] suggesting that proteinases had not significantly affected our results.

The average water content of tibia from normal people of the same age as those with osteogenesis imperfecta was 9.4%, which is higher than the mean adult content of 8.5%. As both young and abnormal bone contain more rather than less water [8], [Quelch and Melick, unpublished] the results, if expressed relative to the dry weight, would increase the differences found.

Discussion

In this study, we have shown that high levels of α_2HS-glycoprotein, albumin, and sialic acid are present in human neonatal bone and that the levels are lower in bone from children and adults. These proteins accounted for 36% of the noncollagenous proteins released by clostridial collagenase digestion of neonatal bone.

Human fetal bone has been reported to contain at least 10 × more α_2HS-glycoprotein than adult bone [12] and we have shown that neonatal bone still contains 7 × more α_2HS-glycoprotein than adult bone. Dickson and Bagga [11] have also shown that the concentration of α_2HS-glycoprotein

progressively falls in bone throughout childhood and adulthood. In contrast, the albumin levels in fetal bone have been reported to be only 5 × the adult levels [12], and in our study the neonatal levels were only twice the adult levels. While the neonatal and adult levels of bone albumin were significantly different, we found, in agreement with Dickson and Bagga [11], that the albumin levels in bone did not fall significantly after childhood. Dickson and Bagga [11] also reported that the albumin levels in adult bone returned to childhood levels after 61 years of age.

The α_2HS-glycoprotein was concentrated relative to albumin in human bone. We calculated an approximate concentration factor of 94 in neonatal bone while Wilson et al. [12] calculated a concentration factor of 70 in fetal bone and of 35 in adult bone. However, albumin was shown by Wilson et al. [12] to be concentrated only 1.4 × relative to immunoglobulin G in human bone. These figures indicate that albumin is concentrated to only a small extent in human bone as immunoglobulin G is probably associated with blood vessels rather than being concentrated in the bone matrix. The factors that determine the levels of α_2HS-glycoprotein and albumin in bone are uncertain but both proteins are incorporated into bone during mineralization [12, 13]. Analysis of human bone of different densities has shown that 96% of the α_2HS-glycoprotein, but only 58% of the albumin, was associated with the mineral phase [11].

Heterogeneity of the sialic acid containing proteins has been reported [4] and Quelch et al. [8] showed that one major and two minor sialoprotein-containing peaks were obtained by DEAE-cellulose chromatography of the noncollagenous proteins of human bone. It is likely that the major peak contained the bone sialoprotein reported by Shetlar et al. [10] and that the minor peaks contained a mixture of sialic acid containing proteins including α_2HS-glycoprotein [14] and possibly osteonectin [15]. As the noncollagenous proteins used in the present study were prepared in the same manner as reported by Quelch et al. [8], it is likely that most of the sialic acid was in the major bone sialoprotein. The α_2HS-glycoprotein probably did not contribute a significant amount of sialic acid to the total concentration of sialic acid in the bone extracts as the concentrations of sialic acid in bone changed much less with age than did the concentrations of α_2HS-glycoprotein in bone.

Bone sialoprotein is synthesized in bone and it is likely that the high concentration of this protein in young bone is the result of increased bone cell activity [10]. We observed, in agreement with Dickson and Bagga [11], that the concentrations of bone sialic acid fell with age in a similar manner to the concentrations of albumin in bone.

The high levels of α_2HS-glycoprotein, albumin, and sialic acid observed in bone from patients with osteogenesis imperfecta were probably due to high levels of bone turnover in the type I and III forms of this disease [16, 17]. The persistantly high concentrations of these proteins during childhood and early adulthood suggested that the bone turnover rate had remained high. Histological studies have shown that the turnover rate of bone in osteogenesis imperfecta does not decline with age [17]. Dickson et al. [18] have also reported high levels of α_2HS-glycoprotein in bone from patients with osteogenesis imperfecta, and abnormally high levels of urinary α_2HS-glycoprotein have been observed in some patients with this disease [19].

The high concentrations of noncollagenous proteins in bone from patients with osteogenesis imperfecta, Paget's disease, and renal osteodystrophy are not likely to represent the underlying defects in these diseases but they are likely to reflect the rate of bone turnover [2, 20]. The primary biochemical defects in osteogenesis imperfecta have been shown, particularly in the type II (lethal perinatal) form of this disease, to involve the primary structure of the type I collagen chains [21, 22].

As all of the normal and some of the abnormal bones were obtained after death, blood samples were not available. The relationship of plasma levels of α_2HS-glycoprotein to the amount deposited in bone is unknown. Plasma taken at the same time as a bone sample may differ from the plasma level when the bone was formed. Plasma levels of α_2HS-glycoprotein have been reported to be reduced in Paget's disease of bone [23] and chronic renal failure [24] and elevated in osteogenesis imperfecta and in children [19]. In all four situations, we found elevated levels of α_2HS-glycoprotein in bone, suggesting that plasma and bone levels may not be related.

Our observation of high conentrations of α_2HS-glycoprotein, albumin, and sialic acid in bone with high rates of turnover is in agreement with the reports that α_2HS-glycoprotein and albumin are incorporated into bone during mineralization [13]. Alpha$_2$HS-glycoprotein has a strong affinity for the mineral phase of bone [2, 11, 13]. However, our results suggest that the rate of bone turnover is more important than the mineral content in determining the concentration of α_2HS-glycoprotein in bone because the highly calcified adult bone [8] and osteopetrotic bone contained the lowest levels of this protein. Albumin has a lower affinity for the

mineral phase [2, 11] and its concentration did not always follow changes in the concentration of α_2HS-glycoprotein in the diseases studied. For example, the lowest concentration of α_2HS-glycoprotein was observed in an osteopetrotic sample of bone in which the albumin levels were significantly raised. Albumin and α_2HS-glycoprotein may, therefore, be bound to different components of the bone matrix. The sialic acid levels were also high in young bone and in diseases in which high rates of bone turnover have been reported.

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