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Variations in the Serum Concentration and Urine Excretion of α_2HS-Glycoprotein, a Bone-Related Protein, in Normal Individuals and in Patients with Osteogenesis Imperfecta

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Summary. The concentration of α_2HS-glycoprotein was measured in the serum and urine of normal individuals and of patients with osteogenesis imperfecta. The serum concentration of α_2HS-glycoprotein was higher in normal children than in adults. In women values showed a progressive age-related decrease, from 632 mg/l at 21–30 years to 573 mg/l at 51–60 years. In men there was no such age-related variation, and values were higher than in women of comparable age; the mean value for men aged 20–60 years was 648 mg/l. Of 48 patients with osteogenesis imperfecta, 11 had an abnormally high concentration of α_2HS-glycoprotein in serum; the cause of this is not clear. In urine of 24 normal individuals the mean value of the ratio albumin: α_2HS-glycoprotein was 20 ± 3; in serum the corresponding ratio was 70. Urine excretion of α_2HS-glycoprotein was lowest in female children (132 ± 29 μg/24 h) and highest in male adults (592 ± 91 μg/24 h); values in patients with osteogenesis imperfecta did not differ from normal.

Key words: α_2HS-Glycoprotein — Bone protein — Serum—Urine—Osteogenesis imperfecta.

α_2HS-glycoprotein is a plasma protein with a molecular weight of about 50,000 daltons. Its physiological function is not yet known, but the results of recent research have suggested that it may have a role in bone metabolism. It is synthesized in the liver and incorporated during mineralization into bone [1, 2], where it constitutes one of the major noncollagenous components of the organic matrix. For this reason, it is logical to determine whether the plasma concentration of α_2HS-glycoprotein is abnormal in patients with bone disorders. The concentration of the glycoprotein in the plasma of patients with Paget's disease has been found to be lower than in that of normal adults; when these patients were treated with a diphosphonate or calcitonin, the concentration of the protein increased toward normal levels [3].

Although α_2HS-glycoprotein may have a role in bone, it may also have a physiological function in blood, possibly in the reticuloendothelial system. In vitro studies have demonstrated that the purified plasma protein possesses opsonic properties [4], and that its concentration was lower than normal in sera from 8 trauma patients [5] and in sera from 23 patients with acute bacterial infections [6].

Estimates of the mean concentration of α_2HS-glycoprotein in serum samples from normal subjects vary between 600 and 960 mg/l [3, 6, 7]. The number of individuals included in these estimates has been small, and hitherto no one has systematically examined the range of concentration in normal individuals or possible age and sex differences. In order to obtain suitable reference data for studies in metabolic bone disease, we have measured the concentration of α_2HS-glycoprotein in serum from 311 normal adults and 23 normal children. We have compared the results with those from 48 patients with osteogenesis imperfecta. In addition, since α_2HS-glycoprotein is more concentrated than most other serum proteins in urine [8], we have measured the urinary excretion of the glycoprotein in 38 normal individuals and 14 cases of osteogenesis imperfecta.

Materials and Methods

For studies of normal adults, serum was collected from the National Health Service Regional Transfusion Centre, Cambridge.

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on the day after it was obtained from voluntary blood donors in various parts of East Anglia. For studies of normal children specimens of serum, obtained for a growth study involving 23 normal children aged 6–14 years, were donated by Dr J. Round. The 24-h urine collections were obtained from volunteers among the laboratory staff and their families; collections from children were closely supervised.

Studies were made of 48 patients with osteogenesis imperfecta. All were members of the Brittle Bone Society, and the majority of specimens, including all of the 24-h urine collections, were obtained at residential meetings of the society's members. All those participating gave informed consent; they had been examined by one of us (CRP) and classified on the basis of clinical features and family history as described by Sillence et al. [9]. Patients in Sillence type I were subdivided according to whether they had normal teeth (Ia) or dentinogenesis imperfecta (Ib) [10].

To measure α_2HS-glycoprotein, serum or heparinized plasma (0.2 ml) was mixed with sterile 0.15M NaCl (0.2 ml) and 5 μl of the mixture pipetted with a Ziptrol capillary pipette (Shandon Southern Instruments Ltd., Camberley, Surrey) into holes in immunodiffusion plates containing antiserum to human α_2HS-glycoprotein (Partigen plates, Behring Diagnostics Ltd., Hounslow, Middlesex). Standard reference solutions of 3 different concentrations (Protein Standard Serum, Behring Diagnostics Ltd.) were used to calibrate the plates. After development for 48 h at room temperature, the diameter of the precipitin rings formed was measured with a Partigen ruler against dark ground illumination. Using this procedure the diameters could be read with mean error of ± 6%.

For 24 h urine collections, the bottles contained no added preservative. The total volume was measured and a portion (50 ml) of the specimen was dialyzed (Visking No. 18 tubing, V. A. Howe Ltd., London) against 10 volumes demineralized water with daily changes of water for 7 days at 5°C. The nondiffusible material was lyophilized and redissolved at 40 mg/ml in 0.1 M sodium bicarbonate solution.

One dimensional ("rocket") immunoelectrophoresis was performed in 1% agarose gels containing antiserum (0.4% v/v) for α_2HS-glycoprotein antiserum or for albumin antiserum; antisera were obtained from Behring Diagnostics on 14 × 11 cm glass plates. The gel and electrophoresis buffers were of similar composition (Tris, 90 mM EDTA; boric acid, 80 mM; pH 8.3). The volume of sample solution applied to wells was 5 μl, and electrophoresis was performed at constant voltage (200 v) for 18 h. The plates were washed with 1% NaCl for 48 h, followed by water (1 h); plates were then dried and stained. The stain solution used was Coomassie Brilliant Blue G250 (0.1%) in methanol:water:acetic acid, 50:30:20 (v:v:v). The plates were destained in water:methanol:formic acid, 69:30:1.

Results

For a comparison of the α_2HS-glycoprotein concentration in serum and in plasma, blood was obtained by venipuncture from 37 fasting adults and one portion was placed in a heparinized tube, the remainder into a plain tube. Serum and plasma were separated and these samples were stored deep-frozen until the date of analysis. A statistical analysis of the measurements of α_2HS-glycoprotein concentration in plasma and serum (results not shown) showed no difference between the two methods of preparation.

The results in Table 1 show that the serum concentration of α_2HS-glycoprotein is high in men during the period of skeletal development, that is, up to 20 years. Between 20 and 60 years, it remains constant, the mean value over this period being 648 mg/l. The mean concentration in men aged 61–65 years is slightly higher (676 mg/l), but the difference was not statistically significant.

The concentration of the glycoprotein in serum of normal females was also higher in children and adolescents than at other ages. The mean value for female children is affected by the inclusion of two exceptionally low values (364 and 325 mg/l, respectively); if these are omitted the mean ± SE and range of the remainder of the group (780 ± 41, 620–1040 mg/l) are similar to those of male children. The values in adults show a small, progressive decline with age; the lowest values are reached at 55–58 years. As with men the α_2HS-glycoprotein values in the 61–65-year-old women were slightly, but not significantly, higher than those in younger women.

Values of serum α_2HS-glycoprotein for each individual with osteogenesis imperfecta (OI) were compared with those for the appropriate age- and sex-matched normal group. Table 2 shows that 26% of 34 affected females and 14% of 14 affected males have values above the 95% confidence limits for normal individuals. An analysis of regression by the t test showed that females with OI as a group were different ($P < 0.01$) from normal females in this respect. Of the 11 patients with abnormally high values, all but one were Sillence type I OI (dominant inheritance, blue sclerae and generally mild disease). All were adults and none had had fractures in the preceding year.

The results of the studies of α_2HS-glycoprotein excretion in normal individuals and in those with OI are shown in Fig. 1 and Table 3. Values are higher in males than in females and higher in adults than in children. There appears to be no difference in the pattern of urine α_2HS-glycoprotein excretion between individuals with OI and normal individuals of similar age and sex. The albumin content of a number of specimens was measured by one-dimensional immunoelectrophoresis to compare its excretion with that of α_2HS-glycoprotein. In 24 normal individuals, the albumin: α_2HS-glycoprotein ratio ranged between 5 and 47 (mean ± SE; 20 ± 3); the corresponding ratio in serum is 70.

Table 1. Variation of α_2HS-glycoprotein concentration with age and sex in normal serum.

Age group	Number in Group	α_2HS-glycoprotein concentration (mg/l) Mean ± SE	Range
Male			
7–14	11	767 ± 29[a]	590–1020
18–20	20	741 ± 25[a,d]	510–960
21–30	35	650 ± 19	420–860
31–40	31	647 ± 18[d]	420–860
41–50	28	655 ± 17[e]	420–780
51–60	27	640 ± 17[d]	470–810
61–65	15	676 ± 28	510–840
Female			
6–14	12	709 ± 60	330–1040
18–20	26	661 ± 21	440–900
21–30	30	632 ± 18	440–810
31–40	28	600 ± 13[b]	470–780
41–50	27	590 ± 18[b]	440–840
51–60	23	573 ± 19[c]	420–780
61–65	21	650 ± 16	520–770

[a] $P < 0.01$ when compared with 21–30 male group.
[b] $P < 0.05$ when compared with 18–20 female group.
[c] $P < 0.01$ when compared with 18–20 female group.
[d] $P < 0.05$ when compared with female group of same age range.
[e] $P < 0.01$ when compared with female group of same age range.

Table 2. Incidence of abnormally high values of serum α_2HS-glycoprotein in osteogenesis imperfecta.

	Clinical subtype				
	IA	IB	III	IV	Combined
Female					
Above 95%	6	2	1		26%
Within 95%	15	5	1	4	74%
Total	21	7	2	4	34
Male					
Above 95%		2			14%
Within 95%	5	1	3	3	86%
Total	5	3	3	3	14

The values shown are the number of individuals having a serum α_2HS-glycoprotein concentration above or within the 95% confidence limits for normal individuals of similar age and sex.

Discussion

Other groups have reported similar values for the concentration of α_2HS-glycoprotein in normal adults. Lebreton and coworkers [6] obtained a mean ± standard deviation of 595 ± 120 mg/l in serum from 38 normal subjects. Ashton and Smith [3] obtained a value of 630 mg/l (SE ± 31; n = 25) in plasma from normal adult males and 668 mg/l (SE ± 34; n = 33) for females; the age of their subjects ranged from 21 to 92 years. Both of these studies used reference serum from the same source as in the present study, and the only study [7] reporting markedly different values (mean ± SD: 960 ± 90; 34 subjects) did not. The range of concentration of the glycoprotein in sera from normal adults is large compared with the age and sex-related differences found in this study. This would explain why these differences were not observed in the previous studies which involved smaller numbers of subjects.

The concentration of a protein in blood reflects the dynamic balance between its synthesis and degradation. For α_2HS-glycoprotein, this balance is also affected by removal of the glycoprotein from the plasma for specific physiological processes relating to its role in bone and in the reticuloendothelial system. Lack of knowledge at present about the factors regulating these processes makes it difficult to interpret empirical observations of the concentration of α_2HS-glycoprotein in different conditions. Thus the decreased serum concentration of the glycoprotein in patients with Paget's disease of bone has been attributed to its increased removal from the circulation through increased bone formation. In contrast, the present study shows that increased bone formation does not necessarily result in decreased serum α_2HS-glycoprotein since in normal children, in whom bone formation is more active than in adults, the reverse is the case.

The mean concentration of α_2HS-glycoprotein in females is slightly lower than in males of comparable age; the difference between the sexes becomes more marked in middle age because of an age-dependent decrease in levels in women. Since the only information available about normal donors is their occupation and marital status, it is not possible to exclude the use of oral contraceptives as a contributory factor to the changes in women, but it seems unlikely. Other workers [6] have reported that the serum α_2HS-glycoprotein was high in a young adult woman taking oral contraceptives, but the information in this present study suggests that this high value would be consistent with random variation in a female population of that age.

As a group, osteogenesis imperfecta (OI) patients differed significantly from normal subjects in their α_2HS-glycoprotein levels. Our findings could be explained by the presence within the OI group of a subpopulation with abnormally high values, the remaining patients being indistinguishable from normal. OI is itself a heterogeneous disorder, and at least 6 subgroups are now recognized on clinical,

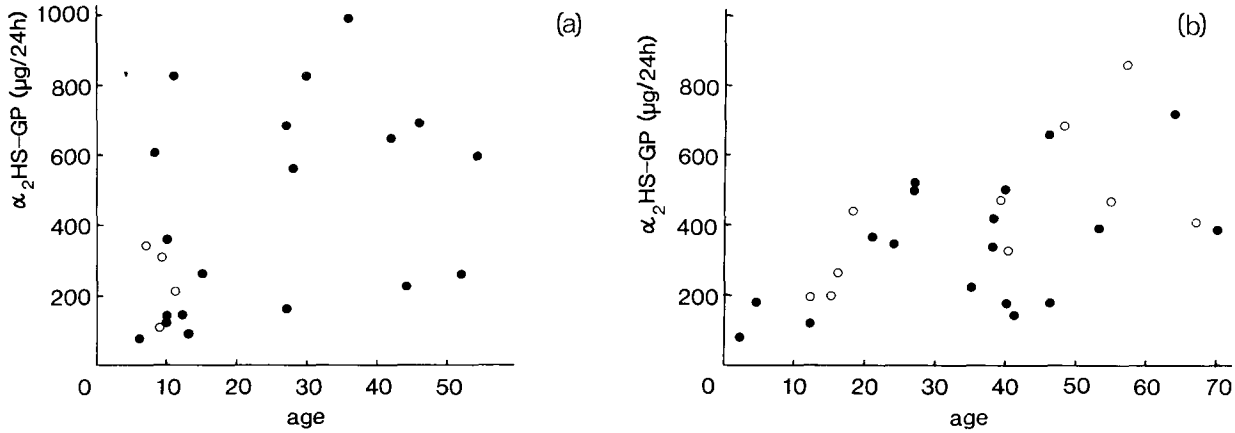

Fig. 1. Urine α_2HS-glycoprotein excretion in normal individuals and patients with osteogenesis imperfecta. **a** values for males; **b** values for females. ● = normal subjects; ○ = cases of osteogenesis imperfecta.

Table 3. Urine α_2HS-glycoprotein excretion in normal individuals.

	α_2HS-glycoprotein (µg/24 h)
Adults	
Male	592 ± 91 (10)
Female	425 ± 66 (16)
Children	
Male	297 ± 87 (9)[a]
Female	132 ± 29 (3)

Values shown are the mean ± SE, with the number of subjects in parentheses.
[a] significantly different ($P < 0.05$) compared to male adults.

radiological and genetic grounds [9,10]. It is likely that this clinical heterogeneity reflects differences in the fundamental molecular abnormalities. At present, there is growing evidence that some of these disorders result from defects in the synthesis or maturation of collagen [11,12].

All but one of the high values for α_2HS-glycoprotein in plasma were found in patients with Sillence's type I OI; in part, this may reflect the total number of patients with this type. Within the group of type I patients the difference between those with and those without high values is not clear; it could reflect a previously undescribed form of heterogeneity. Sillence's type I OI has already been subdivided by Levin and others [10], according to whether or not dentinogenesis imperfecta is also present. We found high values for α_2HS-glycoprotein in both of these groups. Another possible explanation for the different results among Sillence's type I patients is that the findings in some patients have been modified by factors such as previous fractures or intercurrent illness. Further work may help to elucidate this problem.

The studies of the urinary excretion of α_2HS-glycoprotein demonstrate that in this respect, patients with OI do not differ from normal subjects. In normal subjects the range of values is too wide and the numbers studied too small to determine whether there are significant age- and sex-related differences. The results do, however, provide a basis for testing for abnormalities in different types of metabolic bone diseases, and they confirm that α_2HS-glycoprotein excretion in relation to albumin is higher than would be expected from the similarity in molecular size of the two proteins and their relative amounts in plasma.

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