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Clinical Significance of Free Plasma Hydroxyproline Measurement in Metabolic Bone Disease

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Summary: Free hydroxyproline was measured in plasma of 67 normal subjects and in 70 patients with bone disease including primary hyperparathyroidism (n = 19), osteoporosis (n = 18), *Paget*'s disease (n = 14), cancer involving bone (n = 8), chronic renal failure (n = 6), and osteomalacia (n = 5). A good correlation was found between plasma and urinary values of the amino acid in normal subjects ($r = 0.66$; $p < 0.001$). In patients with skeletal disorders a highly significant direct correlation was observed between free plasma hydroxyproline on the one hand and urinary hydroxyproline ($r = 0.92$; $p < 0.001$) and serum alkaline phosphatase activity ($r = 0.86$; $p < 0.001$) on the other, even though there were a few examples of dissociations among these parameters.

Free plasma hydroxyproline decreased in the patients with *Paget*'s disease following chronic administration of salmon calcitonin. Following successful parathyroidectomy, free plasma levels of hydroxyproline decreased in all the cases studied.

Measurement of free plasma hydroxyproline thus appears to provide a specific index of bone metabolism that may be usefully employed as an alternative to the assay of other markers of bone turnover.

Klinische Bedeutung der Bestimmung des freien Hydroxyprolin im Plasma bei Knochenstoffwechselerkrankungen

Zusammenfassung: Freies Hydroxyprolin im Plasma wurde bei 67 Kontrollpersonen und 70 Patienten mit Knochenerkrankungen (primärer Hyperparathyreoidismus, n = 19; Osteoporose, n = 18; M. *Paget*, n = 14; Krebs mit Knochenbeteiligung, n = 8; chronisches Nierenversagen, n = 6 und Osteomalazie, n = 5) bestimmt. Zwischen den Werten für Hydroxyprolin im Plasma und im Harn der Kontrollpersonen wurde eine gute Korrelation ($r = 0{,}66$; $p < 0{,}001$) gefunden. Bei Patienten mit Skeletterkrankungen wurde eine hochsignifikante Beziehung zwischen freiem Hydroxyprolin im Plasma und Hydroxyprolin im Harn ($r = 0{,}92$; $p < 0{,}001$) sowie der katalytischen Konzentration der alkalischen Phosphatase ($r = 0{,}86$; $p < 0{,}001$) gefunden, wenn auch gelegentlich Abweichungen beobachtet wurden. Freies Hydroxyprolin im Plasma fiel bei Patienten mit M. *Paget* nach chronischer Behandlung mit Lachs-Calcitonin. Nach erfolgreicher Parathyreoidektomie fiel das freie Hydroxyprolin im Plasma bei allen untersuchten Patienten.

Die Bestimmung des freien Hydroxyprolin im Plasma ist eine geeignete Kenngröße für den Knochenstoffwechsel, die anstelle anderer Kenngrößen des Knochenumsatzes eingesetzt werden kann.

Introduction

Hydroxyproline exists in human plasma in at least three forms: free, peptide-bound and protein-bound (1). The nonultrafilterable protein-bound form, the so-called collagen-like protein, does not appear to be related to collagen metabolism (2), but to the Clq component of complement (3, 4); the ultrafilterable fraction, comprising both the free and peptide-bound

hydroxyproline is, however, considered to be a reliable biochemical marker of bone turnover, skeletal tissue being the major reserve of collagen in the human body (5, 6, 7).

Generally, the urine of persons eating a collagen free diet contains 95% of peptide-bound hydroxyproline. In contrast, about 50–60% of hydroxyproline in plasma, excluding the nonultrafilterable form, represents the free amino acid (7), which is metabolized mainly in the liver by specific oxidase activity.

The present paper gives the results of our clinical studies on plasma levels of free hydroxyproline in patients with various skeletal disorders, in which measurement of the total urinary excretion of this amino acid is considered as useful.

Materials and Methods

Control values (mean ±SD = 8.65 ± 2.20 µmol/l) of free hydroxyproline in plasma were obtained from 67 normal subjects (21 males and 46 females) aged between 19 and 83 years with no evidence of calcium or skeletal abnormalities from routine history, and physical and biochemical evaluation. Studies under basal conditions were carried out on 19 patients with primary hyperparathyroidism, established by surgery; 15 females with postmenopausal osteoporosis and 3 males with juvenile osteoporosis with radiological evidence of one or more vertebral crush fractures; 14 patients with *Paget*'s disease of bone, four with monostotic involvement, in each of whom the history, physical and biochemical evaluation, X-ray and/or bone-scan findings were diagnostic of this disorder; 8 patients with bone metastases due to cancer of the prostate (3 cases), lung (2 cases), kidney (2 cases), bladder (1 case), confirmed histologically or at autopsy; 6 patients on maintenance haemodialysis therapy who had radiological evidence of osteitis fibrosa; 5 patients with histologically proven osteomalacia, secondary to malabsorption syndrome. Plasma hydroxyproline measurements were also repeated in 8 patients with primary hyperparathyroidism 24 hours after removal of the adenoma and in 3 patients with *Paget*'s disease of bone at 1, 5, and 9 month intervals during treatment with salmon calcitonin (100 MRC U/day in two patients, or every other day in one patient). Blood samples in all patients and control subjects were collected after at least 48 hours on a gelatine free diet. Venous blood, in the maintenance haemodialysis patients, was collected at least 36 hours after the last dialysis. The 24 h total urinary excretion of the amino acid was also measured in all individuals, with the exception of patients presenting bone metastases or renal failure. Finally, blood samples were collected in all patients for the determination of alkaline phosphatase activity. Free plasma and total urinary hydroxyproline were assayed by a standard autoanalyser method (8); separation of free plasma amino acid was carried out according to the method of *Husdan* et al. (9).

The reported values of plasma hydroxyproline are the average of two determinations. Plasma protein and serum alkaline phosphatase activities (10) as well as urinary creatinine, were determined by standard methods used in our laboratory (11). Alkaline phosphatase reference values range between 61 and 171 U/l.

Statistical analysis was performed by linear regression analysis and *Student*'s t test.

Results

Figure 1 shows the correlation between free hydroxyproline in plasma and total urinary excretion of the amino acid, expressed as ratio to creatinine, in the 67 control subjects ($r = 0.66$; $p < 0.001$). Figure 2 shows individual values for free plasma hydroxyproline in 70 patients with various bone disorders. Analysis of the results in the entire series of patients demonstrated a good correlation between plasma hydroxyproline on the one hand and urinary hydroxyproline ($n = 56$, $r = 0.92$; $p < 0.001$) (fig. 3) and serum alkaline phosphatase activity ($n = 70$, $r = 0.86$; $p < 0.001$) on the other. Plasma levels of the amino acid were determined in 8 patients with primary hyperparathyroidism immediately before and 24 hours after removal of the parathyroid adenoma. This demonstrates that after successful parathyroidectomy the plasma levels of hydroxyproline tend to decrease immediately, postoperative values showing a statistically significant drop with respect to preoperative (41.3 ± 28.7 µmol/l vs 17.0 ± 9.6 µmol/l; $p < 0.007$). The effects of salmon calcitonin treatment upon the chosen biochemical markers for bone metabolism, in 3 patients with *Paget*'s disease, are shown in figure 4. It can be seen that, despite small variations, biochemical changes due to pharmacological treatment show a similar trend in all 3 cases; it is of interest to observe that resistance to calcitonin treatment, appearing after five months in the female patient with more severe bone involvement, was adequately correlated with the failure to return to within the normal range of the parameters under consideration.

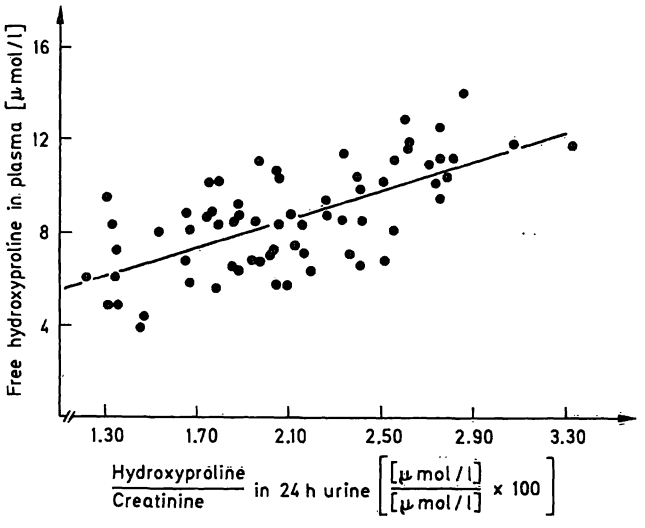

Fig. 1. Correlation between concentration of free hydroxyproline in plasma and total urinary excretion of the amino acid in normal subjects. $r = 0.66$ ($p < 0.001$), $n = 67$.

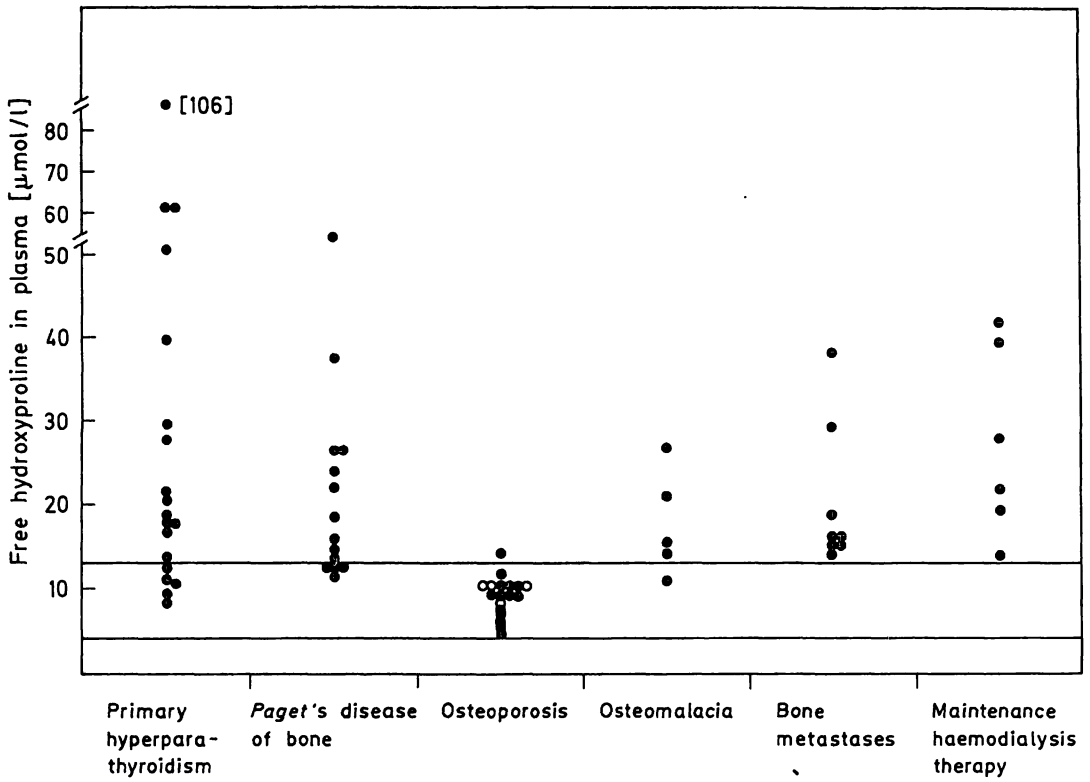

Fig. 2. Individual values for free plasma hydroxyproline in 70 patients with various bone diseases. The horizontal lines indicate mean ± SD for control subjects.
o, indicates three males with juvenile osteoporosis; other patients in this group had postmenopausal osteoporosis.

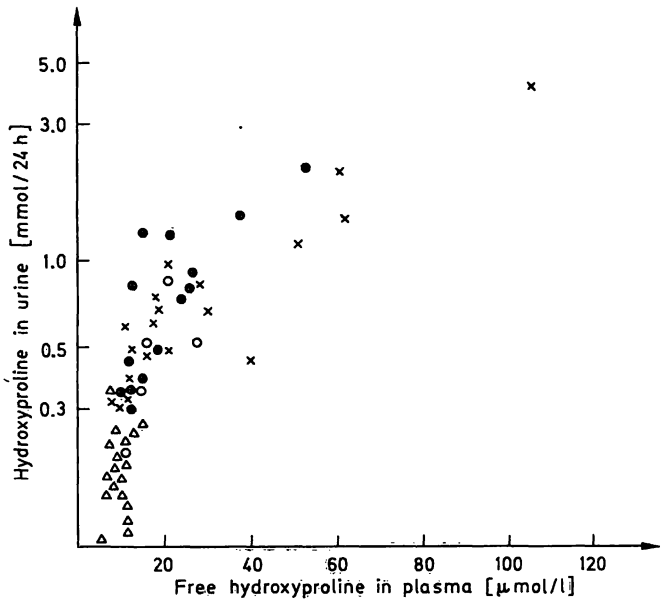

Fig. 3. Correlation between free plasma hydroxyproline and total urinary excretion of the amino acid in 56 patients with various bone disease ($r = 0.92$; $p < 0.001$).
x = primary hyperparathyroidism
△ = osteoporosis
● = *Paget*'s disease of bone
○ = osteomalacia.

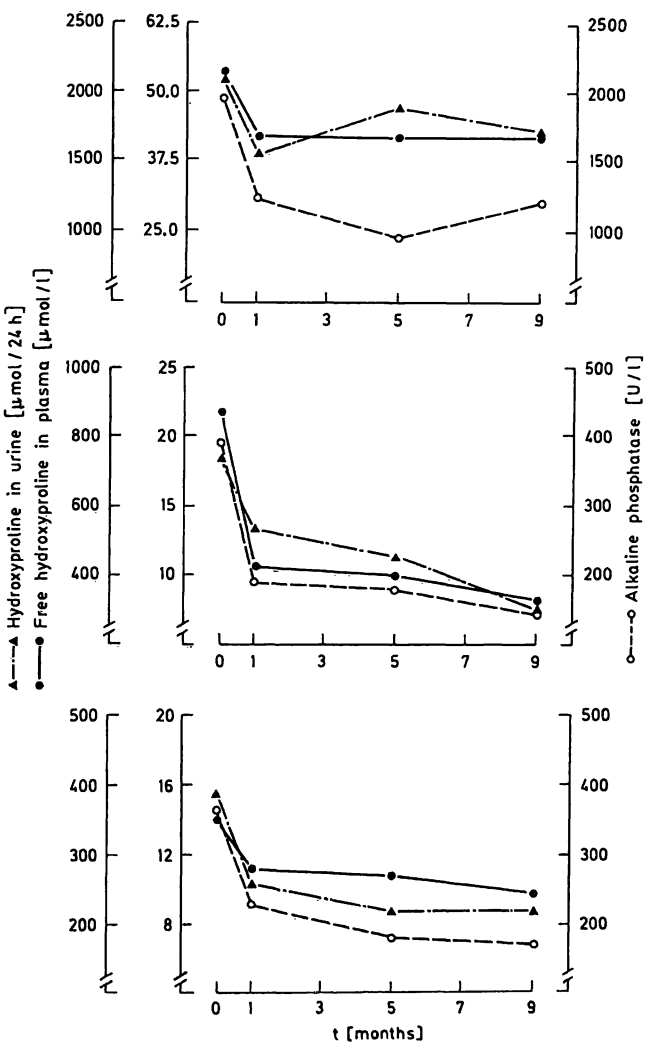

Fig. 4. Changes in free plasma and total urinary hydroxyproline values and serum alkaline phosphatase activity in two women (top and middle) and one man (bottom) with *Paget*'s disease of bone and different degrees of skeletal involvement, during treatment with salmon calcitonin 100 MRC U/day (women) or every other day (man). Represented are the means of duplicate determinations.

Discussion

In the present investigation a highly significant direct correlation was observed between plasma levels of free hydroxyproline and total urinary excretion of hydroxyproline both in the normal subjects and in the patients with various disorders of bone metabolism; this finding appears to confirm that free hydroxyproline in plasma has the same origin as total urinary hydroxyproline, being derived from the metabolism of both soluble and insoluble collagen (7). According to *Laitinen* (7) our data therefore indicate that measurement of the free hydroxyproline fraction in blood may have the same diagnostic value as the determination of total urinary hydroxyproline.

Assay of free plasma hydroxyproline has so far been used in clinical practice only in patients with renal failure (1, 12) to evaluate the degree of bone involvement or the response to therapy; few papers, generally limited to patients with bone metastases (13), have, on the other hand, appeared in the literature to evaluate both the clinical usefulness of plasma hydroxyproline analysis and the changes in plasma amino acid levels in response to treatment in other disorders of bone metabolism. We have, therefore, assayed plasma concentrations of hydroxyproline in 70 patients with the most common skeletal disorders, and, simultaneously, measured total urinary hydroxyproline excretion and serum alkaline phosphatase activity. The results obtained show that the assay of free hydroxyproline in plasma may be usefully employed to evaluate skeletal turnover in metabolic bone diseases, in that free plasma hydroxyproline correlates very significantly with urinary hydroxyproline and serum alkaline phosphatase activity. The finding that four patients with *Paget*'s disease and three patients with primary hyperparathyroidism exhibited plasma levels of free hydroxyproline at the upper normal limits, although not increased to the same extent as the urinary values, may possibly be explained by the fact that in a limited number of cases, plasma levels of the amino acid in the morning may not reflect the entity of the overall bone turnover during a 24 hour period. This hypothesis would appear to be further confirmed by the finding that in two of these patients with primary hyperparathyroidism and in two of the patients with *Paget*'s disease, the serum alkaline phosphatase activity values were also within the normal range. It cannot be excluded, however, that in cases with less severe bone involvement, the assay of all the ultrafilterable hydroxyproline fraction might be more sensitive than assay of the free fraction only. Studies related to this question are currently being carried out. Nevertheless, it should be pointed out that dissociations between total urinary excretion and plasma levels of the free amino acid have already been reported in prostatic cancer patients with bone metastates (14).

Further proof of the importance of free plasma hydroxyproline measurement emerges from findings obtained both in hyperparathyroid patients and in patients with *Paget*'s disease during treatment with salmon calcitonin. The observed rapid decrease in plasma amino acid levels immediately after surgery confirms that measurement of free plasma hydroxyproline may be considered a reliable index of successful parathyroidectomy. Also the response to pharmacological treatment appears to be well documented by the free plasma hydroxyproline measurement. This is showed by a significant decrease in plasma hydroxyproline concentration, which is reflected in a decrease in urinary amino acid excretion and serum alkaline phosphatase activity, in patients with *Paget*'s disease during treatment with calcitonin.

In conclusion, the results presented indicate that assay of free hydroxyproline in plasma may be employed as a reliable marker of bone turnover not only in patients with renal failure but also in those with normal renal function. The advantages with respect to the determination of the amino acid in urine lie in the considerably reduced analysis time and in the absence of urine collection which, particularly in elderly patients, is often inaccurate.

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