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**Research Article**

Bone gamma-carboxyglutamic acid-containing (Gla) protein (BGP, osteocalcin) is a noncollagenous protein of bone present in plasma and removed by the kidney. Plasma BGP has been shown to be elevated in patients with certain bone diseases. The present study evaluates serum BGP (S-BGP), serum alkaline phosphatase (S-AP), and urinary hydroxyproline excretion (U-OHP) in diseases with differing bone turnover rates, and compares the accuracy of these measurements for estimating bone mineralization (m) and resorption (r) rates. S-BGP, S-AP, U-OHP, and creatinine clearance (Clcr) were measured in patients with primary hyperparathyroidism (n = 13), hyperthyroidism (n = 6), and hypothyroidism (n = 6). Bone mineralization and resorption rates were calculated from a 7-d combined calcium balance and 47Ca turnover study. A highly significant correlation (r = 0.69, P less than 0.001) was found between S-BGP and m. Multiple regression analysis disclosed a partial correlation between S-BGP and m when Clcr was taken into account (r = 0.82, P less than 0.001), and between S-BGP and Clcr when m was taken into account (r = -0.62, P less than 0.005). In accordance with this, a stronger correlation (r = 0.89, P less than 0.0001) was found between S-BGP X Clcr and m than between S-BGP and m. A less significant correlation was found between S-AP and m (r = 0.45, P less than 0.05). […]



# Estimation of Bone Turnover Evaluated by $^{47}$Ca-Kinetics

## Efficiency of Serum Bone Gamma-Carboxyglutamic Acid-containing Protein, Serum Alkaline Phosphatase, and Urinary Hydroxyproline Excretion

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## Abstract

**Bone γ-carboxyglutamic acid-containing (Gla) protein (BGP, osteocalcin) is a noncollagenous protein of bone present in plasma and removed by the kidney. Plasma BGP has been shown to be elevated in patients with certain bone diseases. The present study evaluates serum BGP (S-BGP), serum alkaline phosphatase (S-AP), and urinary hydroxyproline excretion (U-OHP) in diseases with differing bone turnover rates, and compares the accuracy of these measurements for estimating bone mineralization (m) and resorption (r) rates. S-BGP, S-AP, U-OHP, and creatinine clearance ($Cl_{cr}$) were measured in patients with primary hyperparathyroidism ($n$ = 13), hyperthyroidism ($n$ = 6), and hypothyroidism ($n$ = 6). Bone mineralization and resorption rates were calculated from a 7-d combined calcium balance and $^{47}$Ca turnover study. A highly significant correlation ($r$ = 0.69, $P$ < 0.001) was found between S-BGP and m. Multiple regression analysis disclosed a partial correlation between S-BGP and m when $Cl_{cr}$ was taken into account ($r$ = 0.82, $P$ < 0.001), and between S-BGP and $Cl_{cr}$ when m was taken into account ($r$ = −0.62, $P$ < 0.005). In accordance with this, a stronger correlation ($r$ = 0.89, $P$ < 0.0001) was found between S-BGP × $Cl_{cr}$ and m than between S-BGP and m. A less significant correlation was found between S-AP and m ($r$ = 0.45, $P$ < 0.05). Furthermore, U-OHP showed a highly significant positive correlation to r ($r$ = 0.78, $P$ < 0.001).**

**Thus, in the studied disorders of calcium metabolism, individual serum levels of BGP depend on both mineralization rate and renal function. Serum levels of BGP corrected for alterations in renal function are superior to uncorrected S-BGP and to S-AP levels in the estimation of bone mineralization rates.**

## Introduction

Bone γ-carboxyglutamic acid-containing (Gla) protein (BGP),[1] or osteocalcin, is a noncollagenous 49-residue protein containing two or three residues of the vitamin K-dependent amino acid γ-carboxyglutamic acid (Gla) (1–4). BGP is synthesized in bone and is present in plasma (5). Experiments in rats have shown that 3 h after injection of warfarin, an inhibitor of γ-carboxylation, the BGP in serum is entirely decarboxylated, suggesting that most, if not all, BGP in serum originates from new cellular synthesis (6), rather than from degradation of bone. The role of BGP in bone metabolism remains unknown, but serum BGP (S-BGP) is of potential interest as a specific marker of bone metabolism. Using a specific radioimmunoassay, plasma BGP levels have been shown to be elevated in patients with increased bone turnover and to correlate with plasma alkaline phosphatase in these patients (7). In theory, it would seem that BGP may be useful for evaluating bone mineralization rate as it does not have the limitations that are inherent for serum alkaline phosphatase (S-AP) and urinary excretion of hydroxyproline (U-OHP), parameters traditionally used as indices for bone turnover. S-AP originates from bone, liver, the gastrointestinal tract, lungs, tumors, and other possible sources (8). Moreover, the different factors affecting degradation of AP are not fully understood. U-OHP has several problems that complicate its interpretation as an index of bone resorption. It reflects dietary intake of gelatin and turnover of nonosseous as well as osseous collagen. A portion of the hydroxyproline in urine is derived from peptides related to collagen synthesis, and many of the hydroxyproline-containing peptides released during degradation of collagen are further metabolized and may not appear as U-OHP (9).

In the present study we have evaluated the relationships between S-BGP, S-AP, and U-OHP, and bone mineralization (m) and resorption (r) rates at the organ level. For this purpose we have chosen groups of patients who have no mineralization defects or fractures, but in whom bone histomorphometry (10, 11) had previously revealed large variations in bone turnover at the tissue level. Moreover, the relative accuracy of the biochemical variables for estimating bone turnover rates was investigated with particular attention to whether the clinical significance of S-BGP measurements was improved when corrected for variations in renal function.

## Methods

25 patients (20 females and 5 males) aged 26–73 yr (mean, 62 yr) with primary hyperparathyroidism ($n$ = 13), hyperthyroidism ($n$ = 6), and hypothyroidism ($n$ = 6) were investigated. The diagnosis of primary hyperparathyroidism was based on elevated serum calcium (corrected for serum albumin) and immunoreactive parathyroid hormone. At a later time, nine of the patients had a parathyroid adenoma removed, whereas the remaining four only have slightly elevated serum calcium levels and have not yet undergone surgery. The diagnosis of hyperthyroidism was based on elevated serum levels of thyroxine ($T_4$) and triiodothyronine ($T_3$) with normal or elevated serum triiodothyronine uptake test ($T_3$-test) and low serum levels of thyroid-stimulating hormone. The diagnosis of hypothyroidism was based on low serum levels of $T_4$ and

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1. *Abbreviations used in this paper:* BGP, bone γ-carboxyglutamic acid-containing (Gla) protein; $Cl_{cr}$, creatinine clearance; CV, coefficient of variation; GFR, glomerular filtration rate; m, bone mineralization rate; r, bone resorption rate; S-AP, serum alkaline phosphatase; S-BGP, serum BGP; S-BGP × $Cl_{cr}$, S-BGP with correction for renal function; $T_3$, triiodothyronine; $T_3$-test, triiodothyronine uptake test; $T_4$, thyroxine; U-OHP, urinary excretion of hydroxyproline.

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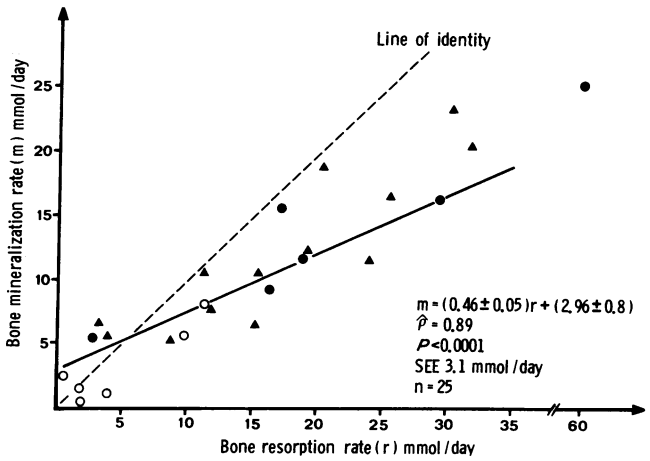


*Figure 1.* Linear regression analysis of the relationship between r and m in 25 patients with hyperparathyroidism (▲), hyperthyroidism (●) or, hypothyroidism (○). SEE, standard error of estimate.

$T_3$-test. None of the patients had signs of concomitant liver disease and all patients had a creatinine clearance ($Cl_{cr}$) > 40 ml/min × 1.73 $m^2$.

Bone mineralization and resorption rates were calculated from a 7-d combined calcium balance and $^{47}Ca$-kinetic study (12, 13) using a modification of the expanding calcium pool model introduced by Burkinshaw et al. (14). The patients, after an equilibration period of 1 wk, were studied while on a diet as similar as possible to their daily fare. The intraindividual coefficient of variation (CV) for m was 15% at a mean value of 4.6 mmol Ca/d, and the CV for r was 20% at a mean value of 5.9 mmol Ca/d (12).

All serum measurements were performed on samples that were withdrawn in the morning after overnight fasting. S-BGP was measured by radioimmunoassay (5) using rabbit antisera to bovine BGP (used at 1: 35,000 dilution). The standard was bovine BGP, where the concentration was established by quantitative amino acid analysis. The tracer was prepared by chloramine-T radioiodination. The assay was 48 h nonequilibrium assay and was terminated by second antibody precipitation with goat anti-rabbit IgG. The detection limit was 0.1 ng, with a CV of 6% in the useful range (0.2–20 ng). The intraassay variation was typically <6%; the interassay variation was <10%. In our laboratory, using standardized restrictions on diet and time of serum collection as described above, the normal range for serum BGP levels is between 6 and 13 ng/ml. Serum BGP levels > 20 ng/ml were established with diluted serum samples, as we have found that this provides a more accurate measurement. The values obtained for BGP are linear, with dilution over the entire useful range of the assay, as long as the volume of serum does not exceed 200 μl.

S-AP was measured spectrophotometrically with *p*-nitrophenylphosphate by the method recommended by the Scandinavian Committee on Enzymes (15). Immunoreactive parathyroid hormone was measured by a radioimmunoassay directed against the COOH-terminal part (65–84) of the parathyroid hormone. Serum levels of calcium, $T_4$, $T_3$, thyroid stimulating hormone, $T_3$-test, creatinine, and albumin were analyzed according to standard laboratory methods. U-OHP was measured spectrophotometrically with *p*-dimethylaminobenzaldehyde substrate according to the manufacturers' (Organon Teknika, B. V. Boxtel, Holland) directions. The individual $Cl_{cr}$ rate was calculated from the mean of 7 d urine collection.

*Correction for renal influence on S-BGP.* Provided BGP is produced in an amount proportional to m, then, production rate of BGP (p-BGP) = $k_1$, a constant. Assuming that the degradation of BGP (d-BGP) is determined by the renal function and serum level of BGP, then d-BGP = $k_2 \times Cl_{cr} \times$ S-BGP, where $Cl_{cr}$, is the creatinine clearance rate; and S-BGP, the serum concentration of BGP. Because of the low molecular weight of BGP (5,800), it is reasonable to assume that BGP is completely diffusible. Consequently, the expression S-BGP × $Cl_{cr}$ is equivalent to the filtered load of BGP.

In a steady state situation with regard to S-BGP, p-BGP equals d-BGP, or m = $k_3 \times Cl_{cr} \times$ S-BGP (where $k_3 = k_2/k_1$). If the assumptions mentioned above are valid, the correlation between S-BGP × $Cl_{cr}$ and m must be superior to the correlation between S-BGP and m. Moreover, as a necessary condition for the accuracy of prediction, $k_3$ (the slope of the regression line) should be independent of the pathophysiological mechanisms (thyroid or parathyroid disorder) responsible for the change in m.

*Statistical methods.* Least squares linear regression analysis was used for testing relationships between parameters without adjustment for covariables. Multiple regression analysis using the GLIM-computer program was performed to evaluate the relationships between (*a*) S-BGP, m, and $Cl_{cr}$; (*b*) S-BGP, m, $Cl_{cr}$, and r; (*c*) U-OHP, r, and m; and (*d*) S-AP, m, and r. Partial correlation coefficients were used to present the results of the multiple regression analysis.

## Results

A close relation ($r = 0.89$, $P < 0.0001$) was observed between r and m (Fig. 1). It appears that with increasing bone turnover, r increases more than m, leading to a progressive negative calcium balance. This was confirmed by a negative correlation ($r = -0.64$, $P < 0.005$) between m and calcium balance. This coupling between r and m to some extent compromises the utility of linear regression analysis to determine the dependence of biochemical markers on bone resorption and bone formation. A significant correlation ($r = 0.69$, $P < 0.001$) was found between S-BGP and m (Table I) in all patients. No relationship was found between S-BGP and $Cl_{cr}$ ($r = 0.06$, $P > 0.60$) using linear regression

*Table I. Linear Regression Analysis of the Relationships between S-BGP, S-BGP × $Cl_{cr}$, S-AP, U-OHP, and m and r in 25 Patients with Hyperparathyroidism (n = 13), Hyperthyroidism (n = 6), or Hypothyroidism (n = 6)**

| | Mineralization rate (m) | | | Resorption rate (r) | | |
|---|---|---|---|---|---|---|
| | *r* | *P* | SEE | *r* | *P* | SEE |
| | | | *nmol Ca/day* | | | *nmol Ca/day* |
| S-BGP (*ng/ml*) | 0.69 | <0.001 | 5.1 | 0.67 | <0.001 | 10.0 |
| S-BGP × $Cl_{cr}$ (*ng/min*) | 0.89 | <0.0001 | 3.2 | 0.87 | <0.0001 | 6.6 |
| AP‡ (*U/liter*) | 0.45 | <0.05 | 6.1 | 0.34 | >0.10 | 12.5 |
| U-OHP‡ (*umol/d*) | 0.74 | <0.001 | 8.5 | 0.78 | $P < 0.001$ | 4.7 |

* *r*, coefficient of correlation; *P*, level of significance; SEE, standard error of estimate of kinetic parameters (m and r) from biochemical measures.
‡ Correlation based on log. transformation.

*Table II. Multiple Regression Analysis* between Biochemical Indices of Bone Turnover S-BGP, S-AP, and U-OHP and $^{47}Ca$-Kinetic Indices of Bone Turnover (m and r), and for the Correlation between S-BGP and Renal Function in 25 Patients with Hyperparathyroidism (n = 16), Hyperthyroidism (n = 6), or Hypothyroidism (n = 6)*

| Parameters tested | Partial correlation coefficient | | |
|---|---|---|---|
| S-BGP vs. m and $Cl_{cr}$ | $r_{13\cdot2} = 0.82$ ($P < 0.0001$) | $r_{12\cdot3} = -0.62$ ($P < 0.0005$) | |
| S-BGP vs. m, and $Cl_{cr}$ and r | $r_{13\cdot24} = 0.49$ ($P < 0.02$) | $r_{12\cdot34} = -0.62$ ($P < 0.005$) | $r_{14\cdot23} = 0.18$ ($P > 0.40$) |
| U-OHP vs. m and r | $r_{53\cdot4} = 0.17$ ($P < 0.40$) | | $r_{54\cdot3} = 0.38$ ($P = 0.06$) |
| S-AP vs. m and r | $r_{63\cdot4} = 0.36$ ($P = 0.06$) | | $r_{64\cdot3} = 0.17$ ($P > 0.40$) |

r, partial correlation coefficient; P, level of significance for deviation of the coefficient from zero.
S-BGP: 1, m: 3, log(U-OHP): 5, $Cl_{cr}$; 2, r: 4, log(S-AP): 6.
* Performed using the GLIM computer program.

analysis. However, multiple regression analysis (Table II) disclosed that S-BGP correlated to m ($r = 0.82$, $P < 0.0001$) when $Cl_{cr}$ was taken into account, and to $Cl_{cr}$ ($r = -0.62$, $P < 0.005$), when m was taken into account. A significant partial correlation was still observed between S-BGP and m ($r = 0.49$, $P < 0.02$) and between S-BGP and $Cl_{cr}$ ($r = -0.62$, $P < 0.005$) when r was considered in the analysis. Linear regression analysis also showed a positive correlation between S-BGP and r ($r = 0.67$, $P < 0.001$). However, the significance of this correlation was lost when the influence of m and $Cl_{cr}$ on S-BGP was taken into account (partial correlation = 0.18, $P > 0.40$) (Table II).

Linear regression analysis demonstrated a significant positive correlation between S-AP and m ($r = 0.45$, $P < 0.05$) (Table I), whereas no significant correlation was found between S-AP and S-BGP ($r = 0.30$, $P > 0.10$) or S-BGP × $Cl_{cr}$ ($r = 0.33$, $P > 0.10$). Moreover, highly significant positive correlations were found between U-OHP and both r ($r = 0.77$, $P < 0.001$) and m ($r = 0.74$, $P < 0.001$) (Table I). Multiple regression analysis (Table II) showed no significant partial correlations between these parameters, although the partial correlation between U-OHP and r almost reached the commonly accepted significance level. A significant positive correlation was found between U-OHP and S-BGP ($r = 0.44$, $P < 0.05$), and between U-OHP and S-BGP × $Cl_{cr}$ ($r = 0.68$, $P < 0.001$).

In accordance with the observation above, that S-BGP depended on both m and $Cl_{cr}$, a stronger correlation was found between S-BGP × $Cl_{cr}$ and m than between S-BGP and m (Fig. 2). In the patients studied m could be predicted from the corrected S-BGP with an average error of 3.2 mmol Ca/d, whereas the error of the estimate was 5.2 mmol Ca/d using the uncorrected S-BGP, and 6.7 mmol Ca/d using S-AP (Table I); r could be estimated with an error of 4.7 mmol Ca/d using U-OHP.

Table III contains the results of linear regression analysis performed to evaluate whether identical relations exist between m and S-BGP, and between m and S-BGP × $Cl_{cr}$ in patients with thyroid and parathyroid disorders. For both types of metabolic disturbances, the level of significance and the standard error of estimate improved after correction of S-BGP for changes in renal function. Furthermore, following this correction, no significant differences were found between the two types of disorders in either the slope or the intercept of the regression lines.

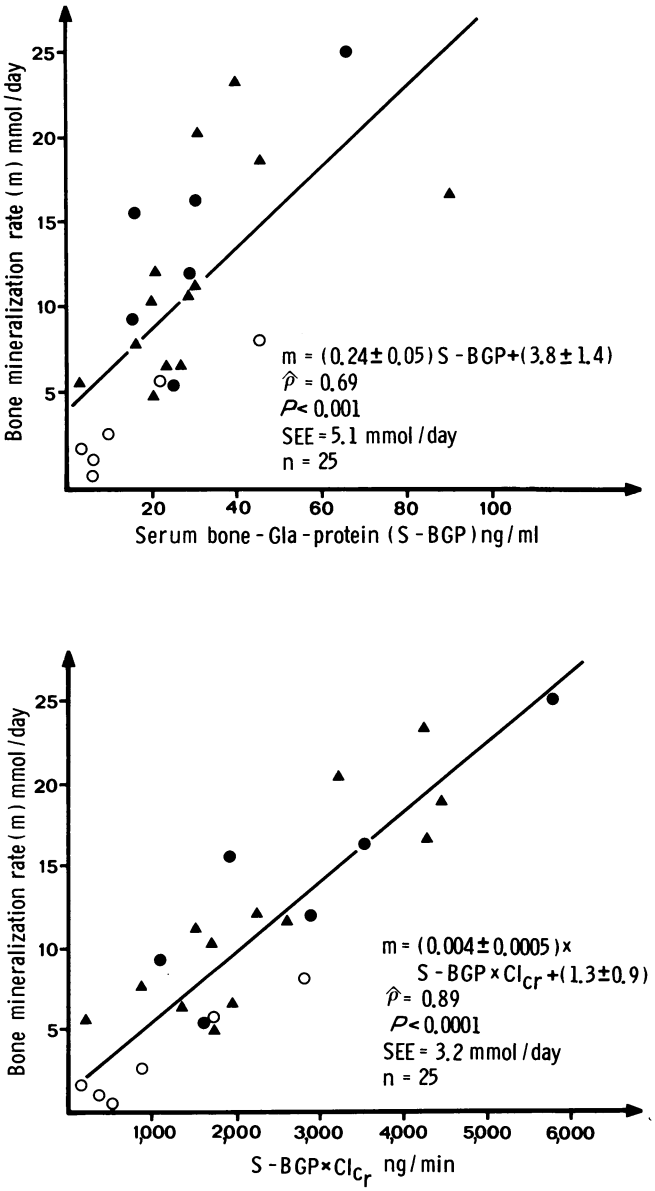


*Figure 2.* Linear regression analysis of the relationship between S-BGP and m without (*top*) and with (*bottom*) correction of S-BGP for renal function by creatinine clearance ($Cl_{cr}$) in 25 patients with hyperparathyroidism (▲), hyperthyroidism (●), or hypothyroidism (○). SEE, standard error of estimate.

## Discussion

Our data demonstrate that S-BGP levels depend on m and renal function in the 25 patients with parathyroid and thyroid disorders

*Table III. Linear Regression Analysis of the Relationship between S-BGP and m, and between S-BGP × $Cl_{cr}$ and m in Thyroid and Parathyroid Disorders**

| | Regression line | | Standard error of estimate of m from the fitted model SEE | r | P |
|---|---|---|---|---|---|
| | Slope $k_3$ (SE) | Intercept $q$ (SE) | | | |
| | | | *nmol Ca/day* | | |
| S-BGP vs. m | | | | | |
| Thyroid disorders ($n$ = 12) | 0.33 (0.08) | 1.05 (2.33)‡ | 4.88 | 0.79 | <0.001 |
| Hyperparathyroidism ($n$ = 13) | 0.17 (0.07) | 6.85 (2.63)§ | 5.18 | 0.57 | <0.01 |
| Comparison between groups ($P$) | NS | <0.01 | NS | | |
| S-BGP × $Cl_{cr}$ vs. m | | | | | |
| Thyroid disorders ($n$ = 12) | 0.0041 (0.0005) | 0.28 (1.50)‡ | 3.23 | 0.91 | <0.0001 |
| Hyperparathyroidism ($n$ = 13) | 0.0038 (0.0008) | 2.82 (1.78)‡ | 3.10 | 0.88 | <0.0001 |
| Comparison between groups ($P$) | NS | NS | NS | | |

* Dependent variable: m; independent variables: S-BGP and S-BGP × $Cl_{cr}$. ‡ Not significantly different from zero. § Significantly different from zero ($P < 0.05$).

evaluated in this study. In a study involving normal human subjects and patients with varying degrees of renal failure, Delmas et al. (16) have shown that plasma BGP varies with glomerular filtration rate (GFR) in an exponential manner (plasma BGP = $5.06 + 66.14\ e^{-0.083} \times$ GFR). That study provided the clinical corollary to tracerkinetic studies in rats in which it was shown that BGP is cleared from plasma by kidneys (6). The available data appear to support the conclusion that the degradation of BGP is dependent on renal function as well as the serum level of BGP. Delmas et al. (16) concluded, however, that plasma BGP should be interpreted in relation to renal function only in patients with GFR values below 30 ml/min × 1.73 $m^2$. In the present study we found the serum level of BGP to be highly dependent on renal function, even at $Cl_{cr}$ rates > 40 ml/min × 1.73 $m^2$. The dependence of S-BGP on renal function within the normal range may to some extent explain the observed increase in S-BGP with age among normal individuals (17, 18).

In the study described here we deliberately chose patients with a wide spectrum of bone turnover rates, but who were not suspected to have mineralization defects, since estimation of m by calcium kinetics may be compromised in patients with osteomalacia or rickets (19). The increased amount of osteoid observed in some hyperparathyroid and hyperthyroid patients (10) is not caused by a mineralization defect with increased osteoid seam width, as is seen in osteomalacia, but rather is secondary to the enhanced activation of new remodeling cycles leading to increased osteoid surface. Furthermore, in the estimation of m, the applied expanding exchangeable calcium pool model compensates for variation in the amount of long term exchangeable calcium (14, 20).

We found no significant partial correlation between S-BGP and r when the influence of renal function and m was taken into account. These findings are corroborated by a histomorphometric study on 26 postmenopausal women with osteoporosis (21) which showed a positive correlation between S-BGP and tetracycline-based indices of bone formation at tissue level, but not to resorption surfaces. Furthermore, in vitro studies on rat osteogenic sarcoma cells (22) and studies on warfarin-treated rats (6) support the concept that BGP is produced by osteoblasts during de novo synthesis of matrix proteins.

S-BGP corrected for the influence of variations in renal function (S-BGP × $Cl_{cr}$) was found to be more accurate than the uncorrected S-BGP in predicting the m. Furthermore, no significant differences were found between the slopes or intercepts of the regression lines describing the relations between the corrected S-BGP and m in either thyroid or parathyroid disorders. Hence, in the investigated patients, the observed relationship appears to be independent of the pathophysiological mechanisms for the altered bone turnover.

The highly significant correlation found between m and r in the present study is consistent with the well-known coupling between resorption and formation at tissue level (23). This coupling insures the integrity of bone mass in spite of large variations in bone remodeling rates. However, a negative correlation between bone balance and m was observed. This may be partly explained by an expansion of the remodeling space in a non-steady state situation with increasing bone turnover due to the sequential separation between bone resorption and formation, where the former always precedes the latter. Also contributing may be an acceleration of an age-related or -induced negative net balance per remodeling cycle. This close coupling between bone resorption and mineralization might explain why S-BGP, S-BGP × $Cl_{cr}$, and U-OHP all showed significant correlations to both m and r, and a positive correlation between S-BGP, S-BGP × $Cl_{cr}$, and U-OHP using linear least squares regression analysis. However, no significant partial correlation was found between r and S-BGP when multiple regression analysis was used to correct for the influence of m and renal function. By linear regression analysis, S-BGP corrected for renal function showed a higher correlation with m than did S-BGP alone. In addition, the correlation between S-BGP and m was improved by conditioning $Cl_{cr}$ in the multiple regression analysis.

In the present study we found S-BGP was superior to S-AP in predicting m, especially after S-BGP is corrected for variations in renal function. We did, however, find a significant positive correlation between S-AP and m, consistent with the observations of Klein et al. (24).

The apparently better predictive value of S-BGP may be due to the fact that alkaline phosphatase is derived from a number of different tissues with several production and degradation rates

(8). In the thyroid disorders, for instance, the degradation of alkaline phosphatase may be enhanced in hyperthyroidism and reduced in hypothyroidism, resulting in a smaller variation in S-AP than expected from the variations in bone turnover.

This study has demonstrated that serum levels of BGP in the studied calcium metabolic disorders depend on both the m and the renal function. Moreover, in the investigated patients, S-BGP corrected for alterations in renal function were superior to uncorrected values and to S-AP in the estimation of m.

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