Serum collagenase-like peptidase activity correlated with bone mineral density

A study of 102 elderly women

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To clarify the relationship between collagenase-like peptidase (CL-peptidase) and bone metabolism, serum CL-peptidase activity and bone mineral density (BMD) in the lumbar spine, measured by dual energy X-ray absorptiometry (DEXA), were determined in 102 women with a mean age of 70 (41–94) years. The serum activity of CL-peptidase increased slightly with age up to the 8th decade, and then decreased. Next, we classified the subjects into the following 2 groups by age: a postmenopausal group 50–69 years, and an elderly group over 70 years. In the younger group serum CL-peptidase activity did not correlate with BMD, while in the elderly it did. Our result suggests that serum CL-peptidase activity may reflect bone resorption in elderly women.

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PZ-peptidase is an endopeptidase that cleaves the synthetic chromatogenic substrate developed for bacterial collagenase. This enzyme is distinct from animal collagenase which attacks native collagen, but there have been several reports suggesting a relationship between PZ-peptidase and the degradation of collagen in vivo. Thus PZ-peptidase is assumed to be a collagenase-like peptidase (Iwase-Okada et al. 1985, Ito et al. 1987). CL-peptidase is assumed to play an important role in collagen breakdown, probably after the initial cleavage of collagen molecules by animal collagenase. Since bone contains much of the total body collagen subjected to high metabolic activity, CL-peptidase may degrade bone collagen metabolites. However, little information is available on serum CL-peptidase in relation to osteoporosis. The recent introduction of dual energy X-ray absorptiometry (DEXA) has made it possible to measure bone mineral density (BMD) at least as accurately and objectively as with previous techniques (Mazess 1989, Fogelman 1990, Ho et al. 1990, Haarbo 1991). Using DEXA, we studied the relationship between serum CL-peptidase activity and BMD to determine whether the enzyme activity reflects bone metabolism.

Material and methods

115 women, 94 patients complaining of lumbago and 21 volunteers, were studied. 6 patients, 2 with rheumatoid arthritis, 2 with hyperthyroidism, 1 with multiple myeloma and 1 with bone metastasis were excluded. 4 patients with hepatic disorders affecting serum activities of CL-peptidase were also excluded. 3 additional patients with apparently inaccurate BMD determinations due to marked spinal osteophytes were also excluded from consideration. None of the subjects had received fluoride or bisphosphonates at any time or had received calcium, vitamin D, calcitonin, or estrogens during the 6-month period preceding the study. The remaining 102 women with a mean age of 70 (41–94) years were evaluated. Informed consent was obtained from these subjects prior to BMD measurements and blood sampling. CL-peptidase activity was measured with Succinyl-Gly-Pro-Leu-Gly-Pro-4-methylcoumaryl-7-amide (MCA) as a substrate, using Kojima's method (Kojima et al. 1979). This method consists of 2 steps. First, the CL-peptidase reaction was done, followed by the dipeptidyl peptidase IV (DPP IV) reaction, after which the released 7-amino-4-methylcoumarin (AMC) was assayed. The reaction mixture (total volume, 0.2 mL) contained 50 µL of 0.2 M Tris-maleate buffer (pH 8.0) with 20 mM $CaCl_2$, 50 µL of Suc-Gly-Pro-Leu-Gly-Pro-MCA, and 100 µL of

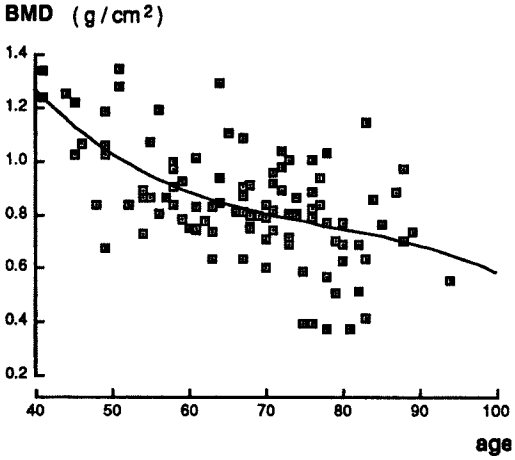

Figure 1. Relation between BMD and age. BMD fell steeply from the age of 40 to the mid-60s and then more slowly (r 0.6, P 0.0001).

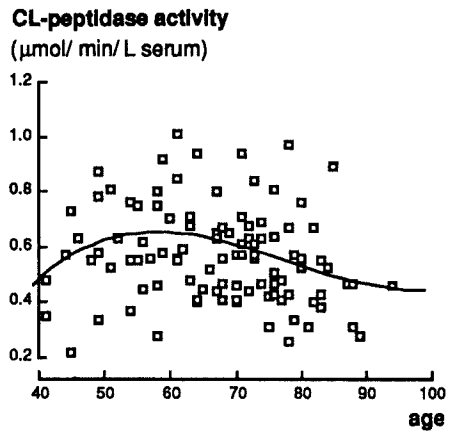

Figure 2. Relation between serum CL-peptidase activity and age. The CL-peptidase activity increased in the post-menopausal period, and then decreased (r 0.3, P 0.03).

human serum. Instead of serum, the blank and standard tubes, respectively, contained 100 μL of water and 90 μL of water plus 10 μL (1.00 nmol) of 100 mol/L AMC reagent. All samples were incubated at 37 °C for 60 min. AMC released by the enzyme reaction was calculated as follows: (Sample-Control)/(Standard-Blank) × 1 nmol/60 min/100 μL. BMD at the 2nd–4th lumbar spinal level was measured by DEXA (DPX, Lunar Radiation Corporation, Madison, WI) in the frontal projection. DPX of the lumbar spine was performed with each subject supine and the lower legs elevated on a block to minimize lordosis.

All values were expressed as means (SE). The relationships between age and BMD, and the serum activity of CL-peptidase were analyzed by polynomial regression analysis. The correlation of serum CL-peptidase activity with BMD was analyzed by simple regression analysis.

Results

The mean age, BMD, and CL-peptidase activity of the volunteers were 65 (3.9) years, 0.886 (0.038) g/cm^2, and 0.606 (0.041) μmol/min/L, respectively, with none of these values differing from the corresponding values of the patients whose chief complaint was lumbago [65 (1.1) years, 0.868 (0.019) g/cm^2, and 0.592 (0.017) μmol/min/L respectively], indicating that the subjects were very similar. Since no significant difference was found in the BMD values between the patients and volunteers, these data were combined and analyzed together. The average BMD was 0.862 (0.019) g/cm^2. BMD fell steeply from age 40 years to age 60 years, and then more slowly after 60 years (r 0.581, P 0.0001) (Figure 1). The average serum CL-peptidase activity was 0.57 (0.02) μmol/min/L. The serum CL-peptidase activity increased in the postmenopausal period, and then decreased in the elderly (Figure 2). Next, we classified the subjects into the following 2 groups by age: a postmenopausal group (50–69 years old) and an elderly group (more than 70 years old) after Riggs and Melton (1983). The relationship between BMD and serum CL-peptidase activity was also calculated by single regression analysis. In the postmenopausal group, serum activity of CL-peptidase did not correlate with BMD (r 0.031, N.S). In the elderly, however, serum CL-peptidase activity showed a correlation with BMD (Figure 3).

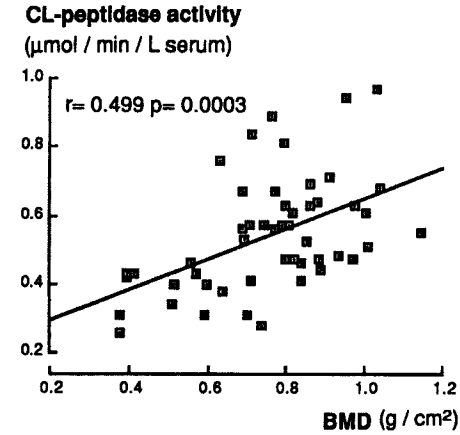

Figure 3. Scatter plot with linear regression line demonstrating a significant correlation between serum CL-peptidase activity and BMD in the elderly group (r 0.5, P 0.0003).

Discussion

Finding a sensitive and specific marker of bone resorption is a challenge, because all constituents of bone matrix are likely to be degraded into minute peptides during osteoclastic bone resorption. Urinary hydroxyproline formed by the posttranslational modification of proline residues in collagen and urinary pyridinium crosslinks generated from lysine and hydroxylysine residues are the most useful, but these markers require further validation (Delmas et al. 1991). In our previous reports, the activity of CL-peptidase in serum was found to be a diagnostic index in some diseases, such as rheumatoid arthritis and systemic lupus erythematosus (Iwase-Okada et al. 1985, Ito et al. 1987). CL-peptidase is thought to play a role in the degradation of peptide derived from collagen, which contains a high proportion of repeating Gly-Pro-X triplets, because CL-peptidase preferentially hydrolyzes the Gly-Pro sequence. Thus, CL-peptidase may process minute peptides released from bone matrix during bone resorption. The result of this study suggests that the activity of serum CL-peptidase, like that of other biochemical markers, increases in the postmenopausal period, and decreases in the elderly (Kelly et al. 1989). There was a significant correlation between serum CL-peptidase and BMD in the elderly group. This result is interpreted as reflecting decreased collagen breakdown in elderly women with low BMD in the lumbar spine. Thus the serum activity of CL-peptidase may reflect bone resorption indirectly. However, in the postmenopausal group, no correlations were obtained in our series, probably because (1) the time of menopause was not precisely determined and (2) in the postmenopausal period bone turnover is not necessarily high, a mixture of low and/or normal bone turnover being found (Delmas et al. 1983, Brown et al. 1984, Delmas 1992). Our result suggests that serum CL-peptidase activity may reflect bone resorption in elderly women whose bone turnover is likely to be homogeneously low.

We conclude that serum CL-peptidase activity decreases with age and has a positive correlation with BMD in the lumber spine of elderly women.

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