Bone resorption in healthy and osteoporotic postmenopausal women: comparison markers for serum carboxy-terminal telopeptide of type I collagen and urinary pyridinium cross-links

Matti J Välimäki1, Riitta Tähtelä2, James D Jones3, James M Peterson4 and B Lawrence Riggs4

Third Department of Medicine1, Helsinki University Central Hospital, Helsinki, Finland; United Laboratories2, Helsinki, Finland; Department of Laboratory Medicine and Pathology3 and Endocrine Research Unit4, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, USA

We compared two highly specific markers for bone resorption—pyridinium cross-links (pyridinoline (PYR) and deoxypyridinoline (DPR)) in urine and carboxy-terminal telopeptide of type I collagen (ICTP) in serum—in 63 healthy postmenopausal women and 63 women with osteoporosis characterized by more bone resorption than bone formation. The ICTP, PYR and DPR levels were all higher, by 24% (p = 0.001), 16% (p = 0.05) and 25% (p = 0.004), respectively, in the osteoporotic women. For the merged groups, there were significant correlations between serum ICTP concentration and urinary PYR (r = 0.667, p < 0.0001) and DPR (r = 0.452, p < 0.0001) excretion: for the osteoporotic and normal women separately, the r values were 0.73 (p < 0.01) and 0.45 (p < 0.01) for PYR and 0.51 (p < 0.01) and 0.22 (p = 0.08) for DPR versus ICTP respectively. Weak correlations in linear regression between ICTP and various indices of bone formation (osteocalcin, bone-specific alkaline phosphatase and carboxy-terminal propeptide of type I procollagen) disappeared when the correlation between ICTP and pyridinolines was accounted for by calculation of partial correlation coefficients in multiple regression analysis. Serum ICTP concentration appears to discriminate between groups of normal and osteoporotic women as well as urinary pyridinium cross-links, which is thus far the most sensitive method for assessing bone resorption.

Matti Välimäki, Third Department of Medicine, Helsinki University Central Hospital, SF-00290 Helsinki, Finland

Pyridinium cross-links (pyridinoline (PYR) and deoxypyridinoline (DPR)) of collagen molecules are present in bone and cartilage and, to a lesser extent, other connective tissues, except skin. They are released into the circulation from bone that is undergoing resorption and then are excreted in the urine. Thus, they hold considerable promise as markers of bone resorption (1, 2) in Paget’s disease, primary hyperparathyroidism, osteomalacia and osteoporosis, particularly for monitoring the response to therapy (3–7).

The structure of type I collagen fibers varies according to the tissue it is in. In bone, the type I collagen molecules are cross-linked by means of three residues of hydroxylysine, lysine and their derivatives, which form a cyclic PYR structure: in loose connective tissues, such as skin, the cross-links contain histidine. The carboxy-terminal telopeptide region of type I collagen (ICTP) in bone contains pyridinium cross-links that are liberated during degradation (8). The ICTP concentration in serum correlates with the bone resorption rate measured either histomorphometrically or by calcium kinetic studies (9, 10). Because it is measured in serum, the ICTP value might be superior to the urinary PYR + DPR value if the information provided by the two tests is equally valid.

In the study in healthy and in osteoporotic postmenopausal women reported here, we compared the serum ICTP concentration and the excretion of urinary pyridinium cross-links with each other and with biochemical markers of bone formation.

Subjects and methods

Subjects

We studied 63 healthy women aged 50–79 years (mean ± SD, 65 ± 6 years) who were 3–40 years postmenopausal. They had no disease and were not using drugs known to affect bone metabolism. No subject in this group had a vertebral fracture evident on anteroposterior or lateral radiographs of the lumbar or thoracic spine. We also studied 63 women aged 50–74 years (65 ± 5 years) who had one or more vertebral fractures and were 1–36 years postmenopausal. All of these fractures had occurred after minimal or no trauma. The subjects had never taken sodium...
fluoride, had not taken estrogens within the preceding 6 months and had not taken calcium within the preceding 3 months. The studies were approved by the Mayo Institutional Review Board.

Methods

Blood samples for the determination of ICTP, bone-specific alkaline phosphatase (BAP), osteocalcin and carboxy-terminal propeptide of type I procollagen (PICP) were taken after an overnight fast. A 24-h urine sample also was collected for measurement of pyridinium cross-links and creatinine. The samples were obtained from subjects in a previously reported study (11) and were kept frozen at −70°C until thawed for analyses made in the present study. Serum concentrations of osteocalcin and BAP were not repeated. However, serum ICTP and PICP and measurements of urine pyridinium cross-links were new. They were done blind in terms of group allocation.

Serum ICTP was measured by a competitive radioimmunoassay (RIA) with a polyclonal antiserum, raised in rabbits, to ICTP from decalcified human femoral bone (Orion Diagnostica, Oulunsalo, Finland) (8). The intra-assay variation was less than 8%; the interassay variation was less than 7%. Serum BAP was determined by ELISA (12) using mouse monoclonal antibody to BAP (interassay variation, 13%; intra-assay variation, 6%). Serum osteocalcin was measured by RIA (12) with a rabbit antiserum to bovine osteocalcin (interassay variation, 10%; intra-assay variation, 3%). Serum PICP was determined by RIA (13) with rabbit antiserum to PICP purified from skin fibroblasts (interassay variation, 5%; intra-assay variation, 3%).

Total PYR and DPR were quantitated in aliquots of urine hydrolyzed with HCl, prefractionated on a cellulose column (14) and analyzed by a modification of the method of Eyer et al. (15). Intra- and interassay variations were both 5%. Pyridinium and DPR standards were kindly furnished by Dr Simon P Robins. The results were expressed relative to creatinine excretion.

Table 1. Values (mean ± so) for biochemical markers of bone turnover in postmenopausal women.

<table>
<thead>
<tr>
<th>Biochemical marker</th>
<th>Osteoporotic (N = 63)</th>
<th>Control (N = 63)</th>
<th>Percentage difference</th>
<th>p</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICTP (µg/l)</td>
<td>3.97 ± 1.65</td>
<td>3.20 ± 1.11</td>
<td>24.0</td>
<td>0.001</td>
<td>1.8–5.0</td>
</tr>
<tr>
<td>PYR (nmol/mmol Cr)</td>
<td>49.1 ± 16.8</td>
<td>42.5 ± 12.1</td>
<td>16.0</td>
<td>0.05</td>
<td>24.7–52.3</td>
</tr>
<tr>
<td>DPR (nmol/mmol Cr)</td>
<td>16.3 ± 6.4</td>
<td>13 ± 4.8</td>
<td>25.0</td>
<td>0.004</td>
<td>6.0–15.6</td>
</tr>
<tr>
<td>BAP (µg/l)</td>
<td>29.1 ± 11.0</td>
<td>21.1 ± 6.3</td>
<td>38.0</td>
<td>&lt;0.001</td>
<td>5.3–28.3</td>
</tr>
<tr>
<td>Osteocalcin (µg/l)</td>
<td>9.9 ± 2.0</td>
<td>8.9 ± 2.0</td>
<td>11.0</td>
<td>0.003</td>
<td>3.3–11.3</td>
</tr>
<tr>
<td>PICP (µg/l)</td>
<td>130.7 ± 50.7</td>
<td>118.1 ± 57.1</td>
<td>11.0</td>
<td>0.305</td>
<td>38–202</td>
</tr>
</tbody>
</table>


b N = 62.
c N = 31.

d

Fig. 1. Serum carboxy-terminal telopeptide of type I collagen (ICTP) concentrations in normal and in osteoporotic postmenopausal women.

Statistical analysis

The relationship between serum ICTP concentration and urinary PYR excretion was assessed by linear regression using the method of least squares. Differences between the normal and osteoporotic subjects were assessed by the Wilcoxon rank-sum method. The Z scores are: observed value minus predicted mean, divided by $S_{r-x}$ (estimate of variability about the fitted regression line). The CLINFO and SAS data analysis systems were used for the computations.

Results

The mean ICTP concentration was 24% higher (p = 0.001) in the osteoporotic women than in the normal women (Fig. 1 and Table 1). The Z score was 0.67 (p < 0.001) for ICTP, 0.54 (p = 0.05) for urinary excretion of PYR and 0.72 (p = 0.004) for urinary excretion of DPR. The differences were 16% for urinary excretion of PYR (p = 0.05) and 25% for
urinary excretion of DPR (p = 0.004) (Fig. 2 and Table 1). Also, serum BAP, osteocalcin and PICP values were higher in the osteoporotic women than in the normal women (Table 1).

There were statistically significant correlations between serum ICTP and urinary PYR (r = 0.667; p < 0.0001) and DPR (r = 0.452, p < 0.0001) in the study population (Figs. 3 and 4). The separate r values were 0.73 (p < 0.01) and 0.51 (p < 0.01) for osteoporotic women and 0.45 (p < 0.01) and 0.22 (p = 0.08) for normal women, respectively. The ICTP concentration was less closely correlated with BAP (r = 0.27, p = 0.002, N = 126), with osteocalcin (r = 0.295, p < 0.001, N = 125) or with PICP (r = 0.24, p = 0.02, N = 93).

Multiple regression analysis showed that the main correlation was between ICTP and urinary PYRs (r = 0.59, p < 0.0001, N = 93). Partial correlation of ICTP with bone formation markers adjusted for PYR were as follows: BAP, 0.05 (p > 0.5); osteocalcin, 0.07 (p = 0.44); PICP, -0.09 (p = 0.38).

Discussion

Our data are consistent with the suggestion that serum ICTP concentration is an index of bone resorption. It correlated well with urinary PYR and DPR excretion, which are generally recognized markers of bone resorption (1, 2), but correlated less well with serum BAP, osteocalcin and PICP values, which are indices of bone formation. The correlation of ICTP with the markers of bone formation disappeared when the correlation with urinary PYRs was held constant by assessment of partial correlation coefficients. Thus, the correlation between ICTP and the bone formation markers was probably a consequence of the well-established coupling of bone formation to bone resorption (16, 17).

Postmenopausal osteoporosis is believed to be associated with an increase in bone resorption relative to bone formation (18). The distinction between groups of normal and osteoporotic women was at least as good with serum ICTP as with urinary PYR and DPR. The mean increase of 16% in urinary excretion of PYRs in osteoporotic women in the present study is of the same magnitude as the increase (18%) in a similar study by Robins et al. (19): the mean ICTP concentration was 24% higher in osteoporotic women than in normal women. However, there was a large overlap, and the correct classification of an individual subject was not possible on the basis of the serum ICTP value. This large overlap also has been found between women with vertebral or hip fracture and age- and sex-comparable controls (20). Also, all markers evaluated thus far (21) have shown a large overlap, probably due to continuum of true values for bone turnover in postmenopausal osteoporosis that range from normal to high (18).
Moreover, the lower skeletal mass in osteoporotic women may have spuriously lowered the values for a given level of bone cell activity. In individual patients with osteoporosis, these assays may be used in selection of the treatment (18, 22) and in monitoring the response to therapy. Patients with a high rate of bone turnover are more likely to respond to antiresorptive therapy than are those with a normal rate of bone turnover (18, 22).

The present study suggests that the serum ICTP concentration may discriminate just as well between populations of normal and osteoporotic women as the excretion of urinary pyridinium cross-links, the latter being thus far the most sensitive method for assessing bone resorption. In fact, serum ICTP has several practical advantages as a marker of bone resorption. First, although ELISA methods for urinary pyridinium cross-links have started to become available (23, 24), hitherto they have been measured most reliably by a more complicated method using high-performance liquid chromatography. Second, it is clinically advantageous to measure markers for bone resorption and bone formation in the same specimen because bone loss is related to an uncoupling between these two processes. The gap between markers for resorption and markers for formation can be used as an index of this uncoupling (11). At present, the two major markers for bone formation, BAP and osteocalcin, must be measured in serum samples (21). Finally, serum samples can be obtained over shorter intervals of time than urine samples, unless the patient is catheterized, and thus may be of value in physiological research studies in osteoporosis. On the other hand, as all resorption markers (25, 26) exhibit a diurnal variation, an integrated value for urinary cross-links from 24-h collections might be superior to serum ICTP. However, this potential advantage is lost when 24-h urine sampling is replaced by morning collections, which is commonly done for practical reasons.

Acknowledgments. This work was supported by AM-04875 and RR-00585 and by a grant from the Yrjö Jahnsson Foundation, Helsinki, Finland.

References

11. Eastell R, Robins SP, Colwell T, Assiri AMA, Riggs BL, Russell RG.

Fig. 4. Relationship between serum carboxy-terminal telopeptide of type I collagen (ICTP) and urinary deoxypyridinoline in the entire study population.

Received October 29th, 1993
Accepted May 2nd, 1994