Increased matrix concentrations of IGFBP-5 in cancellous bone in osteoarthritis

C A Sharp, S J Brown, M W J Davie, P Magnusson, S Mohan

Background: In osteoarthritis cancellous bone adapts to meet altered mechanical loading. These changes may be mediated by insulin-like growth factors (IGF-I and IGF-II), but the matrix bound binding protein, IGFBP-5 has not been investigated.

Objectives: To measure IGF-I, IGF-II, and IGFBP-5 in femoral head bone from non-OA controls and patients with OA, and to relate these to apparent density (ρA) and elastic modulus (Ec).

Methods: ρA, Ec, and IGF system components were measured in cancellous bone from superior and inferior regions of femoral heads from 31 patients with OA and 11 age selected controls.

Results: Ec and ρA were greater (p<0.05) in the superior region of all femoral heads. In primary OA, ρA was increased in the inferior region (p<0.05). IGFBP-5 was increased, about twofold, at superior and inferior regions in primary OA (1.60 and 1.54 ng/mg bone, respectively, both p<0.05) and in Paget’s disease (2.44 and 1.75 ng/mg bone, both p<0.05) compared with controls (0.73 and 0.95 ng/mg bone). In controls, inverse correlations between IGFBP-5 and both ρA and Ec at superior (r = −0.64 and −0.73, both p<0.05) and inferior regions (r = −0.72, p<0.05 and −0.24 (NS)) were seen, but these were lost in OA.

Conclusions: IGFBP-5 may modulate cancellous bone formation by negative feedback. In end stage OA this is disrupted, but has little influence on material properties.

PATIENTS AND METHODS

Proximal femora were obtained postoperatively (mostly OA) and at necropsy (mostly healthy). After removal, all samples were stored frozen and precautions taken to minimise deterioration and dehydration. Visual examination and radiographs of the femoral heads were combined with clinical records, where available, to group the bones into those with no overt bone or joint disease and those affected by OA. OA was defined by the presence of typical visible features, including fibrillation and wear of the articular cartilage, eburnation and pitting of the subchondral bone, and gross changes such as femoral head deformation and osteophytes. The presence of other bone and joint related disorders was identified from clinical notes.

Cancellous bone cores (12 mm diameter) from the superior and inferior regions were taken perpendicular to the axis of coronal sections (approx 16 mm) cut through the centre of the intact femoral head, cleaned by water jetting, and defatted. Physical and biochemical measurements were performed on most of the cores obtained from 42 subjects aged between 50 and 90 years and classified as healthy (n = 11, 7 male/4 sex unknown), end stage primary OA (n = 21, 7 male/11 female/3 unknown), and OA secondary to Paget’s disease (n = 7, 4 male/3 female) and ankylosing spondylitis (n = 3, 1 male/2 unknown) at sites adjacent to, or other than the femoral head. Apparent density (ρA, g/cm³) was calculated from the hydrated tissue weights and gross volumes of the bone cores, and stiffness (Ec, MPa) by unconfined compression tests.

Each bone core was then powdered under liquid nitrogen, defatted, and lyophilised. Chemical analyses were made on weighed samples of dried bone powder. IGF-I, IGF-II, and IGFBP-5 were extracted from washed bone powders by demineralisation under dissociative conditions (0.5M EDTA, 4 M guanidine-HCl, and protease inhibitors in 30 mM Tris-HCl, pH 7.4). This was repeated four times. Extracts were

Abbreviations: Ec, elastic modulus; IGF, insulin-like growth factor; IGFBP-5, insulin-like growth factor binding protein-5; OA, osteoarthritis; ρA, apparent density
pooled and dialysed (Spectraphor No 3, 3500 Mr cut off point) against 20 mM acetic acid. Dialysed samples were transferred to 15 ml polypropylene tubes and their volumes adjusted to 10 ml with 20 mM acetic acid. A 5 ml aliquot was subjected to speed vacuum centrifugation and reconstituted with 500 µl of 1 M acetic acid and subjected to Bio-Spin separation using Bio-gel P-10 to separate the IGFs from their binding proteins.\(^{10,11}\) The IGF pool was then neutralised and used for IGF-I, IGF-II, and IGFBP-5 determination by validated radioimmunoassays.\(^{81,1}\)

Increased IGFBP-5 in OA bone matrix \(^{1163}\) region (table 1). In all groups, values for both concentrations were greater (p = 0.03) in the superior disease groups except for the combined OA group where IGF-inferior regions in either the controls or individual joint system components were found between the superior and examined. No differences in the concentrations of the IGF approximately threefold) than those of IGF-I in all the bones.

**RESULTS**

**Comparison of superior and inferior regions**

IGF-II concentrations were consistently greater (approximately threefold) than those of IGF-I in all the bones examined. No differences in the concentrations of the IGF system components were found between the superior and inferior regions in either the controls or individual joint disease groups except for the combined OA group where IGF-II concentrations were greater (p = 0.03) in the superior region (table 1). In all groups, values for both \(\rho_A\) and Ec were greater (all p<0.05) at the superior region (table 1).

**Comparison of the healthy and joint disease groups**

Approximately twofold more IGFBP-5 was extracted from both superior (p = 0.0002) and inferior regions (p = 0.004) of the combined OA group than from controls. IGFBP-5 was similarly increased in the primary OA (p<0.0001 and p = 0.004 respectively) and Paget’s disease (p = 0.017 and p = 0.037 respectively) groups (table 1). IGFBP-5 in those with ankylosing spondylitis was not markedly different in either region (0.98 (0.4–3.0) and 1.05 (0.4–1.8) ng/mg, respectively) from controls.

In controls, strong inverse relationships were found between IGFBP-5 and \(\rho_A\) at both superior (\(r_s = -0.64, p = 0.048\)) and inferior regions (\(r_s = -0.72, p = 0.013\)) (fig 1). Similar trends with Ec were also found at these sites (\(r_s = -0.73, p = 0.016\) and \(r_s = -0.24, p = 0.48\), respectively) (fig 2). No significant associations were found in either the primary or secondary OA groups.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>IGFBP-5 (ng/mg bone)</th>
<th>IGF-I (ng/mg bone)</th>
<th>IGF-II (ng/mg bone)</th>
<th>Apparent density (g/cm(^3))</th>
<th>Stiffness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Superior</td>
<td>0.73</td>
<td>(0.14–1.10)</td>
<td>0.06</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Inferior</td>
<td>0.95</td>
<td>(0.18–1.90)</td>
<td>0.02–0.11</td>
<td>0.04–0.09</td>
<td>0.05–0.41</td>
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<tr>
<td><strong>Primary OA</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Superior</td>
<td>1.60(^{\text{e}})</td>
<td>(0.75–3.79)</td>
<td>0.09</td>
<td>0.16</td>
<td>0.73(^{\text{e}})</td>
</tr>
<tr>
<td>Inferior</td>
<td>1.54(^{\text{b}})</td>
<td>(0.70–2.96)</td>
<td>0.03–0.24</td>
<td>0.11–0.31</td>
<td>0.47(^{\text{a}})</td>
</tr>
<tr>
<td><strong>Secondary OA—Paget’s disease</strong></td>
<td></td>
<td></td>
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<tr>
<td>Superior</td>
<td>2.44(^{\text{a}})</td>
<td>(0.70–4.99)</td>
<td>0.06</td>
<td>0.20</td>
<td>0.66(^{\text{a}})</td>
</tr>
<tr>
<td>Inferior</td>
<td>1.75(^{\text{a}})</td>
<td>(0.70–3.19)</td>
<td>0.02–0.06</td>
<td>0.19–0.32</td>
<td>0.45–1.15</td>
</tr>
<tr>
<td><strong>Combined OA group</strong></td>
<td></td>
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<tr>
<td>Superior</td>
<td>1.75(^{\text{d}})</td>
<td>(0.38–4.99)</td>
<td>0.08</td>
<td>0.22(^{\text{a}})</td>
<td>0.68(^{\text{a}})</td>
</tr>
<tr>
<td>Inferior</td>
<td>1.54(^{\text{b}})</td>
<td>(0.38–3.19)</td>
<td>0.02–0.24</td>
<td>0.11–0.37</td>
<td>0.41(^{\text{a}})</td>
</tr>
</tbody>
</table>

Data are presented as median values, ranges, and the number of subjects (n). Differences between the healthy controls and OA groups are indicated by *, and between regions by †. Levels of statistical significance are denoted by a, p<0.05; b, p<0.005; c, p<0.001; d, p<0.0005; and e, p<0.0005.

![Figure 1](www.annrheumdis.com)
interaction with binding proteins. IGFBP-4 is the major IGF
normal conditions IGF activities are modulated by their
effects of the IGFs and IGFBP-5 on bone metabolism. Under
stimulates bone cell proliferation14 through both IGF depen-
ture.12 Bony changes may be partly explained by the known
effects of the IGFs and IGFBP-5 on bone metabolism. Under
normal conditions IGF activities are modulated by their
interaction with binding proteins. IGFBP-4 is the major IGF
binding protein produced in vitro by human osteoblasts and a
potent inhibitor of IGF induced bone cell proliferation13 15 and
not measured in this study. In contrast with this, IGFBP-5
not only binds to and stores the IGFs in bone but also
stimulates bone cell proliferation16 through both IGF depen-
dent and IGF independent mechanisms.7 Here, and in
contrast with other reports,7 both IGF-I and IGF-II were
not significantly different in OA, but the relationships
between the individual growth factors were conserved.
However, we found approximately twofold more IGFBP-5
in extracts of femoral head cancellous bone from patients
with end stage primary OA and in those with OA secondary
to Paget’s disease elsewhere in the skeleton than in healthy
bones.

Accumulation of matrix IGFBP-5 may be accounted for by
increased cellular production, reduced destruction, or
decreased removal from bone. Alternatively, differences
between the sources of the material may have influenced
the stability of matrix bound IGFBP-5, leading to degradation
of IGFBP-5 and loss of IGFBP-5 immunoreactivity in the
healthy bones. Under appropriate conditions IGF binding
proteins are degraded by IGFBP proteases, also produced by
bone cells.12 However, it is unlikely that differences in IGFBP-
5 stability alone contribute to the findings reported here for
the following reasons: firstly, much of the IGFBP-5 is
embedded within the mineralised matrix which stabilises it
and limits proteolytic degradation; secondly, the IGFBP-5
assay detects both intact and degraded fragments, and
therefore limited proteolysis should not have significantly
compromised the IGFBP-5 measurements in the necropsy
samples; and thirdly, if IGFBP-5 in the necropsy specimens
had degraded it might be expected that correlations would
not be found between IGFBP-5 and the material properties in
this material.

We can speculate as to the physiological effects of
increased IGFBP-5 in the matrix of OA bone. Firstly, in
healthy bone, for which we have relatively few samples,
inverse correlations between IGFBP-5 and \( \rho_a \) and Ec would
be expected if IGFBP-5 were involved in the modulation
of bone formation through a negative feedback mechanism.
This hypothesis is supported by the loss of these correlations
in OA bone (figs 1 and 2) indicating a breakdown of control,
with increased IGFBP-5 responsible for maintaining \( \rho_a \),
possibly by stimulating bone formation and tissue deposi-
tion. Interestingly, our finding of inverse correlations with
IGFBP-5 and material and mechanical parameters in healthy
bone is supported by a recent study reporting similar
findings between cortical bone IGFBP-5 and bone mineral
density in postmenopausal women with osteoporosis.16
Secondly, if coupled bone turnover is increased, osteoclast
mediated bone resorption may release matrix bound
IGFBP-5 and other anabolic agents such as IGFs and
transforming growth factor \( \beta \), further potentiating bone
cell activity. These factors may provide an environment
which can maintain an active matrix producing osteoblastic
phenotype that can respond to the altered dynamic loads
experienced in joint disease by remodelling cancellous bone
architecture.

In conclusion, we find increased matrix concentrations
of IGFBP-5 at femoral head sites in end stage OA.
Although this may preserve coupled bone remodelling
and maintain bone mass, it does not necessarily improve
bone strength.

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