CONCISE REPORT

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

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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 (ρ_A) and elastic modulus (Ec).

Methods: ρ_A , 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 ρ_A were greater ($p < 0.05$) in the superior region of all femoral heads. In primary OA, ρ_A 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 ρ_A and Ec at superior ($r_s = -0.64$ and -0.73 , both $p < 0.05$) and inferior regions ($r_s = -0.72$, $p < 0.05$ 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.

The osteoarthropathies are common disorders in Western societies, with knee and hip osteoarthritis (OA) affecting 10–25% of people in retirement. OA can be classified as primary when the diagnosis excludes secondary factors, such as Paget's disease, at adjacent skeletal sites that affect the joint. In the hip, end stage OA is characterised by progressive changes to the cartilage, the underlying calcified cartilage, and the cancellous bone network.

Investigations of the material and compositional properties of OA bone suggest increased proportions of less-well mineralised bone^{1,2} with structural changes to the cancellous network that are different from, and in some respects opposite to, those associated with healthy aging.³ The amount of bone present, expressed as apparent density, is important because it is a major determinant of the mechanical competence of bone.⁴ Cancellous bone from femoral heads affected by end stage OA has greater apparent density but is no stiffer than healthy bone.^{1,2}

Gross structural changes to the cancellous network are the result of increased bone cell activity and matrix turnover.^{5,6} The factors that mediate these processes in bone are uncertain. The insulin-like growth factors (IGFs) are some of the most abundant factors produced by bone cells and

have been implicated in maintaining bone mass in OA.⁷ IGFs are stored in human bone matrix mainly through their low affinity interaction with IGF binding protein 5 (IGFBP-5).⁸ In bone, IGFBP-5 not only limits the availability of IGF with its receptor, thus modulating IGF actions at the tissue level, but also has stimulatory effects on osteoblasts that are independent of IGF.⁹ To date the matrix associated IGFBPs have not been studied in OA bone. Here we have investigated bone matrix concentrations of IGF-I, IGF-II and, for the first time, their matrix bound binding protein IGFBP-5 in cancellous bone cores sampled from two differently loaded regions of the femoral head in subjects with primary and secondary OA, and in an age selected healthy control group. These have been related to material and mechanical measurements.

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.² 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 (ρ_A , g/cm³) was calculated from the hydrated tissue weights and gross volumes of the bone cores, and stiffness (Ec, MPa) by unconfined compression tests.²

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; ρ_A , 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.^{8 11}

The Mann-Whitney U test was used to test for differences between and within groups, and Spearman rank for correlations. Biochemical findings are expressed as mass of analyte per mg of dry bone powder (ng/mg bone powder). Data are presented as medians with ranges, and significance defined as $p \leq 0.05$. Groups are defined as healthy controls, primary OA, secondary OA, and a combined OA group—the sum of those with primary and secondary OA. The study was approved by the hospital research committee and was conducted in accordance with the Declaration of Helsinki.

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 ρ_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 the primary OA and combined OA groups median values for ρ_A were increased in both superior and inferior regions compared with controls, but differences were significant only at the inferior regions ($p = 0.01$ and $p = 0.035$, respectively) (table 1). Values for Ec did not differ significantly between groups.

Relationships between IGF system components and ρ_A and Ec

In controls, strong inverse relationships were found between IGFBP-5 and ρ_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.

Figure 1 Relationships between ρ_A of the bone cores and IGFBP-5 extracted from the bone matrix. Triangles = healthy controls, circles = primary OA, diamonds = secondary OA (Paget's disease). Open symbols represent inferior and closed symbols the superior regions. Solid and broken lines illustrate linear correlations in the superior and inferior regions of the healthy control group, respectively.

Table 1 Summary of the material and physical properties of the cancellous bone cores and concentrations of the IGF system components measured in extracts of bone sampled from the superior and inferior regions of femoral heads									
IGFBP-5 (ng/mg bone)		IGF-I (ng/mg bone)		IGF-II (ng/mg bone)		Apparent density (g/cm³)		Stiffness (MPa)	
Superior	Inferior	Superior	Inferior	Superior	Inferior	Superior	Inferior	Superior	Inferior
<i>Healthy</i>									
0.73	0.95	0.06	0.06	0.21	0.21	0.62†c	0.29	247†c	51
(0.14–1.10)	(0.18–1.90)	(0.02–0.11)	(0.04–0.09)	(0.05–0.41)	(0.12–0.34)	(0.40–1.01)	(0.20–0.57)	(69–548)	(37–167)
10	11	10	11	10	11	11	11	11	11
<i>Primary OA</i>									
1.60*e	1.54*b	0.09	0.07	0.22	0.16	0.73†e	0.43*a	226†d	58
(0.75–3.79)	(0.70–2.96)	(0.03–0.24)	(0.02–0.18)	(0.11–0.31)	(0.09–0.32)	(0.44–1.07)	(0.27–0.75)	(40–598)	(8–298)
18	17	18	17	18	17	21	19	21	19
<i>Secondary OA—Paget's disease</i>									
2.44*a	1.75*a	0.06	0.05	0.23	0.20	0.66†a	0.41	239 †b	58
(0.70–4.99)	(0.70–3.19)	(0.02–0.06)	(0.02–0.16)	(0.19–0.32)	(0.17–0.29)	(0.45–1.15)	(0.23–0.84)	(154–791)	(23–151)
6	7	6	7	6	7	7	7	7	7
<i>Combined OA group</i>									
1.75*d	1.54*b	0.08	0.05	0.22†a	0.18	0.68†e	0.41*a	239†e	58
(0.38–4.99)	(0.38–3.19)	(0.02–0.24)	(0.02–0.18)	(0.11–0.37)	(0.09–0.32)	(0.44–1.15)	(0.22–0.84)	(40–791)	(8–298)
27	27	27	27	27	27	31	29	31	29
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, $p < 0.0001$.

Figure 2 Relationships between the compressive modulus of the bone cores and IGFBP-5 extracted from the bone matrix. Triangles = healthy controls, circles = primary OA, diamonds = secondary OA (Paget's disease). Open symbols represent inferior and closed symbols the superior regions. Solid and broken lines illustrate linear correlations in the superior and inferior regions of the healthy control group, respectively.

DISCUSSION

Our finding of significantly increased ρ_A at the inferior sites of OA femoral heads supports previous work,¹ and suggests bone formation within the OA femoral head. This may explain the observed changes in cancellous bone architecture.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 proliferation^{8,13} 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 proliferation¹⁴ through both IGF dependent and IGF independent mechanisms.⁹ 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.¹⁵ 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 ρ_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 ρ_A , possibly by stimulating bone formation and tissue deposition. 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 β ,⁶ 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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