Prevalence and possible pathological significance of calcium phosphate salt accumulation in tendon matrix degeneration

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Abstract

***Objectives*—To investigate the prevalence of calcium phosphate mineral salt accumulation in degenerative supraspinatus 'tendinitis' compared with a normal sample of human tendons, and to determine whether there is an association of calcium salt deposition with pathological changes in the tendon extracellular matrix.**

***Methods*—Cadaver tendons (supraspinatus and common biceps tendons, n = 96) and fragments of supraspinatus tendons obtained during shoulder surgery (n = 31) were analysed for calcium content by atomic absorption spectroscopy, phosphorous content using a spectrophotometric assay, and matrix composition (collagen, glycosaminoglycans and DNA) using standard biochemical techniques.**

***Results*—We established baseline values of calcium concentration in macroscopically normal cadaver tendons (mean 1·1 (SD 0·35) μg/mg dry wt, n = 60) and found that 33% (nine of 27) of ruptured tendons from patients with 'degenerative tendinitis' contained an excess of calcium (more than 2SD greater than the normal sample mean). Five of these specimens had increased concentrations of phosphorous and calcium:phosphorous (molar) ratios consistent with a variety of possible calcium crystals, including calcium pyrophosphate, hydroxyapatite, and tricalcium phosphate, in addition to mixed or amorphous calcium phosphate deposits. Four of these specimens contained normal concentrations of phosphorous, consistent with deposits of calcium oxalate or calcium carbonate, although this was not confirmed biochemically. In contrast, surgical specimens (n = 4) from patients with 'calcifying tendinitis' (radiographically detected calcium deposits) all contained salts with a mineral composition consistent with hydroxyapatite. The presence and identity of crystal deposits was subsequently confirmed in five specimens by radiographic microanalysis. Analysis of the tendon matrix demonstrated a number of significant differences between normal and degenerate (ruptured) tendons, including a reduction in collagen content, an increase in sulphated glycosaminoglycans (predominantly dermatan sulphate) and an increase in DNA (cellular) content. However, there were no significant differences between degenerate tendons that were 'calcified' and those degenerate specimens that contained normal concentrations of calcium.**

***Conclusions*—Although there was a relatively high prevalence of calcium salts in degenerate tendons, which might contribute to the pathological process (such as increased matrix collagen degradation), these data are consistent with the hypothesis that 'dystrophic calcification' of degenerate tendon matrix is a pathological entity distinct from cell mediated 'calcifying tendinitis'. Calcification is probably one possible outcome (or end point) of chronic tendon injury, although the possibilty exists that in many cases, the presence of calcium salts may contribute to the tendon matrix degeneration.**

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Rotator cuff 'tendinitis' is a poorly characterised degenerative condition, common in late middle age, that usually affects the supraspinatus tendon and frequently results in chronic shoulder pain and eventual tendon rupture.[1 2] 'Calcifying tendinitis' has a similar pattern of occurrence and, in common with degenerative tendinitis, most frequently affects the supraspinatus tendon at the critical zone', 1 cm from the bone insertion.[3 4] Despite these similarities, the two conditions are now generally considered to be separate pathological entities.[5 6] Although deposits of calcium phosphate salts such as hydroxyapatite and calcium pyrophosphate have been reported in spontaneously ruptured tendons,[7 8] the association of calcium deposits with the pathological process of tendon degeneration and rupture is unknown at present.

We have previously shown in a histological study that small calcium deposits are occasionally present in degenerate supraspinatus tendons, usually associated with regions of 'fibrocartilaginous change' in the tendon midsubstance.[9] Fibrocartilage is characterised by rounded, chondrocyte-like cells, aligned in rows and embedded in a fibrous (type I) collagen matrix rich in proteoglycans.[10] Tendon fibrocartilage is normally restricted to the bone insertion, but also found within tendons at sites exposed to friction or compressive forces.[9 11] We have previously shown a number of matrix changes consistent with the presence of fibrocartilage in supraspinatus tendons, including

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an accumulation of large aggregating proteoglycans, particularly in the region of the critical zone toward the bone insertion.[12 13] We propose that fibrocartilaginous change and the development of a chondrocyte-like cell phenotype represents a tendon cell (tenocyte) response to local factors such as the shear and compressive forces experienced by tendons of the rotator cuff in the shoulder. In certain pathological circumstances, we hypothesised that tendon cell and matrix changes might lead to matrix calcification, in a cell mediated process of matrix calcification similar to endochondral ossification, as originally proposed by Uhthoff.[6] Consequently, in this study we aimed to establish the prevalence and nature of basic calcium phosphate mineral deposits in the normal and degenerate human supraspinatus tendon and to clarify the association of pathological soft tissue calcification with degenerative matrix changes and 'fibrocartilaginous metaplasia' of the supraspinatus tendon matrix.

Materials and methods

Supraspinatus tendons were obtained from cadavers at necropsy within 48 hours of death. No cadaver had any clinical history of shoulder pain or degenerative tendinitis. Specimens were also collected during surgery to repair rotator cuff defects. Tendon tissue removed was waste material that would otherwise have been discarded. Patients with chronic 'tendinitis' (a rotator cuff tear) had been treated conservatively with at least one corticosteroid injection before surgery. Some specimens were also taken from patients with calcifying tendinitis, identified by clinical and radiographic assessment. All specimens were carefully trimmed to remove muscle, fat, and surrounding connective tissues. The tendons were then freeze dried, powdered under liquid nitrogen in a Spex freezer mill and stored at −20°C until required for biochemical analysis.

Duplicate samples were hydrolysed in Spectrosol grade 6 mol/l hydrochloric acid (HCl) (BDH, Poole, Dorset, UK), diluted in 0·1% lanthanum chloride and analysed for calcium by atomic absorption spectroscopy using a Pye Unicam SP9 spectrophotometer and a calcium absorption wavelength of 422·7 nm. The analysis was reproducible, with an intra-assay coefficient of variation (CV) of 7·7% (n = 5) and an interassay CV of 12·2% (n = 5). A small amount (less than 5%) of calcium was adsorbed to the Pyrex tubes during the extended hydrolysis; this was accounted for in all subsequent determinations by hydrolysis of the calcium standards and the inclusion of internal and external controls. Values for calcium and phosphorous obtained for reference materials were within the ranges quoted and the molar ratios of calcium to inorganic phosphorous obtained for calcium crystal standards were in good agreement with values obtained by *x* ray energy dispersive microanalysis (XRMA) (Dr C Scotchford, personal communication). Inorganic phosphorous was assayed using an ammonium molybdate spectrophotometric assay (Sigma, UK).

The biochemical composition of the tendon matrix was determined as described previously.[12 13] Briefly, tendon tissue was digested with papain 0·125 mg/ml in 0·1 mol/l phosphate buffer containing 10 mmol/l cysteine HCl and 2 mmol/l EDTA. Collagen content was assayed by hydroxyproline analysis, using a microtitre plate modification of the method of Bergman and Loxley.[14] The collagen content was calculated using a multiplication factor of 6·94 (human collagen type I contains 14·4% hydroxyproline). Pepsin extracts and cyanogen bromide digests of tendons were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting with specific collagen type I and type III antisera (Southern Biotechnology, Alabama, USA). Sulphated glycosaminoglycans were analysed by a microtitre plate modification of the dimethylmethylene blue (DMB) assay.[15] Chondroitin sulphate and dermatan sulphate were determined by specific glycosidase digestion with chondroitinase ABC and chondroitinase AC, followed by the DMB assay. Uronic acid was measured using a microtitre plate modification of the carbazole assay as described by Dr S Carney (personal communication).[16] The DNA content of the tendon was measured using a modification of the Hoechst 33258 fluorometric assay.[17] Background fluorescence was subtracted from the fluorescence obtained with the Hoechst dye reagent and the difference was used to calculate the DNA content by reference to a standard curve of calf thymus DNA.

X RAY ENERGY DISPERSIVE MICROANALYSIS (XRMA)

Tendon samples were incubated with 1000 U/ml bacterial collagenase in *N*-Tris (hydroxymethyl) methyl-2-aminoethanesulphonic acid buffer containing gentamycin, pH 7·4, with constant mixing for 24 hours at 37°C. The digest was allowed to settle and clarified by centrifugation at 250 *g* for 10 minutes. The supernatant was centrifuged at 5000 *g* for 20 minutes and the pellet was resuspended in 0·1 mol/l sodium cacodylate buffer (pH 7·4), then centrifuged for 30 minutes at 35000 *g* and either scattered on to piolform coated copper grids or processed through to resin and 100 nm sections collected on to piolform coated copper grids. XRMA was carried out using an EDAX 9800 *x* ray microanalysis system with a Philips CM12 transmission electron microscope. *x* Ray spectra were recorded at 100 kV for 200 live seconds with a tilt angle of 20°C. Calcium:phosphorous (Ca:P) ratios were calculated using a measured standard of sintered hydroxyapatitie that was processed as described above.

MATERIALS

Papain (2x crystallised), chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan

sulphate, heparan sulphate, keratan sulphate, hyaluronan, calf thymus DNA, and inorganic phosphorous standards were purchased from Sigma, Poole, Dorset, UK. Chondroitinase AC, chondroitinase ABC, and keratanase were obtained from ICN/Flow, UK. Calcium standards were from BDH, Poole, Dorset, UK. Calcium and phosphorous reference materials (IAEA 153 and V10) were obtained from the International Atomic Energy Authority, Austria. Calcium crystal samples of hydroxyapatite, tricalcium phosphate, and calcium pyrophosphate were kindly provided by Dr C Scotchford, Department of Experimental Pathology, Institute of Orthopaedics, Stanmore, Middx, UK. All other reagents were analytical grade from FSA, UK, or have been described elsewhere.[12 13]

SPECIMENS OF TENDON

Sixty macroscopically normal supraspinatus tendons (40 from men) were obtained (within 48 hours of death) from cadavers aged between 11 and 95 years at death. Sixteen common biceps tendons (12 from men) were obtained from cadavers aged between 12 and 81 years at death. Although none of the cadavers had any clinical history of tendon lesions, there were limitations as to the quantity and quality of information and medical records available. For example, a total of 20 (out of 80) supraspinatus tendons had macroscopic evidence of previous tendon pathology, but all the specimens reported in this study were macroscopically 'normal' (no visible ruptures or calcium deposits). Twenty seven specimens (16 from men) were obtained from patients aged between 38 and 81 years, all undergoing shoulder surgery for rotator cuff tendinitis, none of which had any calcification detected by *x* ray radiography of the shoulder. Four specimens (three from men) were obtained from patients with clinically diagnosed calcific tendinitis (visible radiographically), aged between 47 and 51. Tendon material was taken directly adjacent to the lesion (calcium deposit or tear) and varied in size from 0·1 to 3·0 g wet weight.

STATISTICAL METHODS

The Mann-Whitney *U* test was used to determine the significance of the difference between means.

Results

Sixty macroscopically normal cadaver supraspinatus tendons and 16 common biceps tendons were used to obtain baseline values for calcium and inorganic phosphorous concentrations in adult human tendons. There was no significant difference between supraspinatus and common biceps tendons and 95% of the estimated values were within 2SD of the sample mean (table 1).

In the supraspinatus tendon sample there was a small but significant increase in both calcium and inorganic phosphorous with increasing age, although there was no change in the molar Ca:P ratio, which averaged 0·67 (SD 0·3) (fig 1). A small but significant increase with age was also found in the sample of common biceps tendons, with a constant molar Ca:P ratio of 0·77 (SD 0·12) (data not shown). We found no significant difference between men and women in the respective tendon samples.

For the purposes of comparison with the pathological tendons, an abnormally increased calcium and inorganic phosphorous content was defined as greater than 2SD of the 'normal' (supraspinatus tendon) sample mean. For calcium, this was greater than 1·8 μg/mg dry weight and for phosphorous it was greater

Table 1 Calcium and phosphorous content of human tendon

Tendon	*Age (yr)*	*Calcium (μg/mg dry wt)*	*Phosphorous (μg/mg dry wt)*	*Molar ratio Ca:P*
Supraspinatus (n = 58)	57·3 [11–95]	1·1 (0·35)	1·3 (0·5)	0·67 [0·26–1·57]
Common biceps (n = 16)	50·2 [12–81]	0·8 (0·2)	0·8 (0·1)	0·77 [0·52–0·96]

Mean values (or ratio) with [range] or (SD).

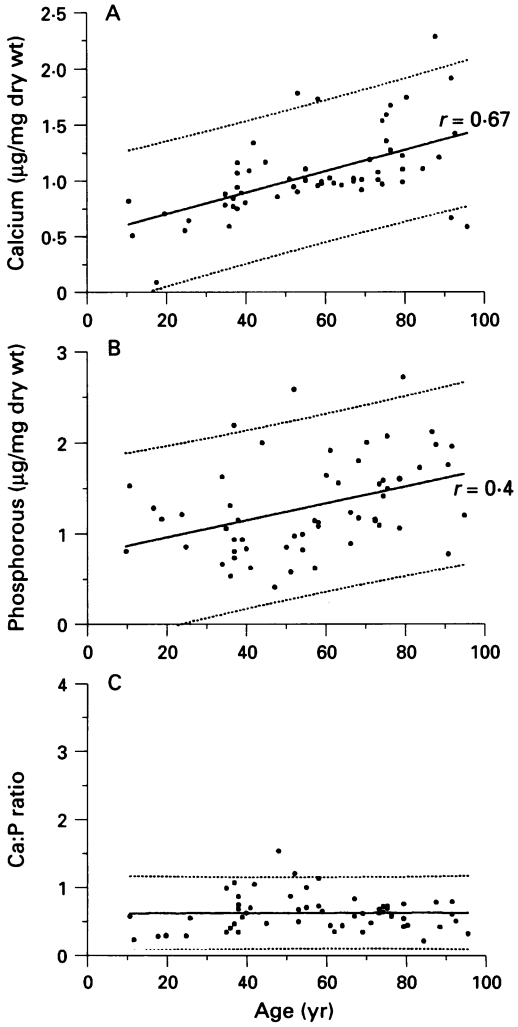

Figure 1 Changes in calcium and phosphorous content with age, in 60 normal cadaver supraspinatus tendons (age range 11–95 at death). A: Calcium analysed using atomic absorption spectroscopy. B: Phosphorous analysed using a commercial spectrophotometric assay (Sigma). C: Molar ratio of calcium to phosphorous calculated for each specimen. Data represent the mean of triplicate assays, expressed relative to the tendon dry weight. Dotted lines represent 95% confidence limits calculated by linear regression analysis.

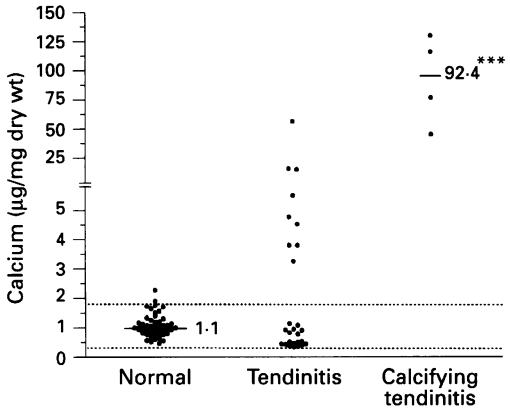

*Figure 2 Calcium content of supraspinatus tendons from degenerate rotator cuff tendinitis (n = 27; age range 38–81), calcifying tendinitis (n = 4; age range 47–51) and normal cadavers (n = 60, age range 11–95 at death) analysed by atomic absorption spectroscopy. Data represent the mean of triplicate assays, expressed relative to the tendon dry weight. ***p < 0·001, Mann-Whitney test.*

than 2·4 µg/mg dry weight. On this basis, nine of the 27 specimens from degenerate rotator cuff tears had an increased calcium content and were subsequently designated 'calcified degenerative tendinitis' (fig 2).

The phosphorous content was also increased in five degenerate tendon specimens (table 2). These were predominantly from women (four of five) and represented the five specimens with the greatest concentrations of calcium. Each had a different molar Ca:P ratio, consistent with a number of possible calcium crystal structures. In particular, one 80 year old tendon (specimen 1) had a composition consistent with crystals of either amorphous calcium phosphate ($Ca_9(PO_4)_6$ (var.) or tricalcium phosphate ($Ca_3(PO_4)_2$). A 66 year old tendon (specimen 2) had a composition consistent with dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$) or dicalcium pyrophosphate ($Ca_2P_2O_7$), and a 65 year old tendon (specimen 3) had a composition consistent with hydroxyapatite ($Ca_5(PO_4)_3OH$). The remaining two specimens contained proportions of Ca:P that were not consistent with any known crystal structure, raising the possibility of mixed or amorphous calcium phosphate deposits.

Our finding of normal concentrations of phosphorous in four of the nine tendons showing calcified degenerative tendinitis was consistent with the absence of any insoluble mineral deposits, particularly as the concentrations of calcium were only slightly in excess of the normal range (table 2). There was insufficient tendon material remaining for XRMA or chemical analysis to establish if these specimens contained some other insoluble calcium salt such as calcium oxalate or calcium carbonate, both of which have been reported in some pathological conditions.[18]

All the four tendons from patients with calcifying tendinitis had, as expected, a greatly increased calcium content, ranging from 45·9 to 130·2 µg/mg dry weight (4·6 to 13% of dry weight) (fig 2). Each of these specimens also contained increased concentrations of phosphorous and in each case the molar Ca:P ratio was slightly greater than the empirical ratio expected for crystals of hydroxyapatite (table 3).

Table 2 Molar ratio of calcium and phosphorous in specimens with degenerative 'tendinitis' and increased calcium (>1·8 µg/mg dry weight)

Specimen	Age (yr)	Sex	Calcium (µg/mg dry wt)	Phosphorous (µg/mg dry wt)	Molar ratio Ca:P
1	80	F	56·7	28·8	1·52 (A)
2	66	F	16·4	12·7	1·0 (B)
3	65	F	15·8	7·5	1·65 (C)
4	55	M	5·6	5·1	0·85 (?)
5	56	F	4·8	3·2	1.15 (?)
6	59	M	4·6	1·7†	2·1
7	67	M	3·8	0·8†	3·8
8	45	M	3·8	2·2†	1·4
9	55	F	3·3	1·5†	1·66

†Levels of inorganic phosphorous within normal range (<2·4 µg/mg dry wt).
Possible basic calcium phosphate (BCP) crystal structures:
(A) Amorphous calcium phosphate [$Ca_9(PO_4)_6$ (var.)] or tricalcium phosphate [$Ca_3(PO_4)_2$]
(B) Calcium pyrophosphate dihydrate [($Ca_2P_2O_7.2H_2O$)]
(C) Hydroxyapatite [$Ca_5(PO_4)_3OH$]
(?) No known BCP composition—mixed mineral deposits?

Table 3 Calcium, phosphorous and molar ratio Ca:P in calcifying tendinitis

Specimen	Age (yr)	Sex	Calcium (µg/mg dry wt)	Phosphorous (µg/mg dry wt)	Molar ratio Ca:P
1	50	M	130·2	59·8	1·69
2	50	M	116·3	47·6	1·89
3	47	F	77·3	27·0	2·22
4	51	M	45·9	14·9	2·39

CONFIRMATION OF CRYSTALLINE IDENTITY BY X RAY MICROANALYSIS

Although our analysis of calcium phosphate crystal standards gave good agreement with the established crystal composition (table 4), this type of chemical analysis was clearly unable to differentiate between soluble and insoluble compounds in the tendon cells and matrix. Three specimens from the cadaver sample and two specimens from the patient sample (all with increased concentrations of calcium) were therefore subjected to XRMA (conducted by Dr C Scotchford, Department of Experimental Pathology, Institute of Orthopaedics, Stanmore, Middx, UK), to establish whether microcrystalline deposits were indeed present. Ca:P ratios were calculated from the mean of at least 10 spectra using a measured standard of hydroxyapatite. This analysis confirmed the presence of crystal deposits in all five specimens, with good agreement between the chemical analysis and the XRMA in three specimens (table 5).

ASSOCIATED BIOCHEMICAL CHANGES OF THE TENDON EXTRACELLULAR MATRIX

Table 6 summarises a number of significant differences in matrix composition observed between normal tendon and calcified or degenerate (ruptured) supraspinatus tendons.

Table 4 Calcium crystal analysis

Crystal	Molar ratio Ca:P	Estimated molar ratio Ca:P (AAS)
Hydroxyapatite	1·66	1·69 (0·15)
Calcium pyrophosphate	1·00	1·04 (0·02)
Tricalcium phosphate	1·50	1·53 (0·1)

Values in parentheses are SD.
AAS = Atomic absorption spectroscopy.

Table 5 Ca:P molar ratio of calcific deposits: comparison of different methods

Tendon specimen	*Chemical analysis (estimated molar ratio Ca:P)*	*XRMA (mean molar ratio Ca:P)*
1	0·94	1·03
2	1·02	1·01
3	0·96	0·96
4	1·52	1·01
5	2·39	1·62

XRMA = *x* Ray microanalysis. Mean of at least 10 spectra.

Calcified and degenerate tendons contained significantly less collagen as a proportion of the tendon dry weight; this was particularly marked in the specimens from patients with calcifying tendinitis. The reduction in the collagen mass was not fully accounted for by the increase in mineral mass or any of the matrix components that we measured.

As reported elsewhere, degenerate (ruptured) tendons contained an increased proportion of type III collagen relative to type I collagen—evidence of a cellular wound healing response to injury.[12] However, there was no significant difference between degenerate specimens with calcium deposits and those without, and analysis of extracts using SDS-PAGE and Western blotting (data not shown[12]) gave no indication of any other collagen type in the matrix (such as collagen type II). In contrast, we found normal amounts of type III collagen in each of the four specimens with calcifying tendinitis, consistent with a lack of previous tendon injury.

Sulphated glycosaminoglycans were significantly increased in degenerate tendons, mainly as a result of an increase in dermatan sulphate. Once again, there was no significant difference between the 'calcifying' and 'non-calcifying' degenerate tendons, which might suggest no specific role for these glycosaminoglycans in the calcification process. In contrast, there was no change in the total glycosaminoglycan content of tendons with radiographically identified calcifying tendinitis, although there was a significant increase in the relative proportion of chondroitin sulphate to dermatan sulphate, essentially as a consequence of a reduction in the dermatan sulphate content.

The uronic acid content was increased in all the patient tendons (calcified and degenerate), indicative of an increase in hyaluronan content and in sulphated glycosaminoglycans. Together with the increase in DNA content, these changes are consistent with the cellular proliferation, infiltration, or both, of inflamed tendons that has been observed in histological studies of supraspinatus tendinitis (Riley *et al*, unpublished observations).

Discussion

In this study, we have established baseline values of calcium concentration in human tendons which are in close agreement with findings from studies of animal tendons.[19] We have also demonstrated a relatively high incidence (33%) of calcium salt accumulation in degenerate supraspinatus tendons. Tendon calcification was not detected in these specimens by *x* ray radiography before surgery, consistent with the small size and diffuse nature of these deposits. Although we recognise that these analytical techniques cannot distinguish between soluble and insoluble calcium salts deposited in the matrix, we obtained confirmation of crystalline deposits in five specimens by *x* ray microanalysis, and a good correlation between the estimated Ca:P ratios—data that support our contention that several different types of microcrystalline calcium deposits are found in some degenerate supraspinatus tendons affected by chronic tendinitis. The reason for the discrepancy between estimated and XRMA Ca:P values obtained for two of the specimens is not known, but may possibly reflect lack of homogeneity in the specimens, as at least two different crystal types (calcium pyrophosphate and hydroxyapatite) were recorded in different *x* ray spectra obtained from the same specimen—further evidence of mixed crystal deposition.

A greater incidence of pathological tendon calcification has been reported elsewhere in the literature. In one study as many as 53% of spontaneously ruptured human tendons contained crystal deposits as determined by electron microscopy, though these studies did not include the supraspinatus or any rotator cuff tendons.[8] However, it must be acknowledged that the presence of calcium salts does not necessarily imply any direct involvement in a pathological or degenerative process, which might occur secondary to any tendon matrix changes or be entirely coincidental. Furthermore, radiographic studies

Table 6 Biochemical composition of normal, degenerate and calcified supraspinatus tendons

	Normal tendon (n = 60)	*Calcifying tendinitis (identified by radiology) (n = 4)*	*Degenerate tendinitis*	
			Non-calcified (Ca < 1·8 μg/mg dry wt) (n = 18)	*Calcified (Ca > 1·8 μg/mg dry wt) (n = 9)*
Age (yr)	58·1 (11–95)	49·5 (47–51)	60·2 (38–81)	60·9 (45–80)
Calcium content (μg/mg dry wt)	1·1 (0·4)	92·4 (38·3)***	0·74 (0·3)	12·8 (17·3)***
Collagen content (% dry weight)	66·6 (5·3)	47·6 (12·5)***	60·2 (7·4)***	59·1 (10·7)***
Type III collagen (% total)	2·8 (3·2)	2·5 (1·7)	10·2 (6·4)***	8·7 (4·4)***
Glycosaminoglycan (μg/mg dry wt)	12·3 (4·3)	11·3 (4·2)	15·0 (4·4)	17·2 (3·4)***
CS (μg/mg dry wt)	6·9 (2·6)	6·8 (2·9)	8·5 (3·2)	8·8 (2·7)
DS (μg/mg dry wt)	2·5 (1·2)	1·4 (0·7)*	3·7 (1·0)***	4·3 (1·4)***
Ratio CS:DS	3·2 (1·5)	5·7 (2·7)**	2·4 (1·2)	2·4 (1·3)
Uronic acid (μg/mg dry wt)	8·5 (1·9)	18·9 (0·3)***	17·1 (5·2)***	18·6 (4·6)***
DNA (μg/mg dry wt)	0·74 (0·3)	1·2 (0·4)**	1·7 (0·8)***	1·8 (0·9)***

Values are mean [range] or (SD). CS = Chondroitin sulphate; DS = dermatan sulphate.
Statistically significant difference from normal tendon sample calculated by Student's *t* test:
*p < 0·05; **p < 0·01; ***p < 0·001.

have reported calcification in up to 7·5% of patients with no history of shoulder pain, and a substantial proportion (65%) of shoulders with calcific deposits are asymptomatic.[20] In addition, in this study we found age related increases in calcium concentration in both 'normal' supraspinatus and common biceps tendons, possibly as a result of an accumulation of calcium deposits within aging cell organelles and necrotic cell debris.[21] The accumulation of these calcium salts with age is unlikely, on its own, to have any pathological significance, as the common biceps tendon is rarely involved in any tendon pathology.

It has been suggested that calcific tendinitis and rotator cuff ruptures are mutually exclusive conditions with different underlying pathologies.[4 6] However, in a large cadaver survey, 6·6% of rotator cuff tendons were found to be calcified, and all these tendons were ruptured.[22] In addition, in a large histopathological study 'calcifying tendinopathy' was the only significant pathological feature in 5% of spontaneously ruptured tendons.[8] A major distinguishing feature of calcifying tendinitis is reported to be the deposition of hydroxyapatite rather than other basic calcium phosphate or calcium pyrophosphate deposits in degenerate or 'dystrophic calcification'.[18 20] In our study, we obtained four clinical cases of calcifying tendinitis, and confirmed that the most likely form of the deposit was indeed hydroxyapatite, even though there was a slight excess of calcium in three of the four specimens. In contrast, we found a variety of possible calcium salts in degenerate tendons, including calcium pyrophosphate, hydroxyapatite, and possibly even calcium carbonate or calcium oxalate, which are occasionally found in some pathological situations.[18] These data would appear to confirm the different aetiology of the two processes ('calcifying tendinitis' versus 'degenerative calcification'), though it is possible that the variety of different crystal structures may represent different stages in the maturation of calcium phosphate crystals, of which hydroxyapatite is the most stable form.[18]

The pathological process of calcifying tendinitis remains the source of some controversy. Recently, it has been proposed that calcifying tendinitis is a cell mediated event resembling endochondral ossification occurring in tendon fibrocartilage, in which the chondrocyte-like cells hypertrophy and calcify the tendon matrix.[6 20] The process is said to be self limiting, with eventual resorption of the calcium deposit and restitution of a normal tendon matrix. More recently, and supporting this proposition, it has been demonstrated that transsected rodent tendon is first converted to cartilage and then to bone, in a model of ectopic endochondral calcification.[23] However, we and others have found no evidence of endochondral calcification, such as matrix vesicles, or type II and type X collagen, in calcifying tendons (GP Riley, unpublished observations).[9 12 13 24] Indeed, fibrocartilage formation is a common finding in both normal and pathological tendons, but often without any associated calcification. We cannot entirely exclude the possibility that an endochondral-type process is involved, as much of the collagen was insoluble and epitopes (of type II collagen, for example) may be inaccessible to antibodies on tissue sections. However, these and other studies would suggest that tendon fibrocartilage formation is not by itself the driving force of tendon calcification, which may require additional local factors such as trauma and tissue hypoxia, causing local cell and matrix changes which can then initiate crystal deposition.[7 9 24 27]

In our analysis of tendon matrix, there was evidence of some alteration in glycosaminoglycan composition associated with the tendon calcification, but with two contrasting patterns in the different (calcified) tendon samples. There was a trend (although not significant) toward greater amounts of both chondroitin sulphate and dermatan sulphate in the calcified degenerate tendons. In contrast, there was a significantly reduced amount of dermatan sulphate in specimens with clinically identified calcifying tendinitis. However, the significance of these observations remains to be determined. Although proteoglycans and other matrix components have been implicated in the mineralisation process, proteoglycans have been shown both to inhibit and to stimulate the formation of calcium crystals in vitro.[25 26] We have previously observed the strong association of calcium deposits with sulphated glycosaminoglycans.[9] Immunohistochemical studies have also identified local changes in glycosaminoglycan type associated with calcific deposits, with an increase in chondroitin-4-sulphate, dermatan sulphate, or both, in the matrix, and the pericellular expression of chondroitin-6-sulphate.[24] These changes may well be significant, as alterations in the size of proteoglycan aggregates can initiate calcification.[26] However, changes in proteoglycan synthesis, degradation, or both, may be secondary, rather than the primary cause of the calcium deposition.[27]

There was no obvious difference in matrix composition between the two groups of degenerate tendons that might explain why some degenerate specimens calcified and some did not. All had an altered matrix composition consistent with cellular proliferation, inflammation, and wound healing.[12 13] Biochemical changes in true (radiographically detected) calcifying tendinitis were also consistent with cellular proliferation and inflammation (increased DNA and hyaluronan), but without evidence of wound healing (no increase in type III collagen), consistent with the absence of any previous tendon injury or trauma.

Although the presence of calcium crystals does not necessarily imply any direct involvement in the degenerative process (as acknowledged above), many different types of crystal are mitogenic and potent stimulators of inflammation,[18 28] which may explain some of the cell and matrix changes we have described. Acute and chronic reactions in soft tissues can be induced by the shedding of crystals from a

deposit, which may occur after trauma.[18] Indeed, microcrystalline deposits of hydroxyapatite and calcium pyrophosphate have been associated with cartilage degeneration and osteoarthritis.[29] Even if the crystals are a secondary phenomenon, it is possible that such deposits cause a concentration of stress and reduce the tensile strength of the tendon.

We did find an association of calcification with a reduced collagen mass—a change that was not completely accounted for by the increase in calcium mineral. Increases in non-collagen proteins and lipids, both of which have been found to increase in aging and injured tendons,[30 31] may account for some of the discrepancy. These glycoproteins or lipids may conceivably have some role in the calcification process.[32] In addition, the reduction in collagen content may also be evidence of an increase in collagen turnover, potentially associated with the ability of many different crystals to induce the expression of matrix degrading enzymes such as collagenase and stromelysin by fibroblasts and macrophages.[33] In conditions such as 'Milwaukee shoulder syndrome' for example, hydroxyapatite deposition in the soft tissues of the shoulder is associated with a severe destructive arthropathy accompanied by a rapid resorption of the cartilage, bone, and rotator cuff tendons.[34] Although degenerative tendinitis is clearly neither as inflammatory nor as destructive, the deposition of microcrystalline deposits of calcium salts within a relatively avascular and hypocellular tendon may result in mitogenic activity and an increase in local collagen degradation in the matrix immediately adjacent to the deposit. This hypothesis is supported by the increased collagenolytic activity found in calcified supraspinatus tendons in explant culture, compared with non-calcified degenerate tendons.[35] It would be appropriate in future studies of degenerate and calcified tendons to test this hypothesis, using immunolocalisation, in situ hybridisation, or both, to examine the cellular expression of such enzymes (and their inhibitors) in proximity to regions of tendon matrix degeneration and any calcific deposits.

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