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Vitamin D metabolites in synovial fluid

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SUMMARY This study has shown that it is possible to measure vitamin D metabolites and vitamin D binding protein (DBP) in synovial fluid as well as serum. Significant amounts of 25-OHD, 24,25-$(OH)_2D$, and DBP are present in synovial fluid. The 25-OHD and DBP maintain a serum:synovial fluid ratio of approximately 2:1 irrespective of the type of joint disease, whereas no such relationship was detected for 24,25-$(OH)_2D$. The possible reasons for these findings are diffusion of the metabolites into synovial fluid or local production from suitable precursors, or both.

Key words: rheumatoid arthritis, osteoarthritis, vitamin D binding protein.

There is a well recognised association between hydroxyapatite deposition and arthritis,[1 2] and hyperparathyroidism may occur with chondrocalcinosis.[3 4] In addition there have been reports of increased values of immunoreactive parathyroid hormone in calcium pyrophosphate crystal deposition disease,[5] chondrocalcinosis,[3] and ankylosing spondylitis.[6] Circulating bioactive parathyroid hormone values may be raised in chondrocalcinosis and ankylosing spondylitis.[7] These observations support a possible role for calcium regulating hormones in the pathogenesis of some types of joint disease.

Two of the most common types of arthropathy, osteoarthritis and rheumatoid arthritis, both show abnormalities of cartilage within the joint. Vitamin D metabolites are probably concerned with normal cartilage integrity, and studies have shown that cultured chondrocytes convert 25-hydroxyvitamin D_3 (25-OHD_3) into 24,25-dihydroxyvitamin D_3 (24,25-$(OH)_2D_3$),[8] and 24,25-$(OH)_2D_3$ enhances sulphation by chondrocytes and stimulates calcium uptake by bone cells.[9] As synovial fluid provides nourishment for cartilage, a study to determine whether vitamin D metabolites are present in synovial fluid was undertaken. If so, what is their relationship with serum values, and do the values differ in different types of arthropathy?

Patients and methods

Samples of synovial fluid and serum were obtained from patients with joint effusions attending the rheumatology outpatient clinic for therapeutic or diagnostic knee aspiration. The samples were stored at −20°C within two hours of being taken from the patient until required for biochemical analysis, which included 25-hydroxyvitamin D (25-OHD), 24,25-dihydroxyvitamin D (24,25-$(OH)_2D$), and vitamin D binding protein (DBP). 25-OHD was determined by a competitive protein binding technique[10] and 24,25-$(OH)_2D$ by high pressure liquid chromatography before competitive protein binding assay (modified from Ref 11). Vitamin DBP was assayed with M-Partigen Gc-globulin radial immunodiffusion plates (Behring Diagnostics, Hoechst House, Salisbury Road, Hounslow, Middlesex TW4 6JH, UK).

The study was undertaken in two parts. Initially, specimens from 57 patients with a wide variety of rheumatological disorders were assayed for 25-OHD. Subsequently the study was extended to the estimation of 24,25-$(OH)_2D$ on a further group of specimens from 27 different patients. Samples from normal control subjects were not available for comparison.

Results

Measurable amounts of 25-OHD were found in all 57 synovial fluid samples assayed. The values ranged from 10 to 200 nmol/l (Fig. 1). The results were subdivided into those from patients with rheumatoid arthritis (diagnosed according to standard American Rheumatism Association criteria for classical or definite disease), osteoarthritis, or miscellaneous arthropathies, e.g., gout, psoriasis. Most values

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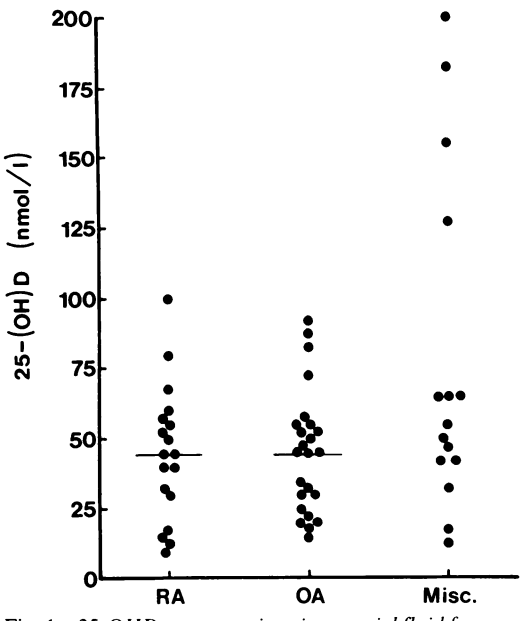

Fig. 1 *25-OHD concentrations in synovial fluid from patients with rheumatoid arthritis (RA), osteoarthritis (OA), and miscellaneous arthropathies (Misc).*

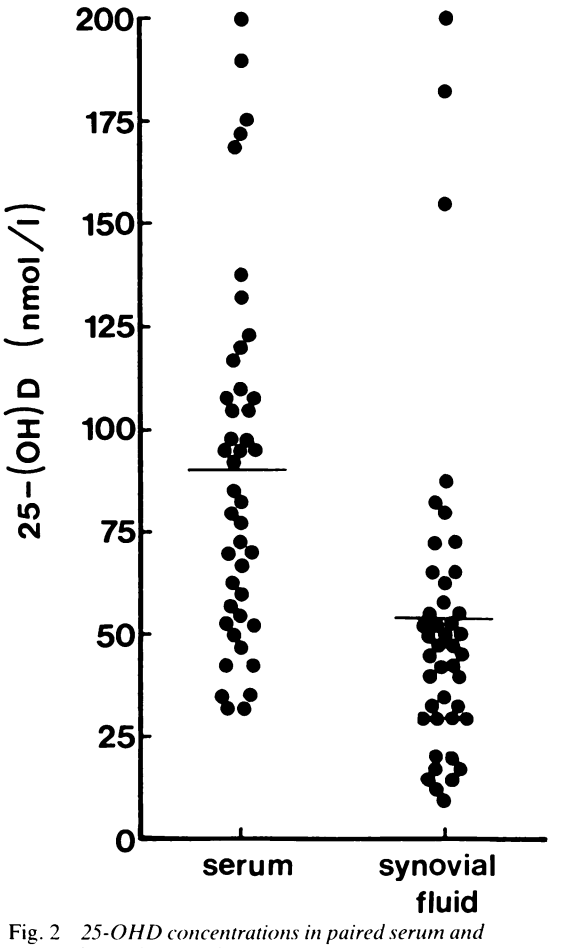

Fig. 2 *25-OHD concentrations in paired serum and synovial samples (n=43).*

were between 13 and 125 nmol/l, with the exception of four specimens from patients in the miscellaneous arthropathy group as follows: one subject with chronic gout (synovial fluid 25-OHD 128 nmol/l), two subjects with polyarthropathy of unknown aetiology (synovial fluid 25-OHD 200 nmol/l, 183 nmol/l), and one patient with an idiopathic knee effusion (synovial fluid 25-OHD 155 nmol/l). Apart from these four unusual patients, which biased the results for the miscellaneous group, there was no difference between the values obtained from patients with rheumatoid arthritis, osteoarthritis, or the miscellaneous group of arthropathies.

Paired samples of serum and synovial fluid were available from 43 patients with rheumatoid arthritis and are shown in Fig. 2. Serum values (mean (SD) 91 (44) nmol/l) were higher than synovial fluid values (54 (40) nmol/l), serum containing approximately twice the concentration of 25-OHD found in synovial fluid (Table 1). Fig. 3 indicates that the serum and synovial fluid values for each pair were closely related (n=43, r=0·87, p<0·001). When the data were examined according to patient diagnosis

Table 1 *25-OHD in paired serum and synovial fluid samples*

	Number	*Mean (SD) 25-OHD concentration (nmol/l)*		*Ratio*	*Correlation coefficient*
		Serum	*Synovial fluid*		
All samples	43	90·8 (43·8)	53·5 (40·3)	1·70	0·87
Rheumatoid arthritis	17	83·5 (47·5)	42·3 (20·8)	1·98	0·87
Osteoarthritis	17	81·0 (33·3)	43·8 (22·8)	1·85	0·89
Miscellaneous arthropathies	9	123·5 (41·8)	92·8 (66·5)	—	—

this close relationship was maintained regardless of whether the patient had rheumatoid arthritis (n=17, r=0·87, p<0·001) or osteoarthritis (n=17, r=0·89, p<0·001). In the miscellaneous arthropathies group the four patients with 25-OHD values >125 nmol/l obscured this relationship. Those remaining showed similarities to the rheumatoid and osteoarthritis groups, though statistical confirmation was not possible because of the small number of samples (n=5).

24,25-$(OH)_2$D was detected in all 27 synovial fluid samples tested except one. The range of values obtained, 1·3–41 nmol/l, was very wide, and five values were much higher than the rest. These specimens came from three patients with rheumatoid arthritis, one with a monoarthropathy of uncertain aetiology, and one with gout. In 15 of the patients providing specimens, paired samples of blood and synovial fluid were available so that the relationship of serum to synovial fluid 24,25-$(OH)_2$D could be examined. Although these 15 pairs of sera and synovial fluid showed the same close relationship (n=15, r=0·88, p<0·001) for 25-OHD that was found in the earlier part of our study, 24,25-$(OH)_2$D did not yield similar results (Fig. 4). Serum concentrations were higher than those in synovial fluid in all except three cases, but not in the previously noted 2:1 ratio, and there was no close relation between the individual pairs (n=15, r=0·23, p=0·40).

Vitamin DBP was measured on these 15 paired samples. The concentration ranges were serum

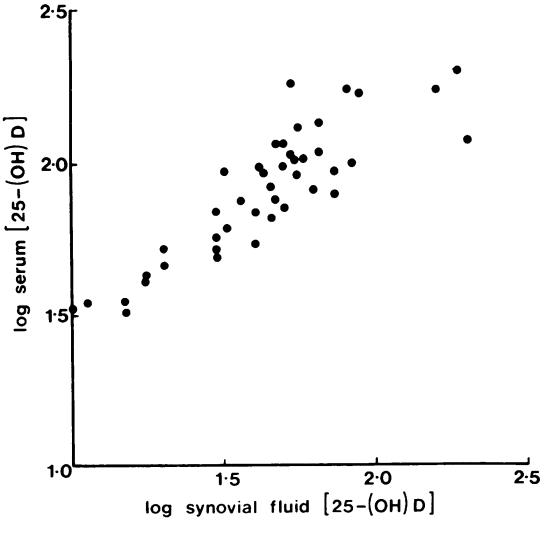

Fig. 3 *Relationship of serum and synovial fluid 25-OHD concentrations (n=43, r=0·87).*

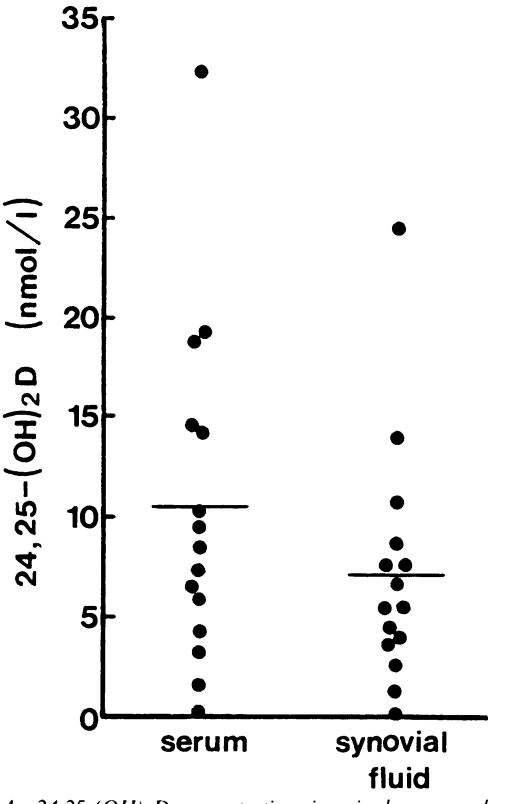

Fig. 4 *24,25-$(OH)_2$D concentrations in paired serum and synovial fluid samples (n=15).*

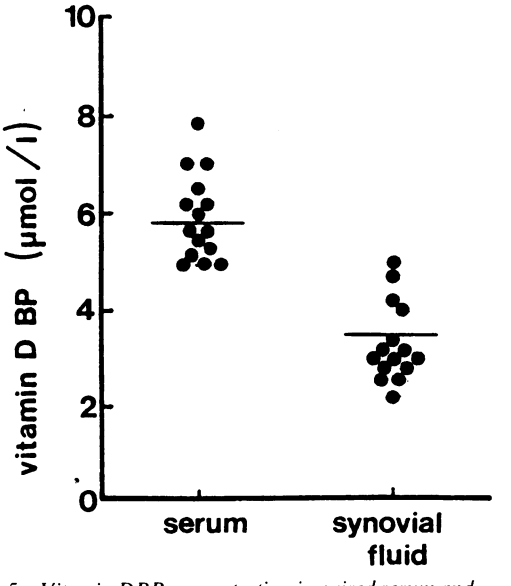

Fig. 5 *Vitamin DBP concentration in paired serum and synovial fluid samples (n=15).*

5·0–7·8 μmol/l and synovial fluid 2·2–5·0 μmol/l. The serum concentrations were higher than those in synovial fluid (Fig. 5) and showed a similar relationship to the 25-OHD values with a serum:synovial fluid ratio of 1·70:1. The individual pairs also showed a significant relationship ($n=15$, $r=0·65$, $p<0·01$).

Discussion

Our studies have shown that significant amounts of 25-OHD and 24,25$(OH)_2$D are present in synovial fluid from patients with pathological knee effusions. A relationship of approximately 2:1 between serum and synovial fluid was found for 25-OHD and vitamin DBP, but this relationship was not found for 24,25-$(OH)_2$D. In the relatively small number of patients studied, no evidence to suggest differences between patients with osteo- or rheumatoid arthritis was apparent.

The composition of synovial fluid is consistent with it being a dialysate of plasma with added mucin from synovial A cells. The presence of vitamin D metabolites in synovial fluid may be simply a reflection of the serum values. Ogston and Phelps suggested that entry of certain substances into body fluids is precluded in proportion to their molecular size and shape, large or globular molecules such as globulin being excluded.[12] This could explain our findings with 25-OHD and DBP, both of which are present in synovial fluid in approximately half the serum concentration. 25-OHD circulates bound to DBP, an α globulin of molecular weight 51 000, and it seems that the size and shape of the molecule inhibits its free passage into synovial fluid.

24,25-$(OH)_2$D does not show a 2:1 ratio for paired serum and synovial fluid samples even though its binding to DBP is known to be equivalent to that of 25-OHD.[13] Garabedian *et al* have shown that in vitro cartilage preparations from articular surfaces and chondrocytes metabolise 25-OHD to 24,25$(OH)_2$D.[8] This metabolite enhances sulphation by chondrocytes and stimulates calcium uptake by bone cells.[9] The 24,25-$(OH)_2$D in synovial fluid may therefore be the result of local production rather than diffusion from the serum.

Differences in local conditions and hence in production of 24,25-$(OH)_2$D may explain the lack of correlation between serum and synovial fluid. The earliest changes in osteoarthritis are microscopic alterations in the articular cartilage, whereas in rheumatoid arthritis chronic synovitis is the major feature, with erosion of the articular cartilage by pannus occurring late in the disease. The stage of the disease may also be important. Mawer has reported that there is no normal level for serum 24,25-$(OH)_2$D but a normal range (0·5–4 ng/ml) which is positively related to the 25-OHD level and is approximately 7–10% of the 25-OHD concentration.[14] This relationship was not reproduced by our small series of serum samples ($n=15$, $r=0·37$), or by the synovial fluid samples ($n=15$, $r=-0·003$).

Our study with 25-OHD showed no differences between patients with rheumatoid arthritis, osteoarthritis, or miscellaneous arthropathies while that with 24,25-$(OH)_2$D was too small to be analysed in disease specific groups. 25-OHD is not the hormonally active form of vitamin D. It is not produced within the joint and is not the metabolite involved in bone mineralisation. Therefore, it can be argued that no changes in its synovial fluid concentration could be anticipated in joint disease. Bird *et al* have compared serum 25-OHD[15] and serum 1,25-$(OH)_2$D concentrations[16] in rheumatoid and osteoarthritis. They found no significant differences in the circulating levels of these hormones, but 1,25-$(OH)_2$D levels mirrored 25-OHD levels.

Since 24,25-$(OH)_2$D appears to be directly involved in bone mineralisation and is known to be produced from 25-OHD by cultured articular cartilage and chondrocytes[8] it is likely that this metabolite will show disease related differences in synovial fluid concentrations. Such differences could relate to increased or decreased local production or utilisation rather than being a reflection of the patient's serum 24,25-$(OH)_2$D status. Further detailed studies of the identification of synovial fluid vitamin D metabolites in a large group of patients are in progress.

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