Bone turnover in early rheumatoid arthritis. 1. Biochemical and kinetic indexes

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SUMMARY Biochemical, hormonal, and kinetic indexes of bone turnover were measured in 17 ambulant female patients with rheumatoid arthritis (RA) of recent onset (mean disease duration 14.2 months) and 19 controls. Mean serum osteocalcin concentration and ⁸⁵Sr accretion rates were reduced and mean urinary hydroxyproline-creatinine ratios were increased in RA, but these differences were not significant compared with control values. Mean total body potassium (TBK), an index of skeletal muscle mass, was significantly reduced in RA, and the ratio of observed to predicted TBK correlated with indexes of bone formation. No abnormality of skeletal metabolism could be shown in early RA, but reduced rates of bone formation associated with diminished muscle mass may influence the development of osteopenia later in the disease.

Key words: calcium kinetics, osteocalcin, total body potassium.

Osteoporosis is a well recognised complication of RA and is generally considered to be of two types. A juxta-articular form of osteoporosis is one of the earliest radiological features of RA¹ and this appears to be mediated by local disease mechanisms.²⁻⁴ More generalised involvement of the axial and peripheral skeleton may also occur, and it has been suggested that this is a manifestation of a generalised disturbance of skeletal metabolism.⁵

Assessment of skeletal metabolism in RA is complicated by effects not only of the disease process but also, as the disease progresses, of disuse due to progressive loss of mobility, chronic therapy with drugs which may affect bone metabolism,⁶⁻⁸ and sometimes poor nutrition and concomitant corticosteroid therapy.

In the present study we have measured biochemical, hormonal, and kinetic indexes of bone turnover in ambulant patients with RA of recent onset and in a group of age and sex matched controls. By choosing such patients we have attempted to reduce the complicating effects of factors such

as loss of mobility and corticosteroid therapy. Since among RA patients who have not been treated with corticosteroids postmenopausal women are the group most at risk of developing osteoporosis,^{9,10} we selected for study predominantly postmenopausal women who had recently developed RA.

Patients and methods

SUBJECTS

Female patients attending two general rheumatology outpatient clinics who had developed seropositive classical or definite RA¹¹ in the preceding three years and who were approaching the menopause or were postmenopausal were asked to participate; patients receiving oral corticosteroids were excluded.

Control subjects were volunteers in good health and not taking drugs known to produce osteoporosis; two were taking non-steroidal anti-inflammatory drugs for minor degenerative joint disease.

Plasma calcium, phosphate, and creatinine were normal in all subjects. Details of patients and controls are shown in Table 1. Informed consent was obtained from each subject and the study received Ethical Committee approval.

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Table 1 Clinical details of patients and controls

Clinical details	Rheumatoid arthritis patients (n=17)	Controls (n=19)
Age (years):		
Mean	55.4	56.5
Range	37-66	41-69
No. postmenopausal	12	15
No. years postmenopausal:		
Mean	11.1	9.4
Range	2-22	1-22
Disease duration (months):		
Mean	15.0	-
Range	5-31	-
Physical activity index (Mean±SD)	30.1±2.6	31.5±2.6

A subjective assessment of physical activity was made in both groups by the Framingham activity index.¹² Disease activity was assessed at the start of the study in patients by the following: Ritchie articular index¹³; joint count (number of active joints); grip strength (mean of three recordings, dominant hand); erythrocyte sedimentation rate (ESR); C-reactive protein (CRP).

LABORATORY STUDIES

Accretion rates were determined using ⁸⁵Sr as an intravenous tracer for calcium and a kinetic model employing the technique of impulse analysis.¹⁴ The ⁸⁵Sr accretion rate provides an estimate of the mass of calcium taken up by the skeleton in one day which remains there for at least 20 days and is not available for short-term exchange. By use of a whole body counter ⁸⁵Sr retention was monitored for five months, and the impulse response function of the skeleton for tracer H(t) was calculated by deconvolution analysis.¹⁵ The impulse response function describes the fate of a 'cohort' of calcium atoms that enter the skeleton in one day. The values of H(t) at 20 and 200 days were calculated from the function, and the fraction of accreted tracer lost in the first six months was calculated as $(H_{20}-H_{200})/H_{20}$.

Urinary calcium, creatinine, and hydroxyproline concentrations were measured in all subjects two hours after an overnight fast. With these quantities expressed in mmol/l a calcium:creatinine ratio greater than 0.45 and a hydroxyproline:creatinine ratio greater than 0.016 were taken to indicate increased net bone resorption.¹⁶

The total body content of potassium (TBK) was determined in each subject, a whole body counter being used to detect the 1.46 MeV gamma rays associated with the decay of naturally occurring ⁴⁰K.¹⁷ TBK results were also expressed as the ratio of observed to expected TBK (TBK O/E), where

predicted TBK was calculated using the empirical formula of Boddy *et al.*¹⁸ which describes TBK in normal women in relation to their weight, height, and age.

Fasting venous blood samples were taken at the start of the kinetic studies and the serum extracted and stored at -70°C until assay. Radioimmunoassays were performed with commercial kits manufactured by Immuno Nuclear Corporation, Stillwater, Minnesota, USA. Serum parathyroid hormone (PTH) was measured by a radioimmunoassay procedure which recognises human parathyroid fragments containing the 44-68 amino acid sequence of the hormone. Purified bovine PTH was used for standard and tracer. Each determination was carried out in triplicate. The sensitivity of the assay was 10 pmol/l. Serum calcitonin was determined by a radioimmunoassay procedure which recognises the 17-32 amino acid sequence of human calcitonin. Pure human synthetic calcitonin was used as antigen for the antiserum, as radioiodinated tracer, and as standard. Non-equilibrium incubation conditions were used to improve sensitivity. Each determination was carried out in triplicate. The sensitivity of the method was 60 pg/ml (ng/l). Serum osteocalcin was determined by radioimmunoassay. Purified carboxylated bovine osteocalcin was used for standard and tracer. Each determination was carried out in duplicate. The sensitivity of the assay was 0.8 ng/ml (µg/l).

Unpaired *t* tests were used to determine significance levels of differences between mean values in the two groups. Radioimmunoassay results that were non-detectable were treated as censored normal data, and mean values were determined by computer by the maximum likelihood theory. Parametric correlation analyses were performed of the various disease activity measures against indexes of bone turnover. For this purpose osteocalcin values were corrected for creatinine clearance and body surface area.²⁰

Results

The results of the studies for patients and controls are shown in Table 2. There was a trend for patients with RA to have lower indexes of bone formation and higher indexes of bone resorption, but these differences were not statistically significant.

Observed TBK values and the ratio TBK O/E were significantly reduced in RA patients compared with controls (*p*<0.05 and *p*<0.001 respectively). The ratio TBK O/E for both patients and controls combined correlated significantly with accretion (*r*=0.47, *p*<0.01), *H*₂₀ (*r*=0.38, *p*<0.05), *H*₂₀₀ (*r*=0.42, *p*<0.05), and serum osteocalcin (*r*=0.51

Table 2 Bone turnover indexes (mean±SD)

	Rheumatoid arthritis patients (n=17)	Controls (n=19)*
Indexes of bone formation:		
Accretion (mmol Ca/day)	5.0±2.1	5.7±2.1
H ₂₀ (mmol Ca/day)	4.0±1.7	4.4±1.5
H ₂₀₀ (mmol Ca/day)	2.7±1.2	3.2±1.5
Serum osteocalcin (ng/ml) [†]	3.2±1.6	3.8±1.6
Indexes of bone resorption:		
Urinary calcium:creatinine	0.37±0.22	0.39±0.15
Urinary hydroxyproline:creatinine	0.021±0.013	0.017±0.007
(H ₂₀ -H ₂₀₀)/H ₂₀	0.32±0.12	0.29±0.12
Calcitropic hormones:		
Serum PTH (pmol/l)	82.3±35.0	68.7±33.7
Serum calcitonin (pg/ml) [†]	60.0±27.0	68.2±29.0
Skeletal muscle mass:		
Observed TBK (mmol)	2184±170	2481±343
TBK O/E	0.94±0.11	1.06±0.09

* Kinetic studies were performed in only 14 controls and serum PTH, calcitonin, and osteocalcin in only 18 controls.

[†] ng/ml=μg/l; pg/ml=ng/l.

p<0.01) but not with the fraction of accreted tracer lost in the first six months. Measures of disease activity did not correlate significantly with any index of bone formation, but urinary hydroxyproline:creatinine ratios correlated with several measures of activity including joint count (r=0.62, p<0.01), ESR (r=0.70, p<0.01), and CRP (r=0.70, p<0.01).

Discussion

Generalised osteoporosis is well known to occur in RA, but uncertainty exists with regard to the mechanism. Duncan *et al.*⁵ suggested that a primary defect in bone metabolism involving the whole osseous system may occur in RA. By histomorphometric analysis of cortical bone in rib biopsy specimens from seven patients these authors found evidence of increased bone resorption, reduced bone formation, and defective mineralisation in RA. As similar histological changes have been observed in bone after immobilisation,²¹ these findings may reflect a form of disuse osteoporosis.

By choosing ambulant outpatients with disease of recent onset we have attempted to examine the effect of the disease process on the skeleton without the additional influence of factors such as loss of mobility in longstanding disease. By the subjective Framingham index our patient and control groups were well matched in this regard, but TBK results showed a reduction in skeletal muscle mass in RA, so that in practice it was not possible to eliminate this effect completely.

Rates of bone formation assessed by kinetic methods in RA showed a trend towards lower values, but this difference was not statistically

significant. All indexes of bone formation correlated with TBK O/E, which is indirectly related to an individual's level of physical activity. This finding is consistent with studies showing that mechanical forces are important determinants of bone formation rates.^{21 22}

Previous kinetic studies of bone turnover in RA are few and the findings conflicting. Dymling²³ measured accretion rates in patients with RA and osteopenia and found a mean reduction in accretion of 16% but considered these values to be normal when related to the diminished skeletal mass. Heaney *et al.* found that local accretion rates in areas of bone with active joint disease were increased two- to fivefold, but that whole body accretion values were normal, suggesting that reduced remodelling was taking place in bone uninvolved in the rheumatoid process. Bergmann *et al.*²⁵ similarly found that accretion rates were increased near involved joints, but that whole body accretion rates were also slightly increased. The latter results were interpreted as evidence for increased bone remodelling in juxta-articular bone but did not support the suggestion that bone turnover was altered in the remainder of the skeleton.

Each of these studies used kinetic methods to determine accretion rates that include ion exchange processes not involved in osteoblastic new bone formation. Since these processes may represent more than half the measured accretion rate,²⁶ these studies tend to overestimate rates of bone turnover. The method we employed allowed a correction to be made for long-term ion exchange processes by extension of the usual study from three weeks to five

months.¹⁵ To calculate the true apposition and resorption rates would have required a simultaneous calcium balance which was not performed, but the quantity H_{200} is a measure of exchange corrected skeletal uptake.

The other index of bone formation we measured was serum osteocalcin. Osteocalcin is a vitamin K dependent bone protein containing three γ -carboxyglutamic acid residues.²⁷ Its function in bone is unknown, but serum levels are increased in diseases characterised by increased bone turnover²⁸ and correlate well with histomorphometric measures of bone formation.²⁹ Two recent studies have found reduced serum osteocalcin levels in RA,^{30 31} but in both studies patients had predominantly longstanding disease. These results together with our findings suggest that any substantial reduction in bone formation in RA is relatively unusual early in the disease and when it occurs may possibly be related to reduced physical activity as the disease progresses.

Increased urinary ratios of calcium and hydroxyproline are thought to indicate increased net bone resorption¹⁶ and have been reported to be both normal³² and increased.³³ In our patients mean urinary calcium:creatinine and hydroxyproline:creatinine ratios were not significantly higher than values for controls, but several patient values were markedly increased, and urinary hydroxyproline ratios correlated with some measures of disease activity. In one previous study urinary hydroxyproline:creatinine levels were found to be higher in patients with more active disease,³⁴ but this may not indicate increased bone turnover, since urinary hydroxyproline is also derived from the complement protein C1q,³⁵ the turnover of which is also increased in RA.

There are few studies of calcitropic hormones in RA. Abnormalities of mineral metabolism suggestive of parathyroid hormone (PTH) excess have been observed in RA,³⁶ but the levels of immunoreactive PTH have been found to be normal in two studies.^{33 37} Catabolism of PTH involves cleavage of the intact 84 amino acid molecule in the neighbourhood of residue 34 to generate an amino terminal fragment with biological activity and a larger biologically inert fragment containing the mid-region and carboxyl region.³⁸ Assays directed against the mid-region may reflect parathyroid secretory activity more specifically than assays against the carboxyl terminal.^{39 40} We were unable to show any disturbance in parathyroid gland activity in RA by a mid-molecule PTH assay.

Kennedy *et al.*³⁷ found no abnormality in plasma calcitonin levels in RA with a relatively insensitive assay (sensitivity 100 pg/ml(ng/l)), whereas Orth *et*

*al.*³⁰ found a reduction in mean calcitonin levels in males but not females with RA. The measurement of calcitonin by radioimmunoassay is difficult for several reasons. Calcitonin is normally present in serum in very low concentrations and declines with age.⁴¹ Circulating immunoreactive calcitonin is heterogeneous, and detection varies with different assays.⁴² The assay we used was more sensitive than that used by Kennedy *et al.*,³⁷ but hormone remained undetected in many samples and it was not possible to establish whether subnormal concentrations occur in RA.

No substantial disturbance in skeletal metabolism could be shown in patients with RA of recent onset. The finding of a small reduction in indexes of bone formation in these patients and the correlation observed between these indexes and skeletal muscle mass suggests that further studies may clarify the role of immobilisation in the pathogenesis of the generalised osteoporosis frequently observed in non-steroid treated patients with long-established disease.

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