

⁴⁷Ca turnover in endemic fluorosis and endemic genu valgum

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1. Calcium turnover was determined after intravenous injection of radioactive ⁴⁷Ca in patients with fluorosis and endemic genu valgum and in age-matched controls. Total Ca turnover in the body, loss of Ca from the body in urine, faeces and sweat (external turnover) and bone mineralization rate were calculated from whole-body retention of ⁴⁷Ca and specific activity of ⁴⁷Ca in serum.
2. Total Ca turnover was significantly higher in younger subjects than in older subjects.
3. Total Ca turnover was significantly higher in patients with fluorosis and in those with endemic genu valgum than in age-matched controls, but the external turnover of Ca was lower in both groups of patients than in controls.
4. Bone mineralization rate was significantly higher in patients with fluorosis and in those with genu valgum as compared to age-matched controls.
5. Total body Ca turnover and bone mineralization rates were significantly higher in patients with endemic genu valgum than in those with fluorosis. The differences persisted even after adjusting for differences in age between patients with fluorosis and those with genu valgum.

Endemic fluorosis characterized by extensive skeletal changes has been reported from several parts of India (Shortt, Pandit & Raghavachari, 1937; Siddiqui, 1955; Singh, Jolly, Bansal & Mathur, 1963) and the neighbouring Ceylon (Senewiratne, Santhi, Hattiarachchi & Senewiratne, 1974). Recently, a new clinical manifestation of fluoride toxicity, endemic genu valgum has been reported from parts of Andhra Pradesh in Southern India, where fluorosis is endemic (Krishnamachari & Kamala Krishnaswamy, 1973). The epidemiological, clinical and radiological features of the syndrome of endemic genu valgum have been reported in detail (Krishnamachari & Kamala Krishnaswamy, 1974). A similar bone disease with osteoporosis due to excess intake of fluoride had been reported from South Africa (Jackson, 1962). While fluorosis is characterized by osteosclerosis and new bone formation, endemic genu valgum is characterized by the simultaneous occurrence of osteosclerosis in some bones (particularly spine) and osteoporosis in bones of the extremities. Metabolic studies in subjects with skeletal fluorosis have indicated increased retention of calcium (Srikantia & Siddiqui, 1965). Turnover studies in such subjects with ⁴⁵Ca have confirmed its delayed excretion and increased bone uptake (Narasinga Rao, Siddiqui & Srikantia, 1968). Differences in the clinical and radiological findings between endemic genu valgum and endemic fluorosis suggest that Ca metabolism and rates of Ca turnover in these two conditions may not be the same. A comparative study of Ca turnover in subjects with skeletal fluorosis and with endemic genu valgum was therefore undertaken using ⁴⁷Ca.

MATERIALS AND METHODS

Subjects

Seven patients with endemic fluorosis, nine patients with endemic genu valgum and ten apparently-normal control subjects volunteered for the study. Six control subjects were of the same socio-economic group as the patients with fluorosis and endemic genu valgum and four subjects were of a higher socio-economic group. Among the control subjects of the low

Table 1. *Details of subjects studied*
(Mean values with ranges in parentheses)

Subjects	No.	Age (years)	Body-wt (kg)
Fluorosis	7	40 (14-55)	42 (25-56)
Genu valgum	9	16 (13-20)	36 (22-50)
Control:			
Low socio-economic group, age-matched for fluorosis.	2	50 (40-60)	44 (40-48)
Low socio-economic group, age-matched for genu valgum	4	21 (18-24)	48 (43-51)
High socio-economic group	4	34 (24-43)	56 (48-64)

socio-economic group, two were matched-for-age with patients with fluorosis while the other four were matched-for-age with patients with endemic genu valgum (Table 1). The mean age of patients with fluorosis was 40 years and that of patients with genu valgum 16 years. All subjects used for radioactivity studies were volunteers who gave informed consent.

All patients with fluorosis and endemic genu valgum came from villages situated in the areas of Andhra Pradesh where fluorosis is endemic. The fluoride content of drinking-water of these villages was as high as 525-850 $\mu\text{mol/l}$. In all subjects radiographs of the left forearm, dorsal spine and both knee joints were taken in the anterior posterior position under standardized conditions of tube length and exposure time.

Materials

The $^{47}\text{CaCl}_2$ (carrier-free) was obtained from the Radiochemical Centre, Amersham, Bucks., UK.

Administration of ^{47}Ca

A solution of 15-20 μCi $^{47}\text{CaCl}_2$ diluted with saline (9 g sodium chloride/l) was injected intravenously to the subjects in the morning after an overnight fast and blood samples were taken 0.5, 2, 4, 8 and 24 h after administration of ^{47}Ca and thereafter daily for the next 10 d. Urine collections (24 h) were made every day during this period. All investigations were performed in a metabolic ward.

Whole-body counting

Body retention of ^{47}Ca activity was determined using a shadow-shield-type whole-body counter (Model IAEA No. 3004; International Atomic Energy Agency, Vienna, Austria) with a scanning geometry (Dudley, 1970). The subjects were scanned twice for 10 min each, forward and backward in the energy range 0.17-1.32 MeV. Each subject was scanned before the injection of ^{47}Ca to establish background levels and within 2 h after injection of ^{47}Ca to obtain the 100 % count. Subsequently the subjects were scanned daily for the next 10 d.

Determination of ^{47}Ca

Blood samples were centrifuged and the serum was separated. ^{47}Ca activity in serum was measured using a well-type scintillation counter (Model No. SC-57A; Tracerlab, 1601, Trapelo Road, Waltham 54, Mass., USA), together with a standard prepared by appropriate

dilution of the injected dose. ⁴⁷Ca activity in urine was measured using liquid-scintillation spectrometer (Model No. 2211; Packard Instrument Co. Inc., 2200 Warrenville Road, Downers Grove, Ill. 60615, USA). Ca in serum was determined by the dye method using o-cresolphthalein complexone (Ray Sarkar & Chauhan, 1967). Urinary Ca was determined by the calcium oxalate method (Henry, 1964).

Evaluation of kinetic measurements

Whole-body ⁴⁷Ca activity was plotted semi-logarithmically and a straight line fitted by the method of least squares. The rate-constant (k) and half-life ($t_{1/2}$) of ⁴⁷Ca loss from the body was calculated graphically. From this the external turnover of Ca ($k \times$ plasma pool, S_2), i.e. loss of Ca through urine, faeces and sweat was calculated. Values for Ca specific activity in serum of these subjects after intravenous injection of ⁴⁷Ca were analysed according to the two-compartment model proposed by Heany (1963). The exponential function: $Y = Ae^{-\alpha t} + Be^{-\beta t}$, where Y is serum ⁴⁷Ca specific activity and A , B are constants, was fitted to serum specific activity values from 5 h to 10 d by the peeling technique (Simon, 1972), observing the correlation coefficient within each component between t and Y is maximum. The goodness of fit was tested by χ^2 test and it was found to be a good fit in all subjects. Various kinetic measurements including total body Ca turnover rate, external turnover of Ca (loss of Ca in urine, faeces and sweat) and bone mineralization (i.e. accretion of Ca by bone) were calculated using the relationships shown in Fig. 1.

Statistical analysis

The statistical significance of difference between groups for Ca kinetic measurements was tested by Student's t test. While comparing patients with fluorosis and those with genu valgum, analysis of covariance was used to correct for differences in age between these two groups to obtain adjusted means for the Ca kinetic measurements.

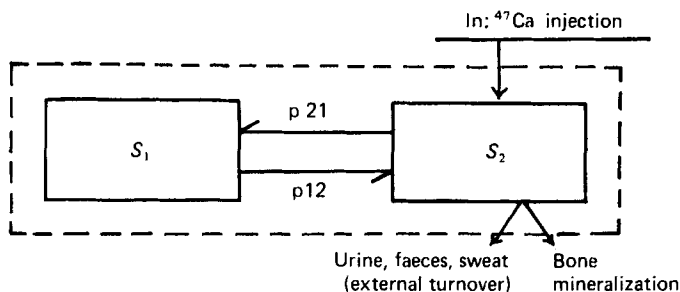
RESULTS

On examination of the radiographs osteosclerosis of the spine was seen in all patients with fluorosis and with endemic genu valgum, and the majority of patients also had calcification of the interosseous membrane of the forearm. Osteoporosis of the lower end of femur and upper end of tibia and fibula was observed in seven of the nine patients with genu valgum, but in none of the patients with fluorosis. This confirms observations already reported (Krishnamachari & Kamala Krishnaswamy, 1974).

Typical curves for the whole-body retention of radioactivity and serum Ca specific activity for control subjects and those with fluorosis are given in Fig. 2, and for control subjects and those with genu valgum are shown in Fig. 3. Values for Ca turnover are given in Table 2.

The external turnover of Ca calculated from whole-body counting of ⁴⁷Ca retention (see Fig. 1) was significantly ($P < 0.001$) lower in both fluorosis and endemic genu valgum, when compared to age-matched control subjects. There was no difference in this measurement, however, between control subjects belonging to the different age-groups. At the end of 10 d, whole-body retention of radioactivity was only 50–60% of the injected dose in control subjects and 80–90% of the injected dose in patients with endemic genu valgum and fluorosis.

There was no difference in Ca turnover measurements between control subjects of the low socio-economic and those of the high socio-economic group. However, there were significant differences ($P < 0.05$) between the old and young control subjects, with respect to total body turnover of Ca and bone mineralization rate. The exchangeable pool size was higher



Equation for plasma specific activity (y) values:

$$Y = Ae^{-\alpha t} + Be^{-\beta t}$$

$$S_1 \text{ (pool size compartment - 1; g)} = \frac{AB(\alpha - \beta)^2 \times 100}{(A + B)(A\beta + B\alpha)^2}$$

$$S_2 \text{ (pool size compartment - 2; g)} = \frac{100}{A + B}$$

$$\text{Intercompartmental flux (g/d) } \rho^{12} = \rho^{21} = \frac{AB(\alpha - \beta)^2 \times 100 \times 24}{(A\beta - B\alpha)(A + B)^2}$$

$$\text{Total system turnover (g/d)} = \frac{\alpha B \times 100 \times 24}{A\beta + B\alpha}$$

$$\text{External turnover (g/d)} \\ \text{(urine + faecal + sweat loss)} = k \times S_2$$

$$\text{Bone mineralization} = \text{total turnover} - \text{external turnover.}$$

Fig. 1. Schematic diagram of two-compartment system and equations used in calcium kinetic measurements. t , time (h); A , B , constants; k , decay constant of loss of ^{47}Ca from body.

in the younger control subjects than in the older control subjects, but this difference was not statistically significant ($P > 0.05$).

Total body Ca turnover rate and bone mineralization rate were significantly ($P < 0.001$) higher in patients with fluorosis as compared to age-matched control subjects. In endemic genu valgum also the bone mineralization rate was significantly ($P < 0.05$) higher than in the age-matched control subjects. The plasma Ca pool was significantly ($P < 0.01$) higher in patients with fluorosis but not in those with genu valgum, as compared to the corresponding control subjects. Intercompartmental flux was higher in patients with genu valgum and those with fluorosis as compared to the corresponding age-matched control subjects, but these differences were not statistically significant ($P > 0.05$).

Comparison of Ca kinetic measurements in patients with fluorosis and in those with genu valgum indicated that values for turnover and bone accretion rates were significantly ($P < 0.05$) higher in patients with genu valgum as compared to those with fluorosis. Patients with fluorosis belonged to the older age-group while those with endemic genu valgum belonged to the younger age-group. Since bone mineralization and total Ca turnover rates were found to be age-dependent in control subjects, an attempt was made to adjust for age while comparing Ca kinetic measurements between patients with fluorosis and those with genu valgum. The regression coefficient for age v . Ca kinetic measurements was not significant between control, fluorosis and genu valgum groups. Analysis of covariance indicated that the turnover and bone mineralization rates were significantly higher in patients with genu valgum than in those with fluorosis, even after adjusting for the age difference between these two groups. The F values between adjusted means were 4.92 for Ca turnover and 5.05 for bone accretion rate, both being significant ($P < 0.05$).

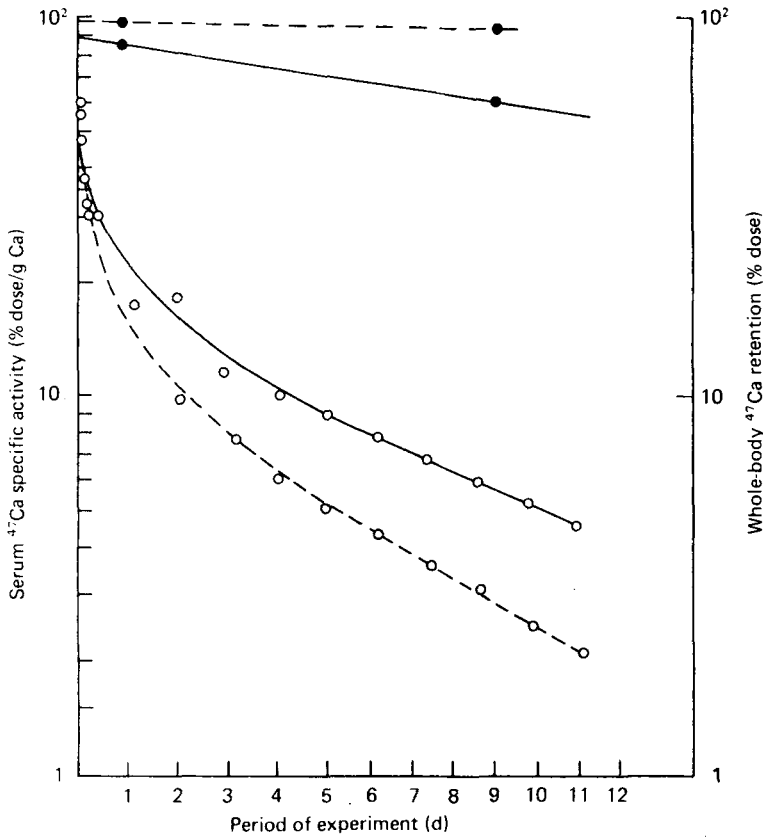


Fig. 2. Whole-body ⁴⁷Ca activity (●) (% dose) and serum ⁴⁷Ca specific activity (○) (% dose/g Ca) in fluorosis (---) and age-matched controls (—) (for details of subjects, see p. 7 and Table 1.) Regression equations for serum ⁴⁷Ca specific activity: age-matched controls: $Y = 26.89e^{-0.18t} + 20.33e^{-0.018t}$, fluorosis: $Y = 7.48e^{-0.14t} + 14.6e^{-0.081t}$, where Y is serum ⁴⁷Ca specific activity, t is time (h).

DISCUSSION

Earlier studies have indicated that both in human subjects (Srikantia & Siddiqui, 1965; Narasinga Rao *et al.* 1968) and in experimental animals (Sriranga Reddy & Narasinga Rao, 1971) chronic fluoride toxicity is associated with increased Ca retention, increased exchangeable Ca pool and increased bone mineralization rate. These changes are in line with the clinical observation that fluorosis is associated with new bone formation as well as increased density of the bone. It has been observed that skeletal fluorosis generally develops in adults after the age of 40 years, while endemic genu valgum has been generally observed in the younger age-group between 9 and 15 years. Clinical observations indicate that while fluorosis is accompanied by exostoses and osteosclerosis throughout the body, endemic genu valgum is characterized by osteosclerosis of the spine and osteoporosis in some of the long bones.

When the values for different Ca kinetic measurements in fluorosis and endemic genu valgum in relation to the corresponding values of the age-matched controls were considered it was observed that the daily loss of the body Ca (external Ca turnover) in fluorosis and genu valgum was less as compared to age-matched control subjects. Total body Ca turnover and bone mineralization rates calculated from the kinetic values indicate that both the turnover and bone mineralization rates are higher in patients with genu valgum than in those

Table 2. ^{45}Ca turnover* in patients with fluorosis and endemic genu valgum after intravenous injection of $^{45}\text{CaCl}_2$

Subjects †	No. of subjects	Retention on 10th day (%)		S_1 (mg/kg)		S_2 (mg/kg)		Flux (mg/kg per d)		Total turnover (mg/kg per d)		External turnover (mg/kg)		Bone mineralization (mg/kg per d)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fluorosis	7	87.5	2.48	53.6	18.9	62.3	5.6	243.8	122	87.5	8.1	0.81	0.20	86.1	8.1
Age-matched control	6	58.7	2.36	15.8	5.2	36.2	3.5	44.1	15.3	33.7	3.3	1.64	0.23	32.1	3.3
Genu valgum	9	86.3	1.73	54.0	12.6	93.6	15.4	244.6	63.7	136.1	18.6	1.06	0.21	134.9	18.3
Age-matched control	4	56.7	2.85	28.1	8.8	86.6	13.8	86.3	38.1	74.1	7.6	2.87	0.75	71.2	7.6

(Mean values with their standard errors)

 S_1 , slow compartment; S_2 , plasma compartment (for details, see Fig. 1).

* For details of measurement, see Fig. 1.

† For details, see Table 1.

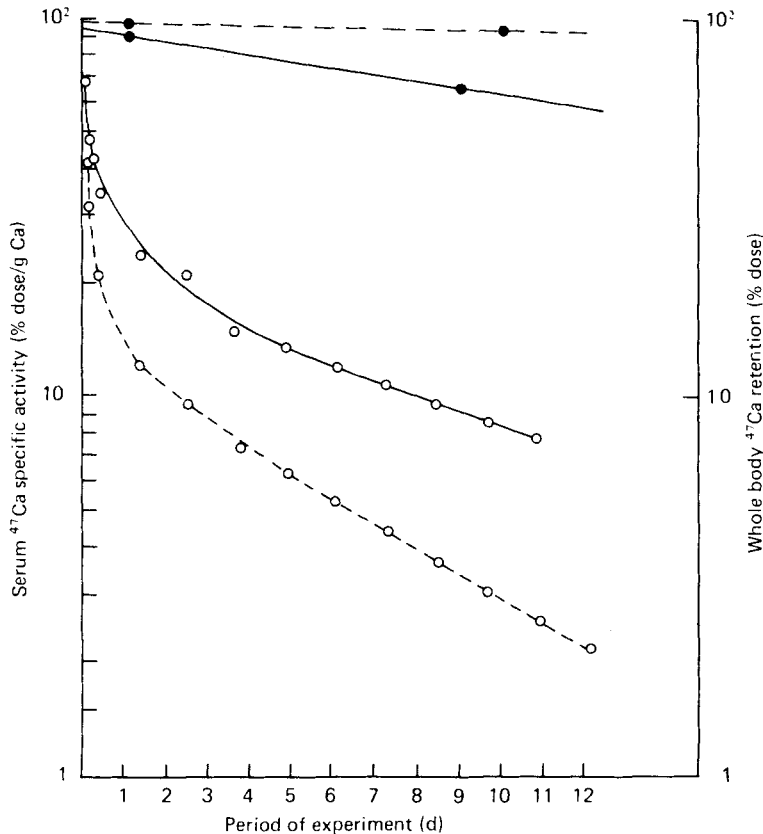


Fig. 3. Whole-body ⁴⁷Ca activity (●) (% dose) and serum ⁴⁷Ca specific activity (○) (% dose/g Ca) in genu valgum (---) and age-matched controls (—) (for details of subjects, see p. 7 and Table 1). Regression equations for serum ⁴⁷Ca specific activity: age-matched controls: $Y = 5.51e^{-0.085t} + 16.46e^{-0.021t}$, genu valgum: $Y = 45.12e^{-0.431t} + 14.4e^{-0.031t}$, where Y is serum ⁴⁷Ca specific activity, t is time (h).

with fluorosis. The patients with endemic genu valgum belonged to the younger age-group. It was observed that these two Ca kinetic measurements were higher in younger subjects than in older subjects. The higher turnover and bone mineralization rates in patients with genu valgum was evident even after the effect of age was eliminated by analysis of covariance. The important fact that emerges from the present study is that the increase in Ca accretion rate which may be attributable to fluoride toxicity is of a greater magnitude in patients with genu valgum than in those with fluorosis.

It is surprising that in genu valgum in spite of osteoporosis, the bone mineralization rate should be higher than in fluorosis. Greater than normal bone mineralization rates have been reported in osteoporosis due to acute immobilization (Heany, 1962), hyperthyroidism (Krane, Brownell, Stanbury & Corrigan, 1956) and acromegaly (Bell & Bartter, 1967). The bone resorption rates also are even higher than bone formation rate in these instances, resulting in osteoporosis. It is thus possible that in genu valgum, also, where osteoporosis co-exists with osteosclerosis, increased bone formation rate may be accompanied by a much greater increase in bone resorption rate. The presence of osteoporosis in only some bones of patients with endemic genu valgum suggests that the increased resorption rate may be confined to these bones. The present kinetic results do not permit evaluation of the resorption

rate in these conditions. A short-term Ca kinetic study such as the present study will not yield information on resorption rate. When Ca balance was done concurrently with a Ca turnover study it was found that the resorption rate was also increased in fluorosis (Narasinga Rao *et al.* 1968), and a similar study is required in endemic genu valgum. Levels of immuno-reactive parathyroid hormone are increased both in fluorosis and in endemic genu valgum (Sivakumar & Krishnamachari, 1976), but the explanation for the simultaneous increased mineralization in some bones and the increased resorption in other bones is not clear.

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