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Calcium metabolism in fluorosis and endemic genu valgum using radioactive tracer, whole body counting and radioimmunoassay

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NATIONAL INSTITUTE OF NUTRITION
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India.

Final Report of work done under the International Atomic Energy Agency Research Contract No. 1749/RS/RB

Contract No.	1749/RS/RB
Title of the project	"Calcium metabolism in fluorosis and endemic genu valgum using radioactive tracers, whole body counting and radioimmunoassay.
Institute where the research was carried out	National Institute of Nutrition Indian Council of Medical Research Jamai-Osmania Hyderabad-500 007, India.
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Time period covered	December 1975 to December 1978

SUMMARY

1. Calcium turnover was determined after intravenous injection of radioactive ^{47}Ca 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 the whole-body retention of ^{47}Ca and specific activity of ^{47}Ca in serum. Calcium balance was simultaneously carried out in some of these subjects.

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.

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6. Based on the ^{47}Ca turnover, and Ca balance, bone resorption rates were calculated. Bone resorption rate was found to be significantly higher in subjects with genu valgum as compared to those with fluorosis. Thus in the case of genu valgum, both bone formation rate and bone resorption rate are exaggerated, perhaps accounting for both osteosclerosis and osteoporosis seen in these cases.

7. Vitamin D nutrition status was determined in cases of endemic fluorosis and endemic genu valgum and compared with age matched controls from the endemic and non-endemic areas. Serum 25-OHD_3 was used as an index of vitamin D nutrition status. No evidence of vitamin D deficiency was found in any of the groups studied. On the other hand serum 25-OHD_3 levels were significantly elevated in genu valgum subjects. It is concluded that vitamin D deficiency may not be one of the factors responsible for bone manifestitations seen in genu valgum.

^{47}Ca TURNOVER IN ENDEMIC FLUOROSIS AND ENDEMIC GENU VALGUM.

Endemic fluorosis characterised by extensive skeletal changes has been reported from several parts of India (Shortt et al, 1937; Siddiqui, 1955; Singh et al, 1963) and the neighbouring Ceylon (Senewiratne, et al, 1974). Recently, a new clinical manifestation of fluoride toxicity, viz. endemic genu valgum, has been reported from parts of Andhra Pradesh in Southern India, where fluorosis is endemic (Krishnamachari and Kamala Krishnaswamy, 1973). The epidemiological, clinical and radiological features of the syndrome of endemic genu valgum have been reported in detail (Krishnamachari and 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 and Siddiqui, 1965). Turnover studies in such subjects with ^{45}Ca have confirmed its delayed excretion and increased bone uptake (Narasinga Rao, Siddiqui and 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 calcium turnover in subjects with skeletal fluorosis and endemic genu valgum was therefore undertaken using ^{47}Ca. This was carried out in two separate studies. In the first study, ^{47}calcium turnover was carried out without any concomitant calcium balance. In the second study, calcium balance and calcium turnover were simultaneously determined in a group of patients of fluorosis with and without genu valgum.

STUDY-1.

Materials and Methods

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 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 μ mol/l. In all subjects radiographs of the left forearm, dorsal spine and both knee joints were taken in the anteroposterior position under standardized conditions of tube length and exposure time.

Materials

The $^{47}CaCl_2$ (carrier-free) was obtained from the Radiochemical Centre, Amersham, Bucks, UK.

Administration of ^{47}Ca

A solution of 15-20 μCi $^{47}CaCl_2$ 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 at 0.5, 2, 4, 8 and 14 hr after administration of ^{47}Ca and thereafter daily for the next 10 d. Urine collections (24 hr) 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. ^{47}Ca 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-cresolphthalenin complexone (Ray Sarkar and Chauhan, 1967). Urinary Ca was determined by the calcium oxalate method (Henry, 1964).

Evaluation of kinetic measurements

Various kinetic parameters including total turnover, external turnover rate of calcium and bone mineralization rate were calculated from the whole body counting data and calcium

specific activity as described by Heany (1963) and shown in figure-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 and Kamala Krishnaswamy, 1974).

Typical curves for the whole body retention of radioactivity and serum Ca specific activity for contol subjects and those with fluorosis are given in figure-2, and for

control subjects and those with genu valgum are shown in figure-3. Values for Ca turnover are given in table-2.

The external turnover of Ca calculated from whole body counting of ^{47}Ca 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 in younger control subjects than in the older control subjects, but this difference was not significantly significant.

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.

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 vs Ca kinetic measurements was not significant between control, fluorosis and genu valgum groups. Analysis of co-variance 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 ($P < 0.05$).

STUDY-2

Subjects

Four patients with endemic genu valgum and 3 patients with endemic fluorosis and seven control subjects from the same socio-economic group as the patients from fluorosis and endemic genu valgum were studied. Among the control subjects four were age matched with genu valgum patients while three others matched for age with endemic fluorosis patients. Details of the subjects are given in table-3.

^{47}Ca turnover

^{47}Ca turnover was studied the same way as described above.

Ca balance

The subjects were admitted into a metabolic ward and fed on a standard diet similar in composition to their habitual diet. Details of the diet fed are given in table-3. A solution of 15-20 µCi of ^{47}Ca, diluted with saline was injected intravenously in the morning on the second day of admission. Blood was drawn at 0.5, 2, 4, 8 and 24 hrs, after the administration of radiocalcium and thereafter daily for the next 10 days. Urine and feces were collected during the last four days of the balance period, ie. on the 8th to 12th day.

Urinary calcium was determined by calcium oxalate method (Henry, 1964).

Results

The results of calcium turnover study are given in table-4. The calcium kinetic parameters were computed from the whole body retention of ^{47}Ca, serum ^{47}Ca specific activity and cold calcium retention data. It can be seen that the relative values for total turnover, external turnover and bone formation rate in genu valgum and fluorosis and the corresponding controls were similar to those observed in the first study, although the absolute values for these parameters were some what different. Thus the results of the second study confirms the conclusions of the first.

Bone resorption rate.

From the bone formation rate and cold calcium retention, bone resorption rate was estimated using the following relationship.

Bone resorption rate (g/d) = bone formation rate g/d - calcium retention g/d.

The bone resorption rates in fluorosis and genu valgum were significantly higher than the figures obtained for the corresponding controls. The bone resorption rate in genu valgum was significantly higher than that observed in fluorosis. The bone resorption rate in the younger age group subjects who form the control for the genu valgum subjects also had a higher resorption rate as compared to older age normals, who form controls for the fluorosis subjects.

Discussion

Earlier studies had indicated that both in human subjects (Srikantia and Siddiqui, 1965; Narasinga Rao et al, 1968) and in experimental animals (Sriranga Reddy and 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 12 and 25 years. Clinical observations indicate that while fluorosis is accompanied by exostoses and osteosclerosis throughout the body, endemic genu valgum is characterized by simultaneous occurence of 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 were

higher in patients with genu valgum than in those 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 inspite 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, hyperthroidism and acromegaly. The bone resorption rate also are even higher than bone formation rate, in these instances resulting in osteoporosis. It is also possible that in genu valgum also where osteoporosis coexists with osteoclorosis, increased bone formation rate may be accompanied by a much greater increase in bone resorption rate. The results of the second study do confirm that the bone resorption rates in genu valgum is considerably increased as is the bone formation rate. We observe however that in genu valgum the bone resorption rate does not exceed the bone formation rate.

It is known that in genu valgum, osteoporosis is present only in some bones, while osteoclorosis in others. In such of the bones, where there is osteoporosis, bone resorption rate will exceed the bone formation rate while the reverse may be true in those bones with osteoclerosis. The net effect of these changes will be an increased rate of both resorption and formation rates. It is observed that even in fluorosis bone resorption is higher than in the corresponding controls. This is in confirmation of the earlier reports by Narasinga Rao et al (1968).

STUDIES ON VITAMIN D METABOLISM IN GENU VALGUM AND FLUOROSIS.

Endemic fluorosis is clinically characterized by osteosclerosis. Endemic genu valgum, which has recently been observed in some endemic fluorosis areas in India is associated with extensive oestoporosis of limb bones, in addition to ostoclerosis of the spine as observed in fluorosis. Genu valgum subjects usually belong to the lower age group, while the fluorosis patients belong to the older age groups. Although the primary etiological factor in the skeletal manifestation of fluorosis and genu valgum is the prolonged ingestion of high levels of fluoride, several other factors including metabolic or dietary may also modify the manifestation of fluoride toxicity. The two factors which may be considered

in this context are parathyroid hormone (PTH) and vitamin D_3 whose role in calcium and phosphorous metabolism and in bone re-modelling are wellknown. It was reported earlier that circulating PTH levels are elevated in fluorosis and more so in subjects with genu valgum (Sivakumar and Krishnamachari, 1976). There is no information on the vitamin D nutrition status of these patients, since these patients in the endemic area are well exposed to sunlight they are likely to have adequate vitamin D. It is not known whether metabolic disturbances due to fluoride toxicity can lead to conditioned vitamin D deficiency. A study was therefore undertaken to determine the vitamin D nutrition status in subjects suffering from endemic fluorosis with and without genu valgum. In the present study, levels of serum 25-hydroxy cholecalciferol (25-OHD_3), a major circulating metabolite of vitamin D, which is known to reflect the nutritional status of vitamin D_3, were determined in subjects suffering from endemic fluorosis and those with genu valgum and in appropriate controls drawn both from the same endemic area and from the non-endemic area.

Materials and Methods

Subjects

Thirty subjects suffering from skeletal fluorosis were selected from an endemic fluorosis area (fluoride content of drinking water was 8 to 12 mg perlitre). Twelve of them were adults (mean age of 40 years) and had features of

osteofluorosis, but did not have genu valgum. The other 18 subjects had genu valgum and were adolescents or young adults (mean age 20 years). In addition, a group of 12 subjects (mean age 20 years) and a group of 8 subjects (mean age 40 years) who resided in the same endemic area but who did not show obvious clinical deformities were also included. Seventeen subjects residing in a non-endemic area where the fluoride content of drinking water was 1.2 - 1.4 ppm served as controls for the above groups. Eleven of them were elderly persons with a mean age of 40 years while the mean age of the other group consisting of six subjects was 20 years.

Fasting blood samples were collected in the respective villages with the consent of the subjects and transported to the laboratory in ice. Serum was separated immediately for the estimation of calcium by Atomic absorption spectrophotometry, inorganic phosphorus by colorimetry (Chen et al, 1956) and alkaline phosphatase by Bodansky's procedure (Bodansky, 1933). Serum 25-OHD_3 was estimated after extraction with ethanol by competitive protein binding assay using normal rat serum as a binding protein (Belsey et al, 1971).

Results and Discussion

Serum values of calcium, inorganic phosphorus, alkaline phosphatase and 25-OHD_3 are given in table-5. Serum calcium

and phosphorus levels were not different in any of the groups studied. Although there were some differences between groups in the serum alkaline phosphatase activity, all the values were however within the normal range. Control subjects from normal control area had significantly lower serum alkaline phosphatase activity as compared to subjects from genu valgum area with or without any obvious clinical signs of genu valgum. The values of serum alkaline phosphatase activity in skeletal fluorosis, was not different from those in the age matched subjects from the area but who showed no obvious clinical symptoms and also from those observed in age matched controls from the non-endemic area.

Serum 25-OHD_3 levels showed vide variations in all the groups. However, 25-OHD_3 levels were significantly higher in genu valgum subjects as compared to controls either from the same area but without any clinical symptoms and or from the non-endemic area. Even in subjects who had been living in endemic genu valgum area but without any obvious clinical symptoms serum 25-OHD_3 levels were higher than in the control subjects of the same age from the non-endemic area. The values of serum 25-OHD_3 levels were not different between subjects of skeletal fluorosis and subjects from the same area, but without obvious clinical signs. However, the values were slightly (not statistically significant), lower than the control subjects of similar age group from non-endemic area. Comparison of serum 25-OHD_3 levels between genu valgum and skeletal fluorosis indicated that, the values are higher in genu valgum than in skeletal fluorosis.

Of the several biochemical parameters studied, only alterations observed were in serum alakline phosphatase activity and 25-OHD_3 levels in various groups studied. Vitamin D nutritional status as judged by the serum 25-OHD_3 levels indicate that none of the groups studied suffered from vitamin D inadequacy, and the values are either within the normal range or higher than the reported values (Haddad and Chyu, 1971; Haddad and John, 1973; Edelstein et al, 1974; Haddad and Stamp, 1974; Stamp et al, 1977; White and Haddad, 1979 and Poskitt et al, 1979)..

One striking observation is that of elevated levels of serum 25-OHD_3 in genu valgum ± as compared to other groups. Such as increase was not observed in skeletal fluorosis, though both the groups of subjects were exposed to high fluoride intakes for a prolonged periods, and perhaps similar exposure to sunlight. One common feature between both the groups is an elevated alkaline phosphatase activity. This may indicate that high fluoride ingestion may activate the osteoblastic activity, while affecting differentially the metabolism of vitamin D. Further work is needed to understand clearly the mechanism of action of fluoride if any on vitamin D metabolism. The present study however suggests that vitamin D deficiency may not be one of the factors in the development of endemic genu valgum.

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Table.1

Details of subjects studied

(Mean values with ranges in parenthesis)

Subjects	No.	Age (years)	Body-wt (kg)
Fluorosis	7	40 (14-55)	42 (25-56)
Genu valgum	9	16 (13-20)0	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)

Table.2

^{47}Ca turnover* in patients with fluorosis and endemic genu valgum after intravenous injection of $^{47}CaCl_2$

(Mean values with their standard errors)

Subject[1]	No.of sub.	Retention on 10th day(%)		S_1 (mg/kg)		S_2 (mg/kg)		Flux (mg/kg/day)		Total turnover (mg/kg/day)		External turnover (mg/kg)		Bone mineralization (mg/kg/day)	
		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
Aged 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

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

* For details of measurement, see Fig.1

[1] For details, see table.1.

Table.3

Details of subjects

Subject	No.	Age (years)	Body wt (kg)	Dietary Ca intake (mg)
Genu valgum	4	17 (15-19)	34.2 (32-39)	402
Control for Genu valgum	4	22 (19-25)	45.9 (41-48)	569
Fluorosis	3	38 (30-48)	45 (37-55)	353
Control for fluorosis	3	36 (25-42)	45.2 (34-52)	685

(Mean values with ranges in parenthesis)

Table.4

^{47}Ca turnover and balance in patients with fluorosis and endemic genu valgum after intravenous injection of $^{47}CaCl_2$

Subjects	No.	Retention 10th (%) day	S1 mg/kg	S_2 mg/kg	Flux mg/kg/d	Total turnover mg/kg/d	External turnover mg/kg/d	B.F.R. mg/kg/d	Balance mg/kg/d	B.R.R. mg/kg/d
Genu valgum	4	93.4 ± 0.84	81.4 ± 13.52	86.0 ±7.19	848.4 ±145.00	212.9 ±27.36	0.52 ±0.091	212.47 ±27.29	4.99 ±0.119	207.5 ±27.37
Control for Genu valgum	4	76.3 ±2.29	28.8 ±4.33	53.3 ±7.31	85.7 ±21.68	67.3 ±5.44	1.00 ±0.192	66.35 ±5.43	4.70 ±0.666	61.7 ±6.04
Fluorosis	3	92.0 ±3.64	42.5 ±17.84	68.3 ±14.31	156.3 ±68.58	71.3 ±14.97	0.81 ±0.293	70.47 ±15.23	3.00 ±0.643	67.4 ±14.62
Control for fluorosis.	3	62.8 ±2.41	81.4 ±18.71	61.7 ±6.62	56.7 ±13.63	20.5 ±4.82	2.95 ±0.257	17.52 ±4.63	6.15 ±3.156	14.9 ±8.63
Genu valgum vs Control P /		0.001	0.02	0.02	0.01	0.01	0.05	0.01	NS	0.01
Fluorosis vs Control P/		0.01	NS	NS	NS	0.05	0.01	0.05	NS	0.05

B.R.R. - Bone resorption rate mg/kg/day; B.F.R. Bone formation rate mg/kg/day
Mean ± SE

Table.5

Serum biochemical parameters in endemic Fluorosis and genu valgum

Group	Serum values			
	Ca (mg/dl)	P(mg/dl)	ALK Ptase	25-OHD_3 (ng/ml)
Genu valgum	9.6 ± 0.27 (17)	5.9 ± 0.35 (18)	11.1 ± 1.96 (15)	82.0 ± 11.87 (14)
Subjects without clinical deformation from genu valgum area.	9.3 ±0.25 (11)	6.6 ± 0.30 (12)	6.2 ± 0.90 (12)	36.9 ± 7.39 (9)
Controls from non-genu valgum area.	9.8 ± 0.65 (6)	6.3 ± 0.65 (6)	3.2 ± 0.76 (5)	20.5 ± 5.84 (3)
Skeletal fluorosis	9.6 ± 0.39 (12)	5.6 ± 0.42 (12)	7.1 ± 1.38 (12)	38.3 ± 8.86 (10)
Subjects without clinical deformation from skeletal fluorosis area.	10.8 ± 0.41 (8)	6.2 ± 1.11 (8)	4.4 ± 0.85 (8)	47.9 ± 24.56 (6)
Controls from non-skeletal fluorosis area.	10.6 ± 0.38 (11)	4.8 ± 0.48 (11)	5.5 ± 0.74 (11)	73.9 ± 20.67 (8)

Mean values ± SE (n)

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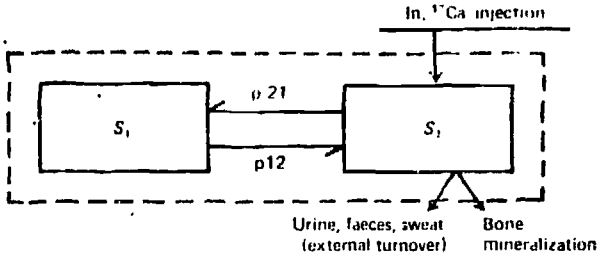

Equation for plasma specific activity (y) values:

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

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

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

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

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

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

Bone *mineralization = total turnover − 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.

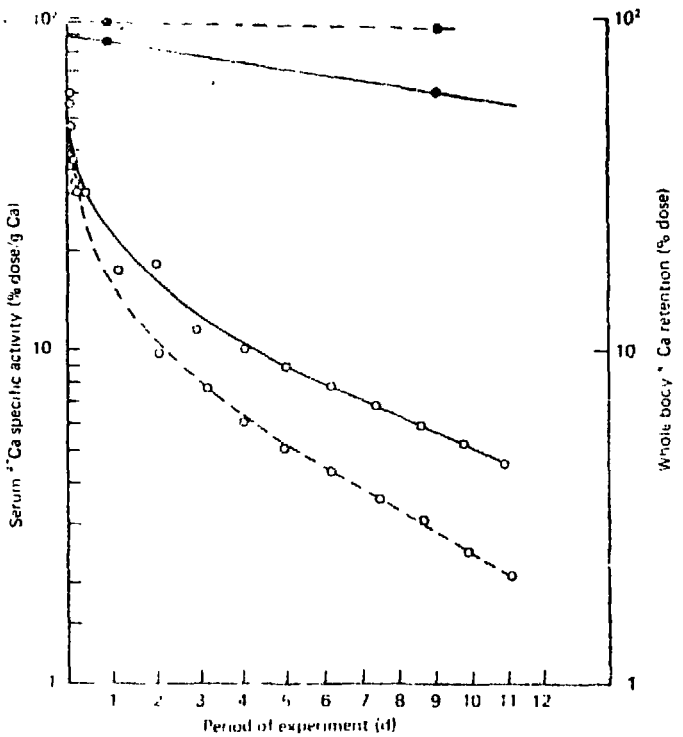

Fig. 2. Whole body ^{47}Ca activity (●) (% dose) and serum ^{47}Ca specific activity (○, ⋯, dose/g Ca) in fluorosis (○—○) and age matched controls (○ ⋯ ○) (for details of subjects, see p. 7 and Table 1). Regression equations for serum ^{47}Ca specific activity: age-matched controls: $Y = 20 \cdot 33e^{-0 \cdot 0188t}$, fluorosis: $Y = 7 \cdot 48e^{-0 \cdot 184t} + 14 \cdot 6e^{-0 \cdot 033t}$, where Y is serum ^{47}Ca specific activity, t is time (h).

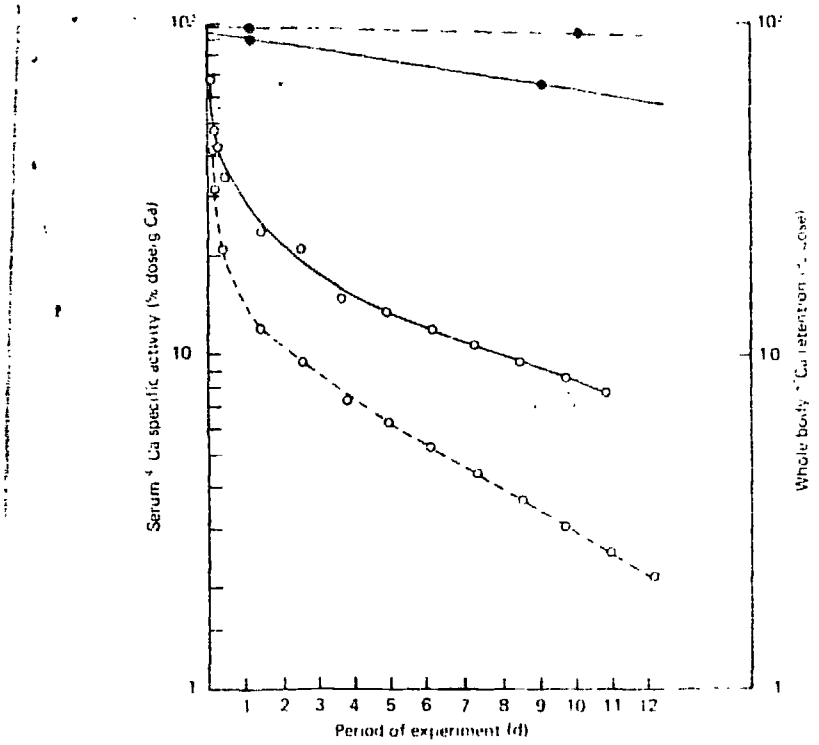

Fig. 3. Whole-body ^{47}Ca activity (●) (% dose) and serum ^{47}Ca specific activity (○) (% dose/g Ca) in genu valgum (——) and age-matched controls (----) (for details of subjects, see p. 7 and Table 1). Regression equations for serum ^{47}Ca specific activity: age matched controls: $Y = 5{\cdot}51e^{-0{\cdot}0854t} + 16{\cdot}46e^{-0{\cdot}0211t}$, genu valgum: $Y = 45{\cdot}12e^{-0{\cdot}1511t} + 14{\cdot}4e^{-0{\cdot}0214t}$, where Y = serum ^{47}Ca specific activity, t is time (h).