

# PARTIAL-BODY CALCIUM MEASUREMENTS BY IN VIVO NEUTRON ACTIVATION ANALYSIS: COMPARISONS WITH X-RAY PHOTODENSITOMETRY MEASUREMENTS OF THE RADIUS

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*A comparison has been made of measurements of bone calcium content of 71 individuals by both x-ray photodensitometry of the radius and in vivo neutron-activation analysis (IVNAA) of the trunk and upper thighs. A good correlation ( $r = 0.76$ ) is found between results of measurements by the two methods though there is wide scatter about the regression line. Twenty-seven patients have been measured sequentially by both techniques to assess changes in bone mineral. Again there is reasonable agreement in the majority (21/27) of cases and there are marked discrepancies in only three cases. It appears that IVNAA measurements on the trunk may show changes in calcium status more quickly than do photodensitometric measurements of the radius.*

The technique of in vivo neutron-activation analysis (IVNAA) allows for the first time quantitative measurements of changes in bone calcium of the total or a large portion of the total body (1-5). The radiologic skeletal surveys sometimes used for this purpose are inaccurate in that changes in bone calcium up to 30% or 40% may not be detected. Quantitative measurements of bone mineral content based on x-ray (6) or gamma-ray (7,8) absorption are considered to be much more accurate but such techniques examine only a small part of one bone which may not be representative of changes in the total skeleton.

The present report concerns an evaluation of bone calcium measurements both by IVNAA and x-ray

photodensitometry. At the University of Toronto, bone calcium measurements have been made on 71 subjects by both these methods. Subjects studied include normal subjects and patients suffering from osteoporosis, osteomalacia, and renal osteodystrophy. Twenty-seven of these patients have been measured sequentially over periods from 3 to 17 months to assess changes in bone mineral as a result of progression of disease or response to therapy.

## METHOD

The IVNAA technique utilizes the fact that natural calcium contains 0.18% of the isotope  $^{48}\text{Ca}$  (i.e., about 1.8 gm of  $^{48}\text{Ca}$  in the total body). When exposed to thermal neutrons,  $^{48}\text{Ca}$  may be converted to radioactive  $^{49}\text{Ca}$  which decays with a radioactive half-life of 8.8 min to  $^{49}\text{Sc}$ , emitting a 3.1-MeV gamma ray. This gamma ray can be measured with a whole-body counter. The measured amount of  $^{49}\text{Ca}$  is then proportional to the amount of calcium exposed to the neutron flux.

The IVNAA facility at the University of Toronto has been reported elsewhere (5,9). Briefly, the subjects are exposed for a 20-min period to 12 sources (5 Ci Pu-Be) positioned above and below the trunk and 12 cm from the surface of the body. The induced  $^{49}\text{Ca}$  radioactivity is measured in a whole-body counter for a 20-min period starting 3 min after the end of the irradiation. The four NaI crystals

Received Feb. 15, 1974; revision accepted May 22, 1974.

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in the whole-body counter are each of 8-in. diam and 4 in. in thickness and they are positioned above and below over the area of irradiation.

The reproducibility of our IVNAA measurements based on sequential measurements in 15 normal volunteers is  $\pm 6.6\%$  (1 s.d.) (5). The  $^{49}\text{Ca}$  count obtained by our procedure will not provide an absolute value for total-bone calcium without appropriate corrections for body size and thickness. The  $^{49}\text{Ca}$  count obtained is dependent on body thickness; both the mean thermal neutron flux and the  $^{49}\text{Ca}$  gamma-counting efficiency decrease proportionately with increasing body thickness. Based on phantom studies reported elsewhere (10), corrections for variations in body thickness may be made based on a 5% decrease in  $^{49}\text{Ca}$  count with each centimeter increase in body thickness. These corrections in the Ca count are probably reliable to within  $\pm 1\%/cm$  body thickness. With respect to body size, it should be noted that the calcium count comes from a fixed area of  $60 \times 30$  cm covering the trunk and upper thighs and therefore is the result of the irradiation of somewhere between one-half and one-third of the total-body calcium depending upon the subject's height. To obtain a measure of total calcium requires a correction for body size; in this paper this particular correction is not applied, comparison throughout being made in terms of calcium count corrected for variations in efficiency dependent on body thickness as noted above.

The technique of bone mineral measurements by x-ray photodensitometry has been reported elsewhere (6,11). The test provides a measurement of the mass of bone mineral (M) expressed as mineral thickness in  $\text{mg}/\text{cm}^2$  through the full thickness of bone at the proximal one-third of the radius, (i.e., the mass of a  $1\text{-cm}^2$  column of bone taken through a diameter of the radius). During the procedure, the arm is immersed in water, which compensates for patient-to-patient variation in soft-tissue thickness. Thus, no corrections as outlined for the IVNAA work are necessary with the x-ray measurements. The reproducibility of measurements on a human radius embedded in plastic is about  $\pm 3\%$ ; the overall error based on the measurement by two film readers of two measurements each on 31 individuals is 5% (1 s.d.) (6).

#### SELECTION OF SUBJECTS

The control subjects were normal volunteers consisting of five men, 34–50 years in age, and eight women, 45–75 years in age (mean 60).

The osteoporotic subjects consisted of nine men and four women all suffering from bone mineral loss and vertebral compression fractures but who all had

normal serum calcium and phosphorus. Two of these subjects (Cases 15 and 16 in Table 1) were studied both before treatment and at intervals up to 14 and 6 months, respectively, on treatment—some data on these two have been reported previously (5).

Thirty-seven patients suffered from renal failure and were on hemodialysis. Either by radiologic assessment or by examination of bone biopsies, all subjects demonstrated some degree of renal osteodystrophy. Twenty-three of these patients were studied while on hemodialysis at intervals ranging from 3 to 17 months (average 9 months).

Two subjects who suffered from osteomalacia were studied before and at intervals up to 9 and 24 months on treatment (Cases 67 and 24 in Table 1). Case 24 was a 19-year-old boy with vitamin D-dependent rickets; some data have been reported on him previously (5). Case 67 had multiple rib fractures associated with the osteomalacia. In addition, two patients with osteomalacia were studied once before treatment.

Bone mineral measurements were also carried out in one woman with adult osteopetrosis and three patients who were investigated but proved to have no metabolic bone disease.

#### RESULTS

A comparison of the partial-body bone calcium measurements by IVNAA with photodensitometric measurements of bone mineral content in the radius for all 71 subjects is shown in Fig. 1. A good correlation is obtained ( $r = 0.76$ ,  $p < 0.01\%$ ) although there is a wide scatter around the regression line. For example, different individuals with the same bone mineral mass value (M) by x-ray photodensitometry can differ by a factor of 2 in their IVNAA count, and vice versa.

Comparisons of the bone mineral measurements between normal, osteoporotic, and osteomalacic subjects are shown for men (Fig. 2) and for women (Fig. 3)—the line drawn is the regression line for all the data (Fig. 1). It will be noted that, in the case of the men, the IVNAA values show a separation between the normal subjects and the patients with metabolic bone disease; there is, however, overlap of M values between these two groups. In the case of the women, IVNAA measurements give a poor separation between normal and abnormal subjects whereas the M values give a complete overlap for these 13 subjects. It should be noted however that these results are for a small number of persons—this is looked into further in the Discussion.

Results of repeat measurements of bone mineral by x-ray photodensitometry and by IVNAA are shown in Fig. 4. Sixteen of the 27 patients showed

no change (less than 10%) in bone mineral by either measurement. These 16 will not be discussed further. The remaining 11 patients (Table 1) showed changes of greater than 10% by at least one method. Five of these (Cases 32, 27, 33, 6, and 24) showed qualitatively similar increases in bone mineral by both techniques. In six patients (Cases 67, 15, 16, 13, 34, and 35) significant discrepancies between the two sets of results were observed. These six will be discussed individually.

In Case 67, the osteomalacic subject with multiple rib fractures, there was radiologic evidence of callus formation at the site of the fractures within 1 month of the onset of treatment. This evidence was reflected by the IVNAA measurements whereas photodensitometric measurements of the radius showed slight decrease in mineral content. For comparison, the other osteomalacic subject, Case 24, was a 19-year-old boy who still had open epiphyseal plates. During treatment, he continued to grow and an ob-

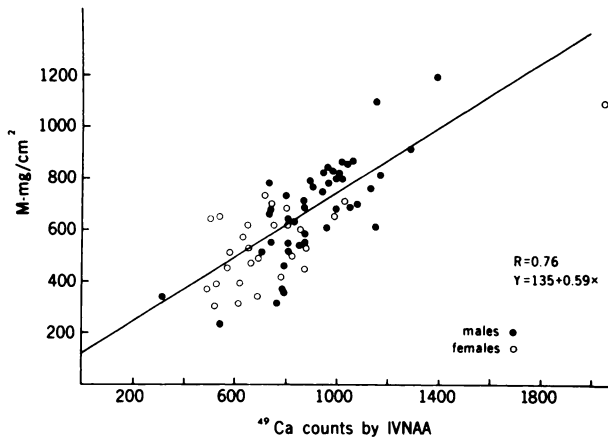
**TABLE 1. 11 PATIENTS SHOWING CHANGES OF GREATER THAN 10% IN BONE MINERAL MEASUREMENT BY IVNAA AND X-RAY PHOTODENSITOMETRY\***

Case No.	Sex	Age	Diagnosis	Treatment	Months on treatment	IVNAA		Mineral mass	
						<sup>40</sup> Ca counts	%	mg/cm <sup>2</sup>	%
32	M	50	Renal failure	HD*	0	946	100	815	100
					3	1003	106	761	93
					11	1060	112	860	106
27	M	25	Renal failure	HD*	0	866	100	711	100
					5	—	—	701	99
					9	979	113	832	117
33	M	47	Renal failure	HD*	0	970	100	835	100
					5	1064	110	830	99
					13	1147	118	928	111
6	M	37	Renal failure	HD* D <sub>2</sub> —50,000 I.U./day	0	879	100	677	100
					5	965	110	—	—
					12	1122	128	773	115
24	M	19	Osteomalacia	D <sub>2</sub> —100,000 I.U./day	0	310	100	340	100
					6	586	189	—	—
					12	687	220	477	140
					24	823	265	724	213
67	M	74	Osteomalacia	D <sub>2</sub> —200,000 I.U./day Ca—2 gm/day P—4 gm/day	0	734	100	785	100
					2	971	132	—	—
					4	1012	138	—	—
					9	1045	142	731	93
15	F	62	Osteoporosis	DCP†—4 gm/day D <sub>2</sub> —50,000 I.U./week Hydrochlorothiazide—100 mg/day	0	491	100	375	100
					3	543	111	—	—
					9	532	109	—	—
					12	507	103	—	—
					14	579	118	265	71
16	M	55	Osteoporosis	DCP†—4 gm/day Methyl testosterone—20 mg/day Ethinyl estradiol—0.5 mg/2 days	0	807	100	517	100
					3	872	108	—	—
					6	996	123	530	106
13	M	33	Renal failure	HD* D <sub>2</sub> —150,000 I.U./day	0	807	100	545	100
					5	808	100	—	—
					10	973	121	—	—
					15	1069	132	519	95
34	M	47	Renal failure	HD*	0	697	100	504	100
					4	906	130	531	105
					11	—	—	567	112
35	F	25	Renal failure	HD* DHT‡—1 mg/day‡	0	687	100	341	100
					4	674	99	385	113
					9	581	85	333	98

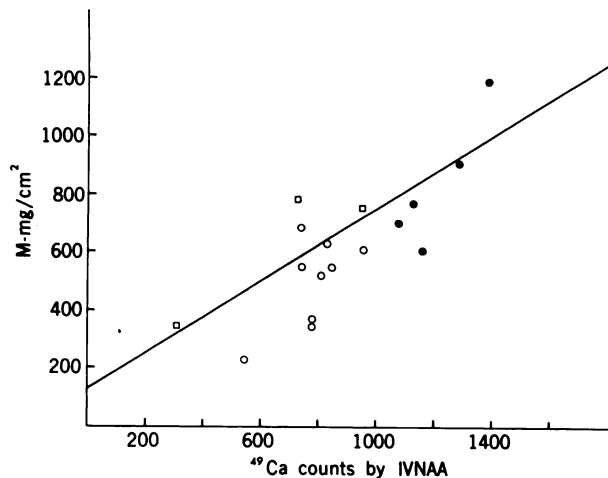
\* All renal failure subjects were on hemodialysis (HD) before and throughout the period of measurements. In subjects whose only treatment is hemodialysis, months on treatment = months since start of measurements.

† DCP, Dicalcium phosphate.

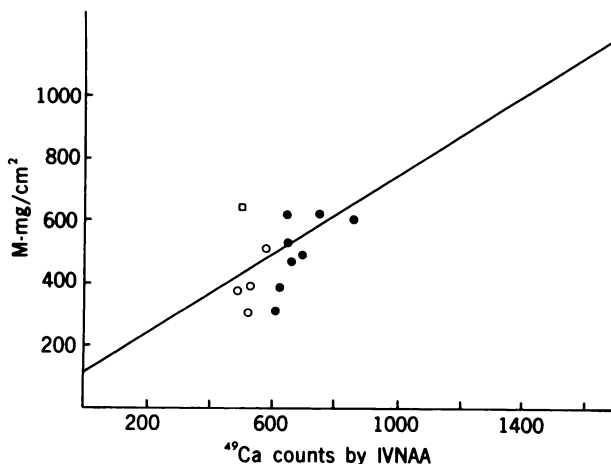
‡ Dihydrotachysterol (DHT): Before and throughout study.



**FIG. 1.** Comparison of partial-body bone calcium measurements by IVNAA with x-ray densitometric measurements of bone mineral content, M.



**FIG. 2.** Comparison of calcium measurements of IVNAA and x-ray photodensitometry for group of male subjects who are either normal (●) or who have osteoporosis (○) or osteomalacia (□). Line drawn is regression line from Fig. 1.



**FIG. 3.** Comparison of calcium measurements of IVNAA and x-ray densitometry for group of female subjects who are either normal (●) or who have osteoporosis (○) or osteomalacia (□). Line drawn is regression line from Fig. 1.

vious increase in both cortical thickness and trabecular bone was apparent by ordinary radiography. A marked increase in bone mineral was observed by both IVNAA and x-ray photodensitometry. It may be noted that the bone calcium results doubled in 12 months by the IVNAA technique and in 24 months by the photodensitometry method.

Neither of the two osteoporotic subjects (Cases 15 and 16) showed change by routine skeletal radiography. The discrepancy between the IVNAA results and those from the radius is unresolved.

The other three subjects had renal failure and were on hemodialysis. In Case 13, the skeletal x-rays showed that the patient had renal osteodystrophy progressive over 6 months prior to this study. At the beginning of the study he was put on vitamin D and during the subsequent 15 months the alkaline phosphatase fell from 525 to 35 I.U. (the normal value is less than 80). The IVNAA results showed an increase in calcium count while x-ray photodensitometry results showed no significant change. Case 34 had no treatment apart from hemodialysis. Skeletal radiography of the hands showed an improvement in renal osteodystrophy over a period of 15 months starting 4 months before the present study; subperiosteal resorption decreased from moderately severe to nondetectable levels. The IVNAA data showed a significant increase of 32% in calcium counts over 4 months whereas photodensitometry showed an increase of only 5%. However, in the following 7 months a further 6% increase in bone mineral mass was found by x-ray photodensitometry. Another patient (Case 35) had suffered from severe renal osteodystrophy for at least 3 years prior to the present study. This had been shown by skeletal radiography. X-ray photodensitometry had shown a fall in bone mineral by 40% in these 3 years. Dihydroxycholesterol therapy was commenced 2 years prior to this study and continued throughout it. The x-ray densitometry showed no real change over the 9 months of the study whereas the IVNAA showed a fall in calcium counts. Thus, the discrepancies in Cases 34 and 35 are more apparent than real as both methods showed a similar trend although not exactly during the same time periods.

#### DISCUSSION

Despite the fact that measurements by the two techniques used studied different parts of the body, the determinations of bone mineral by IVNAA correlate moderately well with measurements by x-ray photodensitometry, i.e., the mineral mass in a small area of peripheral bone in general reflects the min-

eral content in the large area of the central skeleton.\* Good correlations have also been reported between total-body calcium by IVNAA and gamma densitometry of the radius (12,13).

Although in the present work the correlation overall is good, there is a wide scatter around the regression line of Fig. 1. Figures 2 and 3 show data separated by sex for normal subjects and for patients with osteoporosis or osteomalacia. These figures show scatter around the regression line for each subgroup similar to that shown by the total data and indicate that the wide scatter of Fig. 1 is not solely because we are dealing with a mixture of normal subjects and patients with metabolic bone disease.

In part, this scatter is certainly due to the combined effect of the inherent errors of both methods. But variation in bone structure itself from area to area must also contribute to the scatter. This clearly follows from the findings of Horsman, et al (14) who correlated weights of different bones in 23 male skeletons and found, e.g., a correlation coefficient of 0.70 between the weight of the third lumbar vertebra and the radius, a figure not dissimilar to that of 0.76 found in this study between the radius and the trunk.

The apparently good separation between normal and abnormal subjects by IVNAA (Figs. 2 and 3), especially in men, should be interpreted with some reservation. Results on the small number of subjects reported here do not provide a true estimate of the normal ranges. Results of IVNAA measurements on a larger series of both normal and osteoporotic subjects (not reported here because densitometry measurements were not carried out) show that before frame size is taken into account there is significant overlap between normal and abnormal subjects, particularly for postmenopausal women, in agreement with results reported by x-ray and gamma-ray densitometry (7,15).

In the majority of patients (21 of 27), there is a reasonable agreement between the two methods of measuring sequential changes in bone calcium (Fig. 4) but in a few (6/27) there are discrepancies; these, however, are marked only in three cases (67,

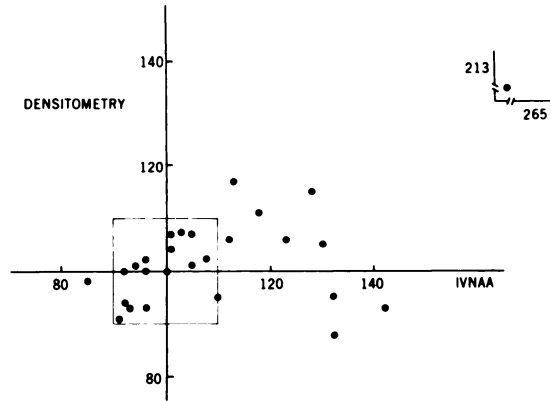


FIG. 4. Comparison of changes with time in calcium bone content as measured by IVNAA or by x-ray photodensitometry. In all cases initial value measured by either technique was taken as 100%, and value shown is comparable bone content measured at some later time. In all cases measurements by IVNAA and by densitometry were made at approximately same time. Note change of axes for point at 265,213. Square around 100,100 point encloses data for those subjects who have not significantly changed in bone content by either technique, i.e., who have changed by less than 10%.

15, 13). We believe that, as a result of progressive disease or therapy, changes in bone calcium may not always be proportional throughout the skeleton. Consequently, as in Case 67, treated for osteomalacia, the results of healing of rib fractures seen when looking at the central skeleton by IVNAA would not necessarily be reflected in values obtained by densitometry of the peripheral bone.

Furthermore, as seen in two renal failure patients (Cases 34 and 35) and one osteomalacic (Case 24) the IVNAA measurements on the trunk appear to show changes in calcium status more quickly than do densitometry measurements on the radius. As trabecular bone appears to be more metabolically active than cortical bone (16), it is possible that this quicker response by the IVNAA method is due to the greater percentage of trabecular bone in the trunk compared with that in the radius.

#### ACKNOWLEDGMENTS

This work was supported in part by the W. Garfield Weston Charitable Foundation and by the Medical Research Council of Canada, Grants M.A. 4142 and M.A. 3889. We wish to acknowledge the assistance of J. Watts and H. Kostalas in taking some of the measurements. We are most grateful for help from D. Fraser and C. Williams in referring patients to us and for discussion of the data.

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\* It may be noted that from the photodensitometry a measure of bone mineral ( $M^*$ ) has been derived in terms of mg/cm, taking into account the total-bone diameter and the combined cortical thickness. This derived value ( $M^*$ ), which quantifies the mineral in a toroidal (donut-shaped) section of bone does correlate better with the partial-body count than does the primary measurement ( $M$ ) used here. As it is the purpose of this paper to compare bone mineral measurements by two published methods, only  $M$  values are considered further. Moreover, when changes in bone mineral are estimated from sequential photodensitometry measurements, percentage changes obtained from the derived values ( $M^*$ ) were the same as those reported here ( $M$ ).

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