A BONE CALCIUM INDEX BASED ON PARTIAL-BODY CALCIUM MEASUREMENTS BY IN VIVO ACTIVATION ANALYSIS

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Measurements of partial-body calcium by in vivo neutron activation analysis have been carried out on normal and osteoporotic subjects. Based on measurements on 16 normal subjects (volunteers less than 55 years of age), a calcium index has been established that takes into account variation in skeletal frame size. On the basis of this index, all osteoporotic patients have bone mineral content less than any of the normal subjects. The normal calcium indices ranged from 0.9 to 1.2, and the osteoporotic indices ranged from 0.41 to 0.83. Thirteen of 22 volunteers over 55 years of age had calcium indices less than 0.9 in agreement with the expected loss of calcium with age.

Measurements of total-body potassium were also made on these same subjects and the calcium/potassium ratios calculated. Although as groups the older volunteers and older osteoporotic subjects had mean calcium/potassium ratios similar to the mean for the normal subjects, the osteoporotic subjects under 55 years of age had a mean calcium/potassium ratio significantly lower, indicating that for this latter group the loss in bone mineral was not associated with a corresponding loss in muscle mass.

In vivo neutron activation analysis (IVNAA) has been used in a number of centers to quantify the amount of calcium in the human body or in a major part of it (*1–5*).

Calcium contains a small abundance of the isotope ^{48}Ca. In normal man, there are approximately 2 gm of this stable isotope of calcium. If the body is irradiated with neutrons, some of this ^{48}Ca may capture neutrons and be converted to ^{49}Ca. Calcium-49 is radioactive with a half-life of approximately 9 min and on decay it emits a 3.1-MeV gamma ray. This gamma ray may be detected externally to the body in a low-background counting facility called a whole-body counter. The number of counts of ^{49}Ca is then a measure of the amount of ^{48}Ca in the body and thus a measure of the total calcium content.

Because of problems associated with the greater absorption of both the incident neutrons and the emergent gamma rays in persons of greater than average thickness, the ^{49}Ca count obtained in a measurement cannot immediately be used as a measure of the amount of calcium in the body.

Based on measurements made with water and with skeletal phantoms, we have been able to correct for variations in overall efficiency related to the thickness of the subject and thus have been able to obtain a calcium count that is related to calcium content of the trunk (*6*).

By itself, however, the corrected count does not tell enough about the calcium status of the individual measured (nor indeed does a measure of the calcium in grams) because different healthy individuals would be expected to have different calcium mass, depending on, for instance, their height and their frame size.

We have measured a total of 16 normal volunteers who have no overt symptoms of bone or other calcium disorders and who are under 55 years of age and we have developed from these data an index of bone calcium status. Results from a group of older volunteers, from a group of osteoporotic patients, and from four unusual cases are compared with those from the younger volunteers in terms of this calcium index.

On the same subjects, we have measured the potassium content of the whole body, which provides

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an estimate of muscle mass. Normal potassium contains 0.001% of ^{40}K, a radioactive isotope that emits 1.46-MeV gamma rays which may be detected in a whole-body counter. Just as with measurements of ^{49}Ca, there is self-absorption of the gamma rays in the body and correction must be made for the different absorptions by persons of different thickness. In the case of ^{40}K, this problem is solved by the use of internal standardization using ^{42}K, a 12-hr isotope that emits 1.52-MeV gamma rays.

The purpose of this potassium measurement is to enable a comparison to be made of calcium (a measure of bone mineral mass) and potassium (a measure of muscle mass) in the volunteers and in the osteoporotic subjects. A number of different workers have compared bone and muscle mass by a variety of techniques and results have been controversial. Both calcium and potassium decrease with age (*7,8*). Cohn, et al (*9*), using IVNAA and potassium counting, report that in osteoporosis the decrease in potassium is proportional to the decrease in calcium. Meema, et al (*10*), using x-ray densitometry, found similar results for osteoporotic men but in osteoporotic women the potassium loss was not as great as the calcium loss. Since ^{40}K measurements were readily obtained on all patients undergoing IVNAA, we have investigated the calcium and potassium ratio in normal and osteoporotic subjects to obtain information on this controversial point.

METHOD

The method of measurement of the calcium has been described elsewhere (*5,11*). Briefly, the subject is irradiated for 20 min by neutrons from 12 Pu-Be neutron sources, each with an output of approximately 10^7 neutrons/sec. These sources are symmetrically distributed around the trunk and upper thighs of the subject, six of the sources being above the body and six below. This irradiation gives a dose of approximately 0.4 rem. The subject is then quickly (3 min) transported to the whole-body counter, where he is counted for 20 min. The detecting system consists of four 8-in. by 4-in. NaI crystals that view the irradiated part of the body. This irradiation and the detection geometry facilitate investigation of a region of the body containing much trabecular bone, an arrangement that appears to show up changes in calcium status more quickly than does investigation of cortical bone (*12*). Depending on the individual, one third to one half of the total bone is irradiated and counted (*5*). The ^{49}Ca count is obtained by measuring the area under the 3.1-MeV photopeak after correction has been made for background and for the effects of incomplete resolution of the photopeaks of sodium and chlorine. Repeated measurements on persons have shown a reproducibility of ±6.6% (*5*). The correction for overall efficiency (neutron flux and gamma counting) has been established from phantom experiments reported previously (*6*). The overall efficiency decreased by 5% for each 1-cm increase in body thickness. Twenty-two centimeters have been chosen as the standard thickness, and the calcium count from each subject has been corrected for variations in overall efficiency resulting from his nonstandard thickness (e.g., a person whose body thickness is 24 cm has his count increased 10%). The effective trunk thickness of subjects has been estimated from measurement of the attenuation of 2.6-MeV gamma rays from an extended thorium source placed above the trunk and measured by the two crystals below the trunk. An attenuation coefficient of 0.41/cm was used, this being the value determined by water tank experiments. For some early measurements, thorium attenuation measurements were not obtained. For these subjects, we have estimated the effective thickness from their weight and height, using the relationship:

$$\text{Effective thickness (cm)} = 1.46\sqrt{W/H} - 6.36$$

where W is weight in grams and H is height in centimeters. This relationship has been established from measurements of thorium attenuation and $\sqrt{W/H}$ on 73 subjects varying in effective thickness from 17 to 34 cm (Fig. 1). This figure shows that, using this relationship, the effective thickness estimated from $\sqrt{W/H}$ data may differ from the corresponding thorium attenuation value by ±4% (1 s.d.). This

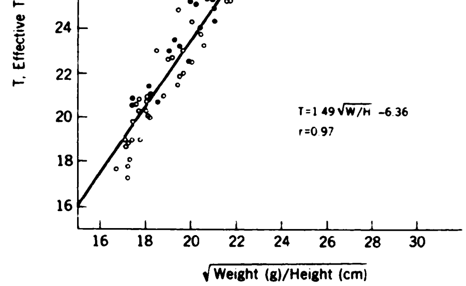

FIG. 1. Relationship between effective thickness (T) measured by thorium attenuation and square root of weight (W) divided by height (H). Males are shown as closed circles, females as open circles.

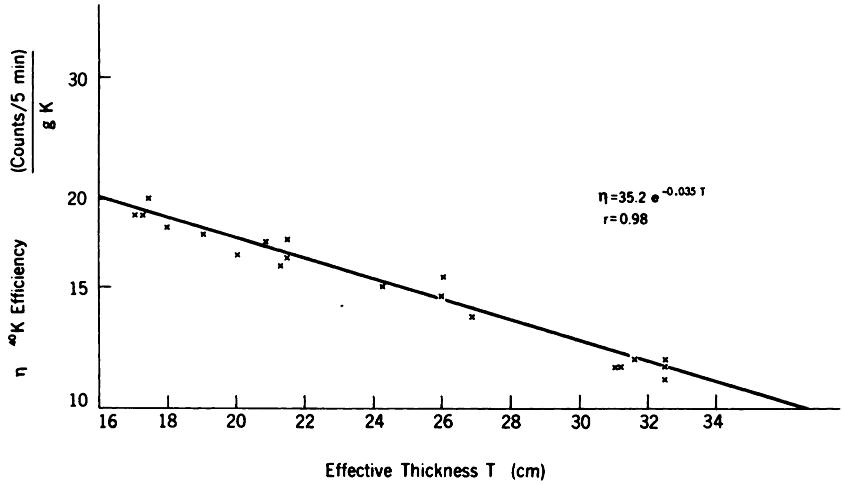

FIG. 2. Relationship between the overall counting efficiency η (^{40}K gamma-ray counts/gm potassium) and effective body thickness (T). Note that this is semilog plot.

error can result in about ±5% error in the calculation of the thickness-corrected calcium count.

Potassium measurements. The counting of gamma rays from ^{40}K gives a measure of potassium content of the body but, as noted before, correction must be made for self-absorption. The relevant correction as a function of thickness has been determined from measurements on 20 persons with thickness ranging from 17 to 32 cm (obtained by thorium attenuation) using an internal standardization procedure (*13,14*).

In this internal standardization procedure, the subject is first measured for ^{40}K using the same geometry as used for the ^{49}Ca measurements above. A bottle containing a standard potassium solution is also measured. The subject is then given 5 μCi of ^{42}K orally and an equal amount is mixed with water in a bottle. This solution has the same geometry as the stable potassium standard. Urine is collected for 24 hr. After 24 hr, the subject is again counted, but this time the ^{42}K (plus ^{40}K background) is measured. Immediately afterwards, the ^{42}K standard is measured and so is the urine (diluted to give the same geometry as the standard). After subtraction of backgrounds and correction of the ^{42}K body count for urine losses*, the mass of potassium in the body may be obtained from the equation:

$$\text{gm K} = \frac{^{40}\text{K body count}}{^{40}\text{K standard count}} \times \frac{^{42}\text{K standard count}}{^{42}\text{K body count}} \times \text{gm K in standard} = (^{40}\text{K body count})/\eta$$

The efficiency η (the ^{40}K counts/gm K) will vary from person to person. A graph of η versus body thickness is shown in Fig. 2. The results show a sim-

* In three cases of steatorrhea, fecal losses were also determined and appropriate corrections made.

TABLE 1. NORMAL VOLUNTEERS LESS THAN 55 YEARS OF AGE

Case (No.)	Age	Sex	Efficiency corrected Ca count	Ht^3 (m^3)	Ca index	K (gm)	Ca count / K (gm)
193	46	F	1,096	4.91	1.06	109	10.1
50	53	F	687	3.40	0.95	77	8.9
49	51	F	784	4.00	0.93	105	7.4
1	44	F	890	4.66	0.91	100	8.9
182	47	F	851	3.80	1.06	88	9.7
183	49	F	1,069	5.18	0.98	106	10.1
19	34	M	1,130	4.57	1.18	121	9.3
21	50	M	1,473	6.75	1.04	148	9.9
88	53	M	1,220	6.33	0.92	130	9.4
140	34	M	1,352	5.45	1.18	161	8.4
138	44	M	913	4.49	0.96	152	6.0
2	44	M	1,306	6.03	1.03	170	7.7
10	34	M	1,156	5.83	0.94	159	7.3
3	39	M	1,187	5.83	0.97	151	7.9
4	36	M	1,181	5.93	0.95	—	—
205	48	M	946	4.83	0.92	119	7.9

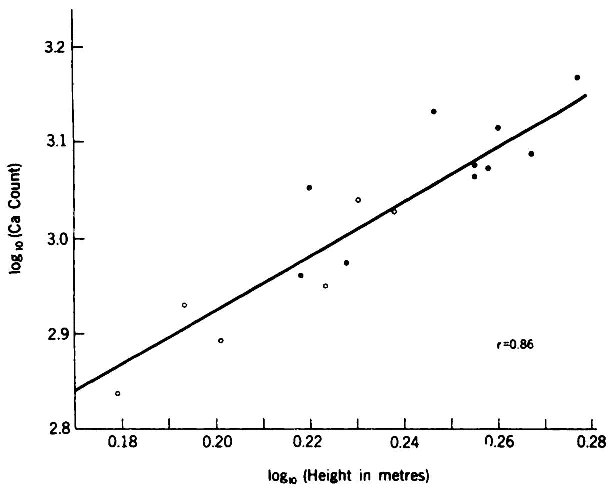

FIG. 3. Plot of $\log_{10}$ calcium counts against $\log_{10}$ height (m) in 16 volunteers less than 55 years of age. Male subjects are shown as closed circles, females as open circles.

ple exponential relationship between thickness and efficiency. For subjects in whom internal standardization was not carried out, the efficiency has been assumed to be that based on their thickness and this exponential relationship and on this basis the potassium mass is obtained from a measurement of ^{40}K count and an estimate of body thickness. Based on the standard deviation of the η v thickness line and on the statistical accuracy of the ^{40}K measurements, the mass of potassium is accurate to $\pm5\%$ (1 s.d.). It should be noted that the standardization procedure depends on knowledge of the ^{42}K retention and on complete equilibration between the ^{42}K and stable potassium at the time of ^{42}K measurement. Subsidiary experiments demonstrated that 24 hr was necessary for equilibration in agreement with reported data (*14*). Because of this complete mixing of the radiopotassium with the stable potassium of the body, measurement of ^{40}K in the trunk alone, together with subsequent measurement of ^{42}K, gives the total stable potassium in the body; in contrast the fixed nature of calcium in the body means that the IVNAA method used here measures calcium in the trunk region alone.

SUBJECTS

For normal controls, the local Human Experimentation Committee allowed the measurement of persons who were colleagues of the authors. These cannot therefore be regarded as a cross section of the whole community, but their results may nevertheless be taken as a yardstick against which to gage the calcium index of other groups of subjects. These 16 volunteers were all between 34 and 53 years of age (mean 44 years). Six were women and ten were men. Fifteen were white and one (man) was of Indian parentage.

A group of 22 older subjects (11 men, 11 women) were measured who were between 59 and 75 years of age (mean 64 years). Twenty-one of them were on a preretirement exercise program. None had any overt symptoms of calcium disease.

Twenty-four persons suffering from osteoporosis were also examined. Eleven of these (eight men, three women) were less than 55 years old (mean 43 years), while two men and 11 women were over 55 (mean 63 years). The diagnosis of osteoporosis was based on the presence of vertebral fractures associated with generalized reduced mineral mass as shown by standard x-radiography and on there being normal values for serum calcium, phosphorus, and alkaline phosphatase.

In addition, studies are reported on four patients who, as x-radiography showed, had increased bone mass. One had osteopetrosis; one had an unusual form of bone fragility with increased bone mass and bone density by radiologic assessment; and two had widespread Paget's disease.

RESULTS

The results for the 16 volunteers whose ages were less than 55 are shown in Table 1 together with other pertinent data. The calcium counts corrected for variations in efficiency due to body thickness are plotted against height on a log/log plot in Fig. 3. A least-squares fit to these data gives a slope of 2.82 and an r value of 0.86 ($p < 0.001$). The calcium count thus varies nearly as the cube of the height. For convenience, we have assumed that the

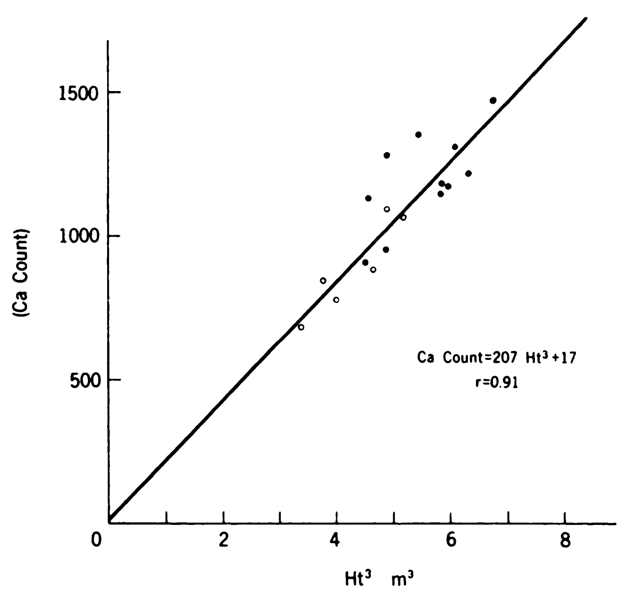

FIG. 4. Relationship between calcium counts of 16 volunteers of Fig. 3 and cube of their heights (m^3).

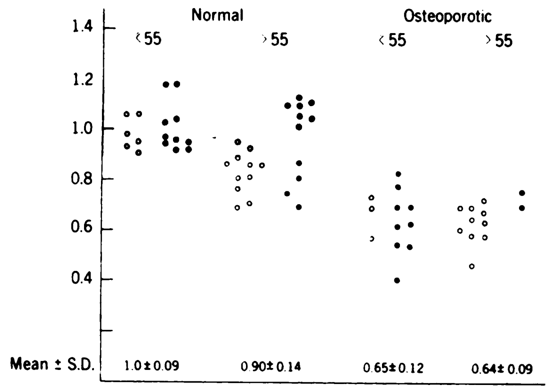

FIG. 5. Calcium indices for normal and osteoporotic subjects separated by sex, age, and clinical condition. Males are shown as closed circles, females as open circles. Shaded area represents range for young normal subjects (volunteers less than 55 years of age).

efficiency-corrected calcium count varies exactly as the cube of the height and by least-square methods have fitted the data to the equation:

$$\text{Calcium count} = A\ (\text{height})^3 + B$$

This fit is shown in Fig. 4. The best values for A and B are 207 and 17, respectively. The r value for this line is 0.91 ($p < 0.001$).

By subtracting from the calcium count of each individual subject the best value for B, 17, and then dividing by 207 $(\text{height})^3$, an index of bone calcium is obtained.* Naturally, the average value of this index found in the 16 volunteers is 1.0. The standard deviation about this mean value is ±0.09. All the 16 volunteers under the age of 55 had a calcium index between 0.9 and 1.2 (Table 1).

The results for the older (>55-years-old) volunteers and for the osteoporotic patients have been treated in the same way, subtracting 17 from the calcium count and dividing by 207 $(\text{height})^3$. The calcium index for these subjects separated by sex, age, and clinical condition, is shown in Fig. 5. The volunteers over 55 years of age had a mean calcium index of 0.90; the osteoporotic subjects had values of 0.65 (≤55) and 0.64 (>55) and all osteoporotic subjects had values below the range for normal subjects less than 55 years of age.

The calcium measurements for the four subjects with other types of bone disease have been treated in the same way, and calcium indices are shown in Table 2. It is seen that these all had high values, varying from 1.55 to 2.37.

For comparison, Fig. 6 shows the calcium counts (corrected only for neutron and gamma attenuation in the thickness of the body) for the normal and the osteoporotic subjects. The calcium counts do not provide the clear separation between osteoporotic and young normal subjects seen when one uses the calcium index (which takes into consideration skeletal frame size).

Table 1 also shows the values of the potassium content for the 16 normal volunteers less than 55 years of age, and the ratios calcium/potassium, taken as the calcium count divided by potassium content in grams. Similar ratios have been obtained for the other subjects. Figure 7 shows these calcium/potassium ratios, again separated by age, sex, and clinical condition, for normal and osteoporotic subjects. The mean calcium/potassium ratios for volunteers were 8.6 (≤55 years) and 8.6 (>55 years), and for osteoporotic subjects over 55 years of age the mean calcium/potassium ratio (7.8, s.e. 0.25) was only slightly different ($p < 0.05$). In contrast, for the young osteoporotic subjects under 55 years of age, the mean value for the calcium/potassium ratio (6.1, s.e. 0.3) was about 30% below the mean control value, indicating a significantly greater loss in bone mineral than in muscle mass. For the four patients with high mineral mass, the calcium/potassium ratios are shown in Table 2. In all cases, calcium/potassium was well above the normal range.

* The height used in these calculations was the maximum height. For many osteoporotic subjects, the height at time of measurement was significantly less than their stated maximum height. The stated maximum height was checked by a measure of arm span.

TABLE 2. PATIENTS WITH INCREASED BONE MASS

Case (No.)	Diagnosis	Age	Sex	Efficiency corrected Ca count	Ht^3 (m^3)	Ca index	K (gm)	Ca count / K (gm)
29	Osteopetrosis	60	F	2,140	4.57	2.37	103.4	20.7
203	*	41	F	1,190	3.18	1.78	79.1	15.0
71	Paget's disease	79	M	1,713	5.36	1.53	105.0	16.3
82	Paget's disaese	61	F	1,162	3.05	1.81	76.0	15.3

* An unusual form of bone fragility with increased bone mass and bone density by radiologic assessment.

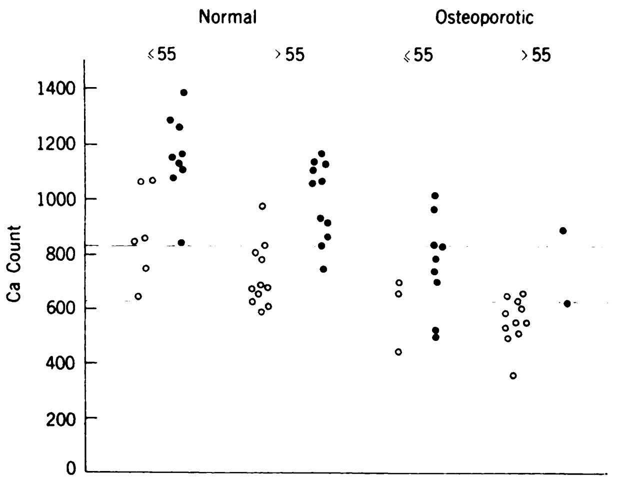

FIG. 6. Calcium counts (corrected only for thickness effects) for subjects of Fig. 5. Lines are drawn below lowest values for normal males (upper line) and normal females (lower line).

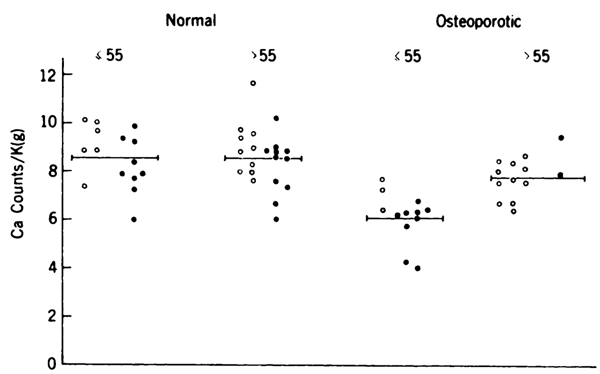

FIG. 7. Calcium/potassium ratios (calcium count divided by potassium in grams) for subjects of Fig. 5. Means and standard error for each group are shown: normals (<55 years) — 8.6 ± 0.3 (s.e.), normals (>55 years) — 8.6 ± 0.3, osteoporotic individuals (<55 years) — 6.1 ± 0.3, osteoporotic subjects (>55 years) — 7.8 ± 0.25.

DISCUSSION

The cubic relationship between calcium count and height agrees with that found by the Washington group (*15*). This agreement is perhaps a little surprising as we measure calcium in only a fixed area of the body and therefore a greater proportion of a small person than of a taller one. However, for the group of 16 volunteers less than 55 years of age, the experimental evidence is quite clear that the cubic relationship gives a good fit in our configuration.

For the younger normal volunteers, the range of indices, 1.2–0.9, is approximately 33% (highest/lowest), with no difference between the sexes (Fig. 5). The calcium counts themselves show a span of a factor of 2 (Fig. 6). Thus, taking into account differences in height by the use of the calcium index reduces the spread of results consequent on individual variations in frame size.

Of greater importance is the clearer distinction that is evident between normal and osteoporotic subjects when the calcium index is used. Using the calcium index, all osteoporotic subjects had values more than 5% below the young normal range. On the other hand, using this same criterion for the case of the calcium counts alone, five osteoporotic women fall within the normal female range and five osteoporotic men fall within the normal male range. In addition, as a group, the older normal women appear to have a greater loss in bone mineral using the calcium index than is apparent using calcium counts alone.

Although a comparison of the younger normal subjects with the osteoporotic ones shows a very marked difference in the calcium indices of the groups, it is appreciated that the number in each group is small and that, with measurements of larger numbers, the separation may become less clear. Nevertheless, the present separation using the calcium index is much better than that using the calcium counts alone. This is in agreement with work of Chestnut, et al (*15*) and Nelp, et al (*16*) using total-body calcium measurements by IVNAA, who have also shown good separation between osteoporotic and normal subjects when skeletal frame size was taken into consideration. It may well be that the IVNAA method, which looks at the central skeleton, is better able to differentiate between osteoporotic and normal subjects than is gamma absorptiometry because the loss of mineral in the central skeleton seen in osteoporosis may not be reflected in a small bit of peripheral bone (*12*).

The mean calcium/potassium ratios for the younger and older normal subjects are the same and are not significantly less for the osteoporotic patients over 55 years of age. These results suggest that loss of bone mineral with age is associated with a corresponding loss in muscle mass. In contrast, the mean calcium/potassium ratio for the young osteoporotic subjects is significantly less than the value for the normal ones, which implies that in young osteoporotic patients the loss of bone mineral is not associated with a corresponding loss in muscle mass. This difference between the older and younger osteoporotic subjects may suggest a difference in etiology.

The results for the patients listed in Table 2 are presented here primarily to show that high calcium indices are obtained in cases where x-radiography shows increased mineral mass. In Cases 29 and 203, in whom there is a generalized increase in mineral mass because of metabolic bone disease, the calcium/potassium ratios are also high. In Cases 71 and 72, the localized nature of the pagetic bone makes difficult any comparison of the calcium mass with the more uniformly distributed potassium.

We conclude that, with our configuration, the use of the calcium index, which takes into account height, can serve as a more useful parameter for assessing calcium status in individuals than calcium counts alone. On this basis, as a group, osteoporotic subjects of all ages are differentiated from the young normal adults (although the older osteoporotic subjects are not differentiated from the older volunteers). Both older and younger osteoporotic groups have the same mean calcium index. However, the results also show that in contrast to osteoporosis associated with aging, young osteoporotic individuals have low calcium/potassium ratios, reflecting loss of bone mineral that is not associated with corresponding loss in muscle mass.

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