Problems in the Interpretation of the In Vivo Measurement of Calcium by the Argon-37 Method: An Investigation of Inert-Gas Elimination in Humans

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An investigation has been made of some physiological problems associated with the interpretation of in-vivo measurements of calcium by the argon-37 method. Inert-gas elimination in humans over a period of several days was studied using i.v. injections of Xe-133. The results imply that the exhalation rate of A-37 formed in bone will be affected by the individual's composition, in particular body fat. Comparison of calcium measurements between individuals and between laboratories is meaningful only if corrections are made for differing individual composition.

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The Argon-37 method, which uses the reaction $^{40}Ca(n,\alpha)^{37}A$ to measure human calcium or bone-mineral mass in vivo, was proposed by Palmer (*1*) and is being developed (*2–4*) as an alternative to the current in-vivo neutron activation analysis techniques, which rely on the $^{48}Ca(n,\gamma)^{49}Ca$ reaction.

The method involves irradiating the subject with fast neutrons, followed by the collection and counting of A-37 exhaled by the subject. The A-37, formed 99% in the skeleton, is chemically inert in the body and is subsequently exhaled. The breath sample is purified and the 2.62 keV Auger electrons from the chlorine daughter are then counted in a proportional counter.

The method and its advantages have been discussed fully elsewhere (*2,3*). In brief, the low dose requirement allows more frequent sequential measurements of individuals and possibly the measurement of children; moreover, the method requires relatively inexpensive irradiation and counting facilities. However, it poses a problem of interpretation, in that although the total A-37 formed provides a measure of body-calcium mass, collection of all the A-37 formed is not practical, since some may be retained and the remainder is exhaled over a period of many hours. A convenient alternative to total collection is to sample the subject's exhaled air, giving the results in the form of an "exhalation curve" (exhalation rate against time). The area under the curve, or under a particular part of it, may then provide a measure of calcium mass.

The curve has been analysed (*2,3,5,6*) to give several exponential components, the half-time of the initial, fastest component ranging between 15 and 40 min for mammals, including humans. Reported values for the half-time of the second component differ considerably, from 156 min to 30 (± 10) hr for human subjects. A third component with a half-time of 7 days has been reported in the exhalation curve of a beagle given a large dose of radiation (50 rads), but has not been detected in human exhalation curves. Discrepancies between the results reported by different groups may be due partly to

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the different breathing circuits employed. Llewellyn et al. (*2*) have reported a good correlation coefficient (0.97) for 16 patients between whole-body calcium (as measured by the Ca-48 technique) and the A-37 exhalation rate at 30 min after irradiation. Correlation with the integral under the curve, however, apparently the most reliable indicator of calcium mass, appeared poor.

The curve's shape may yield physiological information in addition to an index of calcium mass. Ozbas (*7*) suggested that the shape may give information about the blood supply to bone. Llewellyn (*2*) suggested that the two components of the human curve may relate to the two bone compartments, trabecular and cortical. This information may be difficult to extract from the curve, however, in view of the complex nature of inert-gas elimination.

The difficulties stem both from the poor understanding of inert-gas elimination from bone and from the possibility that a significant amount of A-37 will be absorbed by body tissues before being exhaled. Bone as an organ is not readily accessible to investigation, and reported values of bone perfusion vary widely (*11,12*). It is also probable that a fraction of the A-37 produced will be trapped in bone crystals. A study performed by Bigler (*6*) suggests that about 30% of A-37 formed in vivo is trapped in bone crystals indefinitely. Of the fraction of A-37 that does escape from bone into the bloodstream, most may be assumed to be exhaled rapidly. The venous circulation carries it through the heart to the pulmonary capillaries, where rapid equilibration with alveolar gases occurs. Any A-37 remaining in the blood is then recirculated to all body tissues. If a significant fraction of A-37 were taken up by body tissues there would be a potential source of error in the measurements of calcium by the A-37 method. West (*8*) suggests that more than 95% of nitrogen and xenon—physiologically inert gases—diffuse from the bloodstream into the alveoli in the first pass through healthy lungs. The amount of inert gas taken up into tissue and its subsequent rate of release, however, depend on the subject's composition and metabolism (*9,10*).

The study described here was designed to determine what fraction of an inert gas, starting in the venous blood, is taken up into body tissue, and to investigate the rate of exhalation over a period of several days in relation to the subject's physiological characteristics. The inert gas used was Xe-133, since a suitable isotope of argon was not readily available, and Xe-133 provides easily detectable gamma radiation (81 keV). All the rare gases behave similarly in the body, being chemically inert and sparingly soluble. Xenon, however, is more soluble in body tissue than is argon (*9*), so it is likely that more will be taken up into tissue and that elimination will be slower for a particular subject under given conditions.

The Xe-133 in saline was administered intravenously as an impulse injection. There are many reported studies of this type of injection (*11*), but none appear to follow exhalation of the inert gas over the long time scale of interest in A-37 elimination.

METHOD

Each subject received a single impulse injection of Xe-133 dissolved in 1–2 ml of saline. This was delivered into the cubital vein of the arm, the injection taking less than 15 sec. The subjects' "Xe-133 exhalation rate" was measured in one of two ways: 1) by collecting and counting the activity in the exhaled air; and 2) by monitoring the activity remaining in the body using an eight-crystal (12.5 × 10 cm) whole-body counter.

Method 1 involved direct collection of the exhaled air (Fig. 1). The injected activity was 1–2 mCi of Xe-133, which from previous work had been shown to involve a total-body dose of less than 10 mrem. The subject inhaled room air, the exhaled gases being collected in large gas-tight plastic bags. The subject was seated and his thigh was placed adjacent to a collimated Ge(Li) detector, which continuously monitored the activity in the thigh. Each breath sample was later removed from the plastic bag by adsorption onto activated charcoal contained in a glass trap maintained at liquid-nitrogen temperature. The Xe-133 activity in the sample could then be counted with a (7.5 × 7.5 cm) NaI(Tl) detector.

When the exhalation rate had fallen by about four orders of magnitude, the measurements were continued in the more sensitive whole-body counter (Method 2) for several days. For each subject the first whole-body measurement took place before the

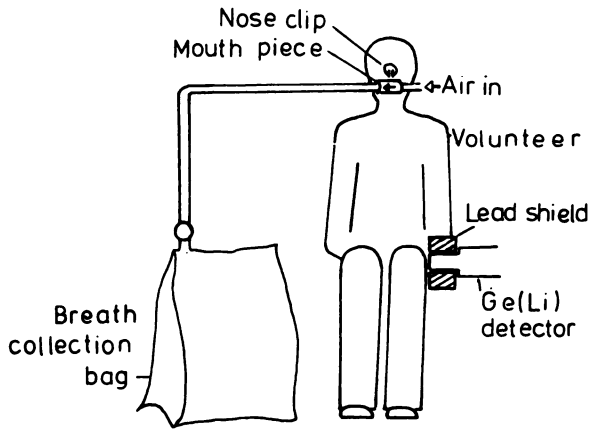

FIG. 1. "Open circuit" breath-collection system. The Ge(Li) detector monitoring activity in subject's thigh is also shown.

TABLE 1. THE SUBJECTS

Subject	Age (yr)	Weight (kg)	Fat (% of body wt)
A	24	65	9
B	27	95	18
C	25	70	22
D	24	68	23
E	64	72	26
F	48	78	34
G	55	89	38
H	29	79	52

last breath sample collection to intercalibrate the two methods.

For Method 2, the subject lay in the whole-body counter during and after injection of ~ 100 μCi of Xe-133, his exhaled air being piped away to prevent contamination of the counter. Breath samples were also collected in bags for the first 3 min after the start of injection. Comparison of the count rate from these bags with that from a "calibration bag" containing a known amount of Xe-133 allowed direct calculation of the percentage of injected Xe-133 breathed out during the first 3 min. Injected quantities (into subjects or calibration bags) were measured by counting the syringe before and after injection.

In order to determine whether the exhalation rate was related to gross physiological variables, data such as age, height, weight, pulse- and breathing-rates, percentage of fat in the body, and a number of lung parameters were measured for each subject. The subjects were all male and considered as "normal" but with widely differing fat content.

RESULTS

The age, weight, and percentage of fat of each subject are listed in Table 1. Three of them (A, E, and G) were measured by Method 1. Subjects A–F and H were measured by the lower-dose Method 2, A and E being measured by both methods.

The measurements obtained from each subject were plotted as an "exhalation curve." Curves from Method 1 are shown in Figs. 2 and 3; note that negligible Xe-133 (~ 10 nCi) remained after the last whole-body measurement. Thus the area under a linear plot would represent the total exhaled, and consequently the total injected, xenon.

The exhalation curves were analysed into component exponentials by a curve-stripping technique. This yielded up to five components in each curve. The biologic half-periods of the components (t_1 to t_5) were similar for all subjects, whereas the percentage of Xe-133 related to each component (p_1 to p_5) differed markedly between subjects (see Table 2). Each component may in a complex way be related physiologically to groups of tissues whose composition and perfusion rates produce similar elimination rates, although the physiological significance of the intermediate half-times is unlikely to be easily identifiable. The diffusion half-times into and out of different regions of a single tissue may vary considerably. Again, the circulation to similar types of tissue in different regions of the body also varies. It may be more useful to consider the data simply in terms of the percentage of the injected Xe-133 exhaled during particular time intervals after injection. These data show more clearly the differences in exhalation rate between individuals. Results for all subjects are given in Tables 3 and 4. (There

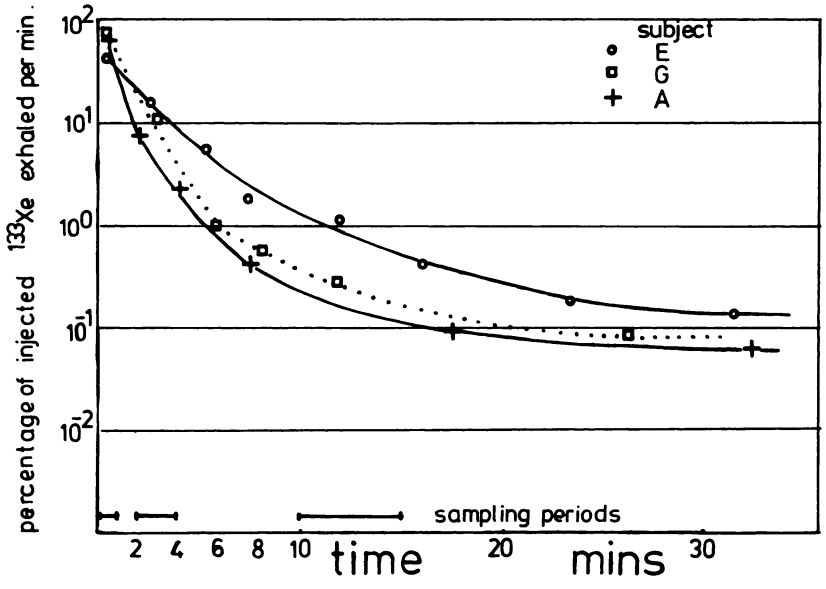

FIG. 2. Xe-133 exhalation curve up to 30 min after injection. Samples from the three subjects were collected using Method 1. Typical sample collection periods are shown. One standard deviation is less than the point size.

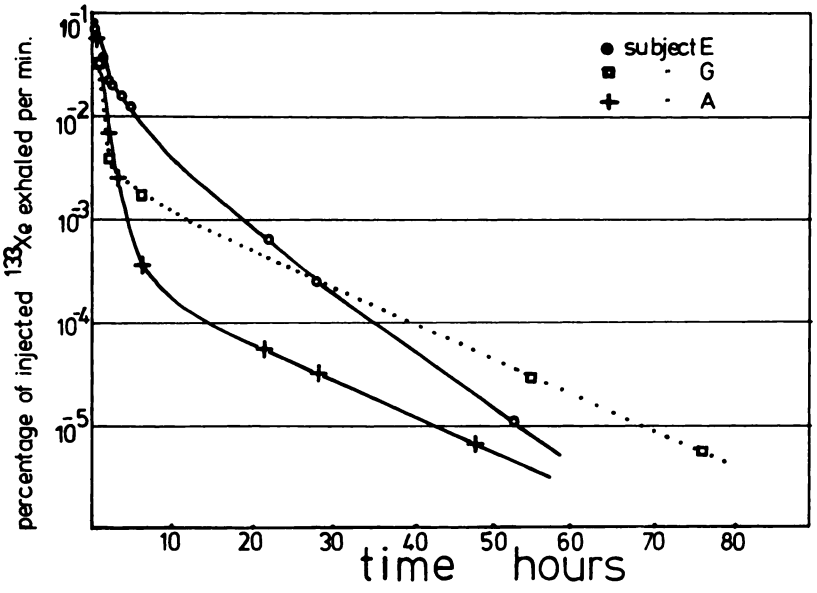

FIG. 3. Xe-133 exhalation curve up to 90 hr after injection. Breath collections (Method 1) lasted 20 min, and later whole-body counter measurements lasted 10 min.

was a large uncertainty associated with the first 3 min of exhalation for subject H.)

The whole-body measurements yielded some additional information. We used the summed output from all eight crystals to plot points on the exhalation curve, but the activity recorded by individual crystals showed that redistribution of xenon occurred within the body, and that half-times in different parts of the body varied accordingly. For instance, the longest half-time in the torso tended to be shorter than that in the knee region. The information obtained from the Ge(Li) detector placed adjacent to the thigh in Method 1 complemented these data.

A number of possible relationships between the physiologic data and the exhalation curves for each subject were investigated. In Fig. 4 the percentages of the injected Xe-133 exhaled in the first 2 and the first 15 min are plotted against percentage of fat. In Fig. 5 the half-times in the torso at about 17 hr after injection are plotted against percentage of fat in the subject.

DISCUSSION

The half-times of the five components in the exhalation curves were similar for all subjects, being approximately < 1 min, 1 min, 20 min, 1–2 hr, and 8–9 hr. These values are consistent with those found by other workers (*9,10*). The percentage of xenon in each component varied between subjects, the greatest differences occurring in the first 15 min, when the majority of the gas is exhaled.

As shown in Fig. 4, the wide disparity between subjects in the amount exhaled in the first 2 min becomes smaller but is still present after 15 min, and the differences appear to be related to the percentage of fat in the subject. Xenon is preferentially absorbed in fatty tissue, the ratio of solubility in fat to that in water being about 20:1 (*9*).

To demonstrate this we performed an experiment on a rabbit. The animal received an injection of Xe-133 into a vein, and breathed in a closed system for about 1 hr. It was then killed and dissected. The Xe-133 activity per gram of tissue was more than ten times higher in fatty tissue than in any other tissue, including blood and bone.

Figure 4 (*exhaled* xenon) suggests that more Xe-133 is retained in the body tissues of subjects with 20–27% fat than in fatter subjects. In part this may be explained by Behnke's proposal (*13*) that there are two types of fat: 'native' fat, of which the

TABLE 2. ANALYSIS OF THE Xe-133 EXHALATION CURVES (METHOD 1, SUBJECTS SEATED)

	Half-time of components (min)					Percentage of gas in each component				
Subject	t_1	t_2	t_3	t_4	t_5	P_1	P_2	P_3	P_4	P_5
A	0.2	1.3	18.0	54	510	44	49	3	4	0.2
E	—	1.5	15.1	158	501	—	86	7	4	3
G	0.8	2.8	23	145	525	83	11	4	1	1

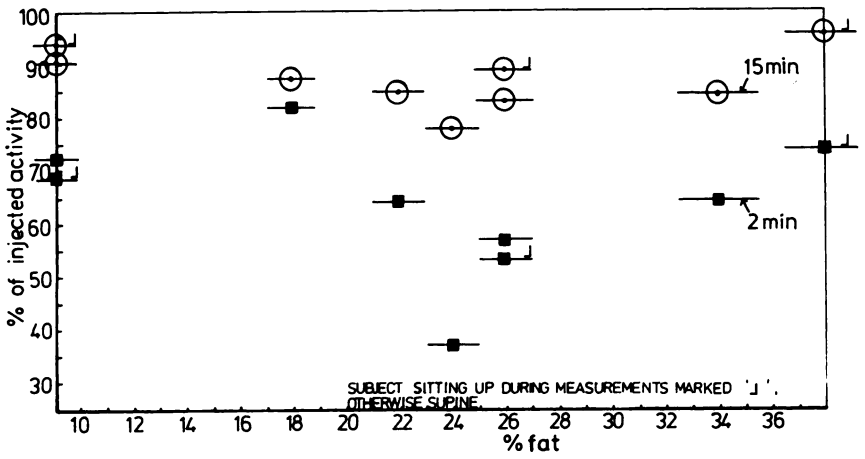

FIG. 4. Percentage of total activity exhaled within 2 min and 15 min of injection, plotted against % body fat.

male body can accommodate about 21%, and additional unhealthy deposits of 'obese' fat. Further, perfusion per unit adipose tissue decreases significantly with obesity (*14*). This would tend to lengthen half-times in obese tissue, as seen in the torso half-times of our subjects (Fig. 5), where the points seem to suggest a change in the nature of fat above ~ 26% body fat. On this basis, uptake in fat can be modelled and a curve similar to that of Fig. 4 generated, if it is assumed that body fat comprises two pools. Uptake occurs mostly in a well-perfused pool, which has a well-defined maximum size corresponding to ~ 26% body fat. Any additional fat is in a poorly perfused pool that absorbs little xenon, but the additional weight places an increased demand on the body, reducing the proportion of body blood flowing to the well-perfused pool. It may be possible, with further information, to predict uptake in fat for any subject.

Other factors beside fat must be investigated—particularly lung characteristics, which must affect the initial part of the curve. All our subjects had normal gas-exchange capabilities, although having differing lung parameters such as functional residual capacity, tidal volume, etc. However, even in healthy lungs the alveolar dead space is relatively poorly ventilated, and it takes several minutes for gas to be cleared from this space. If ~ 95% of the injected Xe-133 enters the alveolar air on the first passage of the blood through the lung, as suggested by West (*8*), a considerable fraction of this must go back into fresh arterial blood and be circulated around the body. Our subjects took at least 15 min to exhale 95% of the Xe-133 injected. Exhalation might be further delayed by pulmonary disease, and the amount of gas recirculated to body tissue would then increase.

Repeat measurements on A and E by Method 2 gave results similar to those of Method 1 with some small differences. This may be due to positioning

TABLE 3. % OF INJECTED XENON EXHALED IN GIVEN TIME INTERVALS (METHOD 1, SUBJECTS SEATED)

Subject	Interval (min)				
	0–2	2–15	15–30	30–300	300–∞
A	76 ± 2	19	1	4	0.2
E	53 ± 1	35	2	6	3
G	73 ± 2	23	1	2	1

TABLE 4. % OF INJECTED Xe-133 EXHALED IN GIVEN TIME INTERVALS (METHOD 2, SUBJECTS SUPINE)

Subject	Interval (min)					
	0–2	2–15	15–30	30–300	300–∞	20–40
A	73 ± 1	18 ± 1	3	5	1	3
B	82 ± 1	6 ± 1	3	7	2	4
C	65 ± 1	20 ± 1	5	7	3	4
D	37 ± 2	41 ± 2	9	10	3	3
E	57 ± 1	26 ± 1	5	9	3	4
F	64 ± 1	21 ± 1	3	10	2	3
H	(— — — 83 ± 10 — — —)		4 ± 2	7 ± 2	6 ± 2	4 ± 2

Percentage error = ±10% where error is not stated.

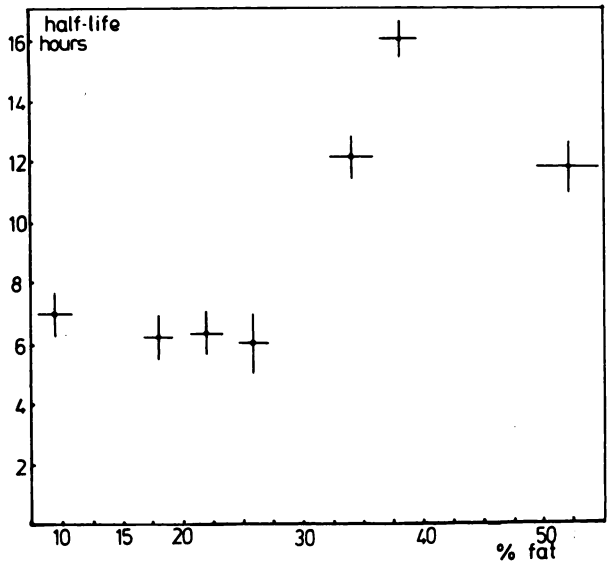

FIG. 5. Longest half-time in torso, against % body fat.

(supine in Method 2, sitting in Method 1) which affects the distribution of blood in the lungs, changing the efficiency of gaseous exchange in different areas of the lung.

Implications for A-37 measurements. Argon is known to behave in a qualitatively similar way to xenon in the body. It seems reasonable to assume that once A-37 escapes from bone into the venous blood, most of it is eliminated rapidly, the elimination curve resembling that for Xe-133. The slow initial rate seen in the A-37 exhalation curve ($t_{1/2} \simeq$ 20 min) must therefore be determined by the rate at which A-37 leaves the bone initially, a rate that is probably governed by the blood supply and composition of bone.

All or part of the A-37 formed is used to provide a measure of the body calcium mass, but the Xe-133 experiments have shown that the proportion of the A-37 exhaled in a given time interval will vary with individual physiology. Some fraction will be retained in body tissues, and the fraction will vary from subject to subject.

A typical breath-collection period used in A-37 measurements is 20–40 min after irradiation (*2*). The percentage of Xe-133 exhaled by our subjects in this (post injection) period is shown in Table 4. The variation is considerable. Moreover, it is possible from the Xe-133 data to estimate the variation in a similar 20-min A-37 collection. To calculate this, we made three assumptions:

1. That an increment of argon in the bloodstream is exhaled at the same rate as Xe-133, elimination being described by the function F (*blood → lung*) which is the Xe-133 exhalation curve for the subject concerned.

2. That the amount of argon formed in the bone during irradiation at time zero is A_0, and its diffusion from the bone into the venous bloodstream is described by a mono-exponential function F (*bone*) $= \frac{dA}{dt} = -A_0 \lambda e^{-\lambda t}$. In this case λ has been assumed to be 0.693/20 min^{-1}.

3. For the purposes of calculation, the exponential F (*bone*) has been divided into 2-min increments, the argon being released as an impulse into the bloodstream at the start of each 2-min interval. Each impulse of argon then leaves the blood in a manner described by F (*blood → lung*), and the percentage of A_0 exhaled in any given period can be predicted. Note that direct collection of breath has been assumed here, as rebreathing has been shown elsewhere to decrease the percentage exhaled (*15*).

A calculation has been made comparing subjects A and E, whose Xe-133 exhalation curves, F (*blood → lung*), are dissimilar. Table 5 shows the results of the calculations. It can be seen that while 50% of A_0 leaves bone during the period 0–20 min, a proportion of it is exhaled between 0–20 min, some between 20–40 min, some between 40–60 min, and so on.

The error introduced into the A-37 breath collections by differences in individual physiology appears to vary according to the collection period chosen. There is ~ 10% difference between A and E in the 0–20 min exhalation period, ~ 4% difference in the 20–40 min period, and ~ 5% difference in the 40–60 min period. Although the result here may be fortuitous, on the basis of these calculations, the

TABLE 5. HYPOTHETICAL ARGON EXHALATION DATA

% of A-37 released from bone interval	%	% of A-37 exhaled					
		0–20	20–40	40–60 min	0–20	20–40	40–60 min
			Subject A			Subject E	
0–20 min	50	43.7	3.3	0.8	39.8	4.4	2.0
20–40 min	25	—	21.9	1.7	—	19.9	2.2
40–60 min	12.5	—	—	10.9	—	—	9.9
Total		43.7	25.2	13.4	39.8	24.3	14.1

20–40 min period seems to minimize the effect of individual physiology. It is apparent that considerable care must be exercised in interpreting exhalation data that have not been deconvoluted. Obviously further investigations of F (*blood* → *lung*) with an argon isotope are required to determine whether the above assumptions are justified.

Intravenous injection studies, while useful, cannot completely determine the physiological factors affecting the elimination of A-37. The bony origin of the A-37 produced by neutron bombardment may pose additional problems: the percentage of A-37 atoms trapped in bone crystals must be predictable, and the possible uptake of argon from bone into bone marrow (a fatty tissue with a slow elimination rate) should be investigated.

None of the problems outlined here are likely to cause difficulties in interpreting successive measurements of calcium by the A-37 method on a single patient, provided that his bone and other body composition remain fairly constant over the period. However, absolute measurements and comparisons of measurements between individuals—or on individuals with rapidly changing bone conditions—cannot be made until the effects of body build and composition have been established.

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