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Symposium: Aging and Body Composition: Technological Advances and Physiological Interrelationships

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In Vivo Animal Models of Body Composition in Aging¹

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ABSTRACT We developed several techniques that provide data on body elemental composition from in vivo measurements in rats. These methods include total body potassium by whole-body counting of endogenous ⁴⁰K; total body calcium (TBCa), sodium and chloride by in vivo neutron activation analysis and total body phosphorus (TBP) and nitrogen (TBN) by photon activation analysis. These elements provide information on total body fat, total body protein and skeletal mass. Measurements were made in 6-, 12- and 24-month-old rats. TBN increased slightly between 6 and 12 months but was significantly lower by 24 months, indicating a substantial loss in total body protein. Working at the National Synchrotron Light Source, we studied rat femurs by computed microtomography (CMT), and the elemental profile of the femur cortex by synchrotron-radiation induced X-ray emission (SRIXE). Although there were no significant changes in TBCa and TBP, indices of skeletal mass, CMT revealed a marked increase in the size and number of cavities in the endosteal region of the femur cortex with increasing age. The SRIXE analysis of this cortical bone revealed a parallel decrease in the endosteal Ca/P ratio. Thus, there are major alterations in bone morphology and regional elemental composition despite only modest changes in total skeletal mass. J. Nutr. 000: 000-000, 1993.

INDEXING KEY WORDS:

• rat body composition • body protein •
microcomputed tomography of bone • X-ray
fluorescence of bone • neutron activation analysis

There have been many aging studies of specific organ systems or cell lines from animals, but there have been far fewer studies of whole body composition, and most of these have relied on carcass analysis end points. Over the past few years we developed a series of techniques to determine a number of body elements in vivo. Carcass analysis usually involves the sampling

of small aliquots of homogenates from whole animals to determine the body elements chemically. Small inhomogeneities and extrapolation from microsample analyses to whole body estimates of chemical composition can lead to large errors. Also, homogenization of the whole animal and chemical analyses are very time consuming. In contrast, in vivo measurement is usually faster, and good accuracy and precision can be achieved by using size- and weight-matched phantom standards.

Whole body counting and in vivo neutron activation analysis have been used as major tools in human body composition studies for many years at Brookhaven National Laboratory (BNL) (Cohn et al. 1980, 1983, 1985). Less well known is the fact that we have conducted parallel in vivo body composition studies in rats. There are obvious experimental limitations when human subjects are involved. For example, the radiation dose to the patient must be low enough to meet the clinical research safety guidelines, whereas with rats, having relatively short lifespans, the radiation exposure need only be limited by doses that would affect the experimental outcome. Also, the use of animal models is the only practical way to achieve a sufficiently uniform population to be able to study one variable at a time without the confounding problems posed by multiple variable systems that are inherent in almost all clinical investigations.

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FIGURE 1 Diagram of the rat whole body counter. Four sodium iodide detectors, each $10 \times 10 \times 40$ cm, are arranged above, below and one on each side of the rat chamber. Standard electronics and a PC-based multichannel analyzer (Nucleus) were used for data collection and analysis.

WHOLE BODY COUNTING

Because rats are small, the absolute amount of each element of interest often falls below the detection limit of the instrumentation used in human studies. For example, the amount of potassium (TBK) in the body of a 70-kg human may be about 130 g; counting a person in the BNL whole body counter for 15 minutes will provide a precision of measurement of approximately 1.8% (Dilmanian et al. 1990). The task of measuring TBK in a 300-g rat that is 230 times lighter than a 70-kg human requires a more sensitive counting system and/or longer counting times. This was achieved by using 4 NaI detectors, each measuring $10 \times 10 \times 40$ cm, by bringing each detector as close as possible to the animal and by arranging the detectors above, below and one on each side of the rat, a counting geometry that approximates a 4π configuration (Fig. 1). Also, the detectors were surrounded by large amounts of shielding material, mostly lead, so the background counts are very low. With this system we can measure TBK in rats with a precision of 3% by counting each animal for 2 hours.

IN VIVO NEUTRON ACTIVATION ANALYSIS

To measure total body sodium (TBNa), chloride (TBCL) and calcium (TBCa) in rats, we constructed a

neutron activation system (Fig. 2) using four Californium-252 sources, two placed on each side of the animal activation chamber (Yasumura et al. 1987). The ^{252}Cf sources are housed in aluminum tubes that can be moved mechanically from a well-shielded storage area to the activation position. Each tube contains two $150\text{-}\mu\text{g}$ ^{252}Cf sources, giving a total of $600\text{ }\mu\text{g}$ of ^{252}Cf in the system. The neutrons are partially moderated with water placed between the sources and the rat chamber; this gives a more uniform distribution of thermal neutron flux through the animal. Our procedure was to activate the rat for 5 minutes, then in 1 minute to transfer the rat from the activation facility to the counting system in the next room and then to count the rat for 15 minutes. The activation and counting systems are sufficiently well shielded so that there is no interference nor increase in the background counting rate due to the proximity of the ^{252}Cf sources to the counter. Bottle phantoms matched in size and weight for each set of rats are used for background corrections and standardization. To determine the background, the bottle is filled with water and the activation and counting procedures are carried out at the beginning and end of each series of animals, typically in the early morning and late afternoon. Standards containing known amounts of Na, Cl and Ca are measured as single-element phantoms and as a mixture of all three elements.

FIGURE 2 Top view schematic of the rat neutron-activation unit. Four $150\text{-}\mu\text{g}$ ^{252}Cf sources, two on each side of the midline, are moved mechanically from their storage locations to positions adjacent to the rat chamber during activation.

TABLE 1
Photonuclear characteristics of the major elements in the human body

Element	Percent of body weight	Threshold energy	(γ , n) Half-life (min)	Daughter emission lines (MeV)
^{16}O	61.0	15.7	2.03	0.511
^{12}C	23.0	18.7	20.40	0.511
^1H	10.0	—	—	—
^{14}N	2.6	10.6	9.97	0.511
^{40}Ca	1.4	15.6	0.015	0.511
^{31}P	1.1	12.3	2.50	0.511
^{32}S	0.2	15.1	0.044	1.27 (0.5%) 0.511
^{39}K	0.2	13.1	7.63	1.27 (1.1%) 0.511
^{23}Na	0.14	2.4	2.6 y	2.17 (99%) 0.511
^{35}Cl	0.12	12.5	32.2	1.27 (90%) 0.511 1.17 (9%) 2.14 (25%) 3.32 (9%)

Note: with 15-MeV photons, N, P, K, Na and Cl can be activated, however, K, Na and Cl contribute very little to the 511-keV peak. Also, the amount of K, Na and Cl in the body is so low that they fall below the sensitivity of this reaction.

PHOTON ACTIVATION ANALYSIS

Total body nitrogen (TBN) and phosphorus (TBP) in rats can be measured by photon-activation (γ , n) analysis using high energy X-rays from a standard radiation therapy unit. The specific radionuclide(s) produced depends on the amount of the parent element in the body and on the reaction (γ , n) energy threshold, as shown in **Table 1** (Wielopolski et al. 1987). Most radionuclides resulting from this bremsstrahlung irradiation are positron emitters. From the differences in energy thresholds and half-life of the radionuclide produced, in combination with known elemental tissue abundance, we can separate and quantify the dif-

FIGURE 4 Curve of successive 30-second counts in the 511 peak over a 30-minute collection. The curve is deconvoluted into its component parts, N and P, by least-squares fitting.

ferent elements. For example, using the 15-MeV radiation therapy unit (Varian), N, P, K, Na and Cl can be activated. However, the amount of K, Na and Cl in the body is quite low, and these elements have very small cross-sections for bremsstrahlung radiation, so the contribution of K, Na and Cl activation products to the gamma spectrum is negligible. The remaining tissue elements that are activated by 15-MeV photons have different half-lives: N = 9.97 minutes and P = 2.5 minutes. **Fig. 3** shows the combined counts in the 511-keV window; a plot of these counts for each 30-second interval over 30 minutes is shown in **Fig. 4**. The measured decay curve is analyzed by a nonlinear least-squares method described by Bevington (1969), and the deconvoluted least-squares curves for N and P are also shown in **Fig. 4**.

ANIMAL STUDIES

In a pilot study using rats from the Gerontology Research Center (National Institutes for Aging, NIH,

FIGURE 3 Gamma ray spectrum from a rat after photon activation with a Varian 15-MeV radiotherapy machine. The peak at 511 keV is characteristic of positron emitters and represents counts from ^{13}N and ^{30}P .

Baltimore) TBK, -Ca, -Na, -Cl, -N and -P were measured by the techniques described above. Three groups of rats were studied: at 6, 12 and 24 months. The rats had been maintained ad libitum since weaning on the NIH open-formula rat chow (NIH-7) containing 1.2% Ca, 0.9% P_i and 23.5% protein. TBK is a measure of body cell mass, and therefore is an index of metabolically active tissue. Body K decreased between 12 and 24 months of age (Table 2) but not in proportion to the loss in body weight, which would suggest that fat is lost at faster rate than lean tissue. Interestingly, despite the body weight loss, TBNa and TBCL indices of extracellular fluid volume, were slightly increased in 24-month-old animals. Table 2 also shows the TBCa values that are not significantly different between the three age groups. Because 99% of the body calcium is associated with bone, this finding indicates that skeletal mass does not change in proportion to the soft tissues (lean and fat) during aging.

When considered together with TBN, which is a direct measure of total protein mass in the body, TBK gives critical information about lean body mass and inferential estimates of muscle mass. Total body protein, as judged by TBN (Table 3), increases slightly between 6 and 12 months of age, but because of the marked increase in body weight during the same period, we conclude that most of the increase in weight can be ascribed to a gain in fat. Between 12 and 24 months, the rats' weight declined dramatically, but this decrease was extremely variable and rapid weight loss was usually followed by death within a month or two. For this strain of rat, 24 months is the age when there is approximately 50% mortality. Total body protein levels were significantly lower at 24 months compared with the 6- or 12-month-old rats. Because the 6-month-old rats were lighter than the 24-month-old rats, the difference in N is not just proportional to weight. This observation is consistent with the expected decline in lean body mass, particularly skeletal muscle loss, associated with very old age.

Table 3 also shows our data on phosphorus. Because more than 85% of the body phosphorus is in bone, like TBCa, it is a good index of skeletal mass. TBP did not change significantly with age, similar to the pattern noted with TBCa.

TABLE 3			
<i>Total body phosphorus (TBP) and nitrogen (TBN) measured by photon activation analysis in male rats</i>			
Age	Body wt.	TBP	TBN
(mo)	(g)	(g)	(g)
6	424 ± 29	3.76 ± 0.16	18.0 ± 1.1
12	578 ± 35	4.15 ± 0.33	19.7 ± 1.2
24	479 ± 24	3.78 ± 0.06	15.6 ± 0.0

Four animals per group. Values are means ± SD.

COMPUTED MICROTOMOGRAPHY OF THE FEMUR

In contrast to the dramatic decrease in the TBN with age, changes in the skeletal mass, judged by TBCa and TBP (Table 3) as noted above, were modest; despite changes in body weight, skeletal mass remained relatively stable. However, on closer inspection of the bone, we saw dramatic changes in the aging skeleton. At autopsy, the femurs from each rat were dissected, cleaned and prepared for computed microtomography (CMT) using an X-ray beam line at the BNL National Synchrotron Light Source (Spanne 1987). There is no particular advantage in using CMT over conventional histological methods once the animal is dead, but CMT can also be used in the living but anesthetized animal by clamping its leg in a fixed position on the micro-manipulator stage before placing the unit in the X-ray beam line assembly. Thus, by using CMT, sequential changes in bone morphology can be studied in vivo in the same animal. The tomographic results clearly show that there is significant endosteal erosion with resorption cavities that increase in size between 6, 12 and 24 months of age (Fig. 5, A-C). These changes are similar to those reported by Kiebzak et al (1988) and are reminiscent of changes that are seen in human subjects during aging (Parfitt 1984). The resorption cavities eventually become so large that they coalesce, increasing the diameter of the medullary space. There is a decrease in the thickness of the femoral cortex but an increase in the cross-sectional circumference and the diameter of the medullary space. Despite these

TABLE 2
Total body potassium (TBK), sodium (TBNa), chloride (TBCL) and calcium (TBCa) measured by whole body counting and neutron activation analysis in male rats

Age	Body wt	TBK	TBNa	TBCL	TBCa
(mo)	(g)	(g)	(g)	(g)	(g)
6	550 ± 21	1.31 ± 0.12	0.53 ± 0.05	0.55 ± 0.04	6.31 ± 0.66
12	616 ± 47	1.34 ± 0.18	0.54 ± 0.07	0.59 ± 0.03	6.83 ± 0.25
24	499 ± 57	1.14 ± 0.14	0.60 ± 0.04	0.65 ± 0.07	6.55 ± 0.35

Five rats per group. Values are means ± SD.

changes, the cross sectional cortical area was similar in the femurs from all three age groups, suggesting that there must have been some increase in periosteal

FIGURE 5 Computed microtomography (CMT) of rat femurs at midshaft for male rats of different age. A) 6 months. B) 12 months. C) 24 months. CMT shows the progressive increase in the number and size of resorption cavities.

FIGURE 6 Ca/P ratio determined by synchrotron radiation induced X-ray emission (SRXEF) of the femur cortex with a resolution of 10 µm. The Ca/P ratio progressively decreases with increasing age at the inner cortical region of the bone.

bone formation with aging. Thus, the skeleton undergoes major changes during senescence, but the changes are not reflected by changes of similar magnitude in the mineral (Ca and P) content of bone.

X-RAY FLUORESCENCE OF CORTICAL BONE

In addition to the CMT results, we took advantage of the synchrotron X-ray source to study the elemental composition of the cortex of the femur. This was done by cutting the femur in cross section into 100 µm slices and measuring the elemental composition of the femur cortex by synchrotron radiation induced X-ray emission (SRXEF) [Jones et al., 1989, 1990]. The elemental profile was determined on several representative slices, scanning across the cortex of the bone from the periosteum to the endosteum, with a resolution of 10 µm, and optimizing the X-ray energy for the measurement of Ca and P. The results, depicted in Fig. 6, show that there is a decrease in the Ca/P ratio at the inner region of the femur cortex with age from 6 to 12 to 24 months. This change in the endosteal Ca/P ratio with age may be related to the loss of mature bone that is relatively calcium rich, compared with the outer surface of the cortex where new bone is being

laid down. A low Ca/P ratio is more typical of immature bone, where crystal (hydroxyapatite) size is relatively small compared with mature bone. These results demonstrate that there is a change in the morphology and regional elemental composition of the skeleton with age.

In summary, we have developed a series of techniques for the in vivo measurement of body elemental composition in rats. These techniques are suitable for a variety of studies where the effect of treatment can be studied longitudinally because the in vivo measurements can be repeated as often as necessary in the same animals. In addition, a more detailed picture of bone can be obtained by CMT, and at autopsy, by using SRIXE for the multielemental analysis of cortical bone.

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