This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

BNL--43714

DE90 007675

DUAL PHOTON ABSORPTIOMETRY: VALIDATION OF MINERAL AND FAT MEASUREMENTS

Steven B. Heymsfield,¹ Jack Wang,¹ Mary Aulet,¹
Joseph Kehayias,² Steven Lichtman,¹ Yakov Kamen,³
F. Avraham Dilmanian,³ Robert Lindsay,⁴ Richard N. Pierson, Jr.¹

¹Department of Medicine, Luke's-Roosevelt and ⁴Helen Hayes
Hospitals, Columbia University College of Physicians and
Surgeons, New York; ³Brookhaven National Laboratory, Upton,
New York; and ²USDA Human Nutrition Research Center on
Aging, Boston, Massachusetts

INTRODUCTION

Photons passing through human tissue undergo attenuation in relation to the specific chemical substances with which they interact. Soft tissues, consisting largely of water and organic compounds, reduce photon flux to a lesser extent than bone mineral, which contains the intermediate-Z element calcium. By selecting two appropriate photon energies and recording their attenuation, the investigator can solve simultaneous equations that subdivide body mass into two components: soft tissue and bone mineral ash (Heymsfield, et al., 1989). Systems are now available that use either a ¹⁵³Gadolinium (¹⁵³Gd) source that generates photons with energies at 100 KeV and 44 KeV or filtered X-rays with energies at about 70 KeV and 40 KeV. Instruments that rely on these sources are referred to as dual photon absorptiometers (DPA) and dual energy X-ray absorptiometers (DEXA), respectively.

Dual photon systems are also capable of further resolving soft tissue into fat and fat-free components (Heymsfield et al., 1989). This is accomplished by use of the R_{ST} , a ratio of attenuations through soft tissue (ST) pixels at the two energy levels (Mazess et al., 1989). The R_{ST} is linearly and inversely correlated with the proportion of soft tissue fat. The patient's percentage fat can be calculated either by internal system calibrations or by using phantoms of known fat content. The latter approach involves serial scans of beef-lard mixtures of known composition, and then using this information to derive the subject's fat mass (Heymsfield et al, 1989; Wang et al., in press).

Fig. 1 shows the dual photon model of body composition. Body weight is divided into three components, total body bone ash (TBBA), fat-free soft tissue (FFST), and fat. Total fat-free body mass (FFM) is the sum of TBBA and FFST.

The aim of this paper is to describe the joint efforts by our group to validate the estimates of body composition derived by dual photon

MASTER

systems. Our initial studies largely involved DPA (Lunar DP4, Madison, Wisconsin), although our discussion will also include the more recently developed DEXA.

MINERAL MEASUREMENTS

The skeleton consists of mineral, protein, water, and small amounts of other compounds (Woodard, 1964). Dual photon systems estimate bone mineral ash, the skeletal fraction remaining after prolonged heating at high temperatures (Heymsfield et al., 1989). The calcium content of bone ash in humans is reasonably constant at 34%-38% of dry mineral weight. The primary focus in our laboratory was to validate TBBA derived by DPA against total body calcium (TBCa), which is found almost entirely within bone mineral. An accurate and precise method of measuring TBCa, delayed gamma neutron activation analysis, is available at one of our Centers (Brookhaven National Laboratory, Upton, NY). This delayed gamma system is described by Cohn et al. (1980).

Fig. 2 presents our study of TBBA versus TBCa in 31 healthy non-obese adults between the ages of 24 and 93 years ($X \pm SD = 58 \pm 20$ years) (Heymsfield et al., 1989; Heymsfield et al., in press). The correlation between bone ash and whole body Ca was highly significant ($TBBA = 3.08 \times TBCa - 432$, $SEE = 238$ g, $r = 0.94$, $p < 0.001$). The ratio of TBCa to TBBA was 0.380 for men, 0.377 for women, and 0.379 for pooled subjects. These results, which confirm earlier initial findings by Mazess et al (1981), support the validity of bone mineral estimates provided by DPA.

An additional point worthy of mention is the relation between bone ash and bone mineral. Mineral, when heated to over 500 C and ashed, loses crystalline water and other volatile components that reduce its mass by 4-5%. We therefore use the limited data available to convert bone ash to osseous mineral (M_o) as (Brozek et al., 1963):

$$M_o = TBBA \times 1.036.$$

DPA MODEL

Fig. 1. Three-compartment DPA model of body composition. F = fat, FFST = fat free soft tissue, and TBBA = total body bone ash. FFM = fat free body mass (FFST + TBBA).

There are two reproducibility issues of concern in relation to bone ash measurements. First, each instrument should be highly reproducible from day to day so that small changes in bone mineral over time are readily detected. The second concern relates to the variety of instruments available that differ in technological features and manufacturer. Optimally, each instrument should provide equivalent estimates of bone ash in the same subject. We performed two pilot studies aimed at examining each of these issues.

In the first study four subjects underwent between four and six DPA scans daily, and the TBBA results were used to calculate a coefficient of variation (CV) (Table 1). The range in CV's was between 0.65% and 1.69%, with an average of 1.34% for the four subjects. We are presently extending this study to DEXA, which reportedly has a CV one-half that of DPA. For comparison, the CV for TBCa estimated by neutron activation, derived on anthropomorphic phantoms, is 0.8%.

The second study involved a cross-comparison between two DPA instruments followed by a comparison of DPA to DEXA (Table 2). Eight subjects underwent DPA scan on our system (#1, Lunar DP4) followed by a scan on a second DPA system (#2, Norland 2600, Helen Hayes Hospital, Havestraw, NY). The mean bone mineral results differed by 2.6% and the regression line of DPA-1 versus DPA-2 was almost identical to the line of identity (Fig. 3). The next phase involved eleven subjects who were first studied using DPA-1 and then underwent study using DEXA (DPX, Lunar). Again the difference in TBBA detected by the two systems was small ($\bar{X}=2.7\%$), with a near-equivalent regression line and line of identity (Fig. 4).

Hence, these initial studies suggest that bone mineral estimates by DPA and DEXA in normal weight, healthy subjects are precise, reproducible between instruments, and compare favorably to the more complex, costly, and less accessible neutron activation method as a means of evaluating bone mineral mass in vivo.

Fig. 2. TBBA on the ordinate versus total body calcium (TBCa) on the abscissa ($TBBA = 3.08 TBCa - 432$, $SEE = 238$ g, $r = 0.94$, $p < 0.001$) (Heymsfield et al., in press).

Table 1 Coefficient of Variation (%) in Subjects Undergoing
4-6 Daily Studies.

	Subjects					
	1	2	3	4	5	$\bar{X}$
DPA						
TBBA	-	1.60	1.69	1.43	0.65	1.34
% Fat	-	2.65	3.73	3.27	4.41	3.52(3.22)+
UWW *						
% Fat	-	5.79	4.18	7.23	-	5.73

* UWW = underwater weighing.

+ Means for 3 subjects with % fat measured using DPA and UWW.

Fig. 3. TBBA measured by DPA-1 (Lunar DP4) on the ordinate versus TBBA derived by DPA-2 (Norland 2600) on the abscissa (DPA-1 = 1.02 DPA-2 + 18.5, $r=0.99$, $p<0.001$).

Table 2. Comparison of Bone Ash (TBBA) Between Dual Photon Systems.

	DPA-1	DPA-2	% Δ
TBBA (n=8)			
$\bar{X}$	2771	2700	2.6%
SD	555	541	
	DPA-1	DPX	% Δ
TBBA (n=11)			
$\bar{X}$	3385	3292	2.7%
SD	648	607

Fig. 4. TBBA measured by DPA-1 (Lunar DP4) on the ordinate versus TBBA derived by DEXA (Lunar DPX) on the abscissa ($DPA-1 = 1.08 DPX + 55.2$, $r=0.99$, $p<0.001$).

These encouraging results represent only an introductory evaluation of bone mineral estimates by dual photon studies. Many technical and biological concerns prevail, including the effects of body thickness, bone marrow fat, and ectopic calcifications on bone mineral results.

BODY FAT

The studies of DPA-estimated fat at our Center involved two phases that differed in methodology and subject number. The first phase involved an extensive series of body composition measurements on the healthy non-obese subjects mentioned above (Heymsfield et al., 1989; Heymsfield et al., in press). In this analysis we compared fat derived by DPA to fat estimated from the following: total body water (TBW), hydrodensitometry (Db), and combined methodologies [Model A: TBW, TBCa, total body nitrogen (TBN), TBK, TBNa, and TBCl; and Model B: TBW, TBBA, Db] (Heymsfield et al., 1989; Heymsfield et al., in press). In the combined methods TBW was established by tritiated water dilution; TBCa, TBNa, and TBCl by delayed gamma neutron activation (Cohn et al., 1980); TBN by prompt gamma neutron activation (Vartsky et al., 1979); and TBBA by DPA (Heymsfield et al., 1989).

Table 3 presents the results of the phase I studies. Fat estimated by DPA was on average similar to, and highly correlated with percentage fat derived by conventional methodologies (all $p < 0.001$). Fig. 5 presents the results for DPA percentage fat versus percentage fat estimated by Model A. This multicompartment neutron activation model is unique in that measured components (TBW, TBCa, TBK, TBNa, TBCl, and TBN) are completely independent from DPA and that assumptions involved are unrelated to age, gender, and ethnicity.

In phase II we evaluated 317 healthy, non-obese Caucasians between the ages of 20 and 93 years. This large subject pool not only included more individuals than in phase I, but the age distribution was younger and more evenly balanced between males and females (Table 4). The protocol in this study was similar to phase I described above, with the exception that neutron activation was not included. As in the earlier phase I, results showed good agreement between DPA percentage fat and fat derived by conventional methodologies. Fig. 6 presents the correlation between DPA % fat and % fat calculated from our multicompartment model B, which is based on TBW, body density, and TBBA ($r = 0.88$, $p < 0.001$). This model has theoretical advantages over the other two-compartment models presented in the Table (4), which fail to adjust fat estimates based on individual differences in hydration and bone mineral.

These studies indicate that DPA is capable of partitioning soft tissues into fat and fat-free components in a manner similar to that provided by conventional methodology.

As with bone mineral measurements, there exists a concern with fat estimated by DPA in the same individual over time and between different absorptiometers. Repeated scans over time of our meat phantoms shows good reproducibility. For example, phantoms #3 and #4 on daily DPA scans have a soft tissue attenuation ratio (R_{ST}) CV of 0.35% and 0.30%, respectively. These CVs translate to ~ 2% on the fat scale for R_{ST} , which ranges between about 1.2 and 1.4 for 100% and 0% fat. When these two meat phantoms were repeatedly studied on the newer DEXA (DPX), their R_{ST} CVs decreased by about 50% to 0.16% and 0.17%, respectively.

Repeated between-day studies for % fat are also available for DPA on the previously mentioned four subjects (Table 1). A parallel comparison of the CV for % fat was made using underwater weighing in

Table 3. Results of Phase I Studies (X $\pm$ SD)

	Males (n=18)	Females (n=13)	Total (n=31)	r(p)**	SEE(kg)
Age	55.2 $\pm$ 18.8	62.5 $\pm$ 22.5	58.3 $\pm$ 20.4	-	-
BMI	23.9 $\pm$ 2.2	22.8 $\pm$ 3.1	23.4 $\pm$ 2.6	-	-
<u>% fat *</u>					
DPA	20.8 $\pm$ 5.1	32.3 $\pm$ 7.3	25.6 $\pm$ 8.3	-	-
TBW	18.9 $\pm$ 7.3	28.6 $\pm$ 7.9	23.0 $\pm$ 8.9	0.91 (0.001)	3.6
UWW	21.6 $\pm$ 5.8	31.0 $\pm$ 8.9	25.6 $\pm$ 8.5	0.88 (0.001)	4.0
Model A	24.1 $\pm$ 7.3	33.0 $\pm$ 9.3	27.9 $\pm$ 9.2	0.90 (0.001)	3.8
Model B	22.6 $\pm$ 6.2	32.0 $\pm$ 8.3	26.5 $\pm$ 8.5	0.93 (0.001)	3.2

* % fat by DPA, 3H2O dilution (TBW), underwater weighing (UWW),
Model A - combined TBW, TBN, TBCa, TBK, TBNa, and TBCl, and
Model B - combined TBW, UWW, and DPA TBBA.

** DPA % fat versus corresponding % fat.

Fig. 5. Percentage fat estimated by DPA on the ordinate versus percentage fat derived by multicompartement neutron activation model on the abscissa [DPA Fat (%) = 0.81 Model A Fat + 3.1, SEE = 3.8%, r = 0.90, p < 0.001].

Table 4. Results of Phase II Studies ($\bar{X} \pm \text{SD}$)

	Males (n=111)	Females (n=206)	Total (n=317)	r(p)**	SEE(kg)
Age	53.7 ± 17.1	52.7 ± 18.5	53.1 ± 18.0	-	-
BMI	24.8 ± 2.7	22.7 ± 2.8	23.5 ± 3.0	-	-
<u>% fat *</u>					
DPA	20.9 ± 5.9	30.9 ± 8.0	27.4 ± 8.9	-	-
TBW	21.1 ± 8.4	29.4 ± 7.8	26.5 ± 9.0	0.79 (0.001)	5.4
UWW	22.7 ± 6.1	29.1 ± 7.8	26.9 ± 9.0	0.81 (0.001)	3.2
Model B	22.0 ± 6.7	29.6 ± 7.3	27.0 ± 8.0	0.88 (0.001)	1.4

* % fat by DPA, $3\text{H}_2\text{O}$ dilution (TBW), underwater weighing (UWW), and Model B = combined TBW, UWW, and DPA TBWA.

** DPA % fat versus corresponding % fat.

Fig. 6. Percentage fat estimated by DPA on the ordinate versus percentage fat derived by multicompartiment model B on the abscissa [DPA Fat (%) = $0.91 \text{ model B Fat} + 1.5$, SEE = 3.2%, $r=0.93$, $p<0.001$].

three of these individuals. The mean CVs for % fat by DPA and underwater weighing were 3.22% and 5.73%, respectively for the three subjects undergoing both studies. The mean % fat in this group was 28.2% by DPA, so the CV represents about 1% total body fat. We anticipate improved precision with the newly introduced DEXA.

The question of between-instrument comparability is an important one that as yet does not have a definitive answer. Our initial studies can be briefly summarized, and they indicate that discrepancies exist between fat estimated by meat phantoms and by internal instrument calibrations, between different instruments, and between DPA and DEXA. These observed differences are worthy of further analysis, and extensive comparative studies are now underway at our Center.

As with bone mineral estimates, future studies need to examine the effects of body size and other conditions, such as state of hydration, on percent fat estimates by DPA.

APPENDICULAR SKELETAL MUSCLE

The fat-free, non-osseous portion of the extremities is almost entirely skeletal muscle, with skin and bone marrow comprising less than 5 percent of soft lean appendicular mass (Heymsfield et al., in press). As DPA software can isolate the extremities, this affords the opportunity to quantify a large portion (70-75%) of skeletal muscle mass in vivo. Our approach was to first develop a simple model of extremity composition. Limb bone ash was converted to skeletal weight by assuming ash comprises 55% of defatted fresh bone mass (Heymsfield et al., in press). Extremity fat was estimated by the beef phantom calibration procedure described earlier (Heymsfield et al., 1989). Appendicular skeletal muscle was then calculated as total limb mass minus fat and bone mass.

The validity of the DPA method for appendicular skeletal muscle mass was evaluated on subjects of the phase I and three additional healthy, lean individuals (n=34) by comparing DPA results to TBK, TBN, and muscle calculated from anthropometric estimates and combined methodologies (Heymsfield et al., in press). The calculated muscle estimates involves use of two models of skeletal muscle mass proposed by Burkinshaw (1985, Burkinshaw et al., 1987). One model requires measurement of TBK and TBN, and the second model is based on TBK and fat-free body mass. Our approach to the second model was to calculate fat-free body mass as body weight minus DPA fat.

Total (upper and lower) DPA appendicular skeletal muscle mass was highly correlated with TBK ($r=0.94$, $p<0.001$; Fig. 7) and summed upper plus lower anthropometric limb muscle plus bone cross sectional areas ($r=0.92$, $p<0.001$). Significant, but weaker correlations were observed between DPA limb muscle and TBN ($r=0.78$, $p<0.001$), and between DPA limb muscle and estimates of whole body skeletal muscle mass based on Burkinshaw's two models (both $r=0.82$, $p<0.001$). These initial results are encouraging and suggest that DPA may also be useful as a means of quantifying a large proportion of skeletal muscle mass in vivo.

APPLICATIONS AND CONCLUSION

The availability of total body bone mineral measurements outside the few neutron activation centers that can quantify TBCa is an important advance in the field of body composition. The traditional two-compartment model of fat and fat-free body mass can be expanded to

further subdivide lean tissues into FFST and TBBA. Moreover, by combining DPA with other methods, such as $^3\text{H}_2\text{O}$ dilution, the investigator could potentially evaluate four (H_2O , mineral, protein, fat) or more body compartments. The door is thus open to answering countless interesting and important questions in human biology previously limited by our inability to quantify skeletal mineral mass.

Similarly, the ability to quantify appendicular skeletal muscle mass, given the limitations of other available muscle-measuring techniques, is a novel and important feature of dual photon systems. The combined capacity to estimate skeletal muscle and bone mineral simultaneously in the appendages further expands the range of questions answerable by these methodologies.

Finally, although fat can be measured using other techniques, dual photon systems offer a new approach independent from classical assumptions or models related to body composition.

ACKNOWLEDGEMENTS

Supported in part by NIH grants AR 39191, DK 41000 and DK 37352, and U.S. DOE contract DE-AC02-76CH00016.

Fig. 7. DPA total appendicular skeletal muscle mass on the ordinate versus TBK on the abscissa ($\text{DPA muscle (g)} = 0.0005 \times \text{TBK} + 5.0$, $\text{SEE} = 1.6 \text{ kg}$, $r = 0.94$, $p < 0.001$) (Heymsfield et al., in press).

REFERENCES

- Brozek, J., Grande, F., Anderson, T., Keys, A., 1963, Densitometric analysis of body composition: revision of some assumptions, Ann. N.Y. Acad. Sci., 110:113-140.
- Burkinshaw, L., 1985, Measurement of human body composition in vivo, in: "Progress in Medical Radiation Physics," Vol. 2, C. G. Orton, ed., Plenum Publishing Corporation, New York.
- Burkinshaw, L., 1987, Models of the distribution of protein in the human body, in: "In Vivo Body Composition Studies," K. J. Ellis, S. Yasumura, and W. D. Morgan, eds., Institute of Physical Sciences in Medicine, London.
- Cohn, S.H., Vartsky, D., Yasumura, S., Sawitsky, A., Zanzi, I., Vaswani, A., Ellis, K.J., 1980, Compartmental body composition based on total body nitrogen, potassium, and calcium, Am. J. Physiol., 239:E524.
- Heymsfield, S.B., Lichtman, S., Baumgartner, R.N., Wang, J., Kamen, Y., Aliprantis, A., Pierson, R.N., Human body composition: comparison of two improved four-compartment models that differ in expense, technical complexity, and radiation exposure, Am J Clin Nutr, in press.
- Heymsfield, S.B., Smith, R., Aulet, M., Bensen, B., Lichtman, S., Wang, J., Pierson R.N., Appendicular skeletal muscle mass: measurement by dual photon absorptiometry, Am J Clin Nutr, in press.
- Heymsfield, S.B., Wang, J., Kehayias, J., Heshka, S., Lichtman, S., Pierson, R.N., Chemical determination of human body density in vivo: relevance to hydrodensitometry, Amer J Clin Nutr, 50: 1282-1289, 1989.
- Heymsfield, S.B., Wang, J., Kehayias, J.J., Pierson, R.N., 1989, Dual photon absorptiometry: comparison of bone mineral and soft tissue mass measurements in vivo with established methods, Am. J. Clin. Nutr., 49:1283.
- Mazess, R.B., Peppler, W.W., Chestnut, C.H., 1981, Total body bone mineral and lean body mass by dual photon absorptiometry. II. Comparison with total body calcium by neutron activation analysis, Calcif. Tiss. Int., 33:361.
- Mazess, R.N., Peppler, W.W., Gibbons M., 1984, Total body composition by dual-photon (153Gd) absorptiometry, Am. J. Clin. Nutr., 40:834.
- Vartsky, D., Ellis, K.J., Cohn, S.H., 1979, In vivo quantification of body nitrogen by neutron capture prompt gamma-ray analysis, J. Nucl. Med 20:1158.
- Wang, J., Heymsfield, S.B., Aulet, M., Thornton, J.C., Pierson, R.N., 1989, Body fat from body density: underwater weighing vs dual photon absorptiometry, Am. J. Physiol., in press.
- Woodard, H.Q., 1964, The composition of human cortical bone, Clin. Orthop., 37:187.