A COMPARISON OF DIFFERING TECHNIQUES FOR THE DETERMINATION OF MINERAL CONTENT IN BONE

A Thesis

by

SAMUEL EVERETT NARROW III

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 1989

Major Subject: Health Physics

A COMPARISON OF DIFFERING TECHNIQUES FOR THE DETERMINATION OF MINERAL CONTENT IN BONE

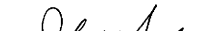
A Thesis

by

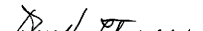
SAMUEL EVERETT NARROW III

Approved as to style and content by:

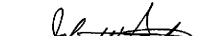
Gerald A. Schlapper
(Chair of Committee)



John W. Poston
(Member)



Dan Hightower
(Member)



John W. Poston
(Head of Department)

December 1989

ABSTRACT

A Comparison of Differing Techniques for the Determination of Mineral Content in Bone. (December 1989)

Samuel Everett Narrow III, B.S. Louisiana State University

Chair of Advisory Committee: Dr. G.A. Schlapper

The medical and veterinary communities are in need of a non-invasive technique to accurately measure bone composition in animals and humans. Common techniques used to determine the structure of skeletons are single-photon absorptiometry, dual-photon absorptiometry, quantitative computed tomography, and neutron activation analysis. Each of these techniques can be used, but each process uses different procedures which might lead to varying results. The results obtained can be used to show mineral content, and over long periods of time can be used to show changes in skeletal composition. Knowledge of composition changes is important in the treatment of pathological diseases such as osteoporosis. Bone diseases such as osteoporosis are thought to be directly affected by bone mineral content in the skeleton. Bone mineral is thought to be proportional to the calcium content in bone volume. When calcium content can be better measured by relating bone strength to calcium content, a clearer understanding of bone diseases as related to calcium content will develop. Data from these experiment showed a correlation between bone mineral density and calcium content in sheep bones. A coefficient of correlation of 0.82 was calculated with a 99.9% confidence interval.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to comittee members Dr. Gerald Schlapper, Dr. John Poston Sr., and Dr. Dan Hightower for their help and guidance in writing this thesis. Also, I would like to express my gratitude to Dr. Dennis James and Brain Poteet for their contributions to my work for this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Determination of bone density dates back to the early 1900's when attempts were made to measure mineral content using X-rays (Hodge et al. 1935). A significant portion of today's research relates to the accurate determination of bone density in humans and animals. Currently, the determination of bone density in the clinical environment relies on the use of single-photon absorptiometry (SPA), dual-photon absorptiometry (DPA), and quantitative computed tomography (QCT). These processes all use photon transmission to determine bone density and bone density variations in skeletal composition. These measurements supply no direct data on the mineral content in bones themselves, because photons do not specifically sample the primary mineral of interest, calcium. Another technique, neutron activation analysis (NAA), can quantify actual calcium content in bone. Neutron activation analysis is discussed in further detail in the literature review and methods sections.

Bone is composed of two types of skeletal material, cortical bone and trabecular bone. The trabecular bone is located in the cavity region of the skeleton. It is porous and is predominantly found in the vertebra, pelvis, long, and flat bones. The cortical bone is found in the shafts of bone and is a compact type of material. Bone is a changing structure of the body that breaks down and replenishes throughout a person's lifetime. It

---

This thesis will follow the style of The Health Physics Journal.

is governed by metabolic and mechanical processes that are not totally understood.

The diagnosis of bone disease is a current topic of interest in the medical and veterinary communities. Methods presently can measure some characteristics of bone makeup but cannot directly measure calcium content. Calcium provides strength to the bones and the lack of calcium is a pivotal indicator of a bone disorder. Calcium depletion of bone is an important factor which is a contributor to bone disease. A significant loss of calcium can lead to a higher fracture risk, lower bone mineral density, and lower bone mineral content.

This research will determine if there is a correlation between bone mineral density (BMD) measured using DPA and calcium content measured using NAA. If such a correlation exists researchers may draw a clearer inference of calcium content in bones. Other researchers currently are developing methods to estimate fracture probabilities for varied animals but are not directly relating this information to mineral content. Their techniques supply a general understanding of bone composition by relating fracture probability to bone density calculations (Firoonzia et al. 1986).

True calcium content will be determined through use of a non-invasive technique, neutron activation analysis. The results from activation analysis will be compared and related to bone density measurements performed by dual-photon absorptiometry. After data are collected from neutron activation analysis and dual-photon absorptiometry, correlations will be examined to determine if a relationship exists between bone mineral density and calcium content. If such a correlation is found, dual-photon absorptiometry could be used to define calcium content in bones in the future and provide a basis that will give doctors and veterinarians added confidence in DPA measurements of skeletal materials. Although NAA is used in this study, it is not a process which should be

repeated more than several times a year. NAA delivers a larger radiation dose than conventional absorptiometry and may cause adverse effects on living subjects if repeated activations occur over short periods of time, less than one year. If NAA is used in a repetitive fashion, radiation doses will accumulate and increase the probability of stochastic risks. Doses could even reach threshold exposures for certain non-stochastic effects. The benefits of using NAA are that it provides a direct measurement of calcium content which can be compared to bone strength in the study of bone disease.

LITERATURE REVIEW

Currently, determination of calcium content of bones is not a direct process and, thus, its accuracy is not insured. For the past ten years the medical and veterinary communities have been relying on techniques such as single-photon absorptiometry (SPA), dual-photon absorptiometry (DPA), and quantitative computed tomography (QCT) to assess skeletal composition in terms of bone density or fracture probability (Firoonzia et al. 1986). These techniques, although of value in relating bone density to fracture probability, do not provide a directly verifiable measure of calcium content in bones. One technique, neutron activation analysis (NAA), does perform a direct measurement of calcium content using a non-invasive method.

These methods of measuring bone characteristics all utilize nuclear processes. Absorptiometry is the measurement of gamma or X-ray transmission through an object. Photons are attenuated in a medium and are not all absorbed in the medium. The photons that do pass through the medium can be detected on the opposite side and analyzed using scintillation detection equipment. Thus, photon transmission can be used to infer certain characteristics of bones such as bone mineral density in g/cm^2 and bone mineral content in grams. The two types of absorptiometry are single photon absorptiometry (SPA) and dual photon absorptiometry (DPA). These processes differ in that SPA has a source which only emits a single monoenergetic photon while DPA has a source which emits photons of two different energies.

Single photon absorptiometry is a simple and widely available procedure that is

used to infer mineral content in the region of the distal portion of the radius (forearm) in units of grams of bone per unit length (g/cm). An I-125 source which emits a 27.5 keV gamma ray normally is used. Results of these measurements, in grams of bone per unit length, generally are characterized as having a 6% accuracy and a 2-3% precision level when measuring a forearm (Firoonzia et al. 1986; Ott et al. 1987). Even though SPA is a helpful tool in the evaluation of bone composition, it has several difficulties. The major limitation of SPA is that the precision of the measurements may be significantly altered if the subject is not precisely repositioned (Firoonzia et al. 1986; Ott et al. 1987). Also, it is an accepted point that in physiological systems expected variations in BMD and BMC values for normal and pathological individuals may overlap. Some BMD measurements of patients with osteoporosis are the same as BMD measurements of patients without osteoporosis. Also, there is a poor correlation of measurements of bone mineral content of the distal radius, the iliac crest trabecular bone volume, and spinal trabecular bone mineral content (Firoonzia et al. 1986; Andersen and Nielsen 1986; Aloia et al. 1988). This occurs because mineral content measurements for the extreme distal radius that contains significant amounts of trabecular bone do not correlate well with data for the iliac crest trabecular bone volume and the spinal trabecular bone mineral content (Firoonzia et al. 1986; Ott et al. 1987).

Dual photon absorptiometry has become the more prevalant clinical technique for diagnosis of osteoporosis, a common bone disease (Firoonzia et al. 1986; Andersen and Nielsen 1986; Aloia et al. 1988; Ott et al. 1987). DPA is very similar in principle to SPA, except that DPA uses a radioactive source that emits two gamma rays with distinct energies. The dominant isotope used in DPA units is gadolinium-153. This radionuclide emits 44 keV and 100 keV photons that are detected, after transmission through the object being scanned, by a scintillation detection assembly as will be discussed in the methods.

The 44 keV photon is a characteristic X-ray produced from Gd-153 and the 100 keV photon is a gamma ray emitted from the nucleus of the isotope. These photons can interact in three major ways: Compton effect, photoelectric effect, and pair production. The Compton effect is the dominant process in the human body because of the large number of low Z elements which makeup the body and the relatively low energy of the photons emitted from the Gd-153.

The process of DPA furnishes a measurement of bone mineral density, BMD. BMD using DPA is a measurement of the attenuation of a sample for a specific surface area and expresses the average density of the scanned bone volume as a two dimensional measurement in units of g/cm^2. These regions of interest then can be combined to provide an overall average BMD.

Quantitative computed tomography (QCT) has an added feature that SPA and DPA cannot provide. QCT scans supply a spatial separation of trabecular bone from cortical bone which allows one to independently measure both types of bone (Firoonzia et al. 1986; Ott et al. 1987). QCT scans can be used to infer with some reliability bone mass, provided that the user employs calibrated equipment and strictly adheres to the calibration specifications of the QCT equipment, to ensure precision of measurement. Calibration is performed using a device that has reference densities which correspond to density characteristics of real bone and soft tissue (Firoonzia et al. 1986). The accuracy of QCT compared to DPA has been determined using fresh cadaver vertebrae with a specially constructed anthropomorphic torso phantom (Firoonzia et al. 1986).

The major advantage of QCT is the capability for three-dimensional localization of trabecular and cortical bone. This characteristic allows the user to obtain a density measurement of a predetermined volume of trabecular and cortical bone. The disadvantage of QCT is the difficulty in repositioning a subject for further measurements.

DPA is preferred over SPA due to the superior accuracy in measuring integral bone density (Firoonzia et al. 1986; Andersen and Nielsen 1986; Aloia et al. 1988; Ott et al. 1987). One of the major advantages of DPA, when compared to QCT scans, is that DPA is affected only slightly by the amount of fat in the volume scanned. In addition, DPA has been used more successfully than QCT in measuring integral bone density in the hip region. The major areas of interest that can be scanned by DPA are the vertebrae, femoral neck, or hip region. DPA is used mainly to scan the hip region because it can be used to provide a superior analysis of the hip over the other techniques discussed (Firoonzia et al. 1986; Ott et al. 1987).

A major drawback of DPA is that the technique does not provide the ability to separate spatially cortical bone from trabecular bone (Firoonzia et al. 1986). Thus, DPA is not prescribed for the evaluation of early stages of trabecular bone loss in the spine. Trabecular bone loss in this area is considered a significant clue to the early manifestations of osteoporosis. What DPA does provide is an analysis of a slice of the surface area scanned and a calculation of BMD for any bone mineral or calcified tissues in that slice. This procedure cannot provide an accurate measurement of trabecular bone in the spine and is the reason DPA is not considered the best method to detect early signs of bone disease when compared to QCT.

In the 1960's, researchers expressed optimism regarding use of the technique of neutron activation analysis to determine trace amounts of chlorine, sodium, calcium, nitrogen and phosphorus in the body. Battelle Pacific Northwest Laboratories (BPNL) and Brookhaven National Laboratory (BNL) conducted studies employing *in vivo* neutron activation analysis to estimate total body calcium. These studies concluded that calcium content could be determined within an accuracy of ± 1.7% in human studies (Cohn et al. 1970) and ± 8% in cadaver and phantom studies (Palmer et al. 1968),

respectively.

The Battelle experiments were performed by irradiating human cadavers and phantoms filled with a skeleton surrounded by tissue equivalent liquid. The subject was irradiated with 2 MeV neutrons produced from the $^{9}Be(d,n)^{10}B$ reaction, and 14 MeV neutrons produced from a Van de Graff positive ion accelerator (Palmer et al. 1968). These studies found that the 2 MeV neutrons produced Ca-49 activity levels approximately 10 times higher than those using 14 Mev neutrons. The lower energy neutrons thermalized inside the body volume and caused a greater number of Ca-48 atoms to be activated. At BNL, live subjects and phantoms were irradiated using 2 MeV neutrons. Both groups emphasized that, to uniformly activate a subject or phantom, the sample must be irradiated bilaterally. This procedure enabled a more uniform deposition of neutrons inside the cadaver or phantom and, therefore, more uniformly activated any Ca-48 atoms present.

Both groups also found that higher energy neutron generators provided a more suitable neutron source than a thermal source because of the deeper penetration of these higher energy neutrons when compared to thermal neutrons. High energy neutrons penetrate the body and lose energy until they reach thermal energies. Once the neutrons reach thermal energies, they only travel a very short distance before they are absorbed and activate an atom. The greatest probability of interaction of neutrons with other atoms occurs in the thermal energy region. The researchers at BPNL and BNL preferred activating phantoms, cadavers, and humans with 2 to 4 MeV neutrons because these neutrons created a more uniformly activated region in the body without unnecessarily endangering other tissues (Palmer et al. 1968). Results showing the differences in activating a cadaver with different energy neutrons are shown in Figure 1. This figure illustrates how higher energy neutrons activate a larger number of Ca-48 atoms. As the

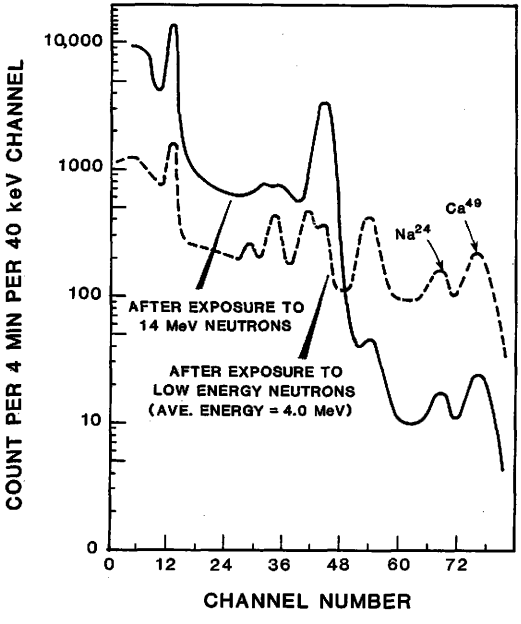


Fig 1. Gamma-ray Spectra for 4 MeV and 14 MeV Neutron Activation

higher energy neutrons activate more calcium atoms, they also can cause greater biological damage and increase the risk of stochastic effects. The lower energy neutrons activate fewer calcium atoms but do produce enough activated calcium atoms to enable the detection of a noticeable Ca-49 peak on a multi-channel analyzer using NaI(Tl) scintillation equipment.

BNL specified several suitable sources for the determination of calcium in humans, ^{238}PuBe sources which yield a 4.5 MeV neutron field, 14 MeV neutrons which are produced from the ^{3}H(d,n)^{4}He reaction, experimental reactors, and portable isotopic sources such as: ^{241}AmBe, ^{238}PuBe, ^{239}PuBe, and Cf-252 (Palmer et al. 1968). ^{241}AmBe and ^{239}PuBe sources are of limited use due to large amounts of the specified isotope required. For example, in order to achieve a usable amount of neutrons for activation analysis of calcium with a ^{239}PuBe source, the mass of plutonium required approaches its critical mass, a fact of significant safety concern (Cohn 1970).

The detection equipment used by BNL and BPNL for *in vivo* neutron activation analysis for determination of total body calcium were first generation whole body counters. The Battelle whole body counter consisted of six 10 cm thick by 23.8 cm diameter NaI(Tl) detectors mounted in a circular array (Palmer et al. 1968). As the subject was scanned by the detector, the rate of movement was slowed to automatically correct for Ca-49 decay. The array of detectors also could be rotated in a circular fashion to provide a uniform cylindrical geometry from one end of the body to the other. This process produced a count rate that was essentially independent of the location of a radioisotope in the body. In 1967, this NaI(Tl) whole-body detector, was considered one of the most sensitive in use (Palmer et al. 1968).

BNL used a 54-NaI detector array, whole body counter with an associated computer facility. This whole body counter had the following characteristics: high

sensitivity and spectral resolution, invariance of counting with respect to body weight, and an ability to find internal localization of a radionuclide (Cohn et al. 1970). Immediately after bilateral activation of a sample, the sample was placed in the detector array and counted for 15 minutes. The elapsed time between activation and counting varied from five to six minutes. Once the count was performed, the data were stored in the computer and the Ca-49 activity was corrected for decay.

Both groups were quite successful in the determination of total body calcium content. Palmer stated that the average skeleton is composed of 1000 to 1500 grams of calcium and that only 12 grams are in the body fluids (Palmer et al. 1968). He noted that the concentration of calcium in the body fluids, atherosclerotic plaques, and kidney stones will vary only by a few grams each year. Activation analysis was found to be capable of detecting an annual change of 2% or more of total body calcium, which corresponds to a variation of approximately 30 grams of calcium for an adult. Thus, a large calcium variation indicates a major change in the overall skeletal composition and does not indicate a small change in calcium due to variation in body fluids or other contributors. NAA was considered a viable technique to determine calcium content in the body and the diagnosis of bone disease. Uncertainties of this method were the uniformity of irradiation of the phantom, cadaver, or human.

Additional research was performed using the same basic procedures by K. Boddy (Chamberlain et al. 1968) and by J. Anderson (Activation 1967). Others have continued to pursue the determination of total body calcium using NAA. Ott used four nuclear techniques, SPA, DPA, QCT, and NAA, to determine how effective each was when used for the study of bones and the diagnosis of bone related disease (Ott et al. 1987). In the early 1980's her experiments focused on total body calcium content determination by NAA and showed the ability to determine significant bone loss in humans as they aged.

Nelp previously measured total body calcium by total body NAA in the same manner with similar results (Nelp et al. 1970).

In summary, since the early 1970's, these methods discussed previously which analyze of skeletal composition have emerged and become procedures of choice for diagnosing bone disorders. These techniques are performed using either X-rays, gamma rays or neutrons. Single-photon absorptiometry (SPA), dual-photon absorptiometry (DPA), quantitative computed tomography scan (QCT) and neutron activation analysis (NAA), each have distinct characteristics which make them useful for analysis of specific components of the skeleton.

NAA is a way to measure non-invasively the actual calcium content in bones. Thus, it can be used to relate calcium content to other measuring techniques such as DPA. NAA is not used widely to measure calcium content directly because of the possibility of the larger radiation doses to the individual and the economic considerations of performing NAA. Also, NAA is not an extremely available procedure at this time. By using data obtained from NAA, DPA can be used to infer calcium content with a much lower radiation dose to the individual and with a lower economic cost.

MATERIALS AND METHODS

The overall goal of this research was to find a way to infer calcium content of bones using a non-invasive technique. Then, these results could be used to determine skeletal changes in humans or animals while incurring the smallest radiation dose to the subject. This study used bones from sheep cadavers. In the future, experiments should be expanded to use live animals to gather total body BMD and calcium content information.

The Texas A&M University College of Veterinary Medicine provided bones from sheep for this study. Each sheep was slaughtered and dismembered. Four leg bones, one from each leg, were sent to the Texas A&M University Nuclear Science Center (NSC). When the experiment first began, each group of four legs from an individual animal were separately packaged, identified, and irradiated at the NSC using the TRIGA reactor facilities. The facilities utilized consisted of Beam Port 4, a neutron radiography port, that has a thermalized beam of neutrons in a field 28 cm by 43 cm (11 inches by 17 inches). This beam was homogeneously distributed by the use of a beam shaper placed between the reactor core and the target area. The samples were carried to the target area by a chain driven cartridge holder connected to a timer. Samples prepared for irradiation were placed in the cartridge holder and the timer was activated which sent the sample into the neutron field for a preset irradiation time.

The bones and phantoms used in this project were all similar in size and shape to minimize any differences in results that were dependent on geometry. The animal bones used were the two front leg bones. The back leg bones were not used because they could not be fully scanned with the DPA unit since it was limited to samples 20.3 cm (8 inches)

in length . The rear leg bones were on average 25.4 cm (10 inches) long and the DPA unit could not provide total BMD and BMC values for the whole bone. The front leg bones were approximately 15.2 cm (6 inches) long with an outside diameter of 2.5 cm (1 inch).

The size of the phantom closely approximated the average size of the sheep bones. It was constructed of a polyethylene tube 15.2 cm (6 inches) long with an inside diameter of 2.5 cm (1 inch) and was filled with 18.745 g of CaO_2. The resemblance of the phantom to the sheep bones kept geometry differences to a minimum and allowed the assumption of similar counting geometry so that comparative neutron activation analysis could be used to determine the calcium content in the bones.

NAA is a more advantageous process because it allows the use of phantoms which closely approximate the geometry, volume and composition of the actual samples. Using a phantom with a known amount of calcium allows the experimenter to calculate the activity of the phantom in counts per gram of calcium. Once the calcium activity is determined, the mass of calcium in the bone can be calculated by dividing the calcium activity of the bone by the specific activity of Ca-49 in the phantom, as discussed later. Comparative NAA enables the experimenter an easier, quicker, and slightly more accurate method than absolute NAA which incorporates activation foils each time a bone is activated. The accuracy of absolute NAA has been noted as unsatisfactory and calibration has been called extremely tedious (Bergerioux et al. 1979; Simonits et al. 1980).

The bones used in this study were stripped of most of their muscle, tissue and skin leaving only trace amounts of flesh and ligament on the exposed bone. This reduced any contribution to 3.07 Mev gamma photopeak of Ca-49 from the activation of different isotopes in the body. The most notable isotope subject to activation in the body tissues is Na-23. This isotope is found throughout the body and can be activated easily to Na-24

which emits a 2.75 MeV gamma ray. The 2.75 MeV photopeak of Na-24 could interfere with detection of the 3.07 MeV photopeak from Ca-49. With most of the flesh stripped from the bones, sodium content is reduced and the difficulties of Na-24 interfering with Ca-49 are reduced.

Each bone was labeled and measured for BMD and BMC using the previously mentioned method of DPA. The table on page 37 lists BMD and BMC as inferred by DPA and total calcium content of each bone as measured by comparative NAA as listed in the results. Calcium content is listed in grams after it was calculated using the activation equations explained in the results. Each BMD is in units of g/cm^2 and BMC is expressed in units of grams of total bone mineral in this table.

The detection equipment consisted of two Harshaw NaI(Tl) crystals, one 12.7 cm (5 inch) and one 7.6 cm (3 inch) crystal, individually connected to a tube base followed by a pre-amp, a Canberra amplifier model 2012, an Ortec single channel analyzer model 550, and a Canberra high voltage supply. Each separate crystal assembly was connected in a summing fashion to a Nuclear Data multi-channel analyzer without a coincidence circuit. Figure 2 shows the configuration of the detection system.

This system was calibrated systematically before any bones were evaluated. A bone phantom was made to the specifications mentioned earlier and filled with 90 ml of aqueous Na_2CO_3 solution. The composition of the mixture was 0.1 g of Na_2CO_3 and 1000 ml of H_2O. Na_2CO_3 was chosen because of its emission of high energy gamma rays and its availability. Na-24 has a 100% emission of a 2.754 Mev and 100% emission of a 1.37 MeV gamma ray per disintegration (Walker et al. 1984). The 2.75 Mev gamma emission is reasonably close in energy to 3.07 Mev gamma from Ca-49 and, therefore, helped define the position of the sodium photopeak stops relative to the calcium

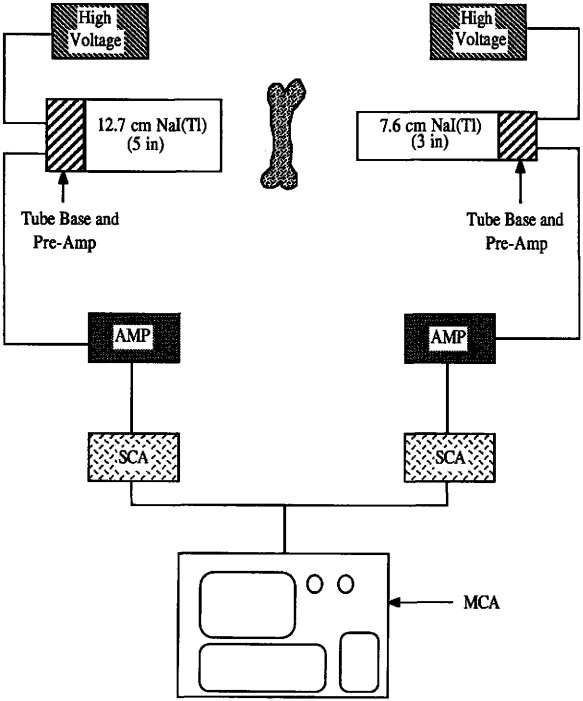


Fig 2. Scintillation Detection Equipment Schematic

photopeak. The MCA channels were preset as 1.0 keV/channel and the region from 2.875 to 3.21 MeV was used to monitor the 2.75 MeV photopeak of Na-24. Using this energy range permitted the calcium activity to be clearly separated from the sodium activity, the sodium photopeak ended at channel number 2850. Once the end of sodium photopeak was found the calcium photopeak was analyzed from channel 2850 to 3245. This enabled the determination of the calcium photopeak to be performed easily and quickly.

The phantom filled with Na_2CO_3 was irradiated in a dry tube in the NSC reactor position A-8 and allowed to decay to a radioactivity level of approximately 10 microcuries before handling. A dry tube is an aluminum tube approximately 152.4 cm (5 feet) long with a 10.2 cm (4 inch) inside diameter. It is air tight when sealed.

Once the original source activity decayed to the specified level, it was taken to the laboratory and counted. The detection system was calibrated for energy in keV per channel. Sets of five counts of the phantom were taken at distances of 2.5, 5.1, 10.2, and 15.2 cm (1, 2, 4, and 6 inches) away from each detector to determine the efficiency of the detectors. The five counts for each distance were averaged. Once the average was determined, calculations to determine the true activity of calcium in the phantom were performed and divided into the net number of counts registered by the detectors. These calculations are discussed later. Then, this product was then multiplied by one hundred to give an efficiency in percent for the detection system.

The number of counts obtained with each detector were summed in the MCA and the background count was subtracted to give the net counts for each sample. The background count was taken by setting a count time for 24 hours with no radioactive samples in the laboratory. Performing this long background count enabled the

determination of background counts from naturally occurring radioisotopes and a more exact background count for the Ca-49 photopeak region from 2.875 to 3.21 MeV.

After the detection system was calibrated, the process of activating the bones and bone phantoms was performed using the TRIGA reactor. The reactor core was placed against the thermal column at the east face of the reactor pool. With the reactor against the thermal column, beam port #4 (BP #4) could be used to activate the calcium in the samples and phantoms to supply approximately 10,000 counts per sample per irradiation. Figure 3 shows the layout of BP #4 and the "sample prep" room. The approximate neutron flux density for BP #4 was estimated in the range of $5x10^6$ n/cm^{2}sec by the NSC staff (Krohn 1989 pers. comm.). Previous experiments by Palmer and Chamberlain, as discussed earlier (Palmer et al. 1967; Chamberlain et al. 1968 Cohn et al. 1969), irradiated both whole body phantoms and cadavers with approximately the same flux density as that obtained in BP#4. The range of the neutron energies used by Chamberlain and Cohn were fast energies, a maximum of 14 MeV but predominantly 4 MeV, down to thermal energies, 0.025 eV. Beam port #4 had a neutron spectra from thermal to fast energies, of these neutrons the predominant energies ranged from 0.025 eV to 11 eV.

The irradiation process started with each bone being individually wrapped in a plastic bag and given an identification number. Once this was accomplished the bones were stored in a freezer until the actual time of irradiation. Before the bones were irradiated, the phantom was activated. Activating the phantom enabled one to determine the flux density for the beam port. Each time the phantom was placed in position it was irradiated for fifteen minutes. The bones were activated in the same fashion. Both the phantoms and bones were taped to the cartridge holder inside the sample preparation room next to the BP #4 cave. Once the length of irradiation was set on the timer in the

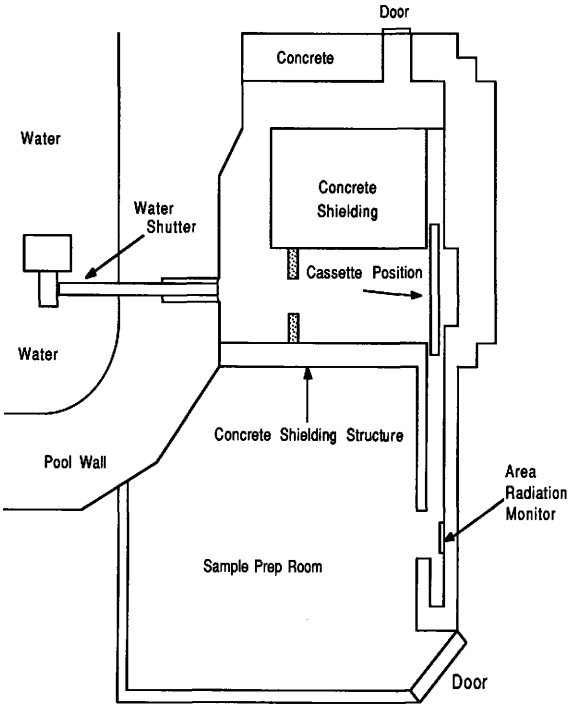


Fig 3. Beam Port #4 Schematic

sample preparation room, the water shutter was remotely opened. The sample was not transferred by the cartridge holder into position in the neutron beam until the flux inside the cave became constant. Determination of a constant neutron flux was accomplished using an area radiation monitor located in the tunnel near the cartridge holder loading position. Once the reading on the area radiation monitor became essentially constant, the flux inside the beam port was considered to be stable with only slight variations of approximately ±5% that were due to natural water convection in the reactor pool. As the water heats, it becomes less dense and the neutron flux will slightly increase due to the decreased density of the water.

After the sample irradiation was complete it was removed from the neutron beam and returned to the sample prep room. The sample was removed from the holder and placed into an unirradiated plastic bag. Immediately after the sample was repacked into the uncontaminated bag, it was rushed to the detection system and counted. When the sample was brought to the detection lab, it was placed on a wooden stand fabricated for this study. The stand positioned the sample at a predetermined height and distance between the detectors. The stand was constructed with sufficient room to permit both bones and phantoms to fit so that a reproducible geometry could be attained simply, easily, and quickly for every sample. Each sample was positioned in the stand horizontally facing the detectors, as shown in figure 2. The distance between the bone and each detector was set at 5.1 cm (2 inches) and was kept constant by the use of a 5.1 cm (2 inch) block placed between the sample and the detector face and removed before the count was started. The bones were counted for forty minutes. These long count times allowed for the collection of approximately 10,000 counts from each activated bone. The phantom needed to be counted for only fifteen minutes to reach this number of counts.

The following paragraphs discuss the second method utilized, dual-photon absorptiometry. The same twelve sheep leg bones were scanned utilizing a dual-photon absorptiometry unit from Lunar Radiation Corporation, Model DP3-B (Lunar 1985). This unit was used to measure the bone mineral density (g/cm^2) and bone mineral content (g) in each bone.

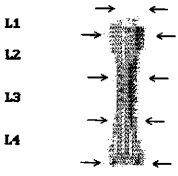
The dual photon absorptiometry unit was calibrated daily before measuring BMD and BMC of the twelve sheep bones. The procedure for calibration, as specified by the manufacturer, was followed in detail (Lunar 1985). A standard containing three blocks of bone mineral regions was scanned five times. Hardware and software supplied by Lunar Corporation with the DPA unit was utilized to determine the width, the bone mineral density (BMD) and bone mineral content (BMC) of the standard. The results were stored in an IBM XT microcomputer with 640K RAM and a 10MB hardisk coupled to the DPA unit. The results from the standard were used as a comparison to any of the measured bone characteristics. The unit employed a 13 mm collimated source that scans at 5.0 mm/sec. The scan consisted of 40 lines, which are incremented 4.5 mm steps (Lunar 1985). An image of each region scanned was displayed on the screen of the computer. It showed the varying regions of density and displayed information such as the calibrated bone mineral density, percent expected of normal for human individuals, and the fracture risk as a function of the input variables of the sample. A printout from the Lunar software is illustrated in Figures 4 and 5.

Each sheep bone was positioned in the same area to retain precision and accuracy. Figure 6 shows a top view of the positioning of the bones on the scanner table. Each bone was positioned in the center of the table below the scanner. Note that prior to each scan all foreign objects were removed from the table top, since any dense object in the path of the beam will attenuate the beam appreciably, causing an error in the measuring

DPX EQUINE FORELIMB RESULTS
VETERINARY NUCLEAR MEDICINE
TEXAS A&M UNIVERSITY, COLLEGE STATION, TX 77840

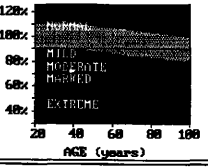
PATIENT ID: NUM12
NAME: NUM12, SHEEP

SCAN: 2.2 5/22/88
ANALYSIS: 1.4 10/2/89



ID: NUM12, SHEEP SCAN DATE: 5/22/88

L2 -> L4 COMPARISON TO REFERENCE MALES



L2 -> L4 BMD (g/cm²)	0.846 ± 0.01
% YOUNG MALES	71
% AGE MATCHED	71
FRACTURE RISK	MODERATE

Age (years).....	19	Large Standard.....	18.19	X-ray Voltage (kVp).....	76.0
Sex.....	Male	Medium Standard.....	13.53	X-ray Current (uA).....	750
Weight (lb).....	154.0	Small Standard.....	9.66	Source Collimation (mm).	0.84
Height (in).....	67	Low keV Air Value...	59526	Sample Size (mm).....	4.5
Ethnic.....	White	High keV Air Value..	58715		
		Average R value.....	1.453		

REGION	BMD[1] g/cm²	% Young[3] Whites	% Age[3] Matched	Fracture[4] Risk
	0.573 ± 0.03	51	51	EXTREME
L2	0.842 ± 0.03	70	70	MODERATE
L3	0.963 ± 0.03	80	80	MILD
L4	0.764 ± 0.03	64	64	MARKED
L1 -> L2	0.722 ± 0.02	63	63	MARKED
L1 -> L3	0.791 ± 0.01	68	68	MARKED
L1 -> L4	0.784 ± 0.01	67	67	MARKED
L2 -> L3	0.892 ± 0.02	74	74	MODERATE
L2 -> L4	0.846 ± 0.01	71	71	MODERATE
L3 -> L4	0.848 ± 0.02	71	71	MODERATE

1 - See appendix B for information on methodologic errors and variables affecting scans. The SD is based on repeated scans (n=5-10) of 6 subjects and 50 scans of 1 subject over 25 days. Statistically 68% of repeat scans will fall within ± 1 SD.
1 - Typical precision of 2.0%.
3 - White Males 2 - Harezs, R.B., et al, (submitted). See Operator's Manual.mot1 - Age matched adjusted for sex, age, ethnic, weight.
4 - Melton, L.J., et al, Am.J.Epidemiol.,1986. See Operator's Manual.

LUNAR DPX System 10

Fig 4. Page 1 of Lunar DPA Printout

DPX EQUINE FORELIMB RESULTS
VETERINARY NUCLEAR MEDICINE
TEXAS A&M UNIVERSITY, COLLEGE STATION, TX 77840

PATIENT ID: NUM12
NAME: NUM12, SHEEP

SCAN: 2.2 5/22/88
ANALYSIS: 1.4 10/2/89

ANCILLARY SPINE INFORMATION**

Region of Interest	BMC (grams)	Area (cm^2)	Width (cm)	BMC/W (g/cm)
	2.25	3.92	1.74	1.29
L2	4.13	4.91	1.36	3.04
L3	3.41	3.54	0.98	3.48
L4	3.66	4.79	1.33	2.76
L1 -> L2	6.38	8.83	1.55	4.12
L1 -> L3	9.79	12.37	1.36	7.21
L1 -> L4	13.45	17.16	1.35	9.96
L2 -> L3	7.54	8.45	1.17	6.45
L2 -> L4	11.21	13.25	1.22	9.17
L3 -> L4	7.07	8.34	1.15	6.13

**Ancillary information is provided for research purposes only and is not documented for clinical use. The methodologic error on individual vertebra will be higher than on the usual L2-L4 sequence. See appendix E for additional information.

LUNAR DPX System 10

Fig 5. Page 2 of Lunar DPA Printout

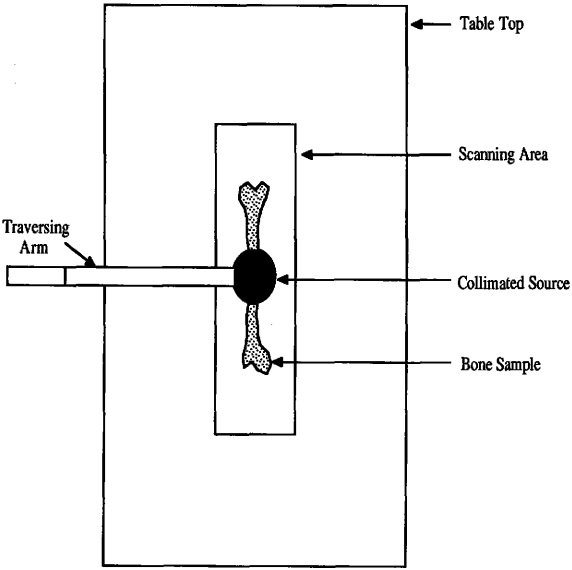


Fig 6. Lunar DPA Table Schematic

process. Once the bone was positioned and all extraneous objects were removed from the scanning path, the process was initiated by entering the appropriate parameters into the computer. The acquisition of data was entirely computer controlled. A manual override could be invoked if necessary to stop the process. After the measurement was performed, the data were stored in the computer for analysis at a later time. Once the requested analysis was completed, the resultant data were stored in a computer file for later use.

The software that was used to compile the information from the dual-photon absorptiometry unit is dedicated to the analysis of human bone mineral. The software was used to calculate parameters such as BMD, BMC, and percent fracture risk from the parameters entered into the program for the specific sample scanned. Therefore, some of the specific information presented in the results may not be fully applicable to the analysis of sheep bones. However, the results can be used to show a correlation between BMD and calcium content. Before any transmission data are obtained, parameteric data must be entered into the computer. The parameters arbitrarily entered for the sheep bones were those of a white male 39 years old, weighing 70 kg, who was 170 cm tall (Poteet 1989 pers. comm.).

Images are processed in the computer and are displayed on the monitor as the DPA unit scans the sample. These images illustrate differences in the density of the bone over the entire volume based on the ratio of the photons emitted from the Gd-153 source to those photons that pass through the target. The 44 keV photons do not normally pass through bone and, thus, do not contribute to the BMD measurements (Lunar 1985). However, the photons are useful because as they pass through skin and muscle and aid in determining the outline of the bone volume. A fraction of the 100 keV gamma rays of Gd-153 penetrate the bone and other dense material in the body. This higher energy

gamma ray is used in the computer analysis to calculate BMD and BMC based on the number of gamma rays detected on the opposite side of the target. This transmitted data is compared to the parameters entered into the computer for the specific sample. These parameters are used in determining the BMD, BMC, and other information such as the fracture risk associated with the BMD and BMC. These risks are shown on the computer monitor and the printout of the analysis. A user friendly chart depicts the regions of normal, mild, moderate, marked, and extreme risk due to fracture based on the calculated BMD. The value for BMD density is plotted on a chart with the subject's age on the X-axis and the BMD plotted on Y-axis. Once the BMD is plotted, the operator only has to read the corresponding Y-axis to find the associated risk for the specific subject. An example of this type of chart is shown in Figure 4. These regions are shaded in different patterns so an individual can easily determine the risk by pinpointing the age and percent fracture risk inside one region.

The Lunar software for this analysis is menu driven with the operator entering data for the sample specific parameters, as previously mentioned. Edge markers on the output of the image define the different regions of interest for each scan. These are labeled L1, L2, L3, and L4. These designations correspond to the lumbar region of the human spine. When the bone mineral content is calculated, it is calculated as an average over that single region. This computer software also averages the BMD and BMC values for combined regions. Combined regions can include two, three or all four regions.

After the bones and phantoms were counted, the data were analyzed to find if a correlation existed between BMD and calcium content, BMD and BMC, and BMC and calcium content. These data are shown and discussed in detail later. The analysis of the data consisted of a linear regression fit for BMD as a function of calcium content, BMD as a function of BMC, and BMC as a function of calcium content. Statistical tests were

performed on a MacIntosh microcomputer using the Statworks software package (Statworks 1985). This software was used to perform calculations and supply data on the F-test, P-value, and coefficient of correlation for each data set analyzed. The values were considered significant if the coefficient of correlation exceeded 0.80 and the P-value for the F-test exceeded 0.95 which relates to a confidence interval of 95%.

CALCULATIONS

The physical characteristics of the bones and phantoms used in this project were all similar in size and shape to minimize geometry effects. The average size of the sheep bones was six inches in length with an average diameter of one inch. The size of the phantom was 15.2 cm (6 inches) in length with an inside diameter of 2.5 cm (1 inch). This minimization of geometrical differences allowed comparative techniques of neutron activation analysis to determine the calcium content in the bones more readily than if absolute NAA were to be employed.

Absolute NAA is a method in which the energy dependent flux density is determined by comparison with a standard of similar geometry and weight of the isotope of interest. The unknowns amount of calcium weight can be calculated from a knowledge of nuclear data such as energy dependent cross-sections for the specific isotope. Some of the major drawbacks of this absolute method occur during placement of the standard such as self-shielding due to the presence of the sample in the neutron beam and the supplemental calculations involved. Limited accuracy in measuring an isotope results from the uncertainties of the base of nuclear data on isotopes. Another uncertainty occurs from the lack of knowledge on the energy spectrum of the neutrons which are used to activate the sample. In the future an improved knowledge base of nuclear data will enhance this technique (Bergerioux et al. 1978). Absolute NAA has an error due to the lack of accuracy in determining the actual flux density. Using comparative NAA techniques eliminates this error in the calculations because flux density does not have to be determined in calculating calcium content when performing relative

techniques. Another problem is the length of calibration of all the components for this technique in order to be successful. The calibration has been noted to be extremely tedious (Bergerioux et al. 1978; Simontis et al. 1980).

The bone phantom was activated six times to determine fluctuation of neutron flux density within the beam port. The average variation of flux density in the beam port during an entire operational day was measured to be ±5%. The phantom was the first sample to be irradiated. It was irradiated to determine the energy integrated neutron flux density at the time of activation. Subsequent irradiations of the phantom were performed after every third or fourth bone was irradiated in beam port #4 to detect any significant variations in the irradiation enviroment present during beam port facility experiments. The length of time between phantom irradiations allowed for the decay of Ca-49 to below detectable levels before the phantom was irradiated and counted again. After the phantom was removed from the cassette holder it was immediately taken to the counting system. Previous experiments illustrated that a longer time between the end of irradiation to the start time of the count created a significant decrease in counts. A two minute decrease in elapsed time for irradiation to counting could increase the number of counts by 20%. An elapsed time of 2.75 minutes, one third of the Ca-49 half-life, was determined to be the minimum time required to remove the phantom from beam port #4 and properly place it in the counting stand. This elapsed time before counting the phantoms was 2.75 minutes for every phantom except one, #5, for which the elapsed time before counting was 2.867 minutes. Each phantom was counted for a total of fifteen minutes. After the counts were recorded, calculations of the activity of each phantom were performed for time at t=o by integrating the activity equation shown below.

$$A(t_1\text{-}t_2) = \int_{t_1}^{t_2} A_0 * e^{-\lambda t}\, dt \qquad (1)$$

Where:

$A(t_1\text{-}t_2)$ = Activity in counts per time interval t_1 to t_2 for a sample removed at t=0 (counts/sec)

A_0 = Initial activity of the sample at t=0 (counts/sec)

λ = Decay constant of isotope of interest in units of per second (1/sec)

t = Time interval for counting period in seconds (sec)

After integration, the equation can be written as:

$$\frac{C}{\lambda} = A_0 * (e^{-\lambda t_1} - e^{-\lambda t_2})\, \zeta \qquad (2)$$

where:

C = Number of counts detected by the counting equipment (counts)

t_1 = Time after irradiation when counting of sample started (sec)

t_2 = Time when sample count stopped (sec)

ζ = Efficiency factor which can be set to unity since relative activity calculations are performed

This equation enables the total number of counts to be calculated for the time integral from the end of irradiation to the end of the count t_2. By dividing the exponential term on both sides of the equation, A_o can be obtained.

$$A_o = \frac{C}{\lambda} * \frac{1}{e^{-\lambda * t_1} - e^{-\lambda * t_2}} \qquad (3)$$

The net counts were obtained by subtracting the background from the gross counts. Then the net counts were substituted in Equation 3 to obtain A_o (activity) at time equals zero.

Calcium content of each bone as listed in the table on page 37 in the results, was calculated using Equation 4. The values for calcium content varied from 7.376 to 13.957 g. Once these values were calculated they were related to BMD in g/cm^2 to determine if there was a correlation of the data.

$$M_{CA} = \frac{A_o}{A_g} \qquad (4)$$

Where:

A_g = Average specific activity in the phantom in (counts / sec / g)

A_o = Average activity of the bone in counts per sec at t = 0 (counts/sec)

M_{CA} = Mass of calcium in the bone in grams (g)

The BMD and BMC values listed in the table on page 38 are averages of each bone's entire volume. These values are taken from analysis performed by Lunar Radiation's dual photon absorptiometry (DPA) unit, model DP3-B which determined values for BMD and BMC of the four combined sections of the scanned bone region. Using parameters such as age, sex, race, height, and weight the DPA unit can accurately measure BMD and BMC within ±2% accuracy and ±1% precision. The four regions designated as L1, L2, L3, and L4, correspond to a person's lower back, the lumbar region of the spine. The value for each individual region's BMD and BMC were computed and averaged for the specific area. These regions can be grouped into any configuration to obtain an average value for combined sections up to the entire four regions L1 through L4.

The exposure imparted to the samples from these two different techniques vary significantly. DPA imparts energy by gamma transmission, while NAA imparts energy by the transmission of a neutron spectrum activating isotopes in the volume of the sample. The technique with the lower dose is preferred for the future determination of calcium content because of the lower risk associated with that exposure. Calculations were performed to illustrate how a DPA unit's dose is considerably less than a dose resulting from neutron activation analysis.

The dose from the Lunar DPA unit is entirely due to the exposure to the Gd-153 source. The source strength of the Gd-153 in the Lunar DPA unit was 1 Ci on November 4, 1988. The Lunar corporation published in their literature, that the exposure from their model DP3-B DPA unit was 10 mrem per 30 minute exposure. This value is well below the non-occupational radiation limits set by the NRC and is considered very acceptable for a medical exposure. The non-occupational limit to the blood forming organs and bone is 500 mrem (10CFR20, 1985). Limits for medical exposures are not as specified because the medical benefit usually outweighs the cost factor for medical treatment. Table 1 lists Texas Department of Health's recommended limits for radiographic exposure limits (TRCR 1989).

The dose from the neutron activation analysis was assumed to be contributed from thermal and epithermal neutrons which activated the Ca-48. The determination of the flux was calculated by averaging the activity of the six phantoms and solving for an average flux using Equation 5. It was calculated to be 4×10^6 neutrons/(sec*cm^2).

$$\Phi = \frac{A}{\sigma * N * (1 - e^{-\lambda * t})} \qquad (5)$$

Where:

Φ = Integral neutron flux (n/cm^{2}sec)

A = Average activity of irradiated phantom at time t (dis/sec)

σ = Energy weighted microscopic cross section of target atoms (cm^2)

λ = Decay constant of radioisotope (sec^{-1})

t = Length of time of irradiation (sec)

N = Number of atoms present in sample

Neutron dose calculations for a sample inside beam port #4 were performed using the flux computed in Equation 5. The absorbed dose from the neutron beam was composed of thermal and epithermal energy neutrons. Evaluation of the ratio of thermal neutrons to epithermal neutrons was performed by the NSC staff. They performed bare and cadmium covered foil irradiations to accurately determine the different fluxes and the

Table 1. Texas Radiographic Exposure and Dose Limits

Technique	Suggested Exposure Limit (mr)	EXPOSURE/DOSE LIMITS Exposure Limit (mr)	Avg. Glandular Dose Limit (mrad)
Chest	20	30	
Abdomen	360	590	
Lumbo-Sacral Spine	550	830	
Cervical Spine	115	170	
Thoracic Spine	340	510	
Full Spine	175	315	
Skull	180	275	
Foot	100	175	
Mammography Screening			1000
Dental Intraoral	400	600	

energy ranges. The ratio of these two energy ranges was 20:1, thermal to epithermal.

Charged particle equilibrium was assumed in the evaluation of the neutron dose. It was hypothesized that all the secondary charged particles that left the volume of sample were replaced by another particle of the same type and energy entering. Equation 6 assumes this and calculates the dose from neutrons by multiplying the flux (rad cm^2/n) times the kerma factor (rad cm^2 / n) times the length of irradiation (sec).

$$D = K = \Phi * F_n * T \qquad (6)$$

Where:

D = Dose (rad)

K = Kerma (rad)

Φ = Flux (n/cm^2*sec)

F_n = Kerma factor (rad*cm^2/n)

T = Irradiation time (sec)

The doses for the thermal neutrons were calculated using Kerma factors quoted by Hurst (Hurst and Ritchie 1961). The Kerma factor for tissue was 2.8×10^{-11} rad cm^2/n. The kerma factor for the bone was not listed in this publication. It was assumed the same as the tissue Kerma factor because bone is approximately twice as dense as tissue and has about one half as many hydrogen atoms in its molecular structure. The dose to the tissue and bone were 95.76 mrad each. The dose to the tissue and bone from epithermal

neutrons were calculated using Kerma factors from Attix's tables for a neutron energy of 11 eV (Attix 1986). The dose to the tissue was 0.261 mrad and 0.229 mrad to the bone. The quality factors used were five for thermal neutrons and 20 for all neutrons other than thermal energy, epithermal and fast (NCRP 1987). These quality factors were used in calculating the approximate dose equivalent to a sample in beam port #4. The doses were then summed and multiplied by the quality factor to determine the total neutron dose equivalent. It was calculated to be 960 mrem.

RESULTS

The phantom was irradiated six times, for fifteen minute intervals. The counts ranged from a low of 10,030 counts for phantom irradiation #3 to a maximum of 10,988 counts for phantom irradiation #4 with an average of 10,381 counts. These values corresponded to activities at t = 0 ranging from 23.74 to 26.00 disintegrations per second per total weight of sample. Once the calcium content had been determined for the phantom, a comparative technique was used to obtain the content of calcium in the individual bones. Equation #4 was solved to determine the mass of calcium in each bone. The average specific activity of the phantom was 24.60 disintegrations per second. The average activity was then used to determine the total calcium content in each bone.

The values of BMD obtained with the Lunar DPA unit ranged from 0.783 to 0.922 g/cm^2 with a standard deviation of 0.050 for the twelve bones as listed in Table 2. Reviewing these measurements in Table 2 shows that the bones with a higher BMD usually corresponded to a larger amount of calcium in the bone. The left leg and the right leg of two animals had considerably less in bone mass in one leg bone as compared to the other leg bone. The corresponding bone numbers are 4,5 and 8,9 in units of grams of calcium as shown in Table 1. The amounts of calcium content varied by 3.76 and 3.12 grams respectively. Their BMD's also varied in the same manner. The values of BMC ranged from 14.20 to 24.97 grams with a standard deviation of 3.566 for the twelve sheep bones. Table 3 and 4 list data on the phantom and bone irradiations.

The relationships between the factors of BMC, calcium content, and BMD were analyzed to determine any statistical error between values such as the coefficient of

correlation or the confidence interval. Linear regressions performed on the data from NAA and DPA for BMD, BMC, and calcium content are illustrated in Figures 7,8, and 9. These figures illustrate linear regressions of the twelve sheep bones plotted as BMC versus calcium content in Figure 7, BMD versus BMC in Figure 8, and BMD versus calcium content in Figure 9. There were no statistical correlations found between BMC and the calcium content of the sheep bones. Statistically these data displayed no correlation between BMD and calcium content. Only half of the smaller animal bones correspondingly increased in BMC and calcium content when compared to the larger bones. The coefficient of correlation was 0.697, and was not considered significant, but

Table 2. Measured Characteristics of the Sheep Bones

Sample	Ca (g)	BMD (g/cm^2)	BMC (g)
1	12.47	0.85	20.15
2	13.96	0.92	22.60
3	12.73	0.92	24.57
4	12.39	0.93	24.97
5	7.38	0.79	16.42
6	11.11	0.90	17.25
7	11.78	0.89	17.18
8	8.63	0.86	18.96
9	14.00	0.91	20.61
10	12.15	0.90	18.90
11	9.15	0.78	14.42
12	9.23	0.84	14.20
		std dev.	std dev.
		0.05	3.57

Table 3. Phantom and Bone Counting Information

Sample #	Counts	t1 (min)	t2 (min)	Ao @t = 0	Ca (g)
Phantom #1	10656	2.75	17.75	25.22	18.745
Phantom #2	10340	2.75	17.75	24.45	18.745
Phantom #3	10030	2.75	17.75	23.74	18.745
Phantom #4	10988	2.75	17.75	26.00	18.745
Phantom #5	10039	2.87	17.87	23.98	18.745
Phantom #6	10235	2.75	17.75	24.22	18.745
Average	10381	2.75	17.75	24.60	18.745
Bone #1	9903	2.25	42.25	16.37	12.47
Bone #2	11085	2.25	42.25	18.32	13.96
Bone #3	10112	2.25	42.25	16.71	12.73
Bone #4	9840	2.25	42.25	16.26	12.39
Bone #5	5858	2.25	42.25	9.68	7.38
Bone #6	8827	2.25	42.25	14.59	11.11
Bone #7	9352	2.25	42.25	15.46	11.78
Bone #8	6587	2.25	42.25	11.33	8.63
Bone #9	10930	2.25	42.25	18.37	14.00
Bone #10	9649	2.25	42.25	15.95	12.15
Bone #11	7170	2.42	42.42	12.01	9.15
Bone #12	7307	2.25	42.25	12.12	9.23
				std. dev. 2.84 (bone)	std. dev. 2.16 (bone)

Table 4. Phantom Counting Data and Activity

Sample #	Net Counts	t1 (min)	t2 (min)	Agm (c/sec/g)
Phantom #1	10656	2.75	17.75	1.35
Phantom #2	10340	2.75	17.75	1.31
Phantom #3	10030	2.75	17.75	1.27
Phantom #4	10988	2.75	17.75	1.39
Phantom #5	10039	2.87	17.87	1.28
Phantom #6	10235	2.75	17.75	1.29
Average	10381	2.75	17.75	1.31
				std. dev. 0.04

the confidence interval for this regression fit was considered significant with a confidence interval of 98.9%. The P-value for the F-test was 0.012 which denotes high confidence interval. The linear regression for this data is shown in Figure 7.

The paired comparison between BMC and BMD show a similar relationship. Figure 8 depicts the linear regression for BMC versus BMD. Values for the BMC increased or decreased respectively as the BMD increased or decreased, but not in proportional amounts. This is seen in Table 2. The coefficient of correlation for BMD versus BMC was higher, 0.754, but was not considered a significant value. The P-value for this data was 0.005 after an F-test was performed. The confidence interval was 99.5%. This P-value indicates that the data points significantly fit in the range of the estimated values using a linear regression and demonstrate a strong level of significance. This information may be useful in the determination of calcium content of bones but is not considered as statistically significant.

The evaluation of calcium content and BMD was performed using a simple linear regression. The regression was performed with the BMD (g/cm^2) on the Y-axis and the calcium content (g) on the X-axis. The values for these two sets of data showed a greater significance than the other two evaluations. The regression shown in Figure 9 shows the data points closely following the line made by the equation for the regression fit of BMD and calcium content. The coefficient of correlation was determined as 0.82. This reflects a significant correlation between the calcium content and BMD. The correlation is not as large as desired, but does show that the BMD (g/cm^2) and calcium content (grams) possess some type of relationship. The P-value for the F-test at 0.001, corresponds to a confidence interval of 99.9%. It was concluded from this data that calcium content can be confidently determined by measuring BMD in g/cm^2.

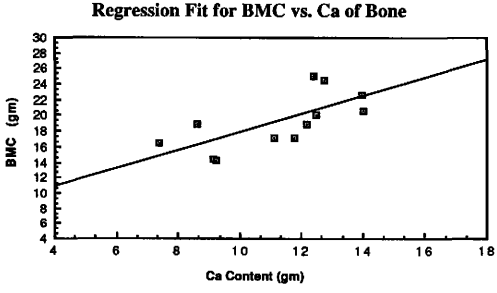


Fig 7. Regression Fit for BMC vs. Calcium Content

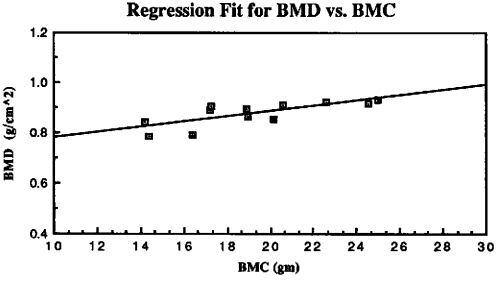


Fig 8. Regression Fit for BMD vs. BMC

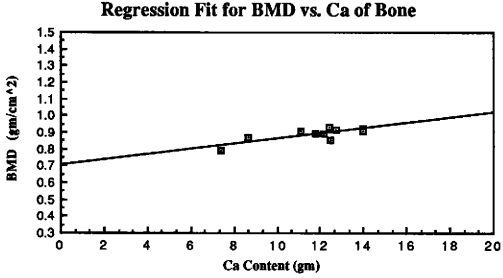


Fig 9. Regression Fit for BMD vs. Calcium Content

CONCLUSION

No previous research has been performed on the specific measurement of calcium content by relating comparative neutron activation analysis to dual photon absorptiometry results. No publications were found comparing BMC or BMD to direct measurements of calcium by using neutron activation. In this project the main goal was to determine if a correlation existed between BMD and calcium content of bones enabling DPA to infer calcium content. The calculations for true calcium content were performed using Equations 1,2,3,4, and 5 using data from comparative NAA experiments discussed earlier. These results were compared to bone density measurements obtained with a Lunar DPA unit. After the data were collected from both techniques, statistical tests were performed to determine the coefficient of correlation between BMD and calcium content. A coefficient of correlation of 0.80 was the minimum goal of this experiment. If such a correlation was found, DPA could be used with more confidence in the future to infer calcium content of bones and would give doctors and veterinarians added confidence in DPA measurements of all skeletal materials. Determination of total calcium content was performed using a standard phantom in comparative NAA experiments. The activity was calculated in units of counts/sec/g of calcium. The phantom was irradiated six times. The subsequent activities were then averaged and used to determine calcium content. The calcium content of the bones were calculated using the standard average specific activity. The calcium content was compared to the BMD and BMC measurements.

This information showed a significant correlation between BMD and calcium content of the bones. A value of 0.82 for the coefficient of correlation for BMD versus

calcium content was slightly larger than the goal of 0.80. This coupled with a confidence interval of 99.9% shows a significant correlation between the two types of measurements. This data also illustrated that calcium is not the only contributor to BMD. If calcium was the only contributor, the linear regression for BMD versus calcium content would intercept the X and Y axis at the point (0,0).

The data discussed in the previous paragraph shows a significant correlation between BMD and calcium content of the front leg bones of sheep. This study creates a small database of information that could be expanded by studies performing experiments using live animals. These live animals would be used to determine whether a correlation between average BMD values and total body calcium content of entire live animals exist. This information could pioneer a technique to indirectly measure calcium content of bones quickly and precisely using dual-photon absorptiometry techniques enabling doctors and veterinarians to easily determine skeletal changes in the study of bone disease.

The medical and veterinary communities are currently seeking methods to perform accurate measurements of calcium loss and bone degradation seen in bone disorders such as osteoporosis. Also, NASA is currently seeking methods to determine bone loss in a zero gravity environment. This information should be beneficial in the determination of bone loss for long term space flight or clinical studies on earth. Animal rights groups and scientists are also interested in using such information to monitor lab animals for skeletal degradation caused from a lack of exercise. This information would be vital in the development of techniques to provide exercise programs to minimize bone mineral loss which is linked to skeletal disorders of these caged animals used in experiments.

REFERENCES

Activation analysis on man, a new tool for medical research? Scientific Research; 1967. (Private Collection S. Narrow).

Aloia J.F., Ashok N., Yeh J.K, Cohn S.H. Premenopausal bone mass is related to physical activity. Arch. Intern. Med. 148; Jan 1988.

Anderson J. and Nielsen H.E. Assessment of bone mineral content and bone mass by non-invasive radiologic methods. Acta Rad. Nov.-Dec.; 1986.

Attix F.H. Introduction to radiological physics and radiation dosimetry. First printing. New York, John Wiley & Sons; 1986.

Bergerioux C., Kennedy G., Zikovsky L. Use of the semi-absolute method in neutron activation analysis. J. Radiological Chem.; 1979.

Chamberlain M.J., Fremlin J.H., Peters D.K., Phillip H. Total body calcium by whole body neutron activation: new technique for the study of bone disease. Brit. Med. J. 7:2:581-583; 1968

Cohn S.H., Dombrowski C.S., Fairchild R.G. *In vivo* neutron activation analysis of calcium in man. Int'l. J. Appl. Rad. Iso. 21:127-137; 1970.

Firooznia H., Rafil M., Golimbu C., Schwartz M.S. Clinical utility of bone mineral content measurements in the diagnosis and treatment of osteoporosis. NY St. J. Med. Dec.; 1986.

Hodge H.C., Van Huysen G., Warren S.L. Factors influencing the qualitative measurement of the Roentgen-ray absorption in toothslabs. Amer. J. Rad. 34:513-538, 678-691, 817-838; 1935.

Hurst G.S., Ritchie R.H. Radiation accidents: dosimetric aspects of neutron and gamma-ray exposures. First edition. Oak Ridge, TN, Oak Ridge National Laboratory ; 1961.

Krohn J. Personal communication. Texas A&M University Nuclear Science Center, College Station, TX 77840, 1989.

Lunar Radiation Corporation. Lunar Radiation DP3 User Manual. Madison, WI 53713. Version 1.0, Dec 1985.

National Council on Radiation Protection and Measurements. Recommendations on limits for exposure to ionizing radiation. Bethesda, MD: NCRP Report No. 91; 1987.

Nelp W.B., Palmer H.E., Murano R. Measurement of total body calcium (bone mass) in vivo with the use of total body neutron activation analysis. J. Lab. Clin. Med. 76:151-162; 1970.

Ott S.M., Kilcoyne R.F., Chenut C.H. III. Ability of four different techniques of measuring bone mass to diagnose vertebral fractures in postmenopausal women. J. Bone and Min. Res. 2:3:201-210; 1987.

Palmer H.E., Nelp W.B., Murano R., Rich C. The feasibility of in-vivo neutron activation analysis of total body calcium and other elements of body composition. Phys. Med. Biol. 13:2:269-279; 1968.

Poteet B. Personal communication. Texas A&M University Veterinary School, College Station, TX 77840, 1989.

Rafferty J., Norling R., McMath C., Tamary R., Morganstein D. Statworks ™ software, Version 1.1. Philadelphia, PA., Cricket Software Inc.; 1985.

Simontis A., Moens L., De Corte F., De Wispelaere A., Elek A., Hoste J. k_0-Measurements and related nuclear data compilation for (n,γ) reactor neutron activation analysis. J. Radiological Chem.; 1980.

Texas Department of Health. Texas Regulations for Control of Radiation. Radiographic exposure limits. Austin, TX: TRCR Part 32.11, Change No. 16; Feb 1989.

U.S. Nuclear Regulatory Comission. Radiation dose standards for individuals in restricted areas. 10 CFR Part 20. Washington DC, U.S. Government Printing Office; 1981.

Walker F.W., Miller D.G., Feiner F. Chart of the nuclides. Thirteenth edition. San Jose, CA, General Electric; 1984.

VITA

Name:	Samuel Everett Narrow III
Date of Birth:	August 2,1964
Parents:	Samuel Everett Narrow Jr.
	Georgia Leah Nees Narrow
Educational Background:	Bachelor of Science in Industrial Technology with Option
	in Nuclear Science, Louisiana State University 1987
Employment:	Graduate Assistant, Department of Nuclear Engineering,
	Texas A&M University
Permanent Mailing Address:	1243 East Bonnie Street
	Gonzales, LA 70737