In Vivo \(^{31}\text{P}\) Nuclear Magnetic Resonance Spectroscopy of Bone Mineral for Evaluation of Osteoporosis

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The mineral content of stationary bone samples can be quantified by \(^{31}\text{P}\) nuclear magnetic resonance (NMR) spectroscopy. The assay can be performed in regions of the anatomy that pose problems for absorptiometric techniques, because the mineral content is measured within a selected volume without concern for the geometry of the bone. In vivo \(^{31}\text{P}\) NMR spectra of the bones in human fingers and wrist are reported. Soft tissue such as marrow and skeletal muscle contributes little to the \(^{31}\text{P}\) NMR spectra of human fingers and wrist and thus should not seriously affect the accuracy of the mineral assay. \(^{31}\text{P}\) NMR spectrometry should prove helpful for confirming rapid bone mineral loss in those at risk and for monitoring response to treatment.

Additional Keyphrases: phosphorus · \(^{31}\text{P}\) · calcium · apatite · bone mineral · osteodystrophy · noninvasive analysis · monitoring bone loss

Two major obstacles have impeded efforts toward prevention of osteoporosis (1–7). First, the underlying causes and the most appropriate prophylaxis and treatment still are not well understood. Second, a method for measuring the presence and extent of osteoporosis is needed, not only for early diagnosis but also to assess the effects of treatment. The work presented here addresses this latter need.

Among the diagnostic procedures currently available for measurement of bone mineral content are single- and dual-photon absorptiometry, quantitative computed tomography, Compton scattering, measurement of total body calcium with neutron activation analysis, and determination of cancellous bone volume from biopsy of the iliac crest (1,3,8–10). Each of these procedures has its technical limitations, and the results tend to correlate poorly with the severity of fracture (4,5,7,10–14).

It was concluded in early 1987 that a better analytical procedure (i.e., easier, cheaper, with lower radiation exposure) was needed (15). Quantitative digital radiography, also called x-ray absorptiometry, has appeared in response to this need (15).

We have taken the alternative approach of measuring the amount of bone mineral (i.e., apatite) directly on the basis of its \(^{31}\text{P}\) NMR spectrum (7,17–19). The relative peak areas in each spectrum from apatite and a reference standard (KPF\(_6\)) are determined by integration. The ratio of these peak areas was shown to be linearly related to the weight of hydroxyapatite within the receiver coil of the NMR spectrometer. The coefficient of correlation, \(r\), for linear least-squares regression analysis was found to be between 0.997 and 0.999 (7).

In theory, \(^{31}\text{P}\) NMR spectra can be recorded in any part of the body. However, the expense of the analysis will be determined in large part by the sizes of the magnet and receiver coil that are required, so measurement of bone mineral content in the extremities would cost less than measurement in the human torso. For this reason, we have begun our research on analysis of the extremities. The recent work of Christiansen et al. (20–22) regarding the suitability of the wrist for monitoring mineral metabolism in the clinical setting and of Dias et al. (23) regarding the incidence of osteoporosis in patients with Colle’s fractures suggests that our current attempts to perform such spectroscopy with the wrist should have clinical application.

Among the responses to our recommendation that \(^{31}\text{P}\) NMR be used for clinical analysis of bone mineral was concern that signals from skeletal muscle and marrow would affect the accuracy of the mineral assay in vivo (24). Thus, three questions are addressed in the work presented here. First, can in vivo \(^{31}\text{P}\) NMR spectra of bone mineral be measured in the human hand and wrist? Second, can surface coils be used to record the spectra? Third, what are the possible contributions to the \(^{31}\text{P}\) NMR spectra from soft tissues such as skeletal muscle and marrow?

Materials and Methods

Materials

Hydroxyapatite and potassium hexafluorophosphate [KPF\(_6\)] were obtained from Bio-Rad Laboratories, Richmond, CA 94804, and Aldrich Chemical Co., Milwaukee, WI 53233, respectively. Skeletal muscle and marrow were excised from dogs immediately after killing (i.e., remains from surgical procedures that had been performed for other research). All experiments with dog tissue and with humans were performed with appropriate review and, where appropriate, informed consent.

Quantification of Apatite

The mineral content of each bone sample was determined from the intensity of its \(^{31}\text{P}\) NMR spectrum relative to that of KPF\(_6\), as previously described (7,17–19). In the in vivo experiments, the KPF\(_6\) reference standard was held in close proximity to the extremity with either a polyethylene sandwich bag or a pair of polyvinyl chloride gloves. \(^{31}\text{P}\) NMR spectra were recorded with a Model NT-150 spectrometer (Nicolet Instrument Corp., Madison, WI 53711) at a resonance frequency of 60.7 MHz. Each sample was kept stationary in a 3.5-T superconducting magnet with an 85-mm internal bore diameter, and one or more single pulses at the resonance frequency were applied with either a Helmholtz coil that surrounded the sample or a saddle-shaped coil that was placed on its surface (7,17–19). The

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time-domain signal was recorded subsequent to each pulse for between 5 ms and 168 ms, depending on the experiment. This signal was apodized by exponential multiplication and then Fourier transformed to obtain the frequency-domain spectra (7). Chemical shifts are given in parts per million (ppm) from $85\%$ H$_2$PO$_4$, which is the accepted method of presentation of $^{31}$P NMR spectra.

A cross polarization, magic angle sample spinning (CP/MAS) $^{31}$P probe from the Nicolet Instrument Corp. was modified for in vivo spectroscopy of the fingers, hand, and wrist. Its coil was replaced with either of two pairs of parallel-connected saddle coils. One Helmholtz coil thus produced had an internal diameter of 55 mm and length of 70 mm, whereas the other had an internal diameter of 75 mm and length of 55 mm. For surface-coil spectroscopy, the CP/MAS probe was modified with only a single saddle coil (55 mm internal diameter and 70 mm length). Observe pulses of 10 $\mu$s to 60 $\mu$s duration were used, depending on the identity of the probe and sample. The magnetic flip angle produced is not known, because a reliable 90° pulse was not possible with our pulse equipment.

Because of the limitations of the superconducting magnet that is available for our work, a significant proportion of the sample in these larger coils lies outside of the "high-resolution" region of the magnet. Narrow peaks are artificially broadened by the inhomogeneity of the field. To circumvent this artifact, we recorded high-resolution in vitro spectra, for comparison, using a probe from the Nicolet Instrument Corp. with a Helmholtz coil that accommodates a stationary 20-mm (o.d.) sample tube with about 7 mL of sample.

Determination of Possible Sources of Interference

Factors that might be expected to interfere with in vivo quantification of bone mineral by $^{31}$P NMR spectrometry include (a) phosphorylated cytosolic metabolites in soft tissue, (b) phosphorylated components of membranes in soft tissue, and (c) the protonated mineral forms that are thought to be involved in the deposition of apatite. The cytosolic metabolites represent only about 5% of the phosphorus of soft tissue; thus they are not expected to make a major contribution to spectral intensity on a per-volume basis. However, the peaks from these metabolites will be quite narrow when the spectra are recorded with a high-resolution magnet. The vast majority of the phosphorus of soft tissue is in membranes in the form of phosphate esters. These ester groups are expected to give rise to broad phosphorus resonances, owing to restricted, anisotropic motion. Just how broad the resonances from a particular membrane will be depends in part on the composition of the membrane (e.g., the relative proportions of the various phospholipids and sphingolipids) and on the rate of motion of the phosphate ester groups of the different membrane components. Although this dependence has been known for some time, it still appears to be a subject of active investigation. For example, in recent papers (25–27) the broad resonance that is seen routinely with brain was attributed variously to (a) "membrane phospholipid," (b) "immobile phosphates," and (c) "mostly calcium phosphate in the skull and some membrane phospholipid." The terms "immobile phosphates" and "calcium phosphate" are poorly used in this context. Truly "immobile" phosphates would give rise to a powder pattern (28), quite unlike the broad peak observed. Furthermore, not just any mineral form of calcium phosphate will give rise to such a peak. The protonated mineral forms that are thought to be involved in deposition of apatite give rise to readily detectable peaks, which are broader than that for apatite. Because the $^{31}$P NMR spectrum of bone is virtually identical to that of hydroxyapatite, the protonated mineral forms appear to be present in adult mammalian bone in only very low concentrations (7, 18).

To clarify the uncertainties of these effects, we recorded high-resolution $^{31}$P NMR spectra (i.e., with the 20-mm probe) of commercial hydroxyapatite and of freshly excised, but otherwise intact, canine skeletal muscle and marrow. Then, to estimate the contribution of soft tissue to the $^{31}$P NMR spectrum of the hand, we compared the total signal intensities from a hand and from freshly excised canine skeletal muscle in the 75-mm (i.d.) Helmholtz coil. The high-resolution spectrum of the hand was simulated by adding together the time-domain spectra of these various components with appropriate weighting factors to approximate the measured contributions.

Magnet Design

Two-dimensional and three-dimensional finite element analysis (29, 30) was used to investigate the feasibility of constructing a permanent magnet for a $^{31}$P NMR spectrometer to be used with the extremities.

Results

In Vivo $^{31}$P NMR Spectrometry

$^{31}$P NMR spectra of the fingers (Figure 1) and of the wrist and hand (Figure 2) were recorded with the 55-mm (i.d.) and 75-mm (i.d.) Helmholtz coils, respectively. The 55-mm coil provided adequate signal-to-noise with as little as one acquisition. However, because of the cylindrical shape of the inner bore of our superconducting magnet, the receiver coil for use with the wrist had to be large enough in diameter and far enough removed from the tune and match capacitors to permit the hand to pass through. The close proximity of the magnet bore to the coil (i.e., 5 mm) required a rake shield, which loaded the coil. This combination of constraints caused the sensitivity of the 75-mm coil to be substantially less than that of the 55-mm coil (e.g., compare the number of acquisitions in Figures 1 and 2). This problem with sensitivity was further exacerbated by the fact that the wrist did not fill the volume within the receiver coil. In addition, a significant proportion of the sample (i.e., fingers, hand, or wrist) lies outside of the "high-resolution" region of the magnet when these larger receiver coils are used, and so the narrow peaks from cytosolic metabolites are broadened by inhomogeneity of the magnetic field. The appearance of the broader peaks from apatite and KPF$_6$ is not affected, but the relative contributions from cytosolic metabolites and bone mineral cannot be ascertained by direct visual inspection of the spectra. These problems could be overcome by using a magnet of appropriate geometry (see below).

Use of Surface Coils

$^{31}$P NMR spectra of a phantom of hydroxyapatite and KPF$_6$ were recorded with the 55-mm (i.d.) saddle-shaped surface coil (Figure 3A–C). These spectra have a very similar appearance but a somewhat lower signal-to-noise ratio than those obtained with the full Helmholtz coil of the same dimensions (Figure 3D).

Possible Sources of Interference

Phosphorus-containing metabolites dissolved in the cytosol of cells in skeletal muscle and marrow exhibit longer
time-domain signals (Figure 4, B and C, left) and thus much narrower natural line widths in the frequency-domain spectra (Figure 4, B and C, right) than do either cell membranes or bone mineral deposits (7, 18, 31, 32). With a high-resolution magnet, these peaks are readily distinguished from the broad resonance of bone in both the time-domain and frequency-domain spectra (Figure 4A–C). The chemical shifts of the peaks from cytosolic metabolites are as follows (ref. 33 and references therein): 2,3-diphosphoglyceric acid (marrow) about +3 ppm; phosphocreatine (muscle) −3 ppm; ATP −5, −10, and −20 ppm; and inorganic phosphate about +3 ppm.

We find that the "high-resolution" $^{31}$P NMR spectrum of about 7 mL of freshly excised, but otherwise intact, dog skeletal muscle exhibits only sharp peaks from phosphorylated cytosolic metabolites; the baseline does not exhibit a hump that could be attributed to cell membranes (Figure 4B). This is true at both 18 °C and 37 °C. The phosphorus resonance from membrane probably is too broad to be detected with these experimental conditions, as was indicated previously by experiments with bovine skeletal muscle (7). (Note that the viability of the muscle tissue used in this experiment is demonstrated by high-intensity peaks from ATP and phosphocreatine and that the entire sample resides in the "high-resolution" region of our superconducting magnet.)

Some indication of a broad hump is apparent in the baseline of the "high-resolution" $^{31}$P NMR spectrum of marrow (Figure 4C). However, this broad resonance is not readily distinguished from the peaks of ATP, and all of the marrow from the entire length of the femur of a large dog (i.e., a volume of about 6 mL) was needed to detect it. The spectrum was essentially the same at 18 °C and 37 °C. (Again note that the entire sample resides in the "high-resolution" region of our superconducting magnet.)

The in vivo $^{31}$P NMR spectra of bone in fingers, hand, and wrist (Figures 1 and 2) are virtually identical to that of stationary hydroxyapatite (Figures 3 and 4A). Variations in homogeneity of the magnetic field over the volume of the sample (e.g., compare the spectra in Figure 4 with those in Figures 1–3) and apodization of the time-domain signal with line-broadening factors ranging from 20 to 400 Hz (e.g., compare the spectra in Figures 2B and 4 with those in Figures 1, 2A, and 3) have little effect on the line shape of the frequency-domain $^{31}$P NMR spectrum of apatite and bone. However, the inhomogeneity of the magnetic field in our superconducting magnet over the volume of the fingers, hand, and wrist gives rise to substantial broadening of the inherently narrow resonances from cytosolic metabolites. Regardless of the line-broadening factor used, the $^{31}$P NMR spectrum of a freshly excised sample of canine skeletal muscle that filled the 75-mm (i.d.) Helmholtz coil appeared as a featureless hump about as broad as the peak from apatite (data not shown). Thus, the spectrum of hand in Figure 2B does not show the narrow resonances of cytosolic metabolites in spite of the use of only a 20-Hz line-broadening factor. The other time-domain signals recorded with the 55-mm and 75-mm coils were apodized with line-broadening factors of 200 Hz and 400 Hz to yield the frequency-domain spectra in Figures 1, 2A, and 3, because.
Magnet Design

Several different permanent-magnet designs were analyzed by three-dimensional finite element analysis with the goal of minimizing size and weight. Our current design would have overall dimensions of 30.5 cm high by 43.2 cm wide by 46 cm deep. Total weight would be 280 kg, 80 kg of which arises from the magnet material. The pole pieces are 11.5 cm in width and 23 cm long and consist of a neodymium–iron–boron magnet material 5 cm in thickness. Each pole piece has a steel face plate, 1.25 cm in thickness, for improvement of field homogeneity. The predicted magnetic field at the center of the magnet, without shimming, is 0.406 T (i.e., 4060 G); thus, $^{31}$P would resonate at a frequency of 6.9 MHz. A mock-up of the magnet is shown in Figure 5.

Because we want to avoid electrical shimming of the magnet, to minimize its cost of operation, the model of Figure 5 was also analyzed by two-dimensional finite element analysis to demonstrate the effectiveness of a mechanical ring shim. Two-dimensional finite element analysis was used for computation instead of three-dimensional finite element analysis because elements of much smaller size can be used with a given size of computer memory. Figure 6 shows flux plots and homogeneity graphs for the magnet with (A) and without (B) a ring shim that is 0.63 cm thick and 1.25 cm wide. The homogeneity factor for the unshimmed magnet is ±484 ppm over a sphere 5 cm in diameter, whereas the much better value of ±16 ppm is obtained with the shimmed magnet. Thus, use of a single mechanical ring shim effects a 30-fold improvement in homogeneity. Multiple shims should yield even better homogeneity, and they will be utilized if required.

Discussion

Measurement of Bone Mineral Weight by $^{31}$P NMR

The weight (i.e., mass) of apatite can be measured directly from the peak area of its $^{31}$P NMR spectrum (7, 17–19). The
relationship between the mass of apatite and the normalized peak area (i.e., relative to an internal standard) is linear. The assay also can be performed in vivo, as demonstrated above, but care must be taken to account for $^{31}$P NMR signals from sources other than apatite.

Although the protonated mineral forms give rise to readily detectable $^{31}$P resonances that are broader than that of apatite, virtually undetectable concentrations of them are present in adult bone (7). Thus, interfering signals are more likely to arise from soft tissues such as skeletal muscle and marrow.

Soft tissues can give rise to two general types of $^{31}$P resonances. The phosphorylated metabolites dissolved in the cytosol give rise to narrow peaks, whereas the phosphate esters in membranes give rise to relatively broad resonances. The apparent line-widths of these latter resonances are determined by the composition of the membrane and the rates of motion of its components, and thus they depend on the identity of the tissue.

The work presented here with freshly excised canine skeletal muscle, in which the viability of the tissue was demonstrated by high-intensity peaks from ATP and phosphocreatine, confirmed our earlier finding (7) that the $^{31}$P NMR spectrum of skeletal muscle does not exhibit a broad resonance under our instrumental conditions (Figure 4B), whether at 18 °C or 37 °C. Because claims that skeletal muscle exhibits a broad resonance (i.e., similar to that observed with brain tissue) arose when investigators started to put whole animals into spectrometers, we would not be surprised if the early in vivo studies had been performed with some bone apatite in the active volume of the receiver coil.

There is some indication of a broad resonance from phospholipids in marrow (Figure 4C). However, all of the marrow that could be surgically removed from the entire length of the femur of a large dog (i.e., a volume of about 6 mL) was required in order to detect its $^{31}$P NMR spectrum. This is much more marrow than is expected to reside in the wrist, yet the number of pulses needed to obtain a usable signal-to-noise ratio with all this marrow was severalfold greater than that needed with an equal volume of bone. A broad resonance from marrow, if it exists, is not expected to interfere significantly with the analysis of apatite. A recent report (31) of high-field $^{31}$P NMR spectra of human marrow did not indicate the presence of a broad peak.

Although our experiments with canine tissue do not permit an exact indication of the contribution of soft tissue (e.g., muscle and marrow) to the $^{31}$P NMR spectrum of human wrist or heel, they do suggest that interfering resonances should arise primarily from dissolved cytosolic metabolites (i.e., primarily phosphocreatine, ATP, and 2,3-diphosphoglyceric acid). These yield much narrower peaks than does apatite under high-resolution conditions, and so their spectral contribution could be ascertained by simple visual inspection or computed deconvolution of the frequency-domain spectra (e.g., compare the spectra in Figure 4, right). The much shorter time-domain signal of apatite compared with those of the cytosolic metabolites (Figure 4, left) also would permit the use of maximum entropy calculations (27). In any case, the contributions from soft tissues in anatomical sites such as the wrist and heel are expected to be relatively small. More will be said of this below.

Assay Requirements

The obvious question then arises of how bone loss might be monitored by $^{31}$P NMR. The answer depends in part on the manner in which the loss occurs (4). The Office of Health Technology Assessment reported (4):

Two broad hypotheses have been proposed for the pathogenesis of primary osteoporosis. The first is that skeletal mass achieved at maturity is the critical determinant for development of clinical osteoporosis later in life. An underlying assumption of this theory is that the rate of bone loss is not significantly increased in persons destined to become osteoporotic... Bone loss is considered a normal manifestation of the aging process. The second theory of pathogenesis is that those determined to become osteoporotic have significantly increased rates of bone loss by comparison with age- and sex-matched controls. The factors responsible for increased rates of bone loss in affected individuals may be the same as those operative during the aging process but are exaggerated. These two theories are not mutually exclusive, and presently it is not possible to state which one of these variables (i.e., bone mass at maturity or accelerated bone loss in later life) is more important.

Fig. 6. Homogeneity graphs (left) and flux plots (i.e., magnetic field lines) (right) in the transverse midplane of the permanent magnet (A) with a 0.63 cm thick by 1.25 cm wide mechanical ring shim, and (B) without mechanical ring shim.

Because of symmetry and mirroring, only the upper right quadrant of each flux plot is shown. See Fig. 5 for the orientation of this quadrant and the relative locations of the magnetic pole piece, steel face plate, flux return, and airgap of the magnet. During the measurement, the wrist or heel would be centered at coordinates $x = 0$, $z = 0$. The homogeneity plots (left) indicate a 30-fold improvement with the shim.

Fig. 5. Mock-up of a permanent magnet that could be used for in vivo analysis of bone mineral density of the extremities by $^{31}$P NMR spectrometry.

The $x$- and $z$-axes indicate the upper right quadrant of the magnet that is represented in the flux plots in Fig. 6.
If accelerated bone loss in later life is found to play an important role in the etiology of osteoporosis, then serial tests will have to be run on individuals to find metabolic changes (34, 35), as is done with the clinical monitoring of other progressive diseases. A primary requirement of such serial tests is that the accuracy and precision of the assay must be sufficiently good to detect small changes that occur over time. The "relative" weight of apatite at a defined locus in a given bone structure would likely be obtained with better accuracy and precision than would calculated values of density that require separate measurement of (a) weight of apatite and (b) volume of bone. The error associated with making two measurements, instead of just one, would be avoided.

If "screening" with a single measurement in midlife (i.e., one measurement of bone mass at maturity) becomes accepted as a valid indicator of possible risk in the patient's later life, however, it generally is assumed that determination of density will be needed to permit a correlation between individuals and a reference population. Therefore, we investigated alternative approaches that might be used to measure mineral density by $^{31}$P NMR.

Measurement of Bone Mineral Density by NMR—Coil Design

A measure of in vivo mineral density could, in theory, be obtained by a combination of NMR spectroscopy and imaging (7). The mass of bone mineral within the receiver coil would be measured by $^{31}$P NMR spectroscopy, and the volume of bone would be determined by proton magnetic resonance imaging via the same coil. The density would be calculated from the mineral mass and bone volume. Because both the mass of bone mineral and the volume of the bone would be measured without moving the patient's extremity, errors in the calculated bone mineral density would be minimized. Furthermore, the exact orientation of the wrist or heel within the instrument would be known. However, the resolution of the image obtained might not be sufficient to provide the needed accuracy and precision of the measured volume and, thus, of the calculated mineral density. An instrument with imaging capabilities would be less routine to operate and substantially more costly to build than a simple spectrometer. A "high-resolution MR imaging" system for use with the wrist (36) and "bone-marrow imaging" of the spine (37) have been described.

Alternatively, one could define the volume to be analyzed within a given structure and then measure the amount of mineral within that volume. For example, the processus styloideus of the ulna has a rather high proportion of trabecular bone with a relatively thin cortical layer, and it is covered primarily by just a layer of skin in thin women (i.e., those at highest risk of developing osteoporosis). A surface coil of appropriate dimensions could be placed such that the signal from material in the active volume of the coil would arise almost exclusively from bone mineral rather than soft tissue. The volume measured and the response of the apatite within that volume to the "observe" pulse would be determined by the dimensions of the coil. This approach would provide a measure of density in a defined volume with minimal contribution from phosphorus in soft tissue. A receiver coil of appropriate shape might also be used in a similar fashion with the posterior and lateral aspects of the calcaneus, and possibly also with the trochanter.

Our demonstration of the use of a surface coil to measure apatite (Figure 3) indicates the technical feasibility of such an approach. However, one must keep in mind the inherent characteristic of surface coils that sensitivity to resonance signals is not constant over the volume of the sample. In addition, the flip angle produced by a given pulse and, thus, the magnitude of the signal intensity obtained varies likewise as a function of the distance from a surface coil. Signal intensities will depend on placement of the surface coil and reference standard relative to each other and to the bone under investigation. This is quite different from Helmholtz and solenoid coils, which produce more uniform fields within their active volumes. However, reproducible placement of the surface coil over a given bone structure and the use of calibration curves will permit accurate and precise measurement of both "relative" weight and density of apatite.

Magnet Design

The spectra in Figure 4 indicate that the $^{31}$P resonances from apatite and soft tissues can be distinguished quite readily with a "high-resolution" magnet. The contributions from naturally "broad" and "narrow" resonances can be distinguished in either the time-domain or frequency-domain spectra.

As indicated above, however, the superconducting magnet available for our use does not have a sufficiently large bore diameter to permit routine work with human extremities. Because the high-resolution region of the magnet is smaller than a hand or wrist, the resonances from cytosolic metabolites in soft tissue appear very broad (e.g., Figure 2B). Furthermore, the close proximity of the magnet bore to the receiver coils for in vivo use seriously compromises sensitivity.

Large-bore magnets that would be capable of reasonably high-resolution spectrometry are available in magnetic resonance imagers. However, such instruments are not readily available for research purposes. Modification of these instruments to perform $^{31}$P NMR spectroscopy of solid samples would be somewhat expensive, and their use for clinical detection of osteoporosis would not be cost effective. Therefore, we have designed a magnet that would be sufficiently inexpensive to build and operate for our research.

The 0.4-T magnet we have designed fits on a desk top (Figure 5). With a single mechanical ring shim, the field exhibits a homogeneity of better than ±16 ppm over a sphere, located at the center of the air gap, that is 5 cm in diameter. This suffices to record the broad resonance of apatite but is not adequate to resolve the inherently narrow peaks of cytosolic metabolites. Better resolution can be achieved with additional ring shims or electrical shim coils, or both, but these would add to the cost of construction. Therefore, we have considered the feasibility of using an inexpensive, medium-resolution version of the magnet in Figure 5 for analysis of the extremities.

Two main considerations are involved. First is the likely contribution from soft tissue to the change over time in the $^{31}$P signal intensity. Second is the possibility of making receiver coils of the appropriate geometry to minimize spectral contributions from soft tissues. We have shown that soft tissue contributes about 15% of the signal intensity of the $^{31}$P NMR spectrum of the human hand. The other 85% arises from bone. By way of comparison, the wrist has a much smaller proportion of soft tissue and, thus, the spectral contribution will be proportionately smaller. If we assume that soft tissue contributes 5% of the spectral intensity of wrist, then the volume of soft tissue would have to change at an annual rate of 20% to produce
an apparent change of 1% per year in the intensity of the $^{31}$P NMR spectrum. Such a large change in soft tissue mass should be readily apparent to the physician on the basis of appearance and weight of the patient. Furthermore, such a large change in soft tissue could be detected in the extremities on the basis of the absolute intensity of its $^1$H NMR spectrum (38). The receiver coil of the spectrometer could be double tuned-and-matched to the resonance frequencies of both $^1$H and $^{31}$P nuclei, and the proton signal would be recorded at the same time that the phosphorus spectrum was obtained. The area under the peak in this broad-line $^1$H spectrum would provide a measure of the amount of tissue in the receiver coil.

The contribution of soft tissue to $^{31}$P NMR spectra of the extremities could be reduced further by appropriate probe design. The experimental results presented above indicate that both surface coils and Helmholtz pairs could be designed such that a minimum of soft tissue would be present in the active volume. These could be placed on or around, for example, the processus styloideus of the radius or ulna, or the posterior or lateral aspects of the calcaneus. The large, accessible air gap of the magnet in Figure 5 was chosen to facilitate the use of such coil designs.

Conclusions

Bone is a complex structural composite of a fibrous organic matrix and an inorganic filler. The inorganic component, primarilyapatite, makes up about 45% of the wet weight of mature cortical bone, whereas the organic substances, mainly collagen, account for about 35%. The remaining 20% is water (39). Mechanical strength (4, 5, 34, 35, 39–47) is determined by a complex interplay among (a) the amount of mineralized tissue present in the bone, (b) the extent of mineralization of the organic matrix, (c) the chemical composition and extent of crosslinking of the organic matrix, (d) architectural aspects of the bone, (e) fatigue from repeated loading of the bone, (f) the rate and direction of deformation of the bone during trauma, and (g) possibly other factors. Thus, it is a gross oversimplification to attempt a predictive correlation of fracture risk based on only one parameter, namely density (4, 5, 10, 34, 35, 39–47).

One must keep in mind that proper deposition of collagen is a prerequisite of mineralization and, thus, a decrease in bone mineralization during later life could arise in part from limitations in the ability to process this protein. The activities of the enzymes involved in synthesis and utilization of collagen decrease with age and are affected by hormone concentrations and other factors (e.g., refs. 48–50 and references therein). This could also result in a decreased rate of repair of microfractures caused by normal use. Whereas some have considered a fracture to be "essential to the diagnosis of osteoporosis—or even diagnostic of it," Nordin (35) has stated that "this practice is not only undesirable but positively misleading." It has come to be realized that fracture risk is a continuous variable that increases as bone density declines, but the relation is not a simple linear one. Thus, if the amount of mineral in a bone is reduced, "osteoporosis must be present (discounting the rare case of osteomalacia), and the main problem is to define the standard against which this reduction should be measured" (35). Although support has been given to the notion of administering estrogen, as replacement therapy, to many if not most postmenopausal women without prior or concomitant bone-mineral analysis (51, 52), Wasnich et al. (53) have calculated that indiscriminate administration of estrogen would not be cost effective. Notelovitz (34) has written, "When one considers the physiology of bone formation and loss, it soon becomes apparent that there is much more to postmenopausal osteoporosis than estrogen replacement. The rate of postmenopausal bone loss needs to be monitored and modulated. Central to the entire issue is the fact that bone is a living organ and needs to be treated as such."

In vivo $^{31}$P NMR spectrometry permits the homeostasis of apatite to be measured in living bone. A measure of relative weight loss and of density of mineral should be possible. Correction can be made for the spectral contributions from soft tissues when high-resolution instrumentation is used or, in the case of the extremities, practicably minimized by appropriate choice of receiver coils.

Use of $^{31}$P NMR would not be limited solely to detection of osteoporosis in the elderly. Difficulties with mineral metabolism in childhood and during space travel also could be monitored. Smaller receiver coils for use with children, and alternative magnet and coil designs for assessing the rapid loss of mineral from load-bearing bones and atrophy of skeletal muscle during space travel (ref. 54 and references therein) are possible.

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References


38. Halbach RV, private communication.


