Femoral Neck Bone Density
Direct Measurement and Histomorphometric Validation

Norman A. Johanson, MD,* Mary E. Charlson, MD,+ Laure Cutignola, AM; Manuel Neves, MD,§ Edward F. DiCarlo, MD,± and Peter G. Bullough, MD*

Abstract: The purpose of this study was to develop a method for directly measuring bone density of femoral neck sections. Three types of density were measured. Real density equals wet weight divided by the actual volume of bone tissue (real volume). Apparent density equals wet weight divided by the total volume occupied by the bone plus the pore spaces (total sample volume). Ash density equals the ash weight divided by the real volume. Corticocancellous cross-sections of the femoral neck were analyzed for density at two levels: level 1, proximal neck and level 3, distal neck. Density measurements were compared with histomorphometric measurements performed on cross-sections at the midportion of the femoral neck (level 2) and with a clinical radiographic measure of bone density (cortical index 3 cm below the lesser trochanter). No correlation was found between apparent density and either real (r = .12, P = .62) or ash density (r = −.09, P = .72) within a given femoral neck section. There was, however, a strong correlation between real and ash density (r = .93, P = .0001). This was expected because real and ash densities are both reflections of bone mineralization. Apparent density showed better correlation, when comparing level 1 with level 3 sections (r = .76, P = .0001), than did ash (r = .57, P = .01) or real density (r = .55, P = .01). There was no correlation between either real or ash density with any histomorphometric parameter. Apparent density was moderately correlated with total bone area expressed as a percentage of cross-sectional area (r = .66, P = .008). This finding tends to validate the direct measurement of apparent density in that both apparent density and total bone area are measurements of the concentration of bone in space. No significant correlation was found between any of the density measurements and the cortical index at 3 cm. This underscores the necessity for precisely qualifying any definition or discussion of bone quality. The success or failure of hip implants may be at least partially determined by the ability of the bone to withstand the insult of implantation of the prosthesis and to adapt successfully to the new mechanical environment. This study represents an early phase of defining parameters that may have prognostic value in long term implant fixation. Key words: femoral neck, bone density, histomorphometry, implant fixation, bone mineral, hip.
The refinement of implant design and the techniques used in performing total joint arthroplasty have led to a more careful focus on the quality of the bone adjacent to the prosthesis.\textsuperscript{6,12} It has become clear, however, that bone quality is difficult to define in a general sense by using specific types of measurements (bone histomorphometry,\textsuperscript{13} clinical bone densitometry\textsuperscript{9}) or focusing on specific anatomic sites (iliac crest, lumbar spine, femoral neck).\textsuperscript{12} It is likely that the issue of bone quality needs to be considered in the context of well-defined clinical conditions such as hip fracture, lumbar-spinal fracture, and implant fixation. Various parameters that may be indicative of bone quality in each of these conditions need to be defined and validated using well-designed outcome studies.

The purpose of this study was to develop a method of directly measuring femoral neck corticocancellous bone density in specimens removed during total hip arthroplasty. The femoral neck was thought to be a good source of bone that could be used to prospectively evaluate bone quality as it relates to long-term femoral component fixation. A technique for performing histomorphometric analysis of femoral neck sections was developed for the validation of direct density measures. It was hypothesized that directly measured density in corticocancellous femoral neck sections would correlate with histomorphometric parameters.

**Materials and Methods**

Pathologic specimens of the femoral head and neck were obtained from 19 hips in 19 patients who underwent total hip arthroplasty. There were 8 men and 11 women with a mean age of 53 years (range, 24–77 years). The diagnoses associated with the hip disease were osteoarthritis (10 patients), rheumatoid arthritis (5 patients), and osteonecrosis (4 patients).

Pathologic specimens were obtained at the Department of Pathology at the Hospital for Special Surgery (New York, NY). Selection was based on availability of an intact femoral neck without damage to the cortical or cancellous bone, and no gross pathological changes in the neck such as cysts or residual osteophytes. Selection was blind with regard to the patient's age, sex, and diagnosis.

Three-millimeter cross-sections containing both cortical and cancellous bone were taken from the femoral neck at three levels (Fig. 1): level 1, at the junction of the head and neck; level 2, at the middle of the neck; and level 3, 1.5 cm above the lesser trochanter. Levels 1 and 3 were used for direct measurements of bone density and level 2 for histomorphometry.

Levels 1 and 3 were analyzed for bone density using modified methods of Arnold et al.\textsuperscript{1-3} and methods of Galante et al.\textsuperscript{7} A similar method was utilized by Carter and Hayes.\textsuperscript{4,5} The terminology used in describing the various weights, volumes, and densities are those used by Galante et al.\textsuperscript{7} The bone marrow was washed out of the cancellous bone using a stream of pressurized tap water. Visual inspection was used to determine if all fat had been removed from the marrow spaces and if all soft tissue had been cleaned from the outer surface of the bone. The submerged weight of the specimens was obtained by weighing them while suspended by a wire from a microanalytic balance and submerged in distilled water. The weight of the wire was subtracted from all weights. While the specimen was submerged, care...
was taken to assure that no air bubbles were trapped within the cancellous portion of the bone. After removal from the water the specimens were centrifuged at 8,000 rpm for 15 minutes to remove excess water. They were then weighed in air, and this was recorded as wet weight.

**Volume Determinations**

The volumes of bone were calculated using the method described by Galante et al. and also utilized by Carter and Hayes. Archimedes' principle states that "a body submerged in water is lightened or buoyed up by a force equal to the weight of the water displaced." Since the weight of water expressed in grams equals its volume expressed in milliliters, the real volume of bone tissue was calculated by subtracting the specimen's submerged weight from its wet weight (real volume (cc) = wet weight (g) - submerged weight (g)).

We developed a technique for the determination of total sample volume (volume of bone plus pores) in an irregularly shaped specimen such as a femoral neck section. Others have studied cubes of trabecular bone and determined the total sample volume using a micrometer. We utilized a vacuum paraffin embedding technique in which the pores in the specimens were first filled with paraffin while enclosed in a heated vacuum. All paraffin that was outside the confines of the specimen was carved away from the outer cortical shell and cut so that it was flush with the two flat surfaces of the section. The sections were weighed while submerged in 100% ethanol and again in air. The weight in ethanol was adjusted by dividing the submerged weight by the density of ethanol. Total sample volume was calculated by subtracting the corrected submerged weight from the weight in air (total sample volume (cc) = weight in air (g) - corrected submerged weight in ethanol (g)).

**Density Determinations**

Density calculations were made as follows. The real density or tissue density equals wet weight divided by real volume or bone tissue volume (real
Density was calculated by dividing wet weight by total sample volume (apparent density = wet weight/total sample volume).

Finally, the sections were ashed in a muffle furnace at 600° centigrade for 24 hours and weighed to obtain the ash weight. Ash density was calculated by dividing ash weight by real volume (ash density = ash weight/real volume).

Histomorphometric measurements were performed on sections from the midneck region (level 2). The sections were decalcified in 5% nitric acid, run through a series of alcohol washes, and embedded in paraffin for histologic sectioning. Four micrometer sections were taken using a Reichert microtome and were stained with hematoxylin and eosin (Fig. 2). Measurements of cortical, cancellous, and total bone areas recorded as a percentage of the total cross-sectional area were performed using the Zeiss Video Plan II (Carl Zeiss, Jena, Germany).

Results

Measurements

Density Measurements. The mean real density (tissue density) for level 1 sections was 2.03 ± .45 g/cc (mean ± SD) with a range of 1.18–2.79 g/cc.
The mean real density for level 3 sections was 2.38 ± .68 g/cc with a range of 1.43–4.13 g/cc.

The mean apparent density for level 1 sections was .77 ± .17 g/cc with a range of .49–1.03 g/cc. The mean apparent density for level 3 sections was .76 ± .17 g/cc with a range of .48–1.04 g/cc.

The mean ash density for level 1 sections was 1.00 ± .33 g/cc with a range of .45–1.75 g/cc. The mean ash density for level 3 sections was 1.17 ± .41 g/cc with a range of .65–2.04 g/cc.

Histomorphometric Measurements. Histomorphometric analysis was performed on level 2 sections. The areas of either cortical, cancellous, or total bone area were expressed as a percentage of the total cross-sectional area. The mean cortical bone area was 16.27 ± 7.61% with a range of 6.4–31.6%. The mean cancellous bone area was 12.9 ± 5.29% with a range of 2.67–21.69%. The mean total bone area was 29.15 ± 8.39% with a range of 15.78–44.00%.

Fig. 5. Comparison of ash density and real density within each specimen at level 3.

Cortical Index Measurements. Cortical index is the percentage of cortical bone occupying the total width of the femoral shaft measured on an anteroposterior radiograph 3 cm below the lowest point of the lesser trochanter. The mean cortical index was 43.1 ± 12.9% with a range of 33–57%.

Correlations

Density Measurements Within Each Specimen. Real density and ash density measurements were compared within specimens at level 1 (Fig. 4) and at level 3 (Fig. 5). There was a strong correlation between real and ash densities ($r = .87, P = .0001$ in level 1 specimens and $r = .93, P = .0001$ in level 3 specimens). No significant correlation, however, was found between apparent density and either real ($r = .12, P = .62$; Fig. 6) or ash density ($r = -.09, P = .72$; Fig. 7).
Density Measurements Between Levels of Each Femoral Neck. There was a moderate correlation between density measurements at level 1 and level 3. Apparent density had the strongest correlation \( r = 0.76, P = 0.001 \); Fig. 8). Ash density (Fig. 9) and real density (Fig. 10) were not as strongly correlated between levels 1 and 3 \( r = 0.57, P = 0.01 \) and \( r = 0.55, P = 0.01 \), respectively).

Density Measurements and Histomorphometric Measurements. Apparent density was the only density measurement found to have a significant correlation with any histomorphometric parameter. There was a moderate correlation between apparent density and total bone volume (the percentage of cross-sectional area occupied by bone tissue \( r = 0.66, P = 0.008 \); Fig. 11). Apparent density correlated less well with cortical bone area \( r = 0.43, P = 0.11 \) and cancellous bone area \( r = 0.43, P = 0.11 \). Neither real density or ash density had any significant correlation with cortical \( r = 0.10, P = 0.73 \); \( r = 0.24, P = 0.39 \), respectively), cancellous \( r = 0.02, P = 0.94 \); \( r = -0.08, P = 0.77 \), respectively), or total bone area \( r = 0.10, P = 0.71 \); \( r = 0.17, P = 0.55 \), respectively).

Density Measurements and Cortical Index. The cortical index was not found to correlate significantly with apparent \( r = 0.16, P = 0.58 \), real \( r = -0.23, P = 0.41 \), or ash density \( r = -0.34, P = 0.22 \).

Discussion

This study has examined several variables that might be expected to help build a definition of bone quality at four different anatomical sites in the proximal femur. The values for real and ash densities reported are in agreement with previously published values. The apparent density values, however, are approximately three times higher than the values...
Fig. 7. Comparison of apparent density and ash density within each specimen at level 3.

recorded for cancellous bone taken from vertebral bodies or from the distal femoral metaphysis.\(^4\,^5\,^7\) They are approximately one half the magnitude of the values of apparent density in cortical bone reported by Keller et al.\(^10\) This discrepancy is explained by the corticocancellous content of the specimens used in this study. The concept of apparent density takes into account the effect that porosity has on bone as a structure in that the denominator for calculating apparent density is the total sample volume (bone plus pores) rather than the real volume of bone tissue. Therefore, much of the variability of apparent density measurements can be explained by the varying contributions of cortical and cancellous bone to femoral neck architecture in relation to total cross-sectional area.

Although many factors may influence the mechanical properties of bone, the stiffness and strength of bone have been frequently compared with bone density. Weaver and Chalmers\(^4\,^6\) and Bartley et al.\(^3\) found positive correlations between human cancellous bone compressive strength and apparent and ash density. Galante et al.\(^7\) showed that in vertebral cancellous bone apparent density varied directly with the compressive strength. These conclusions were supported by Carter and Hayes\(^4\,^5\) who found that the relationship between apparent density and compressive strength could be described by a power function. Moreover, they have noted that this relationship seemed to hold in both compact and porous bone. Other studies have demonstrated power relationships between cortical dry apparent density in the femoral diaphysis and both Young's modulus and bending strength \(R^2 = .79\) and \(R^2 = .80\), respectively.\(^10\) The relationship between real density and mechanical strength is less clear. One study has suggested a negative correlation.\(^7\) In summary, mechanical testing tends to substantiate the im-
Analysis of the strength of the bone specimens was beyond the scope of this study. It was possible, however, to assess the relationship between the various measures of bone density within a given specimen and to observe the correlation of a given measure of bone density at two different levels of the femoral neck within an individual. In this study real and ash density were found to be highly correlated with each other; an expected finding in that they are both representative of the degree of bone mineralization in relationship with the other components of bone (organic matrix and water). This correlation is in agreement with the findings of Mueller et al. who studied the density of cancellous bone and found “hydrated density” to correlate highly with mineral content. Apparent density was found not to be correlated with either real or ash density. Although this is not particularly surprising, it underscores the complexity of addressing the generic issue of bone density or quality.

When examining density along the length of the femoral neck, apparent densities of level 1 and level 3 specimens in a given individual were more strongly correlated than either real or ash density. This was an unexpected finding. In light of the relatively short distance separating level 1 from level 3, one would expect little difference in the degree of bone mineralization, and hence a strong correlation between level 1 and level 3. The dramatic difference in structural characteristics (shape and corticocancellous composition) between the levels (Fig. 1), however, would lead one to expect a much higher degree of variation in the apparent density and a weaker correlation between the two levels. The meaning and significance of this counterintuitive finding is unclear. One might ask the question, “for a given mechanical environ-
Fig. 9. Comparison of ash density in level 1 specimens with ash density in level 3 specimens taken from the same femoral neck.

The lack of significant correlation between the cortical index of the femoral diaphysis and bone density of the femoral neck demonstrates the necessity of defining bone quality according to anatomic site. A cortical index 3 cm below the lesser trochanter might be expected to be a good correlate of apparent density because it is a two-dimensional analog of the concentration of bone in space. The cortical index has been thought to have importance in defining the thickness of cortical bone and to some extent the shape of the endosteal surfaces along the proximal femur. Consequently, this or similar measurements have been utilized in determining bone quality for implant fixation with special regard for the fit of uncemented prostheses. The definition of bone quality in this particular area awaits the outcome of prospective long-term studies. It is possible, however, that appropriate definitions of bone quality of the femoral canal...
Fig. 10. Comparison of real density in level 1 specimens with real density in level 3 specimens taken from the same femoral neck.
Fig. 11. Comparison of apparent density with total bone area.
for implant fixation will not be interchangeable with definitions of bone quality that address other issues such as femoral neck or vertebral-compression fracture.

**Conclusion**

Apparent density as measured in corticocancellous cross-sections of the femoral neck in humans correlates well with the percentage of the cross-sectional area occupied with bone (total bone area) measured in an adjacent section. These two parameters may be valid indicators of the structural properties of the femoral neck, and hence its mechanical quality. Real and ash density do not appear to be as strongly correlated to this type of bone quality. The dissociation between femoral neck density and radiographically measured density in the subtrochanteric region demonstrates the need for a variety of definitions of bone quality depending on the anatomic site and the clinical issue under consideration. Furthermore, these parameters should be evaluated for their prognostic significance in the context of prospective outcome studies.

**References**