Paper

Trabecular bone densitometry using interactive image analysis

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ABSTRACT

Interactive image analysis is a novel application of digital image processing to the densitometry of trabecular bone. Briefly, the exposed surfaces of bleached slices of bone are illuminated so that the trabecular tips are bright in relation to the interstices. The image is then captured by a TV camera and digitized with an interactive image analysis system. A grey scale is then chosen that differentiates the bone at the surface from the background. Finally, a computer program calculates the area fraction of bone within a user-specified box which, by Delesse's principle, is an estimate of the volume fraction of bone. The reproducibility of the technique (expressed as an average SD) is ± 1.5 vol. % and has surface-discriminating capabilities comparable to the traditional point-counting method but is faster and more precise. Most importantly, the technique permits biomechanical testing of the sample after its density has been determined.

Keywords: Trabecular bone, densitometry, digital image analysis

INTRODUCTION

Densitometric analysis of bone samples has been important in many biomechanical studies because of the relationship between density and bone strength[1,2,3]. Trabecular bone densitometry techniques fall into two categories, the non-invasive and the invasive techniques. However, most laboratories have used the latter because they do not require sophisticated scanners and detectors.

Two invasive approaches are available. For the first and more direct method, defatted trabecular bone is weighed and its total volume measured to determine its apparent density[2,4]. Apparent density is the bone mass per total volume of a sample. The second method determines bone density as a volume % by applying Delesse's principle which states that an area fraction measurement of bone can be taken as an estimate of the volume fraction of bone[5]. The volume fraction of bone is equal to the ratio of the apparent to real density where real density is the bone mass per bone volume, excluding pore space. This is the approach commonly used to analyse bone biopsies taken with a trephine[6]. The bone specimen is first dehydrated, defatted, and embedded in methylmetacrylate. The slab of bone is then cut into serial sections which are either stained or microradiographed. Finally, morphometry to determine the bone area fraction and therefore volumetric density can be made manually by point counting[5] and planimetry[3], or automatically by using the Quantimet densitometric scanning device for stained sections[6] and computer-assisted videodensitometry for microradiographs[7]. These two approaches, though very different, have been used in biomechanical studies and have played a key role in the characterization of the physical properties of bone. However, they are both complex and, often, time-consuming.

By coupling stereological principles and digital image processing, we have developed a technique that has surface-discriminating capabilities comparable to the traditional point-counting method, yet is faster and more precise. Hilliard and Cahn[8] have already proved that the point-counting strategy, of all the traditional morphometrical methods, gives the best results for estimating the volume fraction. Densitometry using interactive image analysis should be a useful tool for researchers who need a rapid method for determining the density of trabecular bone.

MATERIALS AND METHODS

A spine obtained fresh at the autopsy of a >70-year-old male was kept frozen until needed for analysis. The vertebrae were stripped of tissue, cut into symmetrical halves with a low-speed Buehler Isomet saw and a Buehler diamond wafering blade (Buehler, Ltd, Lake Bluff, IL, USA), and bleached. The vertebral bodies were then cut transversely at 3-mm intervals, soaked in acetone for 4 h and bleached white.

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Area fraction measurements were obtained in the transverse plane using an interactive image analysis system (IBAS, Kontron Bildanalyse, Germany). The procedures were carried out in a dark room to eliminate stray light. The exposed trabecular surface of the slice was illuminated by a narrow beam of light from a Bausch and Lomb lamp 20 cm away at a 20° angle from the horizontal. Background lighting was provided by a Bogen Quartz light head positioned 30 cm above and about 1 m behind the smaller lamp. Under this lighting configuration, the tips of the surface trabeculae were bright with respect to the inner portions of the bone sample. Using a PAL television camera fitted with a macro lens, the slice images were digitized to a 512×768 pixel spatial resolution ($\times 10$ magnification) and 256 grey levels. A grey scale window which differentiated bone at the surface from the background was then selected by interactively thresholding the colour scale on a high-resolution colour monitor. Finally, an IBAS computer program calculated the area fraction of bone within a user-specified box on the image. By Delesse's principle, this value was a measure of the volume fraction of bone.

Testing was carried out to characterize the technique. The first series of tests determined the reproducibility of the method. Nine 5×5 mm areas from different vertebral slices were chosen and the area fraction determined several times for each. For each trial, the light set–up was dismantled and reassembled to simulate day-to-day variation.

The area fraction measurement obtained by the technique is dependent upon the grey scale window specified by the user to discriminate bone at the surface from bone within the interstices. To determine the reproducibility of operator skill in choosing a grey level window to surface discriminate, surface discrimination was repeated several times for two different 5×5 mm images. Ten area fraction measurements were obtained for each image and compared.

Tests were then performed to determine the accuracy of the surface-discriminating ability of the technique. The area fraction of bone for seven 5×5 mm areas from different vertebral slices was determined several times with interactive image analysis and then, for comparison, with the traditional point-counting method. For this traditional determination, a Zeiss Universal microscope ($\times 12.6$ magnification) fitted with an integrating eyepiece was focused on the surface of the vertebral slice at different randomly chosen sections within the same area measured by IBAS. The slice was, as with the IBAS approach, illuminated by a low-angled Bausch and Lomb lamp to highlight the surface. An estimate of the area fraction was obtained by counting the number of line intersections that fell on surface trabeculae (counting intersections falling on the boundary of bone and marrow space as 0.5 to avoid bias) and then calculating the ratio of this number to the total number of intersections on the eyepiece grid.

To facilitate surface discrimination, the surface of the slice had to be illuminated such that only the tips of the trabeculae were bright. As described above, this was done by shining a narrow beam of light on to the slice from one side and nearly parallel to its surface. A final test was conducted to determine the variation of the measurements due to slice orientation with respect to the light source. An area fraction measurement was taken of a 5×5 mm area. The slice was rotated 90° in the plane of the light beam and a second area fraction measurement was then taken of the same area. This was repeated at 180° and 270° orientations as the slice was rotated a full circle with respect to the Bausch and Lomb lamp. Eight area fraction values were obtained from two different slices.

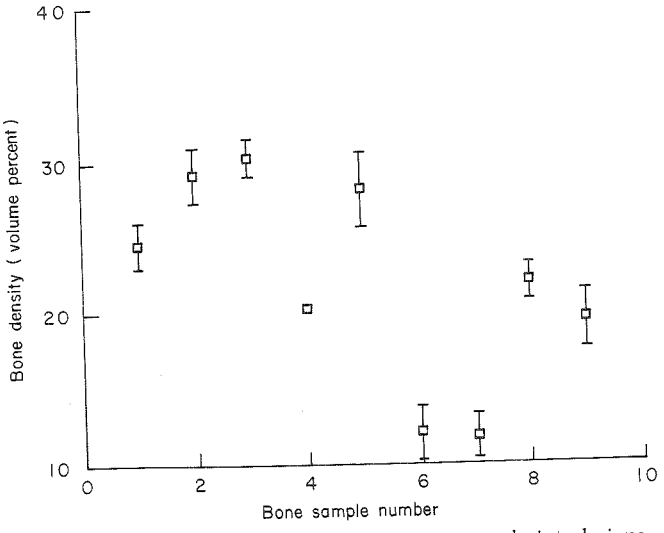

Figure 1 Reproducibility of the interactive image analysis technique. Data was obtained from nine 5×5 mm areas in the transverse plane taken from different vertebral slices. Error bars indicate the standard deviation of the values. The average standard deviation was $1.5 \pm .6$ vol.% with a coefficient of variation of $7.6 \pm 4.1\%$

RESULTS AND DISCUSSION

As shown in *Figure 1*, the reproducibility of the interactive image analysis technique (expressed as an SD) was at most ± 2 vol.%, an acceptable value for most biomechanical applications. Densities which differ by this value, for example, do not show significant differences in bone strength[1,2,3]. More than 50% of the overall reproducibility is due to the variation in the surface-discriminating ability of the operator which can be expressed as an average SD of ± 0.9 vol.% and a coefficient of variation of 3.0%.

The comparison of the means of the volume fraction values obtained by interactive image analysis and by point counting, using the paired-data t-test, demonstrated an insignificant difference between the two types of measurements. Furthermore, there is a highly significant positive correlation between both series of values ($R = 0.98$; $P < 0.005$): $Y = 2.1178 + 0.8443X$ where Y represents the area fraction of bone measured by point counting and X represents the same measurement obtained with interactive image analysis. Finally an F-test to compare the variances of the two techniques showed that interactive image analysis is significantly ($P < 0.002$) more precise than the traditional point-counting method.

The average variation due to slice orientation expressed as an average SD was ± 1.4 vol.%, a value similar to the reproducibility of the technique. Again,

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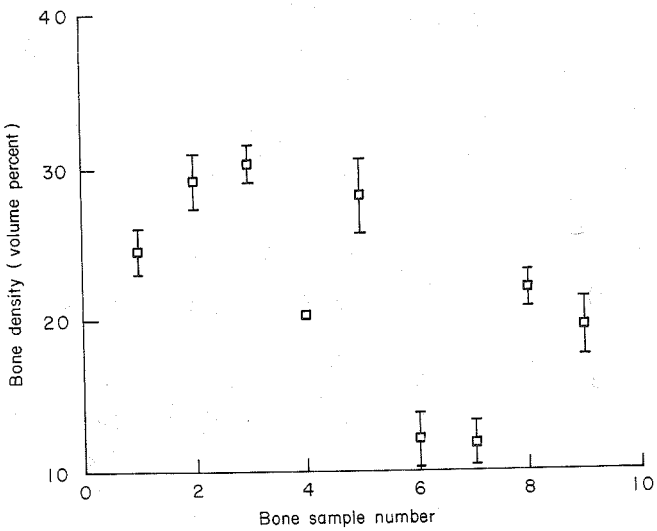

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this is not large enough to affect greatly the significance of the measurements.

The results of this investigation demonstrate that the interactive image analysis technique can be used to determine efficiently and accurately the density of cancellous bone. A study[10] of the density variations within two vertebral bodies which, with one traditional method[9], would have required at least 14 days to complete took only 3 days with the protocol described here. Finally, of primary significance for those interested in the physical properties of bone, it allows the samples to be biomechanically tested after densitometric analysis.

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