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Comments on *Neutron diffraction studies of collagen in human cancellous bone* by Skakle & Aspden (2002)

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Comments are made on a paper by Skakle & Aspden [*J. Appl. Cryst.* (2002), **35**, 506–508] regarding the lateral spacing of collagen in human bone.

Skakle & Aspden (2002) report their determination of the lateral spacing of the collagen in human bone, both compact and cancellous. They question the accuracy of previous neutron diffraction studies of mineralized tissues. One objective of their study was to improve the determination of the diffraction line peak in the presence of considerable noise.

In prior studies, it was assumed that the signal cross section is a Gaussian embedded in noise. The two components are extracted as two functions using a least-squares program. The Gaussian peak is taken to be the peak of the diffraction line. Skakle & Aspden used a computer program that does not assume a shape for the line except that it be similar to a second-order polynomial. They found the lateral spacing of wet tissue human compact bone to be 1.230 nm, and 1.191 nm when dry. Lees *et al.* (1984) reported finding the lateral spacing for cow bone, of density 2.04 Mg m^{-3}, to be 1.24 nm wet and 1.16 nm dry. Skakle & Aspden did not cite the density of their material.

Lees (2003) showed that the lateral spacing d for compact bone collagen is strongly linear with the inverse wet density,

$$d = (0.75/\rho) + 0.871,\ R^2 = 0.98,$$

where ρ is the wet tissue density. There is a different line for the dry tissue spacing,

$$d = 1.467 - (0.639/\rho),\ R^2 = 0.95.$$

The slope of the wet tissue spacing is positive and that for the dry tissue is negative. The largest deviation for either line is less than 0.02 nm. Values for the lateral spacing are listed in Table 1 for both cow and human bone densities. The values of Skakle & Aspden are entered in the last column. The entries for bone of density 2.04 and 1.80 Mg m^{-3} are experimental; the others were obtained from the above equations.

Since the lateral spacing increases with decreasing wet tissue density, and 1.23 nm is a value smaller than that of the other wet tissues in Table 1, the tissue density should be greater than 2.04 Mg m^{-3}. The same argument is employed for dry tissues. When the lateral spacing *decreases* with decreasing density, and 1.191 nm is greater than all other dry tissue terms in Table 1, the wet density again should be greater than 2.04 Mg m^{-3}. Skakle & Aspden were unable to interpret their results because they did not account for density. The difficulty here is that human compact bone is less dense than cow bone. The data-extraction protocol must be consistent since it yields the strong linear dependence on the inverse wet density with an uncertainty less than 0.02 nm

Table 1
Comparison of the lateral spacing of collagen in bone of different densities.

Sample	2.04 Mg m^{-3}	2.01 Mg m^{-3}	1.95 Mg m^{-3}	1.80 Mg m^{-3}	Skakle & Aspden (2002)
Wet spacing (nm)	1.24	1.244	1.256	1.29	1.230
Dry spacing (nm)	1.16	1.149	1.14	1.11	1.191

Lees & Hukins (1992) demonstrated the successful use of X-ray diffraction to determine the lateral spacing of collagen in cow bone of wet density 2.01 Mg m^{-3}. Six adjacent samples of the same bone were obtained. The uncertainty between samples was $\pm$0.03 nm and the uncertainty within a single pattern was $\pm$ 0.01 nm. The lateral spacing (1.22 nm) compares with the calculated value of 1.244 nm, allowing for the uncertainty. No assumptions for the shape of the diffraction line were required. It would be valuable to compare the lateral spacing for the same single specimen by the various known methods in order to evaluate the contributions to the error by the source of neutrons and by the data-extraction process. If possible, the same data should be treated by several processes.

References

Lees, S. (2003). *Biophys. J.* **85**, 204–207.
Lees, S., Bonar, L. C. & Mook, H. A. (1984). *Int. J. Biol. Macromol.* **6**, 321–326.
Lees, S. & Hukins, D. W. L. (1992). *Bone Miner.* **17**, 59–63.
Skakle, J. M. S. & Aspden, R. M. (2002). *J. Appl. Cryst.* **35**, 506–508.

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Response to Lees' comments on *Neutron diffraction studies of collagen in human cancellous bone*

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Some additional comments are made regarding neutron diffraction from bone.

Neutron diffraction has been used in a small number of studies of bone, as it enables the determination of the mean lateral spacing of collagen molecules in mineralized collagen fibres in bone. Previous studies used cortical bone and the main aim of our feasibility study (Skakle & Aspden, 2002) was to see whether usable diffraction patterns could be obtained from cancellous bone and hence provide information on changes in bone structure with disease. In this we were successful. For comparison with the previous studies mentioned above, we also recorded some patterns from a single piece of human cortical bone. We did not, however, measure the density of the bone, and our intention was not so much to obtain definitive values as to test the feasibility of obtaining usable data.

The issue that all these studies raise is how to calculate the mean lateral spacing from the diffraction pattern. We assumed that the whole signal arises from scatter by the liquid-like disorder of the collagen molecules in the equatorial plane and that the position of the peak at $Q \simeq 0.5$ Å^{-1} should therefore be measured as the maximum perpendicular to the equator. The assumption made by Lees (2004) was that the diffraction peak from the collagen comprised a Gaussian superimposed on a polynomial. In this case, the polynomial is assumed to fit a background scatter and, by subtracting this, the peak of the Gaussian is found as the maximum distance perpendicular to the polynomial. At this stage, we would not wish to be dogmatic about which is right and we believe the matter will require a proper theoretical analysis.

However, the different approaches introduce a systematic difference between the results. In Fig. 1, we show a synthetic curve which resembles the shape of the neutron scattering patterns we have recorded and those published by Lees. It may be represented by a Gaussian centred at $Q = 0.5$ Å^{-1}, with a standard deviation of 0.1 and an amplitude of 2, and a polynomial of the form $1/Q^2$. By our approach, the peak would be found at $Q = 0.476$ Å^{-1} (point *A* in Fig. 1) and the corresponding spacing could be calculated from that. Following the analysis of Lees would yield a peak at $Q = 0.5$ Å^{-1} (marked *B* on Fig. 1) and, consequently, an apparently smaller spacing. This could explain the difference in the lateral spacings calculated for dry bone, for which the value we obtain is greater than those found previously. The values for wet bone are harder to explain. In our pilot study, we used a humidity can to try to maintain the bone in a fully hydrated state. However, there were some problems with this and it may have been that the bone was actually partially dehydrated. We included the cortical bone for comparison with the cancellous bone to show that the figures obtained were of the correct order, rather than as a definitive study.

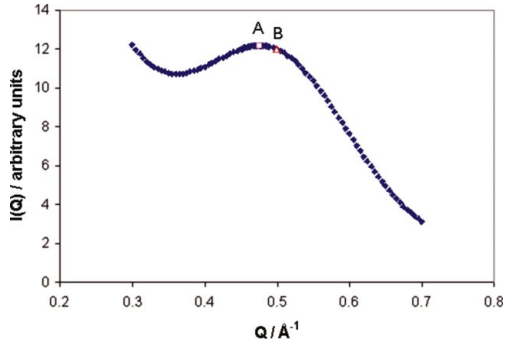

Figure 1
Model of a typical neutron scattering curve from collagen in bone. Assuming it to be a direct scattering function yields a peak at $Q = 0.476$ Å^{-1} (*A*), whereas assuming it to be a Gaussian on a polynomial background yields a peak at $Q = 0.5$ Å^{-1} (*B*).

The differences discussed here will not alter the form of the relationships derived between density and lateral spacing derived by Lees, although the method of calculation used will affect the values of the lateral spacings and hence the coefficients in those relationships. We agree that to compare X-ray data with neutron scattering could be useful, but bone slices for X-ray diffraction need to be less than about 100 µm thick, in contrast with about 1 mm for effective neutron scattering, so it is difficult to use the same sample. Hydration is clearly an important factor. A theoretical analysis is required to explore the form expected for the scattering function. We plan to carry out further studies and to explore these issues.

References

Lees, S. (2004). *J. Appl. Cryst.* **37**, 676.
Skakle, J. M. S. & Aspden, R. M. (2002). *J. Appl. Cryst.* **35**, 506–508.

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