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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.

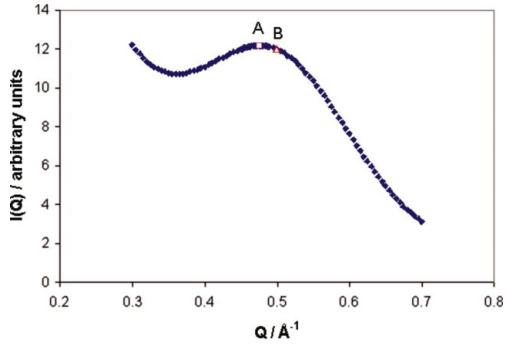

Figure 1
Model of a typical neutron scattering curve from collagen in bone. Assuming it to be a direct scattering function yields a peak at $Q = 0.476$ Å^{-1} (*A*), whereas assuming it to be a Gaussian on a polynomial background yields a peak at $Q = 0.5$ Å^{-1} (*B*).

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

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