Determination of muscle fiber orientation using diffusion-weighted MRI

European Journal of Morphology
1996, Vol. 34, No. 1, pp. 5-10

0924-3860/96/3401-0005$12.00

DETERMINATION OF MUSCLE FIBRE ORIENTATION USING DIFFUSION-WEIGHTED MRI

A. van Doorn[1], P.H.M. Bovendeerd[1], K. Nicolay[2], M.R. Drost[3,1], J.D. Janssen[1,3]

[1]Department of Mechanical Engineering, Eindhoven University of Technology, P.O.Box 513 5600 MB Eindhoven, The Netherlands
[2]Bijvoet Center for Biomolecular Research, Bolognalaan 50, 3584 CJ Utrecht, The Netherlands
[3]Department of Movement Sciences, University of Limburg, P.O.Box 616, 6200 MD Maastricht, The Netherlands

ABSTRACT

Biomechanical studies have shown that the distribution of stress and strain in biological tissue is strongly dependent on fibre orientation. Therefore, to analyze the local mechanical load, accurate data on muscle fibre orientation are needed. Traditional techniques to determine fibre orientation are inherently invasive. Here we used Diffusion Weighted MRI to non-invasively determine, in each image voxel of 0.23 × 0.23 mm, the diffusion tensor of water in the cat semimembranosus muscle. The direction corresponding to the largest eigenvector of this tensor was calculated. This direction was found to correspond qualitatively to the muscular fibre direction, as determined by visual inspection.

KEYWORDS: *fibre orientation - Diffusion Weighted MRI - mechanical anisotropy.*

INTRODUCTION

A characteristic feature of soft biological tissues with a load transmitting function is the presence of fibres, e.g. collagen, elastin, muscle fibres. In general, the mechanical properties of tissue in the fibre direction differ from those in other directions. Especially in activated muscular tissue, the degree of anisotropy is very pronounced. Biomechanical studies have shown that the distribution of stress and strain in muscular tissue depends strongly on muscle fibre orientation (Bovendeerd et al., 1992, 1994; Van Leeuwen & Spoor, 1992). Thus, to analyse the in vivo mechanical loading of muscular tissue, accurate data on muscle fibre orientation are needed.

Traditional three-dimensional anatomic or histologic reconstruction techniques to determine muscle fibre orientation have several disadvantages (e.g. McLean & Prothero, 1992). Firstly, they are invasive and therefore neither permit a series of measurements at different times or loading conditions nor a measurement in vivo. Secondly, in the preparation (fixation) phase, preceding the measurement, the specimen will change shape, causing errors in the subsequent determination of fibre orientation. Thirdly, full three-dimensional anatomic reconstruction is time consuming.

In this paper a technique is presented for determining muscle fibre orientation non-invasively, under in vivo conditions. This technique is based on the assumption that the muscle fibre direction coincides with the direction in which diffusion is least restricted. Diffusion within the tissue is measured with a special form of Magnetic Resonance Imaging (MRI). The technique is illustrated with an application to the cat semimembranosus muscle.

Correspondence to: A van Doorn, Department of Mechanical Engineering, Eindhoven University Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands

METHODS AND MATERIALS

Diffusion in biological tissue. The macroscopic phenomenon of diffusion finds its origin in the Brownian motion of molecules above zero degrees Kelvin. Suppose we would track a water molecule in a water sample. In this isotropic medium, there is no preferred direction of the molecular motion. After a time interval Δt, the probability of the new location of the molecule is a Gaussian distribution, represented by a sphere, with the original position as origin and characteristic radius Δx. The relation between Δt and Δx of the molecule is given by the Einstein equation:

$$\Delta x = \sqrt{2D\Delta t} \tag{1}$$

where D is the diffusion coefficient.

In biological tissue Brownian motion of water is restricted by two major mechanisms (Hazlewood et al. 1991). Firstly, water molecules encounter barriers or obstructions during the time interval of the measurement. Barriers can be formed by permeable or impermeable cell membranes and subcellular compartments. Obstructions can be formed by macromolecules and subcellular organelles. Secondly, water molecules are 'bound' to (macro) molecules and subcellular surfaces.

Especially in brain and muscular tissue, the motion of water molecules is expected to be restricted more severely in directions perpendicular to the fibre direction than in the direction parallel to the fibre. This view is based on experimental findings (e.g. Cleveland et al., 1976; MacFall et al., 1991). This anisotropy is probably caused by the elongated shape of the cells, the structured organisation of extended protein networks and capillaries which parallel the fibre direction.

In contrast to the isotropic situation, discussed above, the most probable locations of a molecule after a time interval Δt must now be represented by an ellipsoid. This ellipsoid has three principal axes. The major axis corresponds to the direction in which the motion of water molecules is least restricted.

Mathematically, diffusion can no longer be described by a single diffusion coefficient, but must be represented by a diffusion tensor $\boldsymbol{D}$. Eq.(1) must be replaced by the Einstein relation for three-dimensional situations:

$$\frac{(\vec{\Delta x}).\, \boldsymbol{D}^{-1}.\, (\vec{\Delta x})}{2\Delta t} = 1 \tag{2}$$

where $\vec{\Delta x}$ represents the characteristic displacement vector of the particle in a time interval Δt. The diffusion tensor is symmetric and possesses three real eigenvalues with three corresponding real eigenvectors. The eigenvector associated with the largest eigenvalue, corresponds to the direction in which diffusive motion is largest.

With respect to an arbitrary coordinate system, the symmetric diffusion tensor may be represented by a symmetric 3×3 matrix. In this study the six unknown components of this diffusion matrix were determined using Diffusion Weighted (DW) MRI. The eigenvalues and corresponding eigenvectors of this matrix were calculated. The direction of the eigenvector corresponding to the largest eigenvalue is assumed to be the direction of the muscle fibres.

Diffusion Weighted MRI. MRI is a non-invasive technique that produces computerized images of internal body tissue and is frequently used in clinical diagnosis. MRI is based on the Nuclear Magnetic Resonance (NMR) property of hydrogen protons within the body. In its basic application, image contrast is determined largely by the spatial variations in two specific MRI parameters, spin-spin (T_2) and spin-lattice (T_1) relaxation times, and to a lesser extent by variations in proton density. Influences of the T_1 and T_2 relaxation times on the NMR signal are described by the Bloch equations (Bloch 1946). In recent years water diffusion has become a well known additional MRI contrast parameter, especially in brain studies (e.g. Verheul et al. 1994). Torrey (1956) has extended the Bloch equations to describe the influence of isotropic diffusion on the NMR signal. Stejskal (1965) included anisotropic, restricted diffusion. Based on these equations, Basser et al. (1992, 1994) have derived an equation relating all terms of the diffusion tensor to the NMR signal intensity. With this equation they estimated the 'effective' diffusion tensor $\boldsymbol{D}^e$ for each image voxel. The suffix 'e' indicates that the diffusion tensor represents a mean diffusion matrix measured over a time interval in the order of 20 ms. To estimate all six coefficients of the diffusion tensor it is necessary to acquire a series of images, which are diffusion weighted in at least six

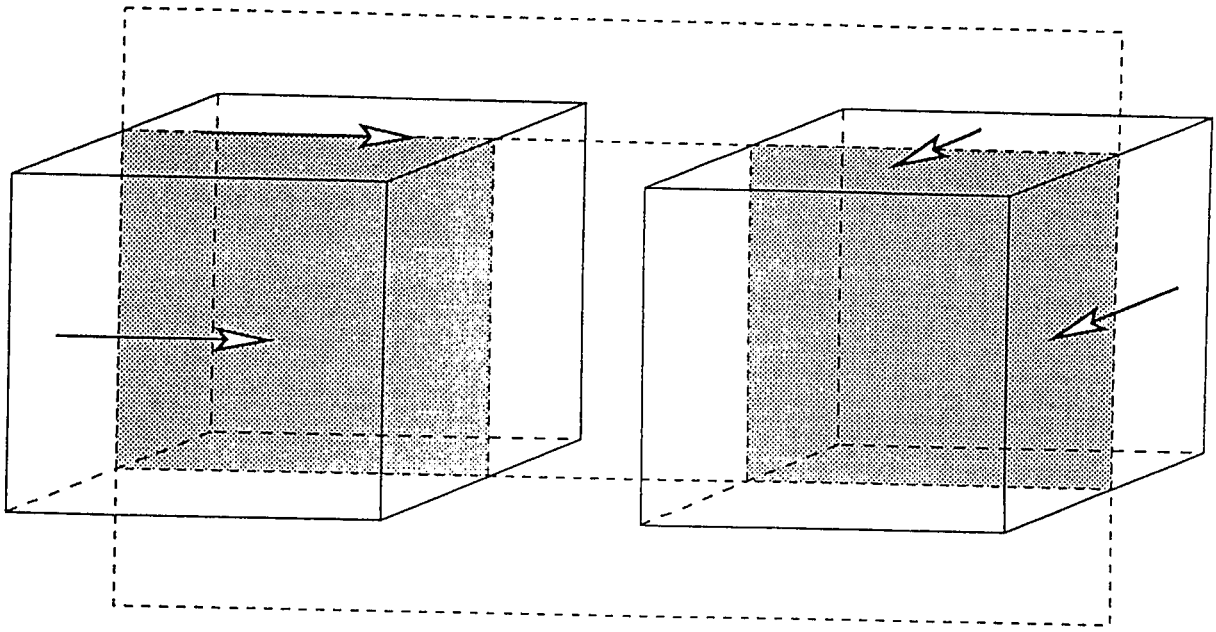

Fig. 1. THE ARROWS INDICATE THE PREDOMINANT DIRECTIONS OF THE FIBRES ON THE OUTER SURFACE OF THE BLOCKS AS DETERMINED BY VISUAL INSPECTION. MRI images were obtained in the marked plane.

different directions (Basser et al. 1992). In each direction several images with increasing diffusion weighting have to be measured.

Material and experiment protocol. The semimembranosus muscle was removed from a cat, which had been perfusion fixated with buffered formaldehyde (pH 7.2). The muscle was cut into blocks of about 8 × 8 × 8 mm. Fibre orientation at the outer walls was determined by visual inspection. Two blocks were glued on a perspex strip, with muscle fibres orientated approximately perpendicularly, as indicated in Fig. 1.

The two pieces were preserved in a container filled with 5% buffered formaldehyde (pH 7.2). Before the MRI experiment, most of the formaldehyde was removed. To prevent desiccation of the tissues some formaldehyde was left and the container was closed. The blocks were placed in the MR-apparatus.

Diffusion weighted images were obtained with the MR settings, described in appendix A. Within a field of view of 30 × 30 mm, 128 × 128 voxels were created. This yields a voxel size of 0.23 × 0.23 × 1 mm, since the slice thickness was 1 mm. Total duration of the experiment was approximately three hours. The diffusion tensor was calculated from the NMR signals for each voxel. The calculation procedure was based on the technique described by Basser (1992, 1994). To get an impression of the material also traditional 'high resolution' MRI scans were made, essentially as above, except that diffusion weighting was omitted and that an echo time of 15 ms was used.

RESULTS

Using the DW-MRI technique, diffusion tensor were measured in the formaldehyde fixated cat semimembranosus muscle in voxels of 0.23 × 0.23 × 1 mm. Characteristic largest and smallest eigenvalues of the tensors were found to be $1.37 \times 10^{-3} \pm 0.31 \times 10^{-3}$ mm^2/s (mean ± SD) and $0.93 \times 10^{-3} \pm 0.31 \times 10^{-3}$ mm^2/s respectively. In most image voxels the ratio of the largest to the smallest eigenvalue was in between 1.3 and 1.8. Only at the borders of the material and at places with loose connective tissue (see arrows fig. 2) the ratios were not within this range. A ratio between 1 and 1.3, indicating nearly isotropic diffusion within the voxel, was considered too small to yield a well determined direction of least restricted diffusion, in view of the noise in the MR-signal. Ratios of the eigenvalues of more than 1.8 were considered unrealistic. For the voxels with a ratio in between 1.3 and 1.8, the eigenvectors corresponding to the largest eigenvalues were plotted on top of the high resolution image as shown in fig. 2 and separately in Fig. 3.

Fig. 2. A HIGH RESOLUTION MR IMAGE THROUGH THE TWO MUSCULAR PIECES OF THE SEMIMEMBRANOSUS MUSCLE (Fig. 1). A projection of the eigenvectors corresponding to the largest eigenvalues, as determined by diffusion tensor imaging, is depicted on top of this image. The coordinate system corresponds to the coordinate system of the magnet. In the block on the left the muscle fibres are almost in the zx-plane. The muscle fibres in the block on the right are approximately aligned to the y direction. The arrows point to areas with loose connective tissue. The region with bright signal intensity in the middle, bottom part of the MR image is due to remaining fixation buffer.

The blocks in Fig. 2 correspond to those in Fig. 1. Due to the homogeneous muscular material there is little contrast in the high resolution image. Size of the voxels of the high resolution scan is approximately $0.08 \times 0.08 \pm 1$ mm. This is too large to discriminate individual fibres (40 μm). Between the two muscular tissue blocks, at the bottom, is a bright area due to remaining fixation buffer.

The eigenvectors were given a unit length in 3-D space. In the pictures (Fig. 2,3) a projection of these eigenvectors on the zx-plane is shown. In the left muscular tissue block, line lengths are close to the unit length, indicating that the eigenvectors have only small y-components. The directions of the eigenvectors form a regular pattern, except at the borders of the tissue. In the block on the right, the small lengths of the lines, suggest that the largest principal diffusion direction lies in the y direction. These results are in agreement with the fibre directions as observed visually.

DISCUSSION

The range of eigenvalues found in the experiment corresponds with values reported in literature. Cleveland et al. (1976) measured in the tibialis anterior of mature male rats, without chemical fixation, diffusion coefficients of 1.39×10^{-3} and 1.0×10^{-3} mm^2/s for directions parallel and perpendicular to the fibres.

Determination of the muscle fibre direction using the DW-MRI technique is based on the assumption that the major principal direction of the diffusion tensor equals the direction of the mean muscle fibre direction within a voxel. Indeed, as shown in the results, the largest principal directions of the block on the left and on the right are perpendicular to each other. These directions qualitatively correspond to the predominant muscle fibre direction as determined by visual inspection at the outer surfaces. At the border of the tissues the direction of several vectors

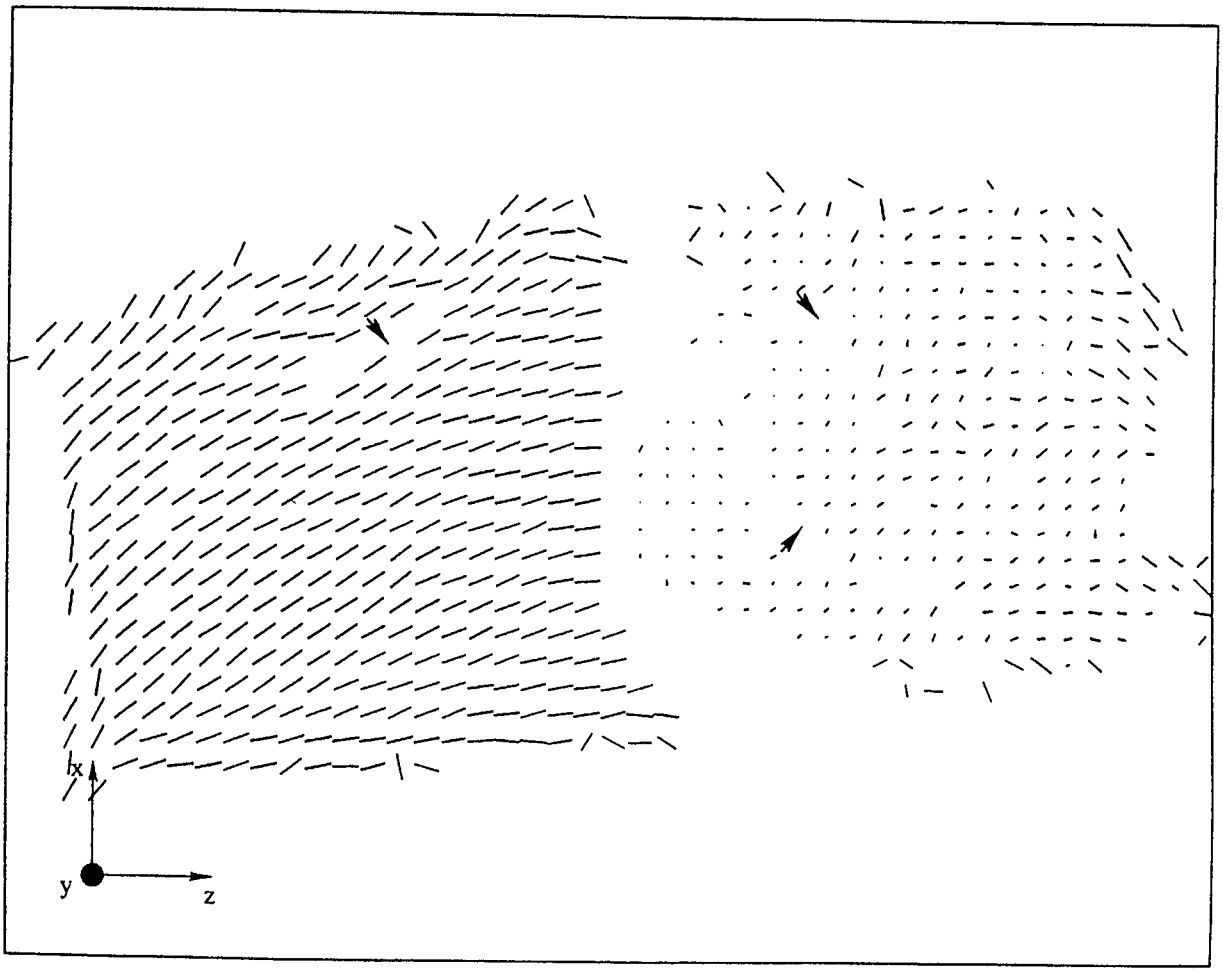

Fig. 3. PROJECTIONS OF THE UNIT LENGTH EIGENVECTORS, CORRESPONDING TO THE LARGEST EIGENVALUES ON THE *ZX*-PLANE ARE PLOTTED. The length of the eigenvalues in the block on the left is close to the unit length, suggesting only small components perpendicular to the *zx*-plane. On the other hand, the length of the vectors in the block on the right suggest that the largest principal diffusion is in the *y* direction. In the regions marked by the arrows, the ratio of the largest to the smallest eigenvalues was not within the required range. The coordinate system corresponds to the coordinate system of magnet.

deviates from the global pattern. Deviations are attributed to cutting artefacts and low signal intensity from partially filled voxels. The latter phenomenon probably also caused the unrealistic ratios of the eigenvalues of the diffusion tensor in some voxels near the border. Also a quantitative comparison of principal directions of diffusion and fibre directions is currently attempted. To test the assumption, the fibre orientation is determined by a fully three-dimensional anatomic reconstruction. It remains to be established whether the accuracy of the fibre direction determined in this manner is sufficient to validate the assumption.

Pilot experiments have shown that the described method can also be applied in vivo (van Doorn & Nicolay, unpublished observations). To prevent artefacts due to motion or anaesthesia, measurement times should be kept as short as possible. The used gradient and imaging sequence is robust, but slow. Therefore, other pulse and gradient sequences might be considered, which enable much shorter measurement times (e.g. Ordidge et al., 1994; Becker et al., 1994). These methods can generate a single DW image in a total experimental time of a few seconds or even a fraction of a second. However, the accuracy of the diffusion tensor estimation may be compromised.

CONCLUSION

Using DW-MRI, diffusion tensors were determined within formaldehyde fixated semimembranosus muscle tissue. Diffusion in muscular tissue was found to

behave anisotropically. The largest principal direction of the diffusion tensor qualitatively corresponds to the muscular fibre direction determined by visual inspection. A comparison of the MRI findings with muscle fibre direction, as determined with traditional anatomic reconstruction techniques, will enable the assessment of quantitative relationship between water diffusion and tissue fibre anisotropy. MRI is a non-invasive imaging method. The DW-MRI experiment can therefore be applied to the intact tissue under in vivo conditions.

ACKNOWLEDGEMENTS

The NMR part of this work was carried out at the Netherlands in vivo NMR facility (Bijvoet Center, Utrecht University), which is supported by the Netherlands Organization for Scientific Research (NWO).

REFERENCES

BLOCH F. (1946) Nuclear induction. Phys. Rev 70 (7 & 8): 460-474

CLEVELAND GG, CHANG DC, HAZLEWOOD CF, RORSCHACH HE (1976) Nuclear magnetic resonance measurements of skeletal muscle; Anisotropy of the diffusion coefficient. Biophysical Journal 16, 1043-1053

BASSER PF, LEBIHAN D, MATTIELLO J (1992) Diagonal and off-diagonal components of the self-diffusion tensor: their relation to and estimation from the NMR spin-echo signal. Book of abstracts SMRM 1992, 1222

BASSER PF, LEBIHAN D, MATTIELLO J (1994) MR Diffusion Tensor Spectroscopy and Imaging. Biophysical Journal 66, 259-267

BECKER CR, LORENZ WJ, SCHAD LR (1994) Measurement of diffusion coefficient using a quick echo split NMR imaging technique. Magnetic Resonance Imaging 12:8, 1167-1174

BOVENDEERD PHM, ARTS T, HUYGHE JM, RENEMAN RS, VAN CAMPEN DH (1992) Dependence of local left ventricular wall mechanics on myocardial fiber orientation: a model study. J Biomech 25, 1129-1140

BOVENDEERD PHM, ARTS T, HUYGHE JM, RENEMAN RS, VAN CAMPEN DH (1994) Influences of endo-epicardial crossover of muscle fibers on left ventricular wall mechanics. J Biomech 25, 1129-1140

HAZLEWOOD CF, LIN C, RORSCHACH HE (1991) Diffusion of Water in Tissue and MRI. Magnetic Resonance in Medicine 19, 214-216

VAN LEEUWEN JL, SPOOR CW (1992) Modelling mechanically stable muscle architectures. Phil. Trans. R. Soc. Lond. B 336, 275-292

MACFALL JR, COFER GP, HEDLUND LW, JOHNSON GA, MAKI JH (1991) Pre- and Postmortem Diffusion Coefficients in Rat Neural and Muscle Tissues. Magnetic Resonance in Medicine 20, 89-99

MACLEAN M, PROTHERO J (1992) Determination of Relative Fiber Orientation in Heart Muscle: Methodological Problems The Anatomical Record 232, 459-465

ORDIDGE RJ, KNIGHT RA, HELPERN JA, HUGG JW (1994) A low flip angle spin-echo technique for producing rapid diffusion weighted MR images. Magnetic Resonance Imaging 12:5, 727-731

STEJSKAL EO (1965) Use of spin echoes in a pulsed magnetic-field gradient to study anisotropic, restricted diffusion and flow. J Chem. Phys. 43 (10) 3597-3603

TORREY HC (1956) Bloch equations with diffusion terms. Phys. Rev. 104 (3): 563-565

VERHEUL HB, BALÁZS R, BERKELBACH VAN DER SPENKEL JW, NICOLAY K, TAMMINGA KS, TULLEKEN CAF, VAN LOOKEREN CAMPAGNE M (1994) Comparison of Diffusion-Weighted MRI with Changes in Cell Volume in a Rat Model of Brain Injury. NMR in Biomedicine 7, 96-100

APPENDIX A

Images were obtained with a 4.7-T SISCO 200/400 system operating at 200 MHz for protons and equipped with Oxford gradients (maximal gradient strength of 110 mT/m). Diffusion of water was measured by incorporating the pulsed gradients of Stejskal and Tanner (1956) into a spin-echo imaging sequence. Pulsed gradients for diffusion weighting were placed symmetrically around the 180° refocussing pulse and could be oriented in any desired direction. The read-out gradient rephasing lobe was placed directly before the echo acquisition in order to prevent cross coupling with the diffusion gradients. Gradient strength was increased from 0 to 103 mT/m in six steps (0, 26, 52, 69, 86, 103 mT/m) to measure the influences of the gradient strength on the spin echo signal intensity. For an echo time (TE) of 40 ms the diffusion gradients had a duration of $\delta = 10$ ms and a separation of $\Delta = 20$ ms. Repetition time (TR) was 2 seconds. Within a field of view of 30 × 30 mm, images were obtained containing 128 × 128 voxels. The slice thickness was 1 mm. Measurements were performed with directions of diffusion weighting in x, y, z, xy, xz and yz direction, respectively.

Received: June 1995
Accepted after revision: August 8, 1995