Changes in diffusion tensor imaging (DTI) eigenvalues of skeletal muscle due to hybrid exercise training

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Target Audience This information will be beneficial for musculoskeletal biologist, hepatologist, and endocrinologist.

Purpose A number of recent studies have used diffusion tensor imaging (DTI) to study skeletal muscle anatomy at the microscopic level, exploiting the eigenvalues calculated by DTI as indices of intracellular water diffusion within the myocyte. The current consensus is that the sarcocemena represents the main restriction to water diffusion in skeletal muscle, with \( \lambda_1 \) reflecting mainly diffusion parallel to the long axis of this, while \( \lambda_2 \) and \( \lambda_3 \) represent diffusion orthogonal to \( \lambda_1 \). By contrast, in a comparison of calf muscle in young female athlete and non-athlete groups, we had predicted that all eigenvalues would be higher in the athletes, assuming their larger muscle cross-sectional area would reflect a larger and less diffusion-restricted intracellular space. However, we found the opposite: all eigenvalues were lower in the athlete group. This points to the possible role of other intracellular components in restricting water diffusion, such as mitochondria, the sarcoplasmic reticulum, macromolecules, and myofilament lattices. We had hypothesized that increased amounts of such intracellular components might be responsible for the lower eigenvalues we observed in the athlete group. The idea that an increase in contractile protein density due to muscle training might be a major factor in restricting water diffusion led us to examine prospectively the timecourse of diffusion changes in response to a training programme.

In our hospital, non-alcoholic fatty liver disease (NAFLD) patients had been participating in a training program designed to improve their metabolic condition using the so-called "hybrid training" (HYBT) developed in the University of Kurume, Japan. We took this opportunity to study changes of muscle DTI eigenvalues due to training, and to compare the findings with previous comparisons between athlete and non-athlete groups.

Methods Patients. Twenty-one NAFLD patients aged 24–61 years averaging 50.2 years were recruited. All were diagnosed by an internal medicine specialist using standard criteria for NAFLD. Written informed consent was obtained from all of the volunteers, and the study had approval by the institutional medical ethics board. Patients underwent HYBT for 30 minutes per day, twice a week, for six months.

Assessment of training effects. Measurements were undertaken at baseline and after 6 months HYBT, and compared by paired t-test. Blood tests included liver function tests (AST, ALT, and albumin), lipids (triglyceride and free fatty acid) and markers of liver fibrosis (hyaluronan and type 4 collagen). Thigh muscle hypertrophy was assessed using anatomical T1-FFE to measure the maximal dimension area (DA) of each thigh muscle, including biceps femoris (BF), intermediate vastus muscle (IV), lateral vastus muscle (LV), and rectus femoris muscle (RF). We measured on this thickest slice.

Hybrid training. As previously described patients performed HYBT in a sitting position without feet touching the floor. Low impedance gel-coated electrodes were placed on the motor points of the bilateral VM and VL of the anterior thigh; for the posterior thigh, electrodes were placed on the motor points of the medial and lateral hamstrings; hamstrings are electrostimulated when the knee was voluntarily extended, while quadriceps are electrically stimulated when the knee was voluntarily flexed.

MR data acquisition and DTI data analysis. We scanned both thigh muscles simultaneously using a 3.0 Tesla clinical MR machine (Nova Dual release 2.6, Philips, Best, the Netherlands). The 16-channel sensitivity encoding (SENSE) body coil (45×30 cm for parallel imaging) was wrapped around the anterior and posterior aspects of both thighs. Diffusion tensor imaging images were acquired using a single-shot echo planar imaging (EPI) sequence with the following parameters: b values of 0 and 500 s/mm², field of view (FOV) 380 (mm), rectangular FOV 100%, matrix size 256 × 256, slice thickness 6 mm without gap, interval number of slices 12 (7.2 cm of the length of scan range), TR = 4,000 ms, TE = 67 ms, SENSE factor 2, number of motion probing gradient (MPG) directions 6, number of excitations 6, and total scan time is 16 ms. The center of the scan range was positioned at the thickest part of the thigh, generating the coronal scout image in each volunteer. We also acquired T1-FFE images for anatomical mapping using the following parameters: matrix size 256 × 184, slice thickness 6 mm, number of slices 12, TR = 13 ms, TE = 2.3 ms, SENSE factor 1.4, and total scan time 2 min 58 sec. Using the thickest slice, we measured FA, \( \lambda_1 \), \( \lambda_2 \), and \( \lambda_3 \) bilaterally for BF, MV, IV, LV, and RF muscles.

Data were analyzed by free software developed by the University of Tokyo (dTV).

Results & Discussion

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<thead>
<tr>
<th>Table 1</th>
<th>Table 2</th>
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<tr>
<td>MV</td>
<td>BF</td>
<td>LV</td>
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<tr>
<td>DA Pre Post</td>
<td>DA Pre Post</td>
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<td>Pre</td>
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<td>Pre</td>
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<tr>
<td>FA</td>
<td>0.32(0.05)</td>
<td>0.31(0.06)**</td>
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<tr>
<td>( \lambda_1 )</td>
<td>2.10(0.07)</td>
<td>2.10(0.07)**</td>
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<tr>
<td>( \lambda_2 )</td>
<td>1.47(0.07)</td>
<td>1.52(0.07)**</td>
</tr>
<tr>
<td>( \lambda_3 )</td>
<td>1.08(0.07)</td>
<td>1.10(0.07)**</td>
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There was no statistically significant pre-post difference in the patients weight (mean 69.1 pre- vs 68.2 kg post-HYBT) or BMI (28.1 vs 27.6 kg/m²), in liver function tests (AST 46 vs 44 and ALT 57 vs 57 U/L, albumin 43 vs 44 g/l), in blood triglyceride (118 vs 113 mg/dl), or in circulating markers of liver fibrosis (hyaluronan 73 vs 66 mg/ml, Type 4 collagen 158 vs 150 mg/l). There was a statistically significant decrease in circulating free fatty acids (0.87±0.34 vs 0.73±0.21 mEq/l (mean±SD); P<0.05). Mean values of FA, the three eigenvalues, and DA of pre- and post-HYBT of BF, MV, IV, and RF are shown in Tables 1-5 (*P<0.05, **P<0.01). The overall picture is of increase in the eigenvalues and a decrease in FA post-HYBT, with some detail which will be discussed below. DA increased significantly in all muscles, as in previous reports, the percentage increases of DA in mean (right and left) BF, MV, IV, LV, and RF were 2.5%, 5.5%, 5.9%, 4.1%, and 5.6%, respectively. When the eigenvalues increase in parallel with DA, as here, the most straightforward hypothesis is that the changes in diffusion properties are due to skeletal muscle hypertrophy. However, these results are apparently inconsistent with our previous (cross-sectional) findings of higher eigenvalues in a trained athlete group compared to untrained controls. It is well-known that myofilaments increase in size and number as a result of muscle training. It might be the HYBT in the present study was of insufficient intensity to increase myofilament number enough to affect this major water diffusion restricting factor. Our previous study showed big differences in maximal DA of the calf (23% larger in the athlete group than in the non-athlete group), much larger than the effects produced by training in the present study (from 2.3% to 6.6%). There might be some threshold effect between these two.

One might speculate about another reason for the increasing eigenvalues. Our subjects had NAFLD, and although this HYBT protocol produced no recovery of liver function and markers of liver fibrosis by blood test, circulating free fatty acids did decreased post-HYBT. Fatty acids are metabolized mainly in mitochondria of the liver and the skeletal muscle. Although we did not measure this, it is tempting to suggest that intramyocellular lipid (IMCL) might have been decreased by HYBT. Although this has not been directly verified, it is conceivable that these hydrophobic droplets contribute to water diffusion restriction, and that a training-induced decrease in IMCL might contribute to increasing eigenvalues.

Conclusion Diffusion properties of thigh muscle in NAFLD patients showed an overall increase in response to hybrid training. This could be due to skeletal muscle hypertrophy, as suggested in previous reports, but if so the effect is not apparently overridden by an increase in intracellular components like myofilament lattices such as may be seen in more intensive strength-training programs.

References
7) Biophys J 2000;79:2084–2094
12) J Gastroenterol Hepatol 2006;21:77-85
13) J Gastroenterol Hepatol 2007;22:775-777