Proprioceptive neuromuscular facilitation training induced alterations in muscle fibre type and cross sectional area

N Kofotolis, I S Vrabas, E Vamvakoudis, A Papanikolaou, K Mandroukas

Methods: Twenty four male university students were divided into two equal groups: PNF training and isokinetic training (ISO). The training regimen for the PNF group consisted of three sets of 30 repetitions against maximal resistance, alternating two patterns of sequential movements of the right lower extremity: (a) toe flexion and ankle plantar flexion and eversion; (b) knee extension and hip extension, abduction, and internal rotation. The ISO group performed three sets of 30 repetitions alternating knee extension and flexion of the right leg at angular velocities of 180 and 90°/s in an isokinetic dynamometer (Cybex). Both groups trained three times a week for a total of eight weeks. Muscle biopsy specimens were obtained from the right vastus lateralis muscle before and after training.

Results: The mean percentage area of type IIB fibre was significantly decreased (p<0.01) after eight weeks of PNF training, whereas that of type IIA fibre was significantly (p<0.05) increased. The mean percentage area of ISO trained type IIAB fibres exhibited an augmentative pattern (p<0.01) with a parallel reduction (p<0.05) in type IIA. Percentage fibre type distribution exhibited a similar pattern.

Conclusions: Both PNF and ISO training alter fibre type distribution and mean cross sectional area. These changes occur in the type II fibre subgroup.

METHODS
Subjects
Twenty four male university students were informed of the test procedure, purpose, and known risks before giving their informed consent to participate. The subjects were divided into two equal groups: (a) PNF training (mean (SEM) age 22.2 (1.3) years, height 178.2 (5.3) cm, weight 74.5 (9.2) kg); (b) isokinetic training (ISO; age 20.5 (1.7) years, height 178.9 (6.7) cm, weight 73.3 (9.5) kg).

PNF training
The training regimen for the PNF group consisted of three sets of 30 repetitions against maximal resistance, alternating two patterns of sequential movements of the right lower extremity: (a) flexion-adduction-external rotation with knee extension; (b) extension-abduction-internal rotation with knee flexion. The first pattern consisted of the following movement components: toes flex and adduct (lateral toes more than medial) toward fibular side; foot and ankle perform plantar flexion with eversion; knee flexes with tibia internally rotating on femur; hip extends, abducts, and internally rotates. The second pattern consisted of the following movement components: toes extend and adduct (medial toes more than lateral) toward fibular side; foot and ankle dorsiflex with inversion; knee extends with tibia externally rotating on femur; hip extends, adducts, and externally rotates. Rest intervals of 30 and 60 seconds were allowed after the completion of 10 repetitions for each pattern and between sets respectively.

Isokinetic training
The ISO group followed a similar pattern of training, performing three sets of 30 repetitions alternating knee extension and flexion at angular velocities of 180 and 90°/s.

Abbreviations: MHC, myosin heavy chain; PNF, proprioceptive neuromuscular facilitation
extension and flexion of the right leg at angular velocities of 180 and 90°/s in a speed controlled isokinetic dynamometer (Cybex 6000; Lumex Inc, Ronkonkoma, New York, USA). The same rest intervals as described above were allowed. Both groups trained three times a week for a total of eight weeks.

Muscle biopsies
Needle biopsy specimens (100–150 mg) were taken by suction from the middle portion of the vastus lateralis from the subjects’ right leg.7 The muscle specimens were trimmed, mounted, and frozen in isopentane, which was cooled with nitrogen at −80°C, and analysed histochemically.

Histochemistry
Serial transverse sections (10 μm) were cut in a cryotome at −20°C. The sections were mounted on coverslips and stained for myofibrillar ATPase after preincubation at pH 4.3, 4.6, and 10.4 for the classification of fibre type distribution. Fibres that were stable at pH 10.4 and 4.6 but labile at pH 4.3 were classified as type IIB (fast twitch glycolytic fibres) or type IIA. About 400 fibres were classified in each sample. Fibre areas from the ATPase stained sections were analysed. Fibres were counted and classified in each sample. Fibre areas from the ATPase IIAB, depending on their staining intensities at pH 4.6 (type IIC) or type IIAB, depending on their staining intensities at pH 4.3 (type IIIB or type IIC, respectively) were classified as type IIB (fast twitch glycolytic fibres) or type IIA. Percentage fibre type distribution after eight weeks of PNF training with a concomitant decrease in type IIB fibres in the PNF trained group had significantly (p<0.01) decreased, and that of type IIA fibres in the ISO trained group had significantly (p<0.01) increased (table 1). The mean percentage area of ISO trained type IAB fibre exhibited an augmentative pattern (p<0.01) with a parallel reduction (p<0.05) in type IIA.

Results
The mean percentage area of type IIAB fibre was significantly (p<0.01) decreased after eight weeks of PNF training, whereas that of type IIA fibre was significantly (p<0.05) increased (table 1). The mean percentage area of ISO trained type IAB fibre exhibited an augmentative pattern (p<0.01) with a parallel reduction (p<0.05) in type IIA.

Percentage fibre type distribution exhibited a similar pattern, with a significant (p<0.05) decrease in type IIB after eight weeks of PNF training. Percentage fibre type distribution after ISO training exhibited an augmentative pattern (p<0.01) in type IIB with a parallel significant (p<0.05) reduction in type IIA (table 2).

The cross sectional area of type IIB fibres in the PNF trained group had significantly (p<0.01) decreased, and that of type IIA fibres in the ISO trained group had significantly (p<0.01) increased (table 3).

Discussion
Overview of principle findings
This study compared the effects of PNF and isokinetic training on the fibre type distribution and cross sectional area of vastus lateralis muscle. We hypothesised that PNF, like isokinetic training, would affect muscle fibre type predominance and mean area, and that these changes would occur in the type II fibre subgroup. The mean percentage area of type IIB fibre was significantly decreased after eight weeks of PNF training with a concomitant increase in type IIA. Percentage fibre type distribution exhibited a similar pattern. Isokinetic training induced a significant increase in type IIB fibre type distribution with a concomitant decrease in type IIA. Hence these data clearly indicate that PNF training alters fibre type distribution and mean area and that these changes occur in the type II fibre subgroup and follow a unidirectional pattern of transformation (fast to slow), partially supporting our initial hypothesis. In contrast, isokinetic training induced alterations seem to follow the opposite directional pattern from type IIA towards type IIB. A detailed discussion of these and related issues follows.

Fibre type distribution and mean area
Studies of transformation of fibre types have suggested that the response of human skeletal muscle to intensive endurance training is a transformation from histochemical type II fibres to type I fibres.9 10 Other studies have also suggested that strength training induces a histochemical fibre type change from type IIB to type IIA.7 11 12 These transformations which occur with different kinds of training or detraining inflicted on the human skeletal muscle therefore seem to follow a unidirectional pattern from fast to slow (IIB→IIA→I) or vice versa.10 13 14 Our findings support the unidirectional pattern of transformation occurring in the type

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**Table 1** Percentage muscle fibre area before and after training

<table>
<thead>
<tr>
<th>Training</th>
<th>Fibre type</th>
<th>Before</th>
<th>After</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNF</td>
<td>I</td>
<td>44.49 (8.9)</td>
<td>40.12 (8.5)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>39.99 (8.7)</td>
<td>46.34 (9.5)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>5.67 (4.0)</td>
<td>8.25 (7.4)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>8.85 (7.9)</td>
<td>5.08 (19.9)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>IIAB</td>
<td>2.36 (4.0)</td>
<td>5.11 (7.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Isokinetic</td>
<td>I</td>
<td>37.26 (10.8)</td>
<td>37.46 (9.3)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>46.61 (9.1)</td>
<td>37.06 (12.6)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>5.10 (6.7)</td>
<td>17.24 (7.1)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>10.28 (9.5)</td>
<td>6.56 (8.4)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IIAB</td>
<td>1.83 (1.5)</td>
<td>1.93 (3.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD).

**Table 2** Fibre type distribution (%) before and after training

<table>
<thead>
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<th>Training</th>
<th>Fibre type</th>
<th>Before</th>
<th>After</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNF</td>
<td>I</td>
<td>47.50 (10.5)</td>
<td>43.88 (10.1)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>36.18 (8.8)</td>
<td>42.31 (10.8)</td>
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</tr>
<tr>
<td></td>
<td>IIA</td>
<td>5.81 (4.2)</td>
<td>8.97 (7.6)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>9.68 (9.6)</td>
<td>5.67 (2.2)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>IIAB</td>
<td>2.12 (3.5)</td>
<td>4.62 (6.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Isokinetic</td>
<td>I</td>
<td>40.03 (10.3)</td>
<td>39.69 (10.2)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>42.43 (9.9)</td>
<td>32.87 (11.5)</td>
<td>0.040</td>
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<td></td>
<td>IIA</td>
<td>5.40 (7.9)</td>
<td>17.92 (8.7)</td>
<td>0.003</td>
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<tr>
<td></td>
<td>IIB</td>
<td>10.76 (9.4)</td>
<td>7.85 (10.3)</td>
<td>NS</td>
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<tr>
<td></td>
<td>IIAB</td>
<td>1.87 (1.5)</td>
<td>1.99 (3.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD).

**Table 3** Cross sectional area (μm2) of fibres before and after training

<table>
<thead>
<tr>
<th>Training</th>
<th>Fibre type</th>
<th>Before</th>
<th>After</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNF</td>
<td>I</td>
<td>4728.0 (1298)</td>
<td>4829.0 (1038)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5503.0 (1110)</td>
<td>5828.0 (1251)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>3541.0 (2364)</td>
<td>2951.7 (2582)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>3406.8 (2287)</td>
<td>1106.5 (2492)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>IIAB</td>
<td>5341.0 (2364)</td>
<td>5406.4 (3384)</td>
<td>NS</td>
</tr>
<tr>
<td>Isokinetic</td>
<td>I</td>
<td>4204.4 (727)</td>
<td>4670.1 (1014)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5118.6 (937)</td>
<td>5647.2 (1451)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>2052.3 (2223)</td>
<td>4695.4 (1594)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>IIB</td>
<td>3373.4 (2386)</td>
<td>2389.7 (2709)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IIAB</td>
<td>3685.8 (2212)</td>
<td>2754.5 (2617)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD).
II fibre subgroup, as eight weeks of PNF training caused a significant decrease in type IIB fibre type distribution and eight weeks of isokinetic training caused a reduction in type IIA. The differential response of the muscle to the two different types of stimulus imposed in this study is not well understood.

One possible explanation may lay in the fact that the biopsy specimens in our study were taken from the vastus lateralis muscle, on which the two different kinds of training probably imposed different loads. It is well known that PNF patterns have a spiral, diagonal direction in line with the topographical arrangement of the muscles and are very similar to the actions and movements found in various sports, facilitating the activation of biarticular muscles. The subjects in our PNF group were trained against maximal resistance, alternating two patterns of sequential movements of the right lower extremity: (a) toe flexion and ankle plantar flexion and evasion; (b) knee extension and hip extension, abduction, and internal rotation. It is logical therefore to reason that these movements recruited a lot of different synergistic muscles and imposed a different load on the quadriceps and hamstring muscles from that imposed by knee extension and flexion respectively, performed in a speed controlled isokinetic dynamometer, with stabilisation straps at the trunk, thigh, and tibia to prevent extraneous joint movement. The higher intensity imposed on the vastus lateralis muscle during isokinetic training compared with PNF training may therefore explain the opposite directional pattern of transformation from type IIA towards type IIB fibres evidenced by the histochemical analysis.

Another possible explanation that cannot be excluded is that the isokinetic training stimuli administered in this case had a combination of resistance and endurance characteristics, imposed under constant speed throughout the range of motion. In contrast, during PNF training, the resistance to the limb was imposed by the physiotherapist without any control of the speed. Similarly to our findings, increased expression of myosin heavy chain (MHC) isoform IIA due to sprint training, which was related to bidirectional transformation from both MHC isoforms I and IIB towards MHC isoform IIA, was reported by Andersen et al. These authors analysed the MHC composition of single fibres from the vastus lateralis muscle of a group of male sprinters, before and after a three month period of intensive strength and interval training. Our findings are only partially in line with the above as isokinetic training induced a transformation that occurred from type IIA towards type IIB fibres with no significant reduction of type IIB. Further study is required to resolve this issue.

Changes in cross sectional area

Several studies have reported hypertrophy in muscle fibres of athletes participating in extremely strength demanding events. Longitudinal studies have also shown enlargement of muscle fibre area after strength training of sedentary subjects. Our findings that PNF training caused significant decreases in type IIB cross sectional area are in line with these studies.

Isokinetic training caused significant increases in type IIA cross sectional area, with a tendency towards reduction in type IIB muscle fibres. As discussed above, the two different kinds of training probably imposed different loads on the muscle examined. Also, the isokinetic training had a combination of resistance and endurance characteristics, imposed under constant speed throughout the whole range of motion.

Data on quadriceps muscle strength of our subjects, not shown here but published previously, showed that eight weeks of isokinetic training at 180 and 907/s, as well as eight weeks of PNF training, produced significant increases in peak torque at speeds slower and faster than the training velocity (60 and 3007/s). These findings are in line with those of Caiazzo et al. It is logical therefore to reason that mostly type IIA fibres were recruited during this type of stimulus and thus the cross sectional area of these fibres was affected, a postulate that is supported by our findings. This contention is also supported by the results of Houston et al. who reported that 10 weeks of high resistance training increased the cross sectional area of type II fibres by 20%, and the results of Brown et al. who reported that 13 weeks of resistance training increased the cross sectional area of type II fibres by 30%. These findings do not agree with those of Andersen et al. who reported no significant increase in the muscle fibre area in any of the three histochemically determined fibre types after a three month, high resistance knee extensor strength training programme in soccer players. The reason for the absence of any significant hypertrophy in that study may be that the amount of conducted strength training was too small.

In conclusion, the findings of this study clearly indicate that PNF training alters fibre type distribution and mean area, and that these changes seem to appear in the type II fibre subgroup and follow a unidirectional pattern of transformation (fast to slow). Similarly, isokinetic training induced alterations appear in the type II fibre subgroup, but the opposite pattern is followed, from type IIA to type IIAB, revealing a differential type of loading on the vastus lateralis muscle.

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