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

Objectives: To compare the effects of proprioceptive neuromuscular facilitation (PNF) and isokinetic training on fibre type distribution and cross sectional area of the vastus lateralis muscle.

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 evasion; (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 increased (p<0.05) after eight weeks of ISO training. The mean percentage area of ISO trained type IIB 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 1.78.2 (5.3) cm, weight 74.5 (9.2) kg); (b) isokinetic training (ISO; age 20.5 (1.7) years, height 1.78.9 (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 evasion; 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 abduct (medial toes more than lateral) toward fibular side; foot and ankle dorsiflex with inversion; knee extends with tibia externally rotating on femur; hip flexes, 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.

Isookinetic training

The ISO group followed a similar pattern of training, performing three sets of 30 repetitions alternating knee flexion. The ISO group followed a similar pattern of training, performing three sets of 30 repetitions alternating knee flexion.

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. 8 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 IIC (slow), partially supporting our initial hypothesis. In contrast, isokinetic training alters fibre type distribution and mean area and that these changes would appear in the type II fibre subgroup. The mean percentage area of type IIB fibre was significantly decreased after eight weeks of PNF training, whereas that of type IIA fibre was significantly increased (table 1). The mean percentage area of type IIA 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 IIA 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 IIB 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 appear 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. 6–8 Other studies have also suggested that strength training induces a histochemical fibre type change from type IIB to type IIA. 7–12 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→IIB→I) or vice versa. 10–13 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 I</td>
<td>44.49 (8.9)</td>
<td>40.12 (8.5)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>39.99 (8.7)</td>
<td>46.34 (9.5)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>5.67 (4.0)</td>
<td>8.25 (7.4)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>8.85 (7.9)</td>
<td>0.58 (19)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>IIC</td>
<td>2.36 (4.0)</td>
<td>5.11 (7.2)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

**Values are mean (SD).**
PNF, Proprioceptive neuromuscular facilitation.

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**Table 2** Fibre type distribution (%) 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 I</td>
<td>47.50 (10.5)</td>
<td>43.88 (10.1)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>36.18 (8.8)</td>
<td>42.31 (10.8)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>5.81 (4.2)</td>
<td>8.97 (7.6)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>9.66 (9.6)</td>
<td>0.67 (2.2)</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>IIC</td>
<td>2.12 (3.5)</td>
<td>4.62 (6.3)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

**Values are mean (SD).**
PNF, Proprioceptive neuromuscular facilitation.

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**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 I</td>
<td>4728.0 (1298)</td>
<td>4829.0 (1038)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>5503.0 (1110)</td>
<td>5828.0 (1251)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>3541.0 (2364)</td>
<td>2951.7 (2582)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>3406.8 (2287)</td>
<td>1106.5 (2492)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>IIC</td>
<td>2368.4 (3533)</td>
<td>3406.4 (3384)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

**Values are mean (SD).**
PNF, Proprioceptive neuromuscular facilitation.

RESULTS
The mean percentage area of type IIB 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 IIA 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 IIB fibres in the ISO trained group had significantly (p<0.01) increased (table 3).
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 eversion; (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.17 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 these results as the isokinetic training imposed different loads. It is well known that the isokinetic training stimuli administered in this case had a combination of resistance and endurance characteristics, imposed under constant speed throughout the whole range of motion.

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 IIB, revealing a differential type of loading on the vastus lateralis muscle.

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**REFERENCES**

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