Facts, Myths, and Misunderstandings: New discoveries on function and age-related morphology in human spinal muscles

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Background

Physiotherapy 1991
BSc (Physiology) 1992
DMPhty (Manip. Phty) 1995
MSc (Anatomy) 2003
PhD (Anatomy) 2008
PGCertTertT (Teaching) 2011

Founder / owner of PhysioMed physiotherapy clinics, 1995 – 2002

Senior Research Fellow, Faculty of Law and Department of Anatomy, University of Otago

Editor Australasian Medical Journal

Boards: Programme Autopsy for Rare Cancer; Society for Death Studies NZ

1. Spinal muscle function
2. Human tissue use is science, education
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Dunedin and Otago Peninsula

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1. Spinal muscle function
2. Human tissue use is science, education

Duathlon World Championships

Ironman NZ

NZ Cycling Team
Seminar outline

- Functional morphology of the spinal muscles
  1. Morphology
  2. Fibre type
  3. Spatial Distribution
  4. Age related changes and cell death
Functional morphology of the spinal muscles
Functional morphology of the spinal muscles
Functional morphology of the spinal muscles

• What is ‘functional morphology’?

*Identification of elements that improve our understanding of muscle function*

- Attachment points
- Shape
- Density of proprioceptors
- Size / cross section
- Fibre type
- Fibre architecture
Functional morphology of the spinal muscles

• What is ‘functional morphology’?

*Identification of elements that improve our understanding of muscle function*

• Why investigate functional morphology?

*Form guides function*

*Knowledge of functional role important*

*Guide and inform diagnosis, therapy*
Functional morphology of the TSP* muscles

T = Transverse Process
SP = Spinous Process

* TSP – transversospinal
Functional morphology
TSP muscles

**Background**

- Deepest, most medial spinal muscles

- Suggested role in spinal pathologies (through EMG, biopsy, ultrasound, MRI findings)

- Knowledge on morphology required for diagnosis, treatment
Functional morphology
TSP muscles

Background

Problems with existing knowledge:
• Thoracic and lumbar regions morphologically similar yet described as dissimilar
• Thoracic: 4 different muscles
  Lumbar: 1 muscle
• Description of ‘superficial’ and ‘deep’ lumbar multifidus (EMG) (Moseley et al. 2002)
Functional morphology
TSP muscles

Aim

Investigate functional morphology of thoracolumbar TSP muscles to clarify form, elucidate function in order to guide diagnosis, therapy and intervention
Gross morphology

Methods

• Microdissection using magnification (surgical microscope)
• 8 sides (different cadavers, 64-89 years, 4 male) from T6 – sacrum
• Each muscle and attachments identified, removed (400 muscles)
• 4 dissected cranial to caudal; 4 caudal to cranial

Lateral view of thoracolumbar spine
Gross morphology

Results

- Attachment between adjacent muscles
- Lack of clearly delineated epimysium
- Contradicts current textbook descriptions of ‘individual muscle’

Epimysium – encloses muscle

Lateral view of dissected thoracic TSP muscles

Cornwall, Stringer, Duxson Spine 2011
Gross morphology

Results

• Organisation / pattern same throughout thoracic / lumbar regions
• Thoracic semispinalis extends to L4 (not previously described)
• Each vertebral level ‘blended’ with adjacent levels
• Few cleavage planes

Lateral view single muscle ‘sheet’ removed from T7

Cornwall, Stringer, Duxson Spine 2011
Functional morphology TSP muscles

Discussion

- Homogeneous arrangement
- No distinct / consistent cleavage planes
- Muscles ‘blended’ together from each level of origin (no distinct sheath of epimysium)
- Fibre arrangement: all in-parallel and multipennate
- Arrangement suggests ‘fine tuning’ function

Lateral view single muscle ‘sheet’ removed from T7

Cornwall, Stringer, Duxson *Spine* 2011
Conclusion

- Anatomical texts could be reviewed:
  - muscles all the same form / different names
  - thoracic semispinalis
  - definition of individual muscles (and epaxial)

- Medical intervention: precise injection of neuromuscular junctions, electrical stimulation difficult

- Diagnosis: accurate EMG, biopsies, muscle cross-section (MRI, US) difficult
Fibre types - anterior cervical muscles

Background

• Examination of anterior cervical muscles (ACM):
  Longus colli
  Longus capitis
  Scaleni (anterior, medius, posterior)

• Function altered in various conditions: chronic cervical pain, whiplash, anterocollis, acute calcific tendonitis, scalenectomy

Modified from Drake et al. (2009) Gray’s Anatomy for Students
Fibre types - anterior cervical muscles

- Current physical therapy targets anterior cervical muscle ‘postural retraining’ with exercise regimens; inconsistent outcomes

- Limited understanding of ACM function as few studies assess fibre types

- Skeletal muscle, fibre types help determine function.
  - Type I fibres: aerobic, tonic
  - Type II fibres: anaerobic, phasic

**Aim:** Quantify ACM fibre types to improve understanding of function
Fibre types - anterior cervical muscles

Methods

• Muscles from 5 cadavers (average age 87; 4 male) sampled at multiple vertebral levels (total 106 sections)
  6 Longus colli, Longus capitis
  5 Scalenus anterior, medius, posterior
• Tissue blocks paraffin embedded
• 5µm sections immunohistochemically stained for type I (1A), type II (MY32) skeletal muscle fibres
Fibre types - anterior cervical muscles

Methods
Fibre types - anterior cervical muscles

Methods
Fibre types - anterior cervical muscles

Methods

- Stereology (random systematic sampling of whole section):
  a) fibre type proportions (total numbers, counting minimum 4% total section area)
  b) cross-sectional area (CSA) occupied by each fibre type

- Muscle section: 5 orange fibers, 3 green
- Larger area occupied by orange
Fibre types - anterior cervical muscles

Methods
Data analysed by ANOVA

• Within each muscle:
  Between each specimen
  Between vertebral levels

• Between different muscles
Fibre types - anterior cervical muscles

Methods

Data analysed by ANOVA

• Within each muscle:
  Between each specimen
  **Between vertebral levels**

• Between different muscles
Fibre types - anterior cervical muscles

Methods

Data analysed by ANOVA

- Within each muscle:
  - Between each specimen
  - Between vertebral levels

- Between different muscles
Fibre types - anterior cervical muscles

Results

• 69,572 fibres counted to assess proportions (650 / slide)
• 556 counted per section to assess cross-sectional area

Within each muscle – ANOVA (post-hoc Sidak):

• No significant difference proportion of fibre types between or within most specimens; 2 longus capitis specimen differed from other specimens
• No significant difference CSA occupied by type I between or within most specimens; 1 longus capitis specimen differed from other specimens

Cornwall, Kennedy, Stringer. (Under review)
# Fibre types - anterior cervical muscles

**Results:** Between different muscles - raw data:

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Proportion of type I fibres</th>
<th>Area occupied by type I fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longus colli</td>
<td>48.8%</td>
<td>63.5%</td>
</tr>
<tr>
<td>Longus capitis</td>
<td>53.9%</td>
<td>63.3%</td>
</tr>
<tr>
<td>Scalenus anterior</td>
<td>73.9%</td>
<td>84.9%</td>
</tr>
<tr>
<td>Scalenus medius</td>
<td>64.8%</td>
<td>78.1%</td>
</tr>
<tr>
<td>Scalenus posterior</td>
<td>57.2%</td>
<td>75.1%</td>
</tr>
</tbody>
</table>

Cornwall, Kennedy, Stringer. *(Under review)*
**Fibre types - anterior cervical muscles**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>L.capitis</th>
<th>L.colli</th>
<th>Sc.ant</th>
<th>Sc.med</th>
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Between different muscles – ANOVA (post-hoc Sidak)
Significant differences in proportion of type I fibres

* denotes significant difference (p>0.05)

Cornwall, Kennedy, Stringer. *(Under review)*
## Fibre types - anterior cervical muscles

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**Between different muscles – ANOVA (post-hoc Sidak)**
Significant differences in percentage of CSA occupied by type I fibres

* denotes significant difference (p>0.05)

Cornwall, Kennedy, Stringer. *(Under review)*
Fibre types - anterior cervical muscles

Discussion

- Longus colli / capitis similar to phasic muscles (e.g. hamstrings, 65% type I)

- Scaleni more highly aerobic, similar to other postural muscles (e.g. lumbar multifidus, 85-95% type I)

- Significant differences CSA / proportion type I longus capitis specimens: perhaps indicates more type II atrophy

Cornwall, Kennedy, Stringer. *Under review*
Fibre types - anterior cervical muscles

Conclusion

• First study assessing whole ACM sections, from multiple levels

• Challenges views all ACM ‘postural’ (elderly); scaleni more ‘postural’, other muscles more ‘phasic’

• Individual ACM likely to have different roles

• Treatment regimens targeting postural ‘function’ for all ACM should be re-examined

Cornwall, Kennedy, Stringer. *(Under review)*
Fibre type spatial distribution

Background

• Section from previous work showed three interesting fibre type distribution

Blue - ‘Normal’ random distribution of type I and II fibres

Green – type I fibres increased

Red – type II fibres increased

Multifidus muscle (whole section T2)
Fibre type spatial distribution

Background

- Non-random distributions were noted in anterior cervical muscle sections from fibre type investigations (elderly samples)

‘Normal’ random distribution

Type II aggregation

Dogma – increased type I fibres

Type II aggregation

Fibre type distributions, cervical muscles
Fibre type spatial distribution

Background

• Understanding spatial distribution important

• Age-related changes

• Physiology, force distribution

Aim: Assess cervical muscle fibre type spatial distributions

‘Normal’ random distribution

Type II aggregation

Dogma – increased type I fibres

Type II aggregation

Fibre type distributions, cervical muscles
Fibre type spatial distribution

Methods

• Assessing 96 pre-processed sections from 5 muscles (previous investigation), whole section

• Anterior cervical muscles, 5 cadavers

• Aggregation = clusters of >10 type II fibres

Type II fibre aggregation

Cornwall and Sheard *Clinical Anatomy* 2012 (Abstract)
# Fibre type spatial distribution

## Results

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Sections clustering / total sections</th>
<th>% sections with clustering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longus colli</td>
<td>16 / 18</td>
<td>89</td>
</tr>
<tr>
<td>Longus capitus</td>
<td>17 / 27</td>
<td>63</td>
</tr>
<tr>
<td>Scalenus anterior</td>
<td>5 / 11</td>
<td>45</td>
</tr>
<tr>
<td>Scalenus medius</td>
<td>15 / 28</td>
<td>54</td>
</tr>
<tr>
<td>Scalenus posterior</td>
<td>8 / 12</td>
<td>67</td>
</tr>
</tbody>
</table>

Type II fibre aggregation

Cornwall and Sheard *Clinical Anatomy 2012 (Abstract)*
Fibre type spatial distribution

Conclusion

• Challenges sarcopenia dogma on increasing, uniform type I proportion and aggregation

• Normal process / distribution?

• No readily available quantitative method to examine type I, type II spatial relationships

Cornwall and Sheard *Clinical Anatomy* 2012 (Abstract)
Quantifying Spatial Distribution

Background

• Observations on fibre type distributions suggested difference to ‘normal’ / expected (non-random)

• No method available for testing distributions statistically

• **Aim:** Develop mathematical method for assessing spatial distribution

Davies, Cornwall, Sheard  Statistics in Medicine 2013
Quantifying Spatial Distribution

Methods

• Generate point data from photomicrograph pre-processed sections (x3) (Fovea Pro)

• Import data to R-stats programme

• Determine parameters for testing

• Create algorithm to interpret and test data

A
‘Random’

B
Type II aggregation

C
Type II aggregation

Davies, Cornwall, Sheard  Statistics in Medicine 2013
Quantifying Spatial Distribution

Result

Analysis includes -

Light and dark fibres

\[ \hat{\rho}(y) = \log \left[ \frac{\hat{f}_D(y)}{\hat{f}_L(y)} \right]; \quad y \in W, \]

Kernel smoothing

\[ \hat{f}_\alpha(y) = n_\alpha^{-1} \sum_{x \in X} 1[m(x) = \alpha] \frac{K_b(y - x)}{c_b(W, y) w_\alpha(x)} \]

Weighting

\[ w_\alpha(x) = \frac{|x|}{\sum_{z \in X} 1[m(z) = \alpha]|z|} \]

Davies, Cornwall, Sheard  Statistics in Medicine 2013
Quantifying Spatial Distribution

Result

Kernel smoothed distributions of three samples

Significance testing of three samples; red line indicates difference to ‘random’ distribution

Davies, Cornwall, Sheard  Statistics in Medicine 2013
Quantifying Spatial Distribution

Discussion

• First method to quantitatively assess and significance test two different fibre populations in samples (Kernel density, random Markov binary field methods most appropriate)
• Development of novel bio-mathematical application
• Application to not only muscle fibre types; other biological distributions
Age related change in spinal muscles

- Formation of Otago Muscle Biology Group

A/P Phil Sheard
Department of Physiology

A/P David Rowlands
Massey University, Wellington

Dr Tania Slatter
Department of Pathology

Navneet Lal

John Brady

Kathrine Neilsen

Ash Gillon
Sarcopenia:
1. Loss of fibres
2. Loss of fibre size (atrophy)
3. Aggregation of fibres
DEVILs: Dystrophin encircled vacuoles & invaginations with intracellular localisation

Scale bar = 100 μm

Anti-Dystrophin

Mouse skeletal muscle

Navneet Lal
Age related change in spinal muscles

Sarcopenia:

**Loss of fibres**
Loss of fibre size (atrophy)
Aggregation of fibres

Position of DEVILs within mouse Soleus and EDL

Navneet Lal
Age related change in spinal muscles

Position of DEVILs within mouse Soleus and EDL

Human spinal muscle
Age related change in spinal muscles
Age related change in spinal muscles
Functional morphology of the spinal muscles

Summary

• Studies have investigated form and function of TSP, anterior cervical muscles

• Data informs function – useful for diagnosis, intervention

• Investigations now focused on determining how age related change occurs (molecular pathways)
So what?

‘Facts, myths, and misunderstandings’

• There is no ‘one’ anatomy textbook that is correct about everything

• Muscle form and function are important yet sometimes poorly understood

• Aging effects all of our skeletal muscle; still little is known about biological mechanisms
Thank you

Otago University Clocktower

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