



SoGoS
Centre for Society,
Governance and Science



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



Background

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

Dunedin and Otago Peninsula



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'?

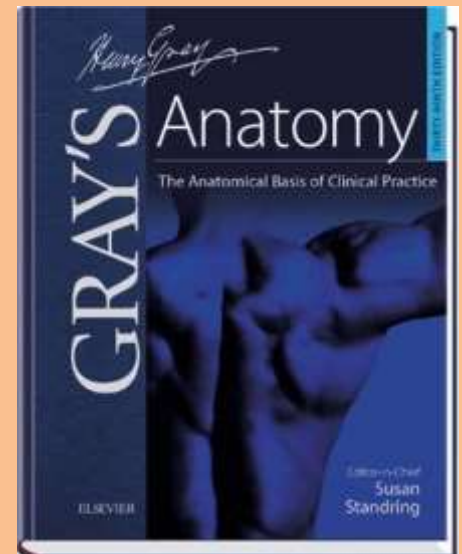
Identification of elements that improve our understanding of muscle function

- Why investigate functional morphology?

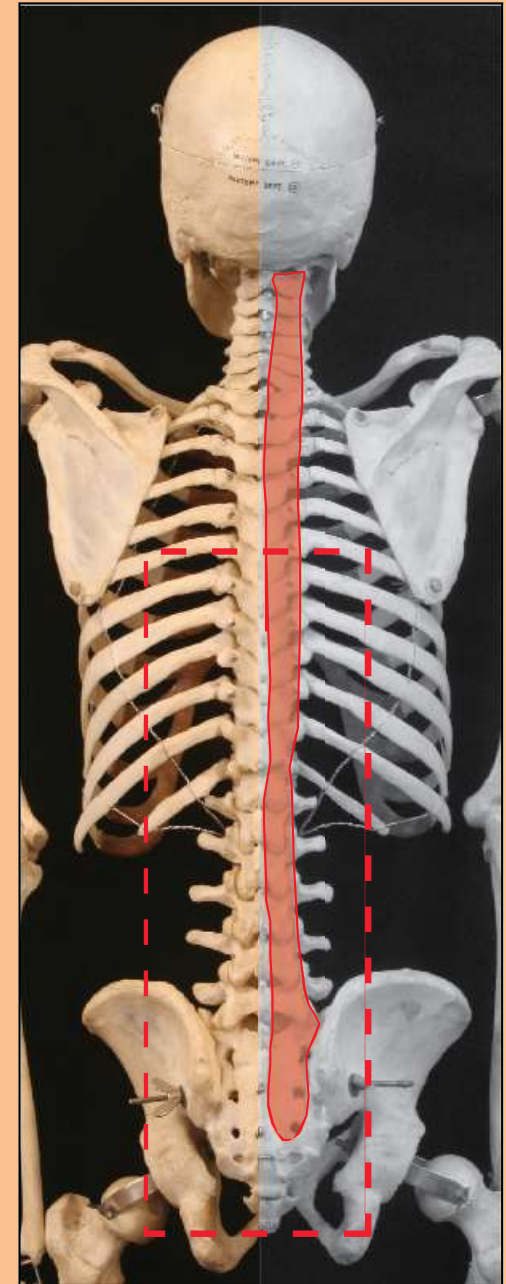
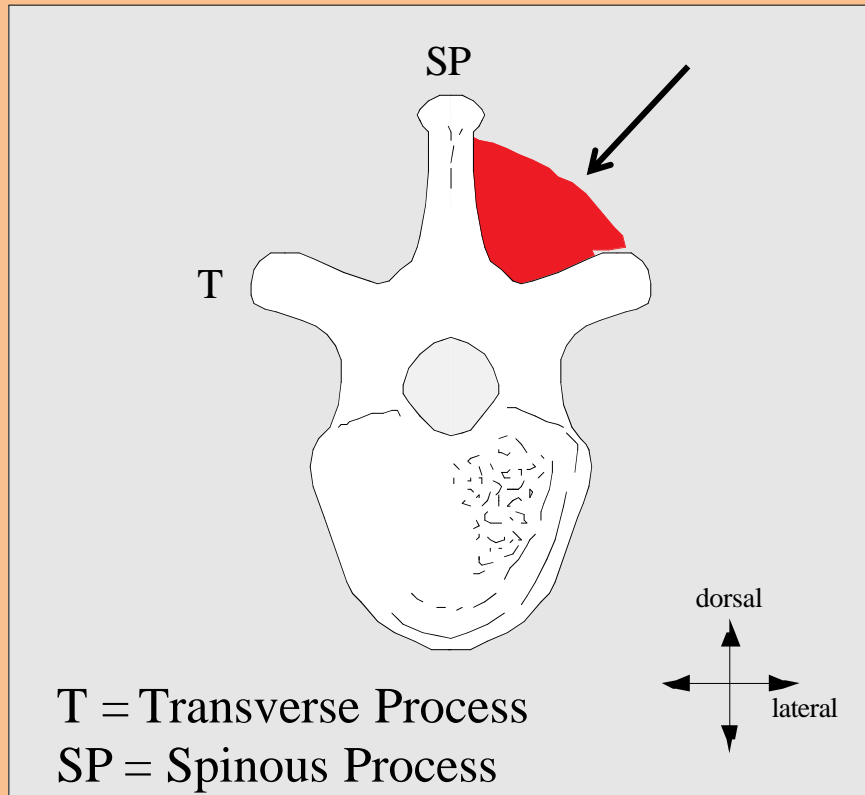
Form guides function

Knowledge of functional role important

Guide and inform diagnosis, therapy



Functional morphology of the TSP* muscles

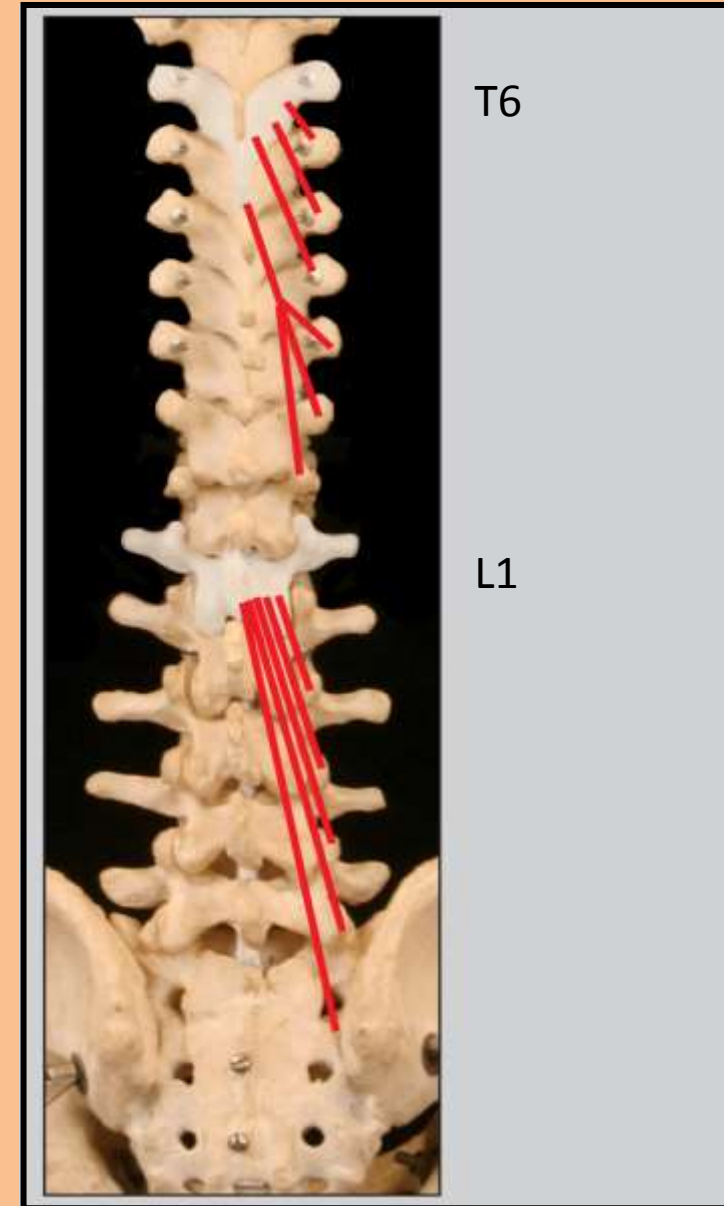


* TSP – transversospinal

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



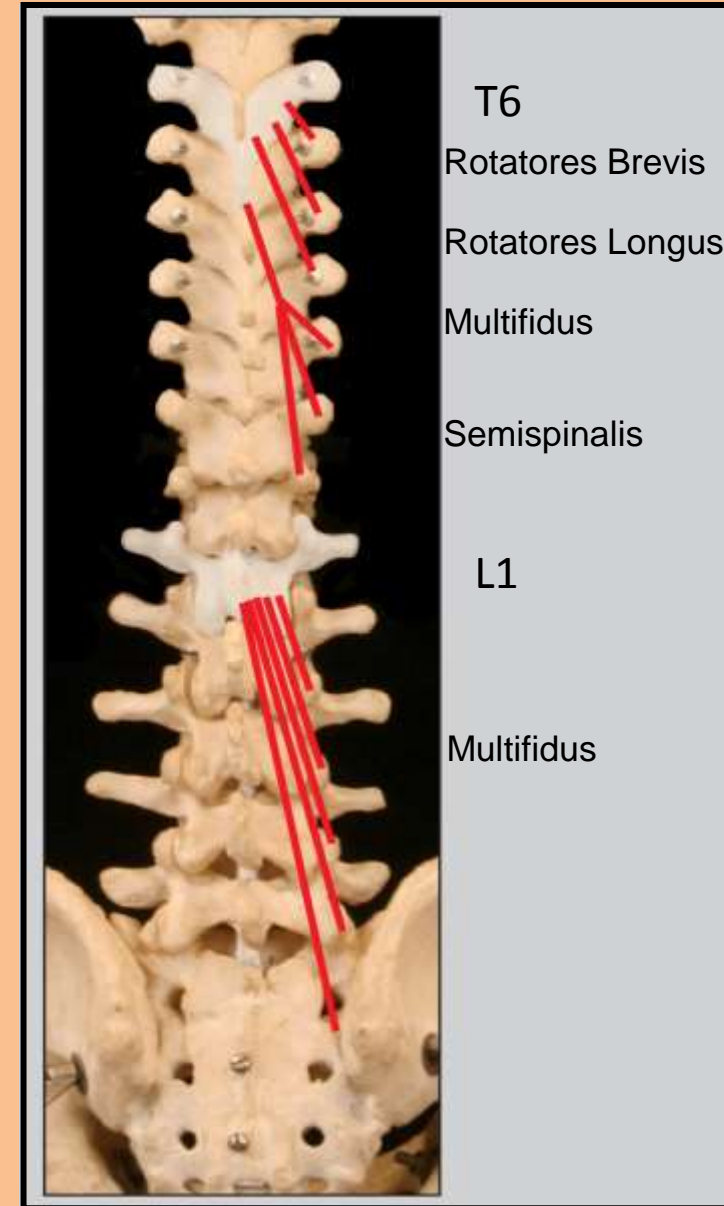
TSP muscle location

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)

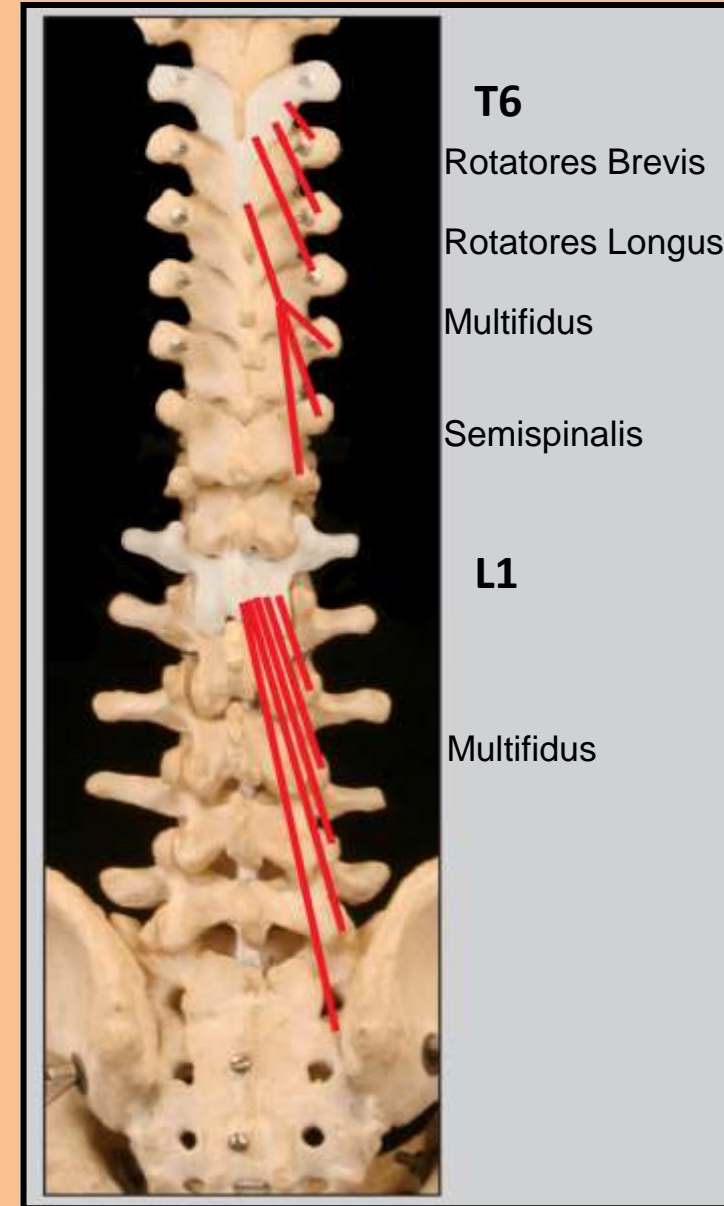


TSP morphology – modern
textbook consensus

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

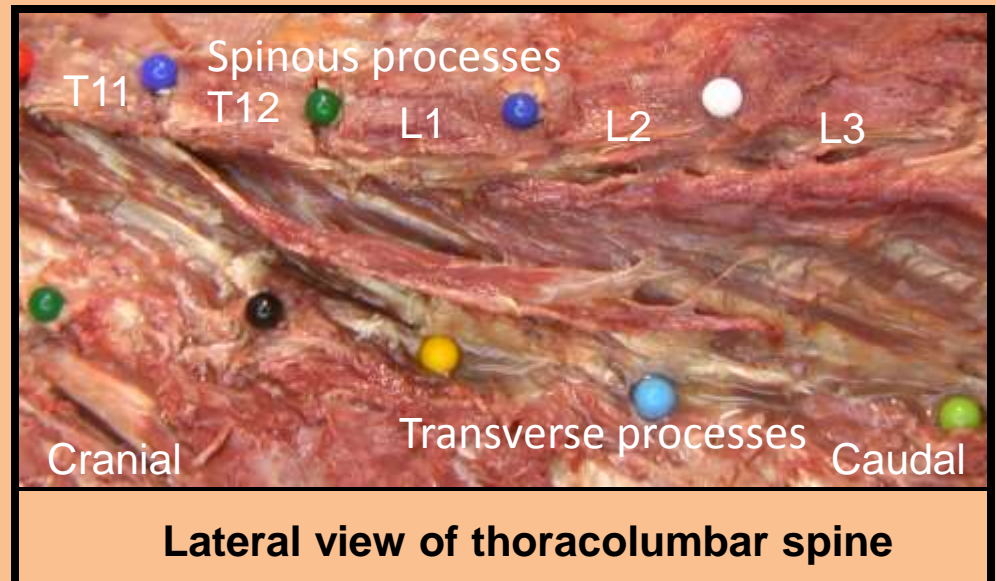


TSP morphology – modern
textbook consensus

Gross morphology

Methods

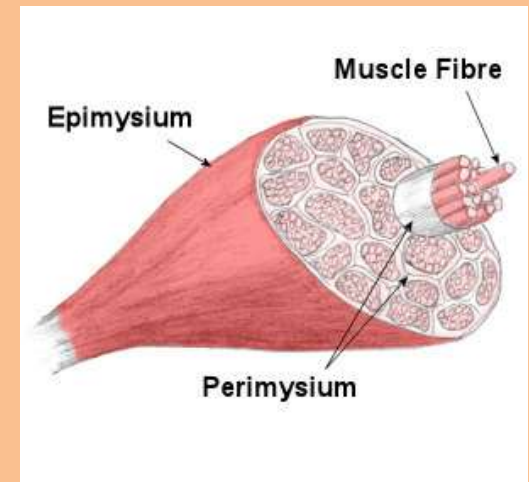
- Microdissection using magnification (surgical microscope)
- 8 sides (different cadavers, 64-89 years, 4 male) from T6 – sacrum
- Each muscle and attachments identified, removed (400 muscles)
- 4 dissected cranial to caudal; 4 caudal to cranial



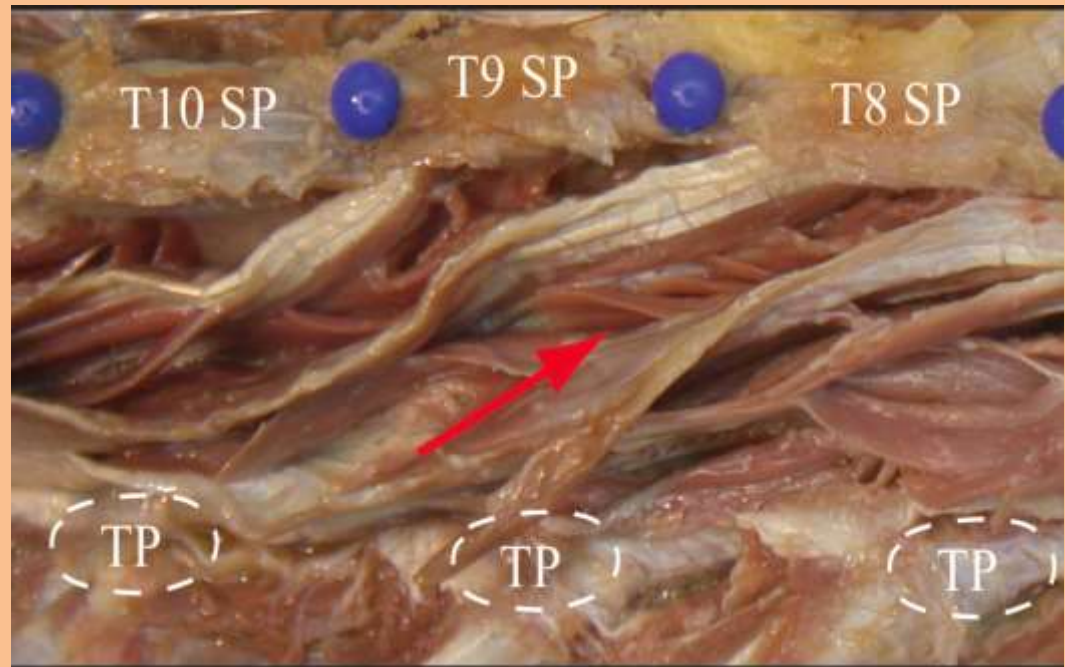
Gross morphology

Results

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



Epimysium – encloses muscle
http://www.teachpe.com/anatomy/structure_skeletal_muscle.php

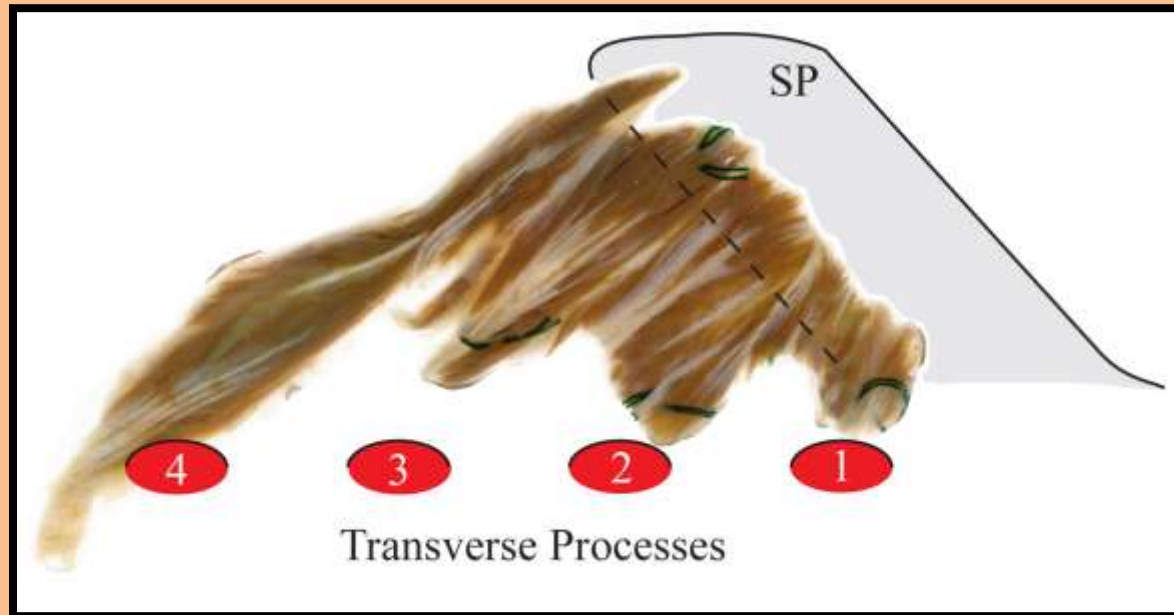


Lateral view of dissected thoracic TSP muscles

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

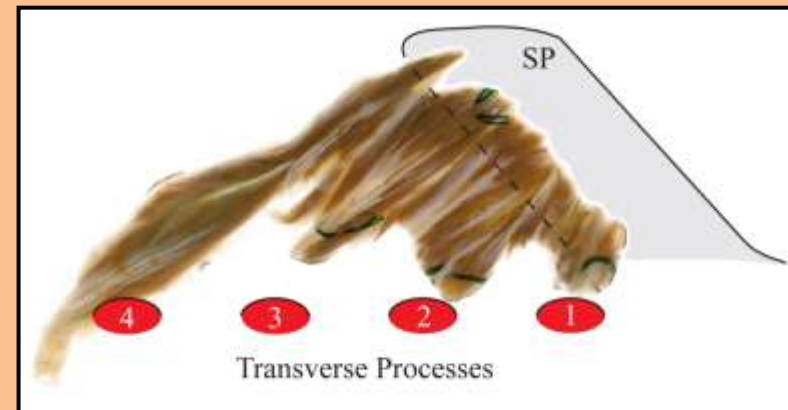


Lateral view single muscle 'sheet' removed from T7

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

Functional morphology TSP muscles

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

Spine

SPINE Volume 36, Number 16, pp E1053-E1061
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ANATOMY

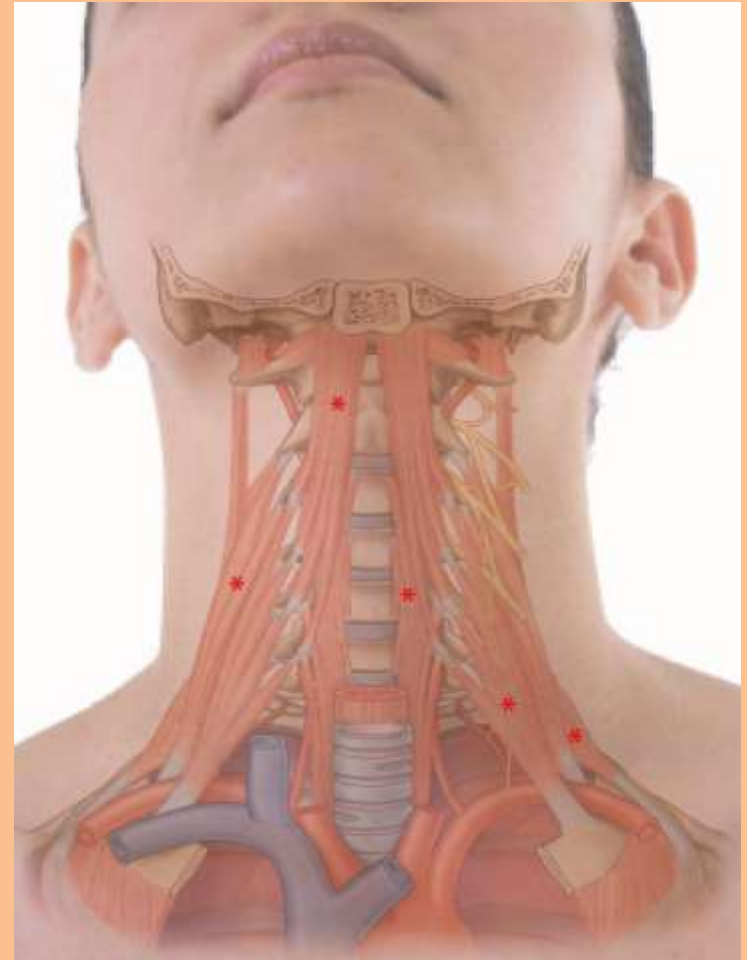
Functional Morphology of the Thoracolumbar
Transversospinal Muscles

Jon Cornwall, PhD, DMPhy, Mark D. Stringer, MS, FRCS, and Marilyn Duxson, PhD

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



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

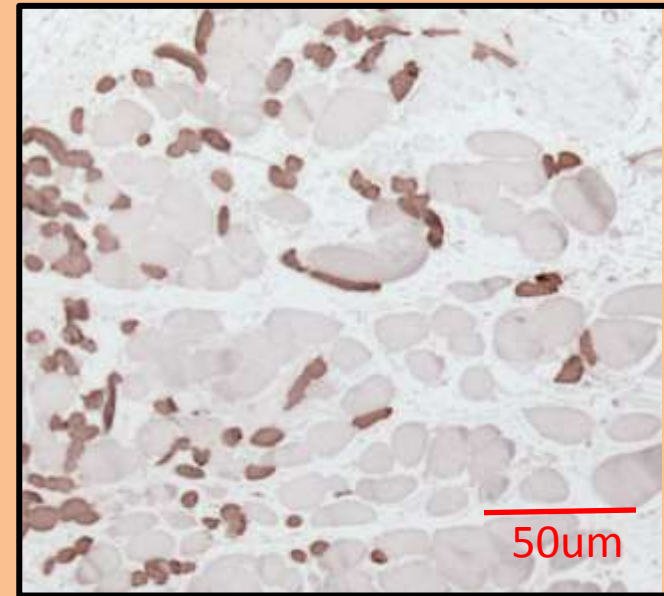


Aim: Quantify ACM fibre types to improve understanding of function

Fibre types - anterior cervical muscles

Methods

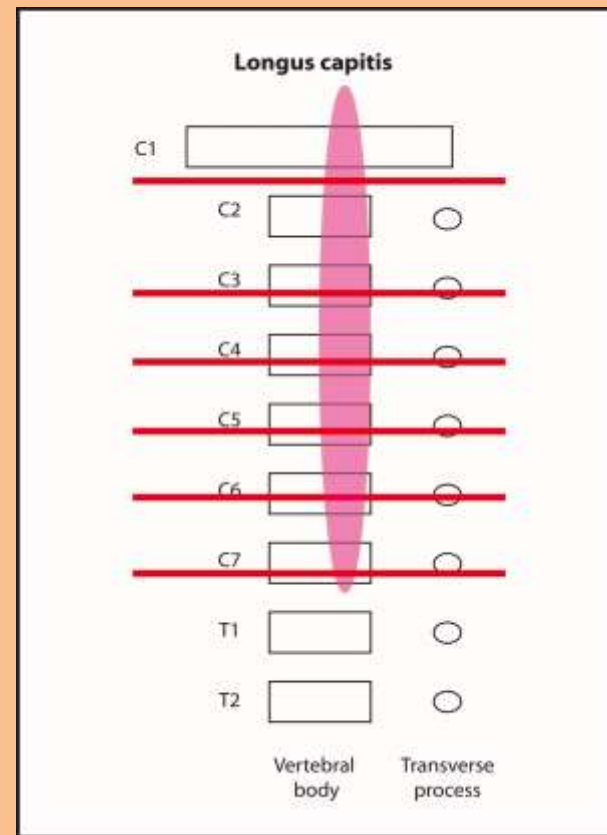
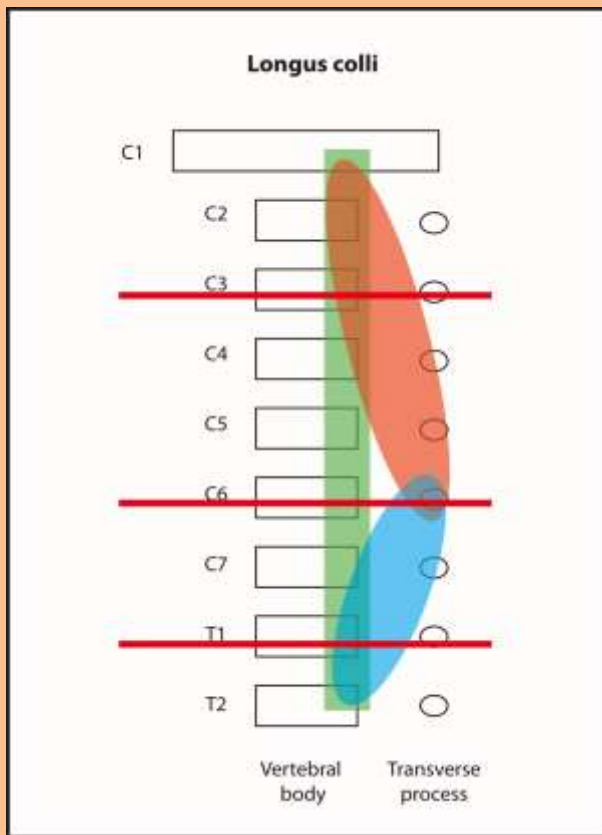
- Muscles from 5 cadavers (average age 87; 4 male) sampled at multiple vertebral levels (total 106 sections)
 - 6 Longus colli, Longus capitis
 - 5 Scalenus anterior, medius, posterior
- Tissue blocks paraffin embedded
- 5µm sections immunohistochemically stained for type I (1A), type II (MY32) skeletal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



Processed section of anterior cervical muscle; type II – dark, type I - light

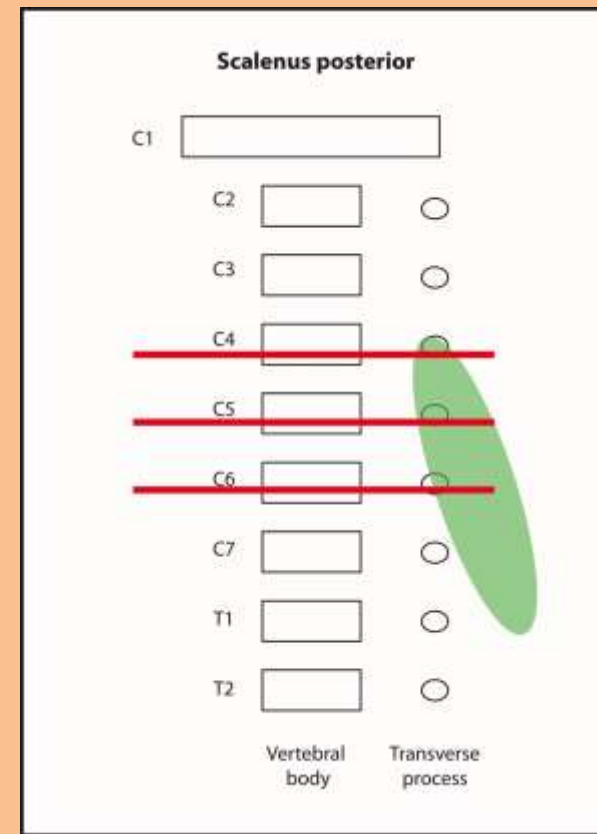
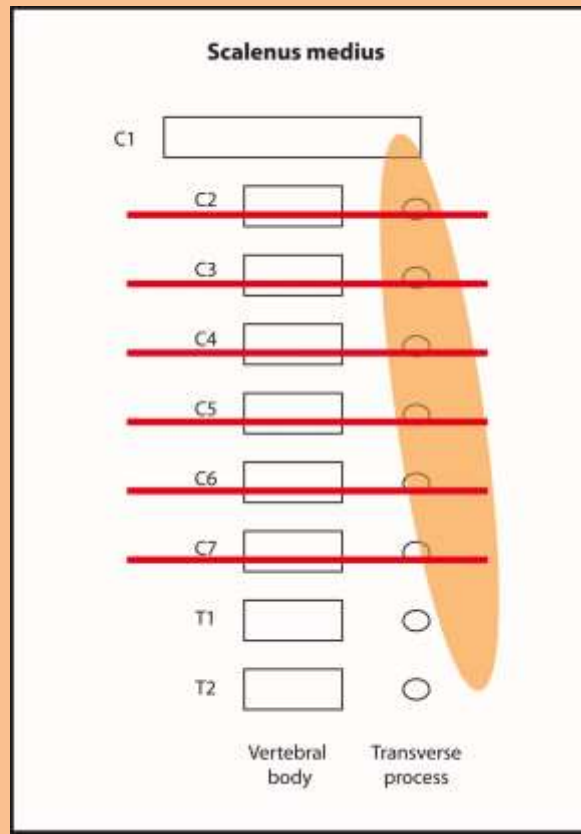
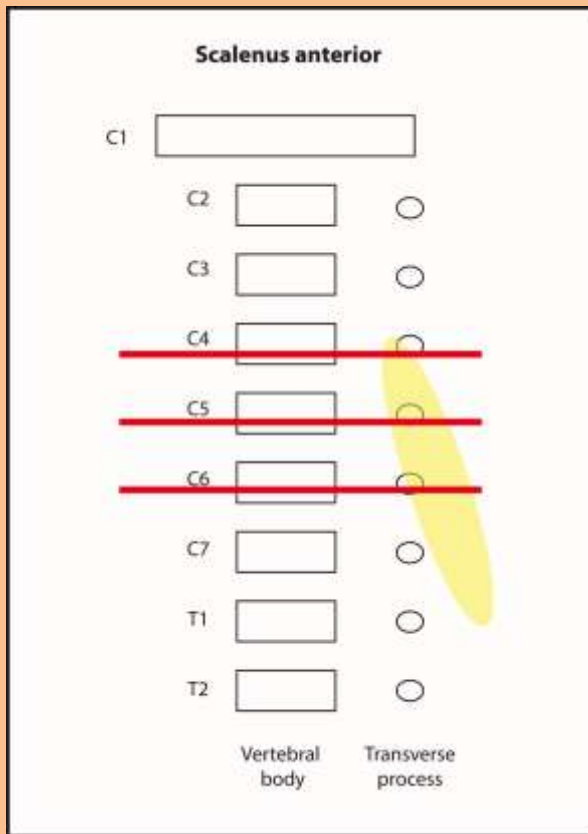
Fibre types - anterior cervical muscles

Methods



Fibre types - anterior cervical muscles

Methods



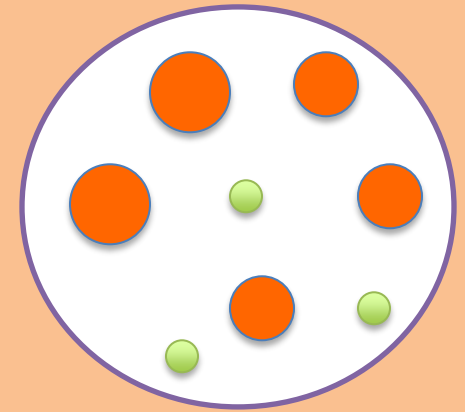
Fibre types - anterior cervical muscles

Methods

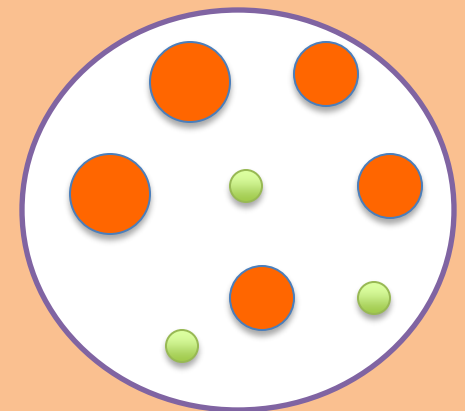
- Stereology (random systematic sampling of whole section):

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



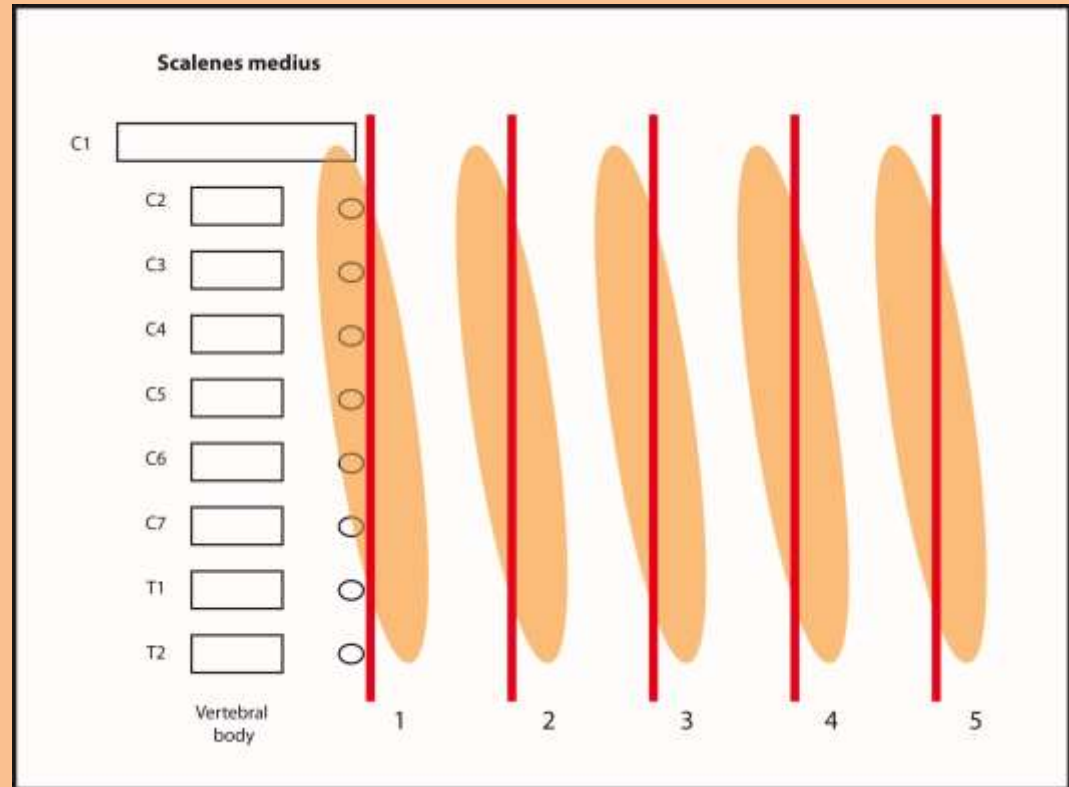
Larger area occupied by orange

Fibre types - anterior cervical muscles

Methods

Data analysed by ANOVA

- Within each muscle:
 - Between each specimen**
 - Between vertebral levels
- Between different muscles

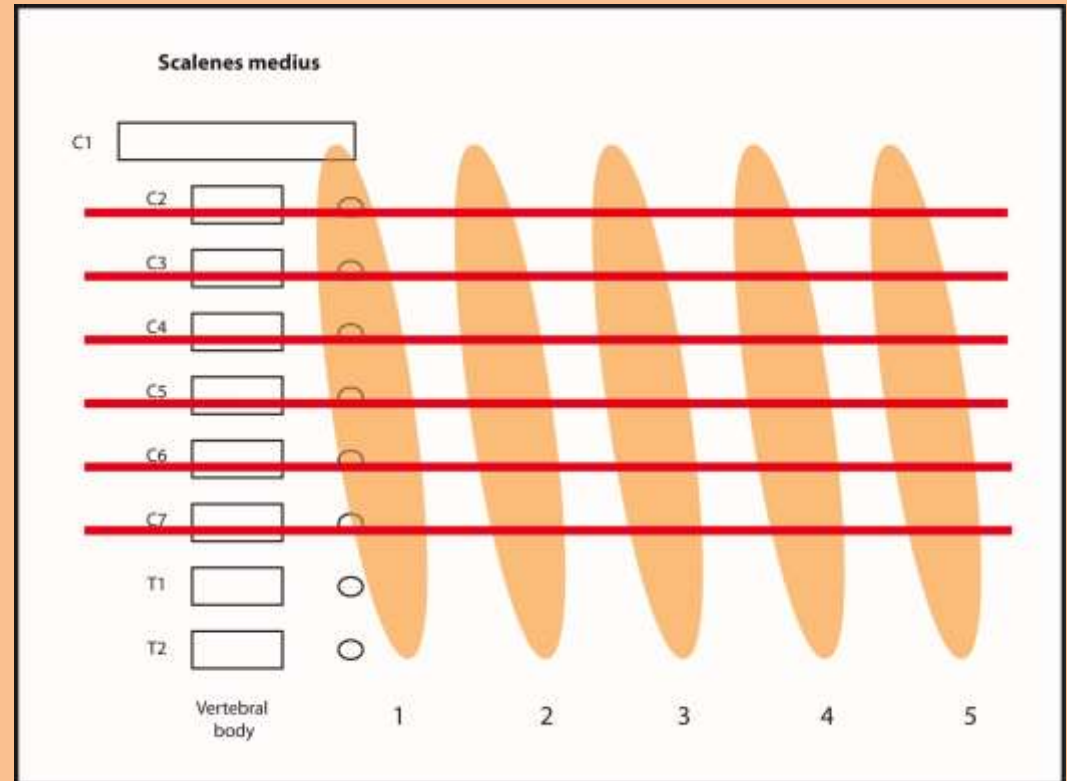


Fibre types - anterior cervical muscles

Methods

Data analysed by ANOVA

- Within each muscle:
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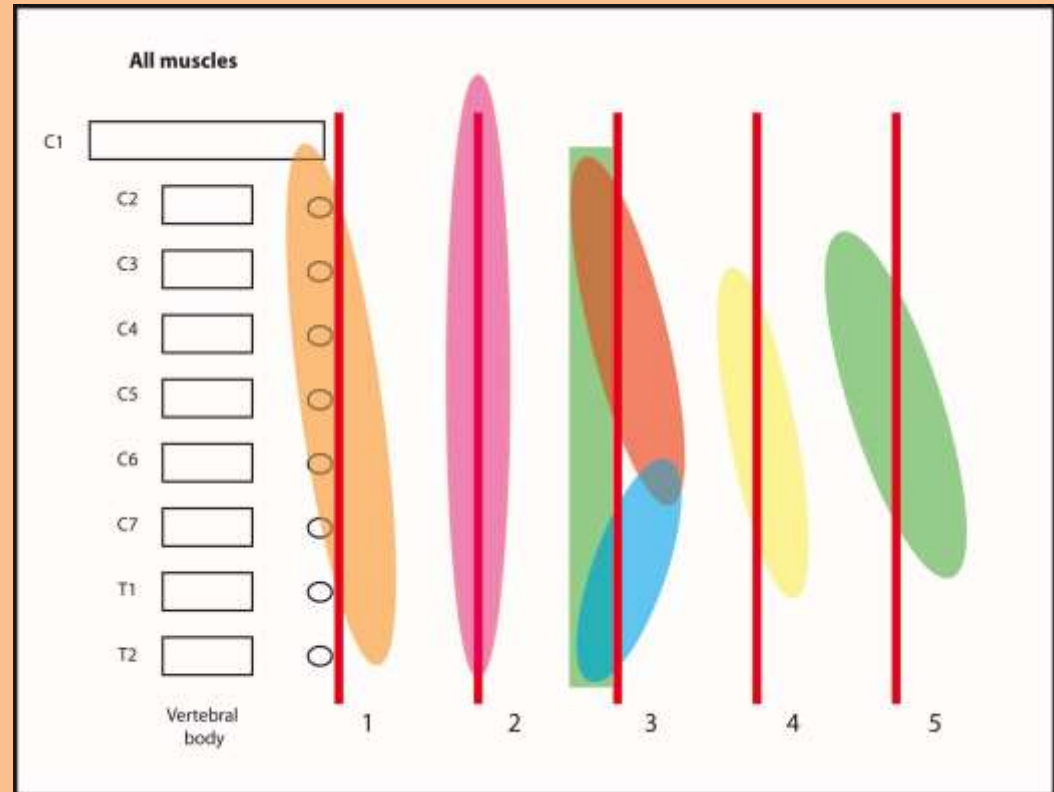


Fibre types - anterior cervical muscles

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

Fibre types - anterior cervical muscles

Results: Between different muscles - raw data:

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

Fibre types - anterior cervical muscles

Muscle	L.capitis	L.colli	Sc.ant	Sc.med
L.colli				
Sc.ant	*	*		
Sc.med	*	*		
Sc.post				

Between different muscles – ANOVA (post-hoc Sidak)

Significant differences in proportion of type I fibres

* denotes significant difference ($p > 0.05$)

Fibre types - anterior cervical muscles

Muscle	L.capitis	L.colli	Sc.ant	Sc.med
L.colli				
Sc.ant	*	*		
Sc.med	*	*		
Sc.post	*	*		

Between different muscles – ANOVA (post-hoc Sidak)

Significant differences in percentage of CSA occupied by type I fibres

* denotes significant difference ($p > 0.05$)

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

Fibre types - anterior cervical muscles

Conclusion

- First study assessing whole ACM sections, from multiple levels
- Challenges views all ACM 'postural' (elderly); scalmi 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

Fibre type spatial distribution

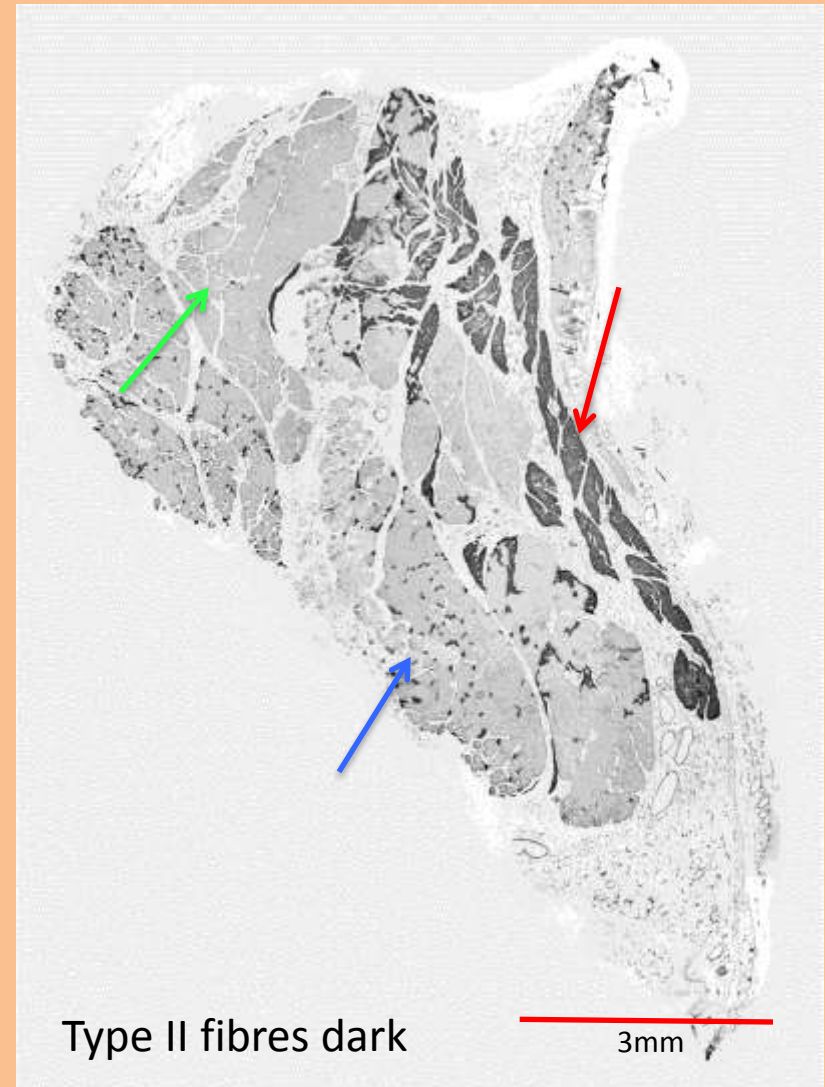
Background

- 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

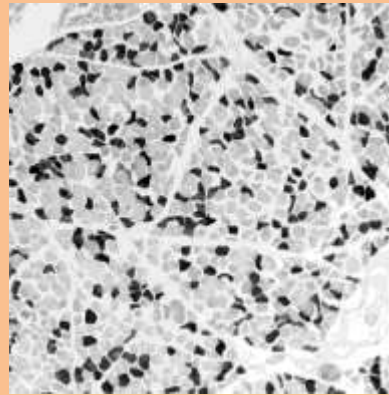


Multifidus muscle (whole section T2)

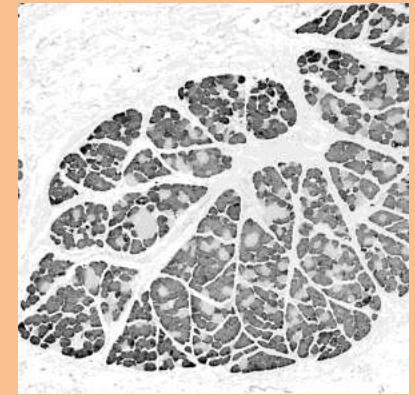
Fibre type spatial distribution

Background

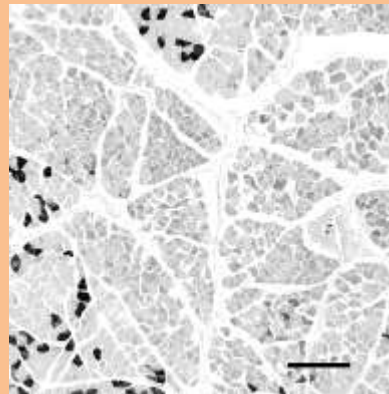
- Non-random distributions were noted in anterior cervical muscle sections from fibre type investigations (elderly samples)



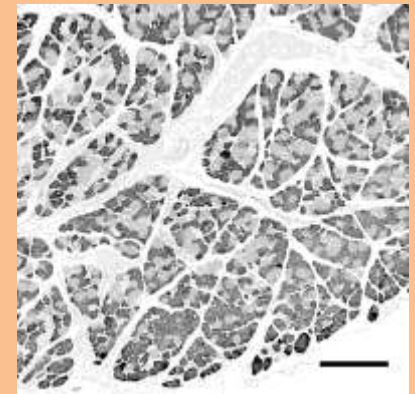
'Normal' random distribution



Type II aggregation



Dogma – increased type I fibres



Type II aggregation

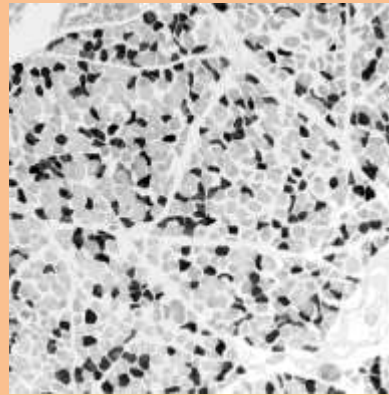
Fibre type distributions, cervical muscles

Fibre type spatial distribution

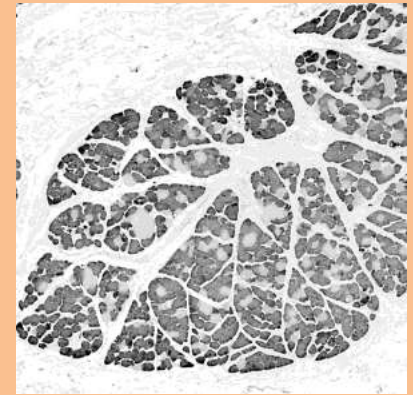
Background

- Understanding spatial distribution important
- Age-related changes
- Physiology, force distribution

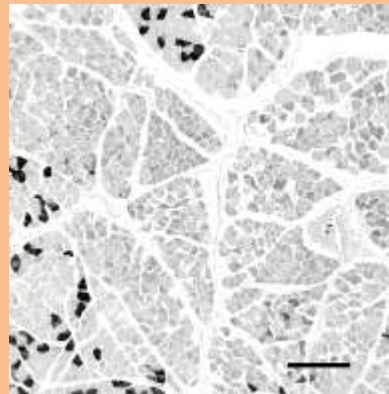
Aim: Assess cervical muscle fibre type spatial distributions



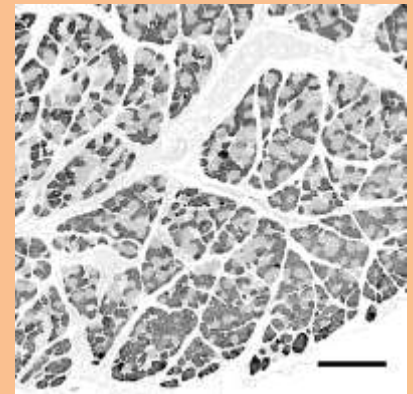
'Normal' random distribution



Type II aggregation



Dogma – increased type I fibres



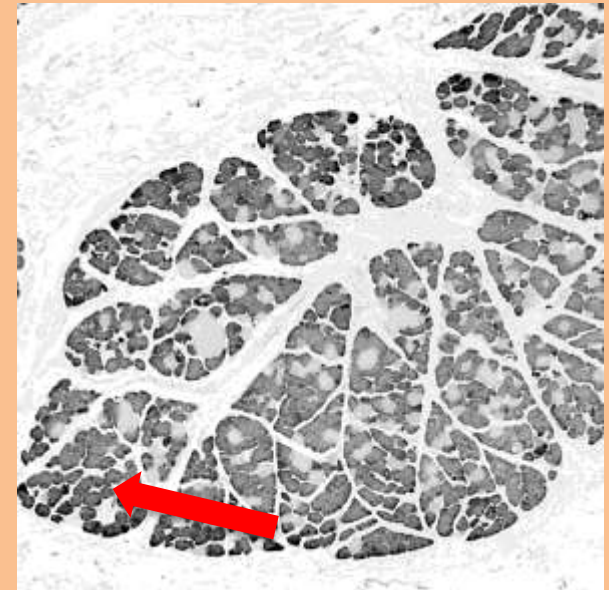
Type II aggregation

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

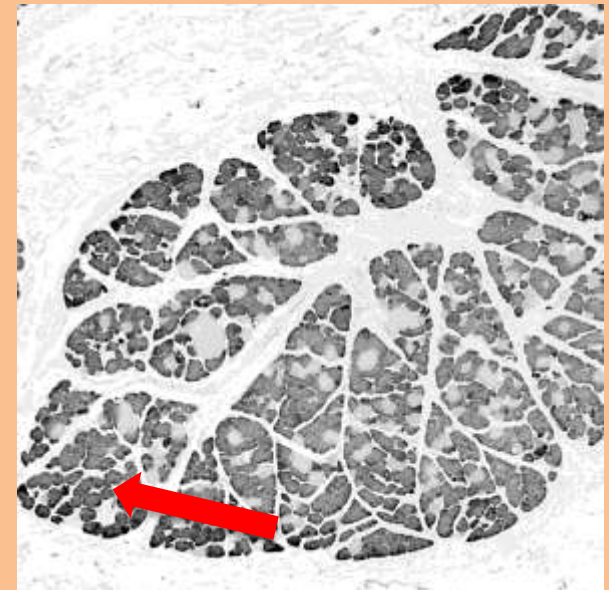


Type II fibre aggregation

Fibre type spatial distribution

Results

Muscle	Sections clustering / total sections	% sections with clustering
Longus colli	16 / 18	89
Longus capitus	17 / 27	63
Scalenus anterior	5 / 11	45
Scalenus medius	15 / 28	54
Scalenus posterior	8 / 12	67



Type II fibre aggregation

Fibre type spatial distribution

Conclusion

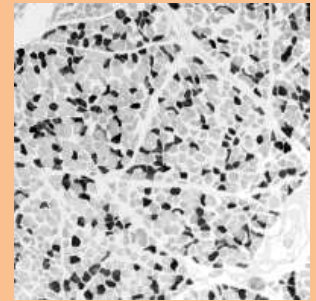
- Challenges sarcopenia dogma on increasing, uniform type I proportion and aggregation
- Normal process / distribution?
- No readily available quantitative method to examine type I, type II spatial relationships

Quantifying Spatial Distribution

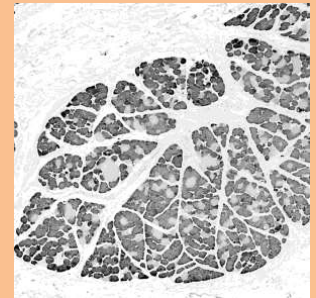
Background

- 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**

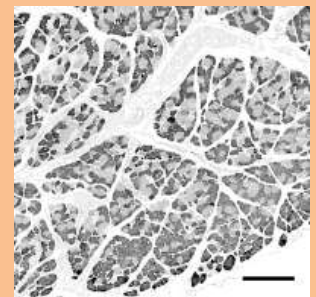
A
'Random'



B
Type II
aggregation



C
Type II
aggregation

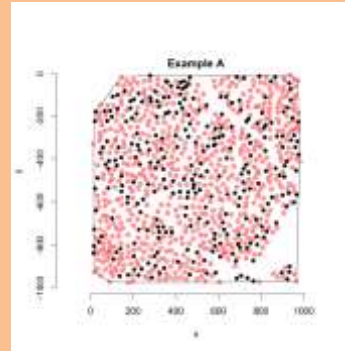


Fibre type distributions,
cervical muscles

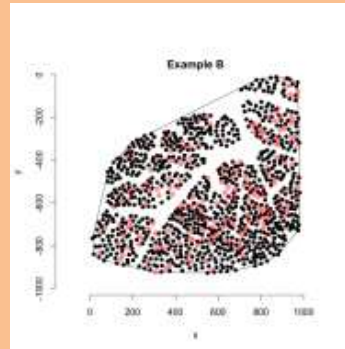
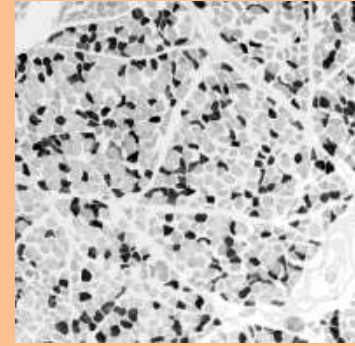
Quantifying Spatial Distribution

Methods

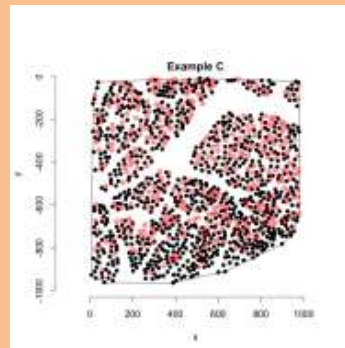
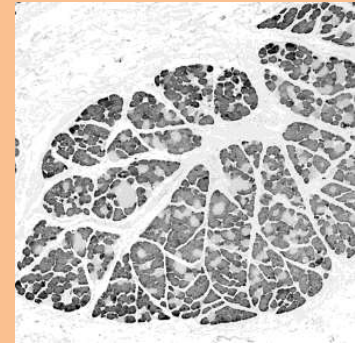
- Generate point data from photomicrograph pre-processed sections (x3) (Fovea Pro)
- Import data to R-stats programme
- Determine parameters for testing
- Create algorithm to interpret and test data



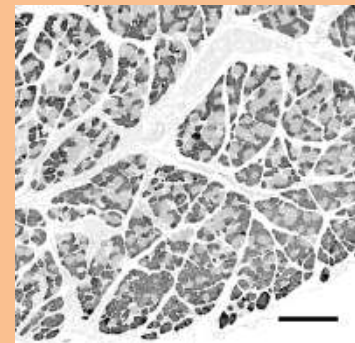
A
'Random'



B
Type II
aggregation



C
Type II
aggregation



Quantifying Spatial Distribution

Result

Analysis includes -

Light and dark fibres

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

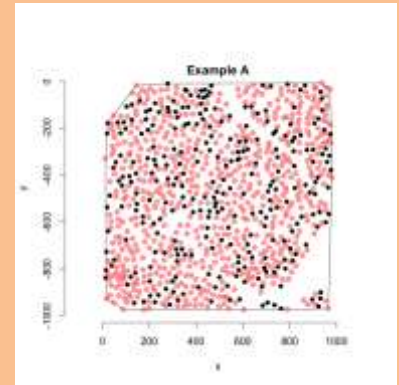
Kernel smoothing

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

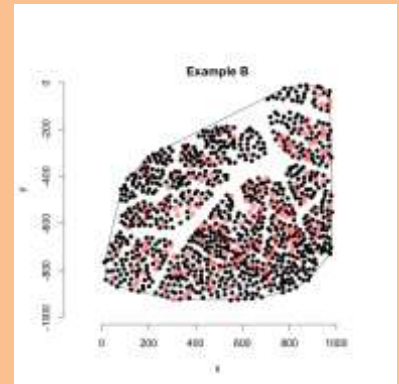
Weighting

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

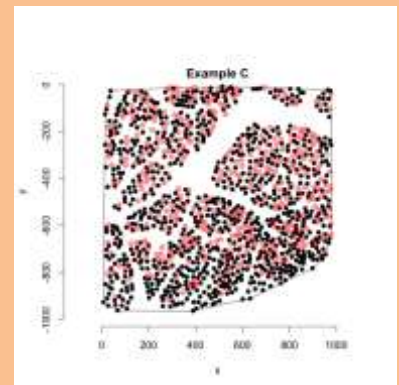
A



B



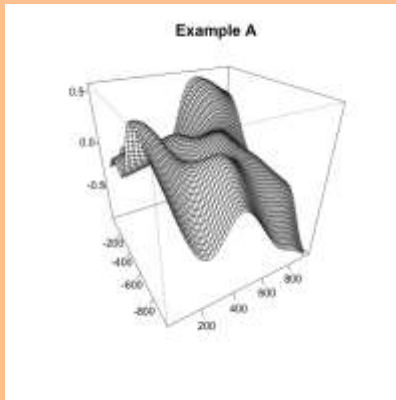
C



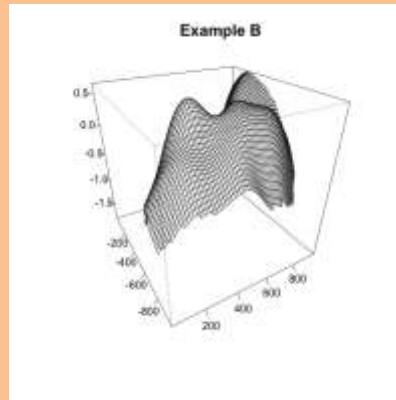
Quantifying Spatial Distribution

Result

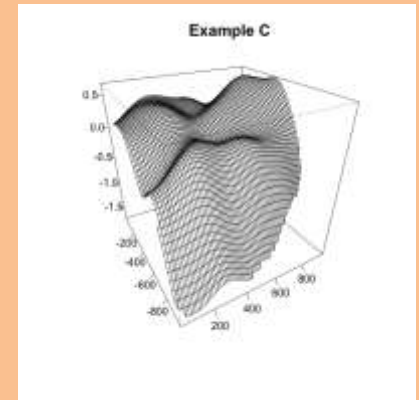
A



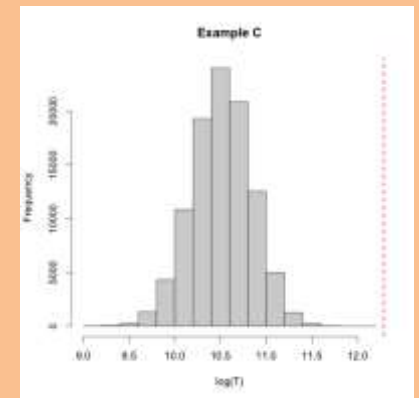
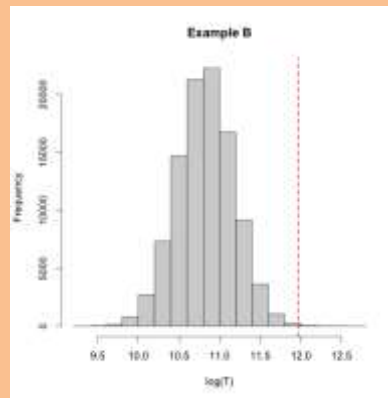
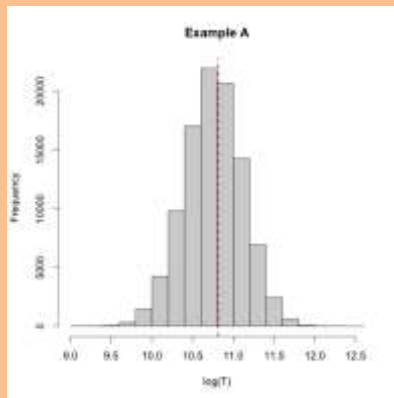
B



C



Kernel smoothed distributions of three samples



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

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

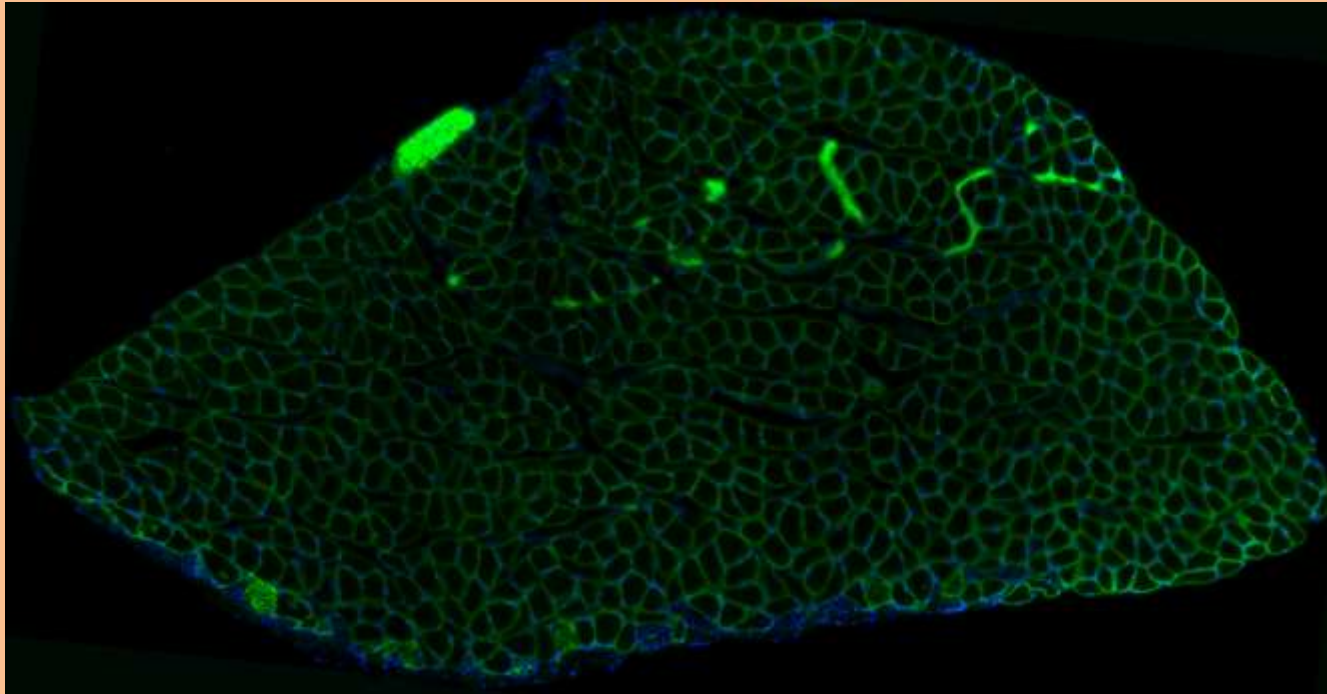


Kathrine Neilsen



Ash Gillon

Age related change in spinal muscles



Cross-section of mouse soleus muscle stained with dystrophin

Sarcopenia:

1. Loss of fibres

2. Loss of fibre size (atrophy)

3. Aggregation of fibres

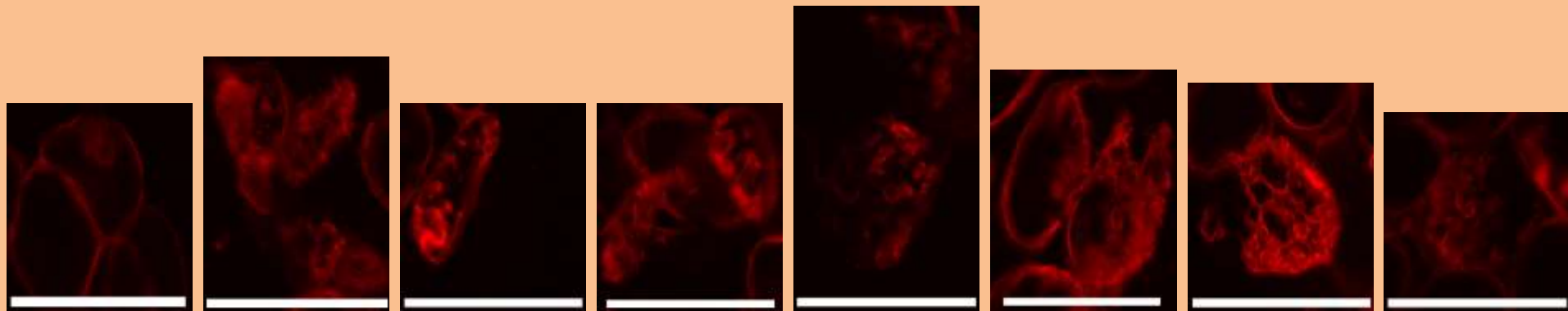


Navneet Lal



DEVILs:

Dystrophin encircled vacuoles & invaginations with intracellular localisation



Scale bar = 100 μ m

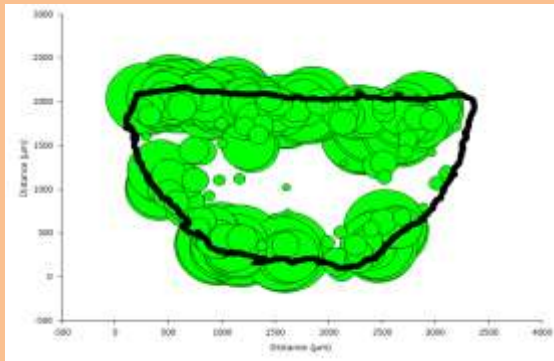
Anti-Dystrophin

Mouse skeletal muscle



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Age related change in spinal muscles

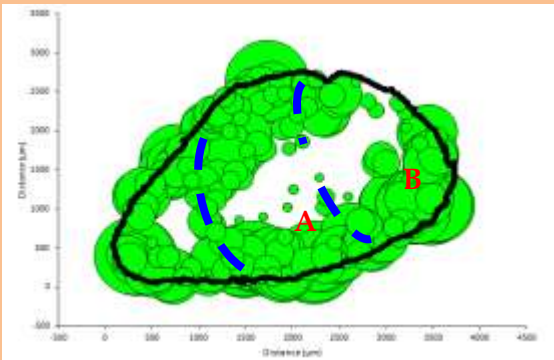


Sarcopenia:

Loss of fibres

Loss of fibre size (atrophy)

Aggregation of fibres

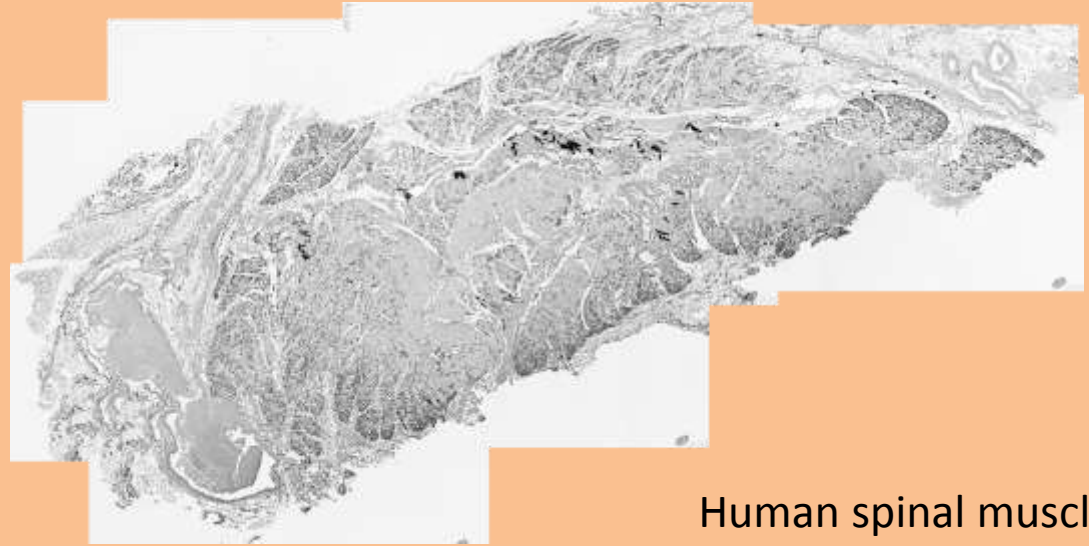
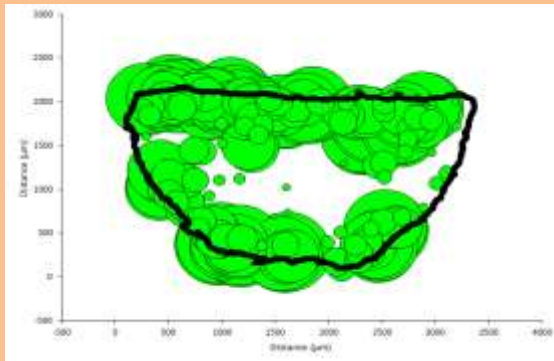


Position of DEVILs within mouse
Soleus and EDL

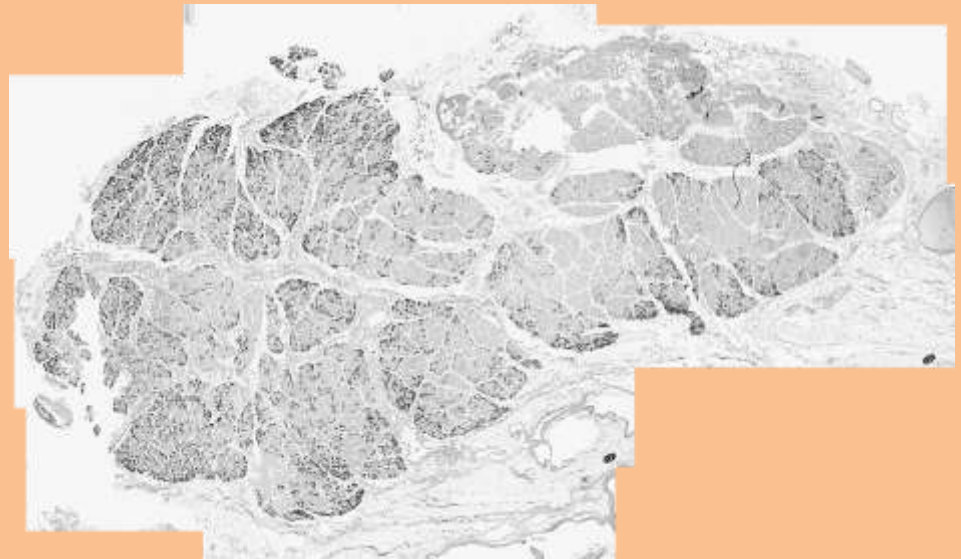
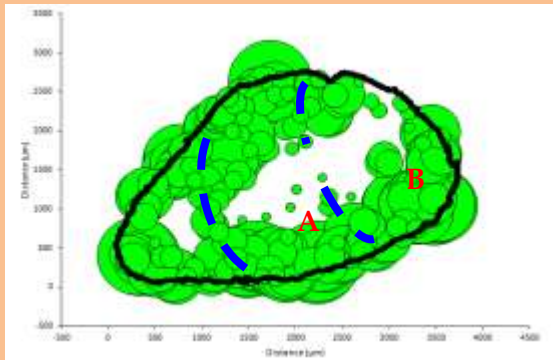


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Age related change in spinal muscles

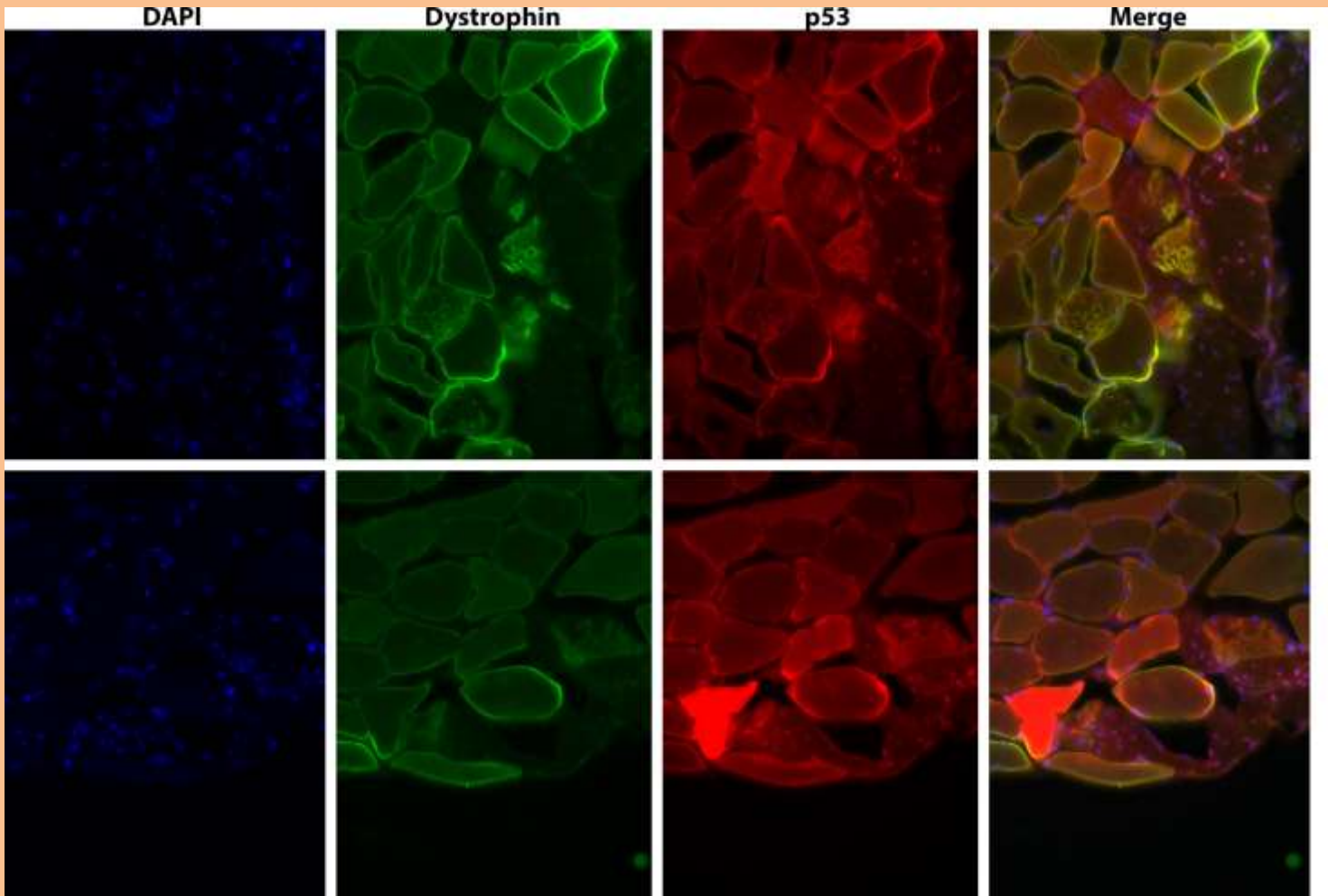


Human spinal muscle

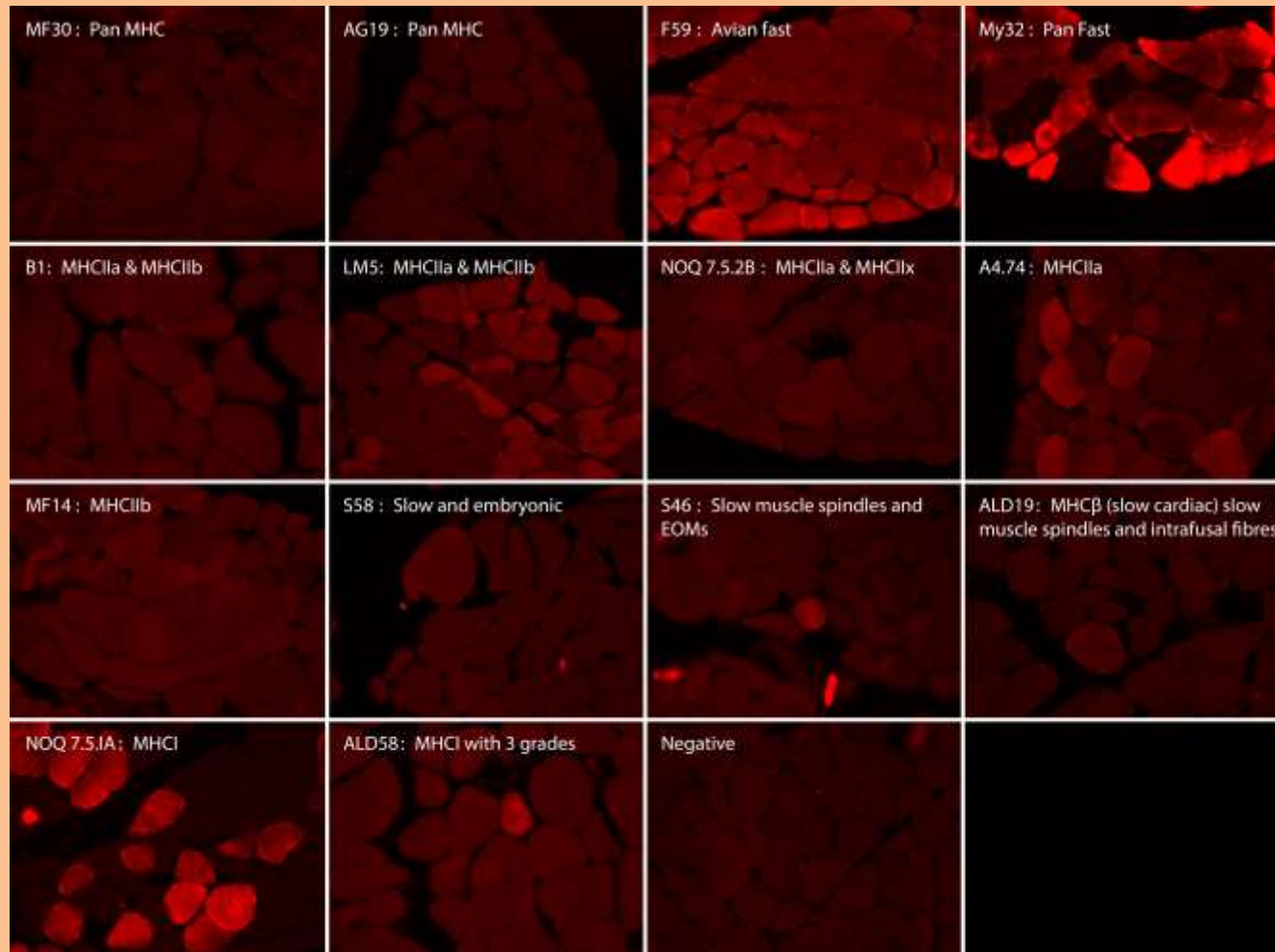


Position of DEVLs within mouse Soleus and EDL

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’

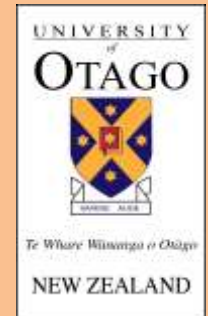
- There is no ‘one’ anatomy textbook that is correct about everything
- Muscle form and function are important yet sometimes poorly understood
- Aging effects all of our skeletal muscle; still little is known about biological mechanisms

Thank you

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