III WORKSHOP APTITUDE PAMPLONA

The ageing muscle: from stem cells to athletic performance

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King's College London

aptitude ACTUAR PARA LA PREVENCIÓN TRANSPIRENAICA DE LA DEPENDENCIA DE LAS PERSONAS MAYORES

Interreg POCTEFA UNIÓN EUROPEA UNION EUROPÉENNE

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Ageing Research at King's (ARK)

The ageing muscle: from stem cells to athletic performance

MRC-ARUK Birmingham 2019

Presentation Outline

Part 1

- The “typical” older muscle, inflammation, cell senescence and the potential role of muscle-derived precursor cells in contributing to this phenotype

Part 2

- Heterogeneity of older phenotypes. Comparing the inactive and active older muscles. What we can learn from master athletes and life-long exercisers

Acknowledgement

Professor Emeritus Norman Lazarus

Steve Harridge

Implications of a progressive age-related decline in muscle function

Modified from Young (1995)

Loss of muscle size and quality in ageing (sedentary)

31 yr

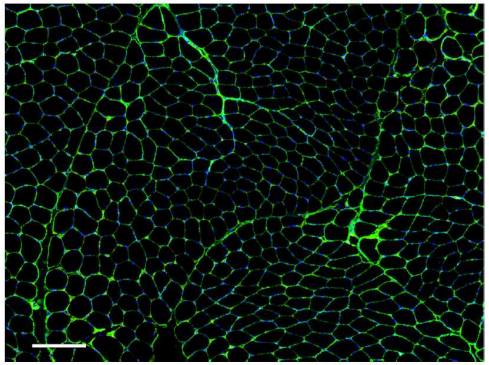
85 yr

Parise & Yarasheski (2000)
Curr Opin Clin Nutr Metab Care,

Sarcopenia

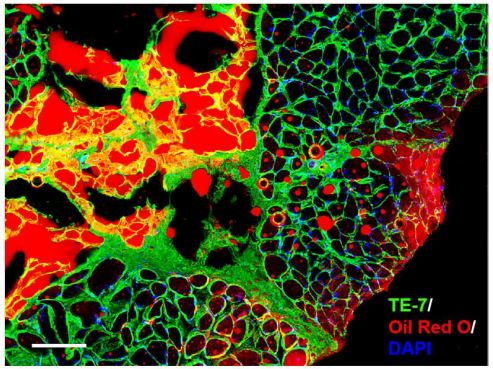
Sarcopenia characterised by:

Young



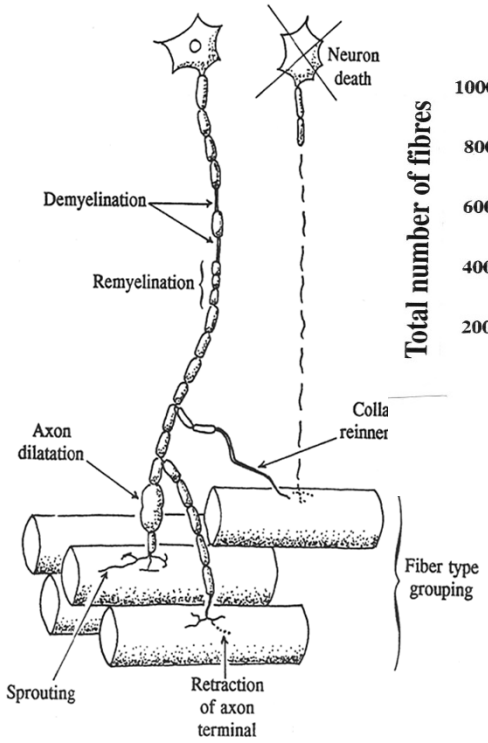
TE-7/ Oil Red O/DAPI

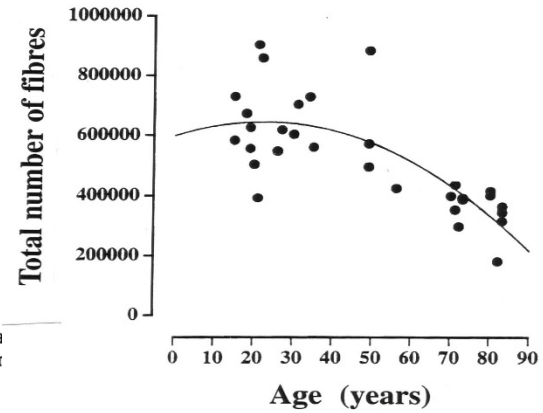
Old



Agley *et al.* 2013

Infiltration of fat and connective tissue (probably an extreme example)





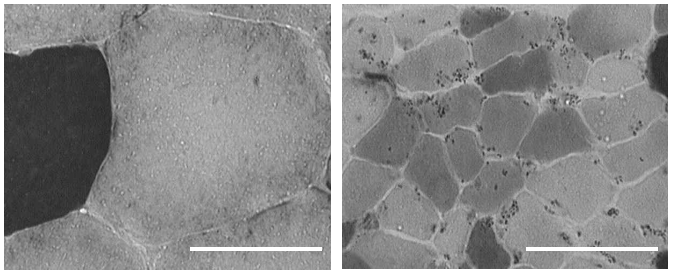
Lexell *et al.* 1988

Motor unit loss & remodelling

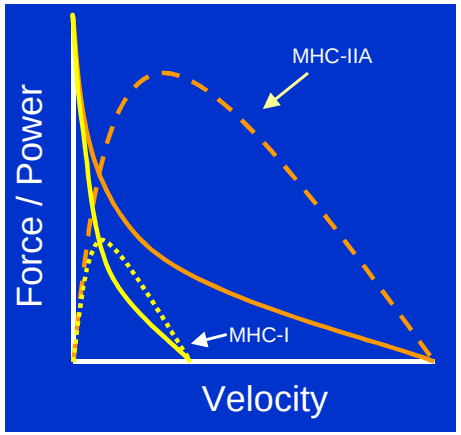
Young

Old

Andersen *et al.* 2003



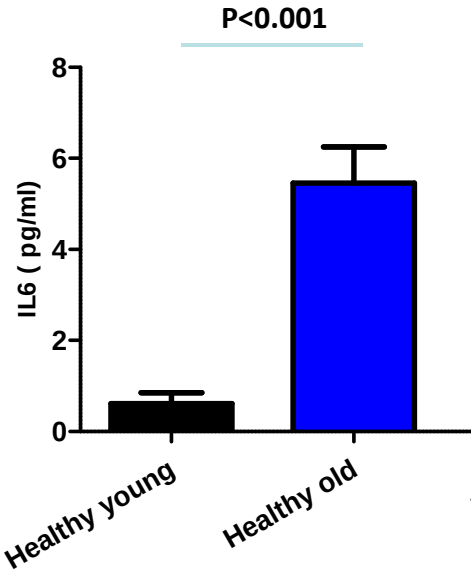
Type II fibre atrophy & reduction in MHC-II isoform expression

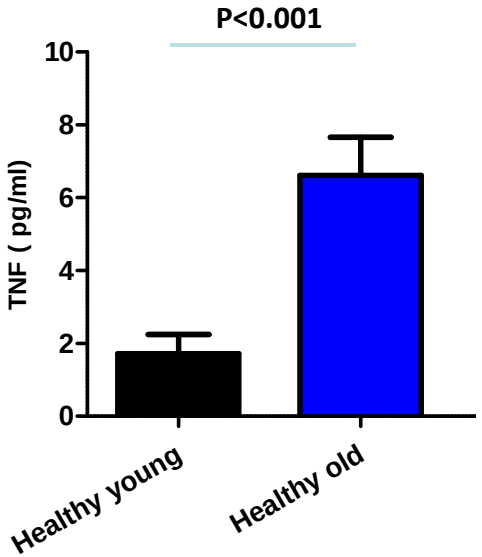


Factors associated with sarcopenia..

- “Anabolic resistance” to feeding and exercise
- Changes in circulating “anabolic” hormones
- Metabolic dysregulation
- Inflammation (“inflammageing”)
- Cellular senescence
- Impaired regeneration from exercise-induced damage

“Inflammageing”

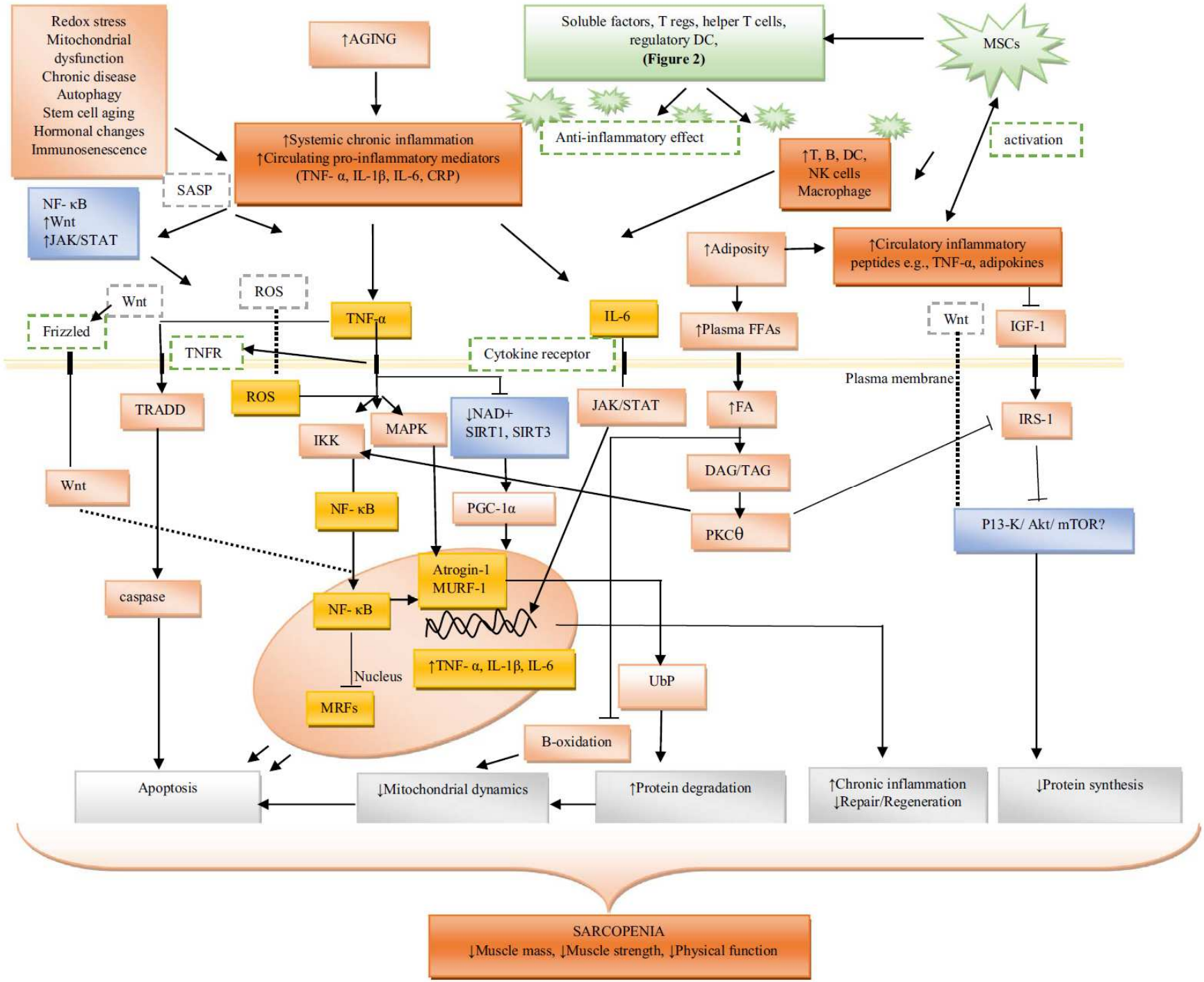




Young 20-36 yrs, n = 55 (30,m 25f)

Healthy Old (sedentary) 57-80yrs, n= 75 (43m, 31f)

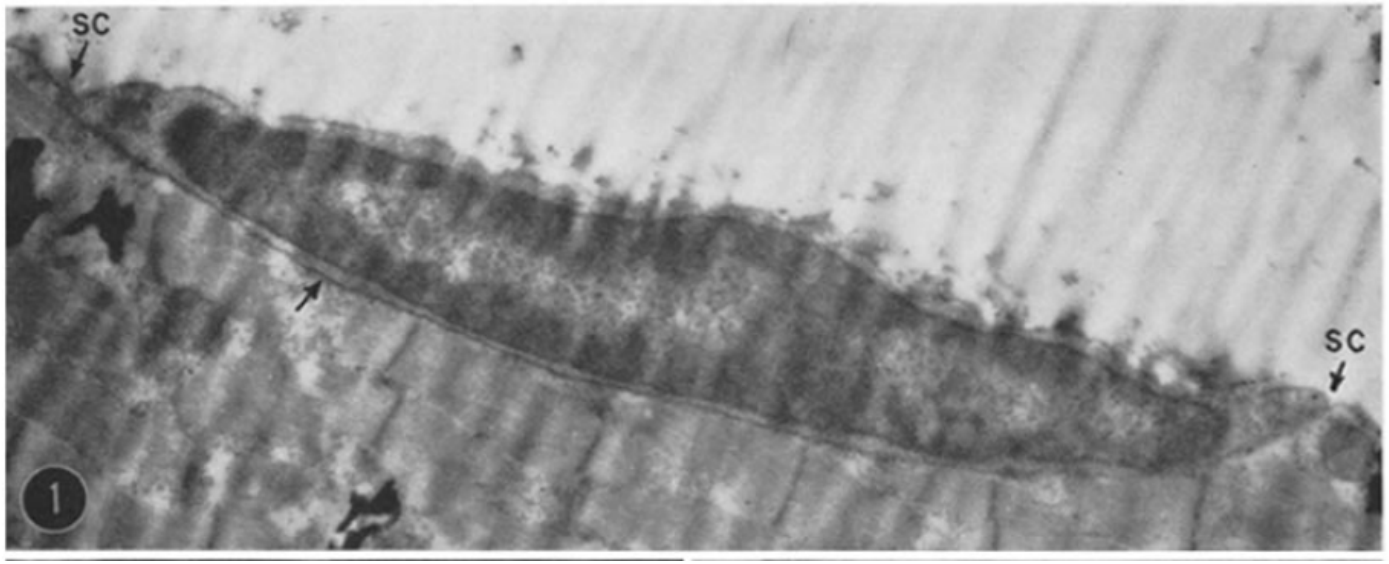
Duggal *et al. (2018)*
Ageing Cell


Redox stress
Mitochondrial dysfunction
Chronic disease
Autophagy
Stem cell aging
Hormonal changes
Immunosenescence
↑AGING
Soluble factors, T regs, helper T cells, regulatory DC, (Figure 2)
MSCs
Anti-inflammatory effect
activation
↑Systemic chronic inflammation
↑Circulating pro-inflammatory mediators
(TNF- α, IL-1β, IL-6, CRP)
↑T, B, DC, NK cells Macrophage
SASP
NF- κB
↑Wnt
↑JAK/STAT
↑Adiposity
↑Circulatory inflammatory peptides e.g., TNF-α, adipokines
Wnt
ROS
TNF-α
IL-6
↑Plasma FFAs
Wnt
IGF-1
Frizzled
TNFR
Cytokine receptor
Plasma membrane
TRADD
ROS
↓NAD+ SIRT1, SIRT3
JAK/STAT
↑FA
IRS-1
IKK
MAPK
DAG/TAG
Wnt
NF- κB
PGC-1α
PKCθ
P13-K/ Akt/ mTOR?
caspase
Atrogin-1 MURF-1
NF- κB
↑TNF- α, IL-1β, IL-6
Nucleus
MRFs
UbP
B-oxidation
Apoptosis
↓Mitochondrial dynamics
↑Protein degradation
↑Chronic inflammation
↓Repair/Regeneration
↓Protein synthesis
SARCOPENIA
↓Muscle mass, ↓Muscle strength, ↓Physical function

Factors associated with sarcopenia..

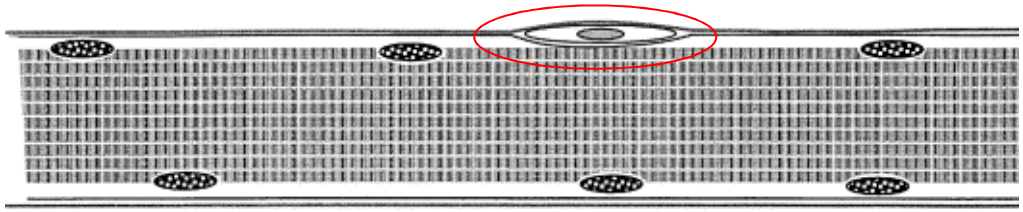
- “Anabolic resistance” to feeding and exercise
- Changes in circulating “anabolic” hormones
- Metabolic dysregulation
- Inflammation (“inflammageing”)
- Cellular senescence
- Impaired regeneration from exercise-induced damage

Satellite cells – the muscle's resident stem cells



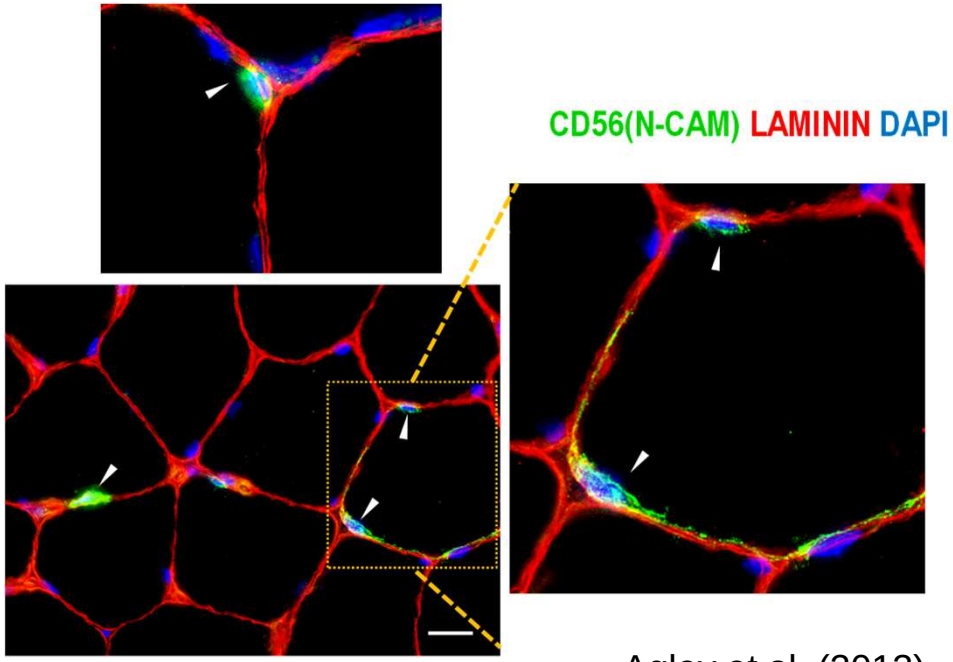
Mauro 1961

Identification of human satellite cells …

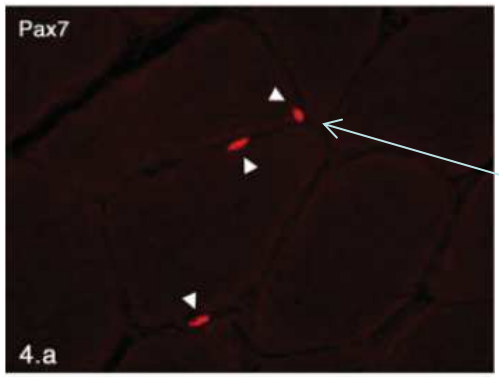


Quiescent satellite cell

Expresses: Pax7, NCAM/CD56

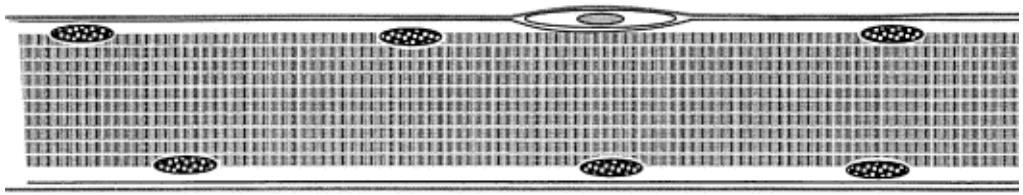


Agley et al. (2013)



Mackey et al. (2010)

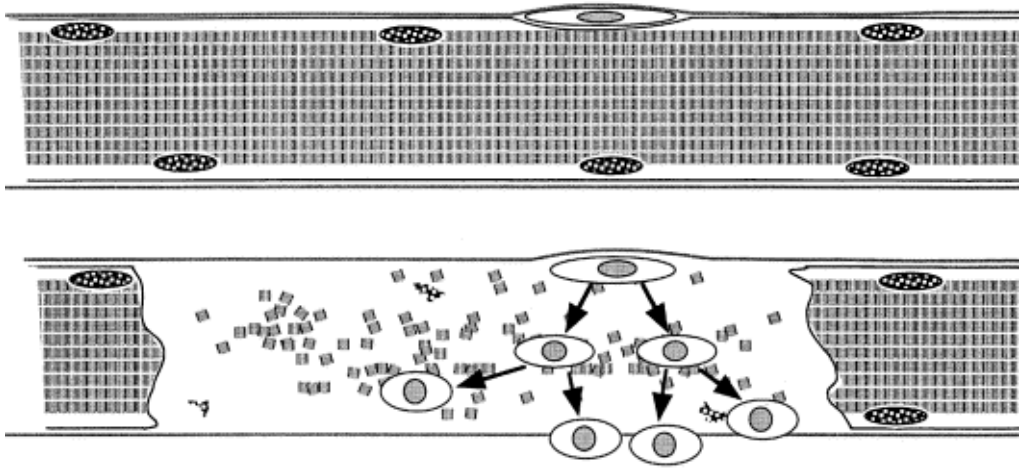
satellite cells →



Quiescent satellite cell
Expresses: Pax 7, CD56 (NCAM), Myf5

Morgan & Partridge (2003)

satellite cells → proliferating myoblasts (muscle precursor cells)

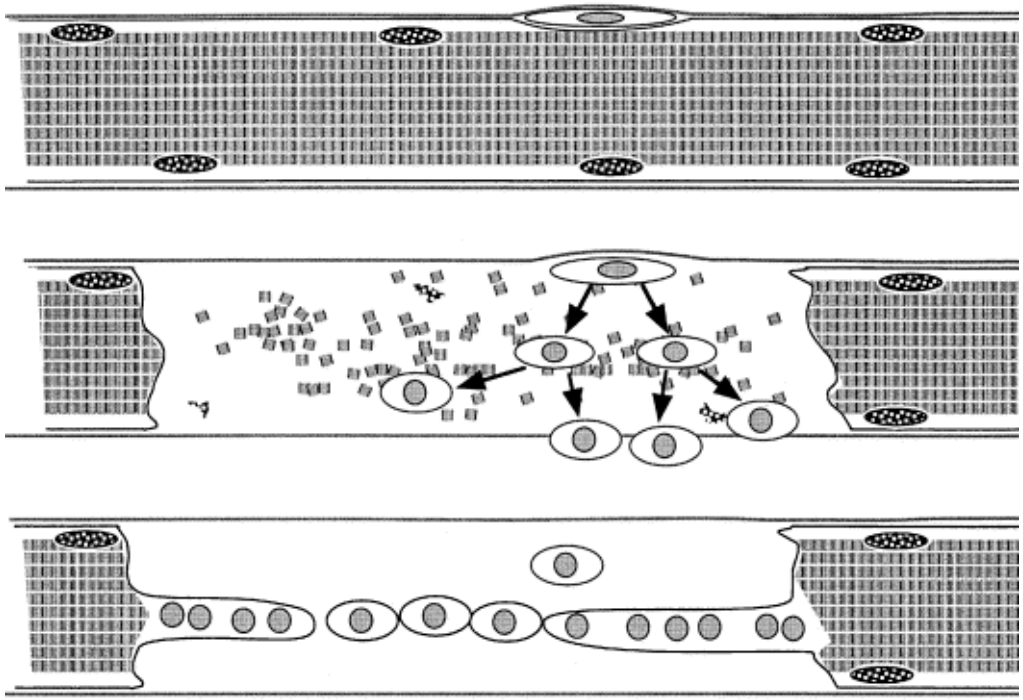


Quiescent satellite cell
Expresses: Pax 7, CD56 (NCAM), Myf5

Activated Satellite cell –
(proliferation and commitment)
Expresses: Pax7, MyoD, Myf5, Desmin

Morgan & Partridge (2003)

satellite cells → proliferating myoblasts (muscle precursor cells) → fusion & differentiation...



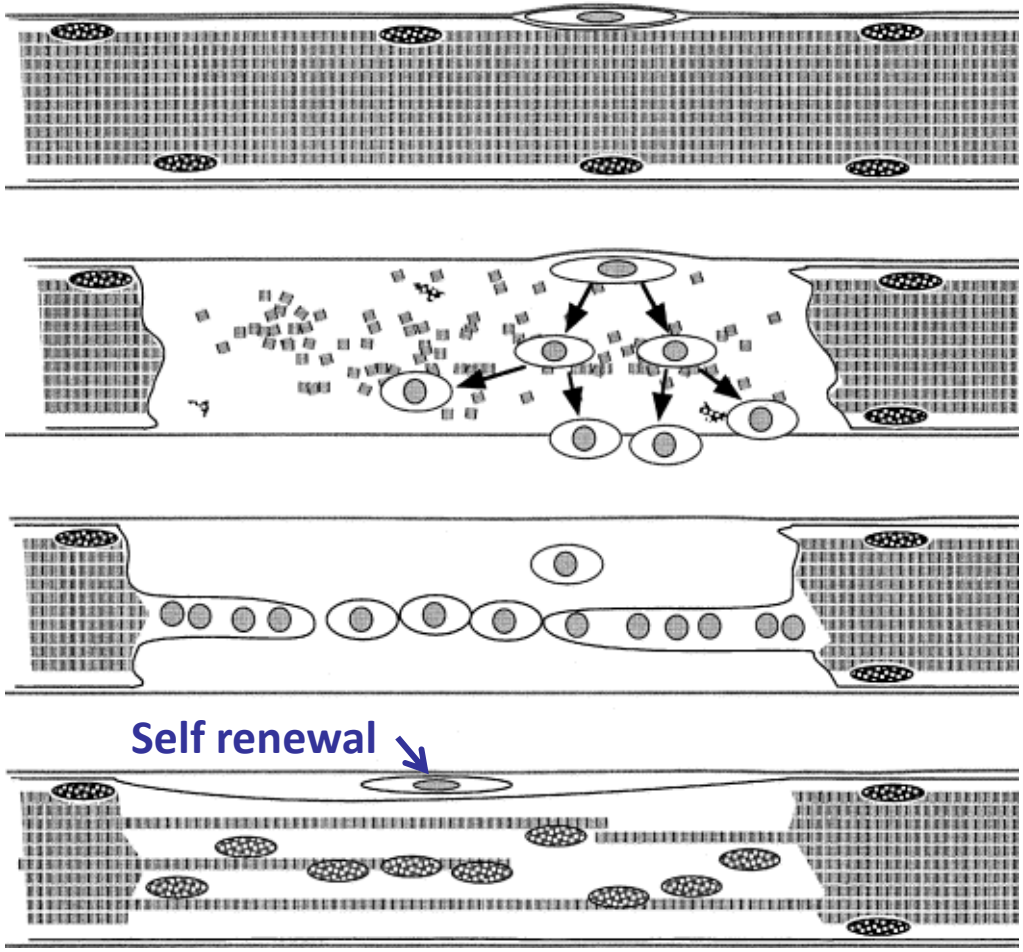
Quiescent satellite cell
Expresses: Pax 7, CD56 (NCAM), Myf5

Activated Satellite cell –
(proliferation and commitment)
Expresses: Pax7, MyoD, Myf5, Desmin

Fusion
Expresses: CD56, MyoD, MRF4, Desmin, Myogenin,

Morgan & Partridge (2003)

satellite cells → proliferating myoblasts (muscle precursor cells) → fusion & differentiation...



Quiescent satellite cell
Expresses: Pax 7, CD56 (NCAM), Myf5

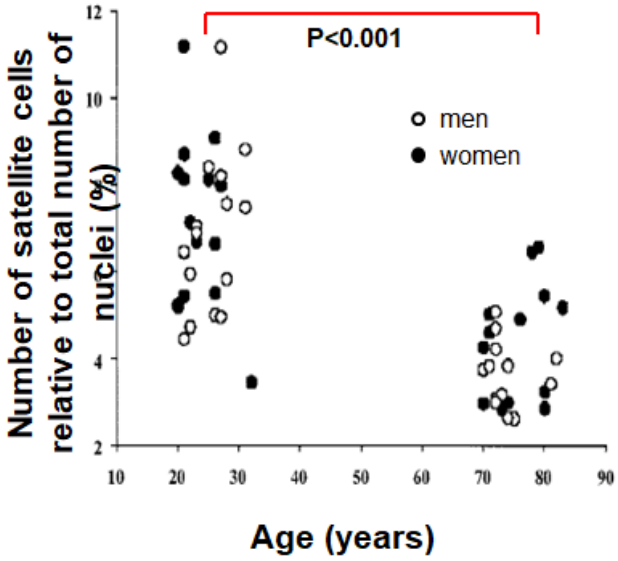
Activated Satellite cell – (proliferation and commitment)
Expresses: Pax7, CD56, MyoD, Myf5, Desmin

Fusion
Expresses: CD56, MyoD, MRF4, Desmin, Myogenin,

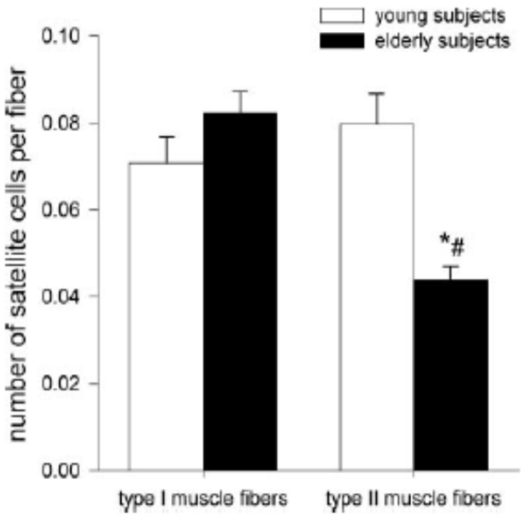
Terminal differentiation - (in culture forming myotubes)
Expresses: CD56, Desmin, myogenin and MHC

Morgan & Partridge (2003)

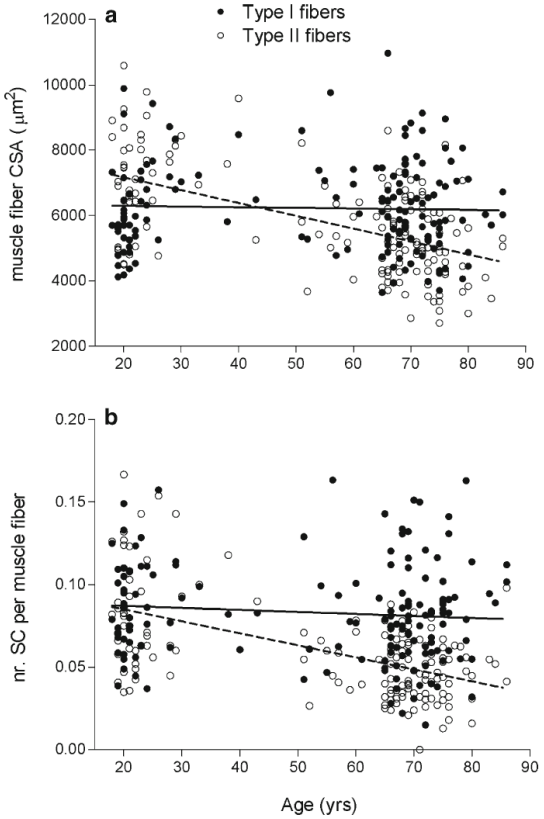
Are there fewer satellite cells in older muscle?



Charifi et al. (2004)

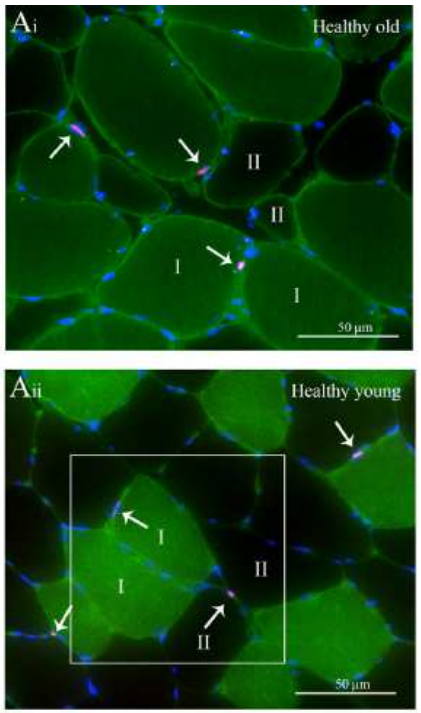


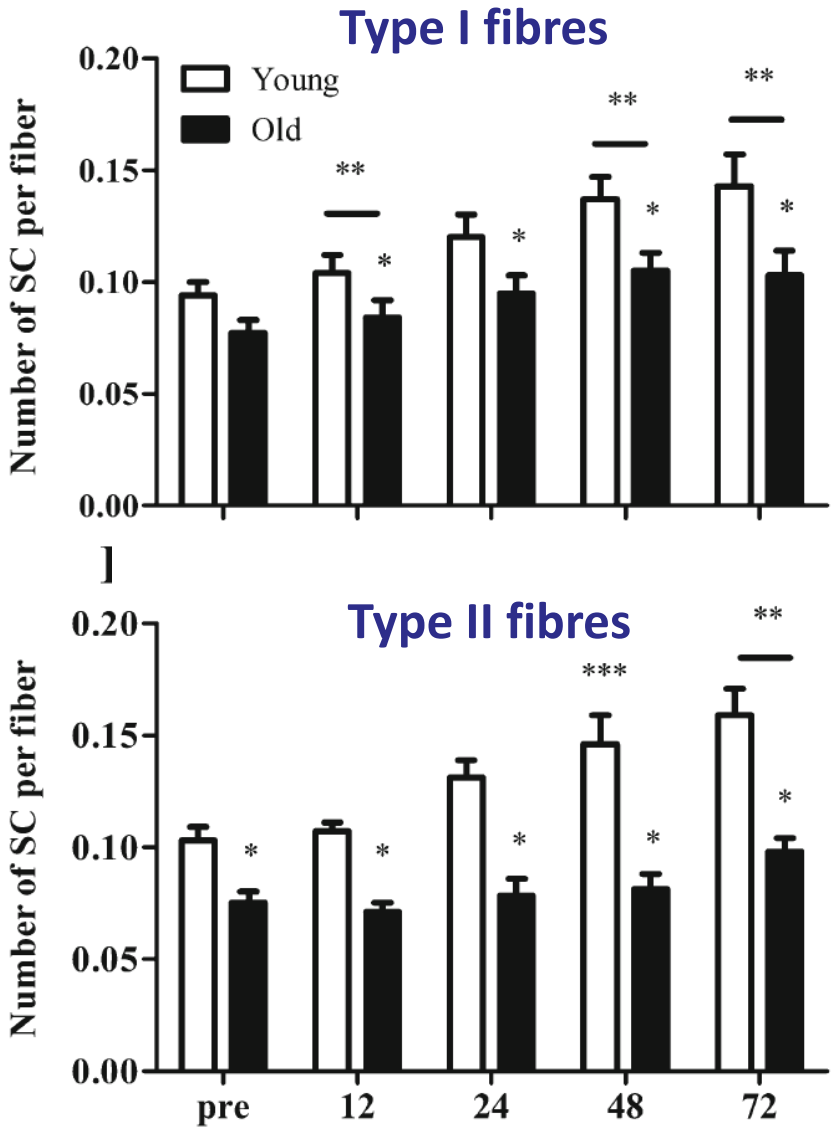
Verdijk et al. (2007)



Verdijk et al. (2014)

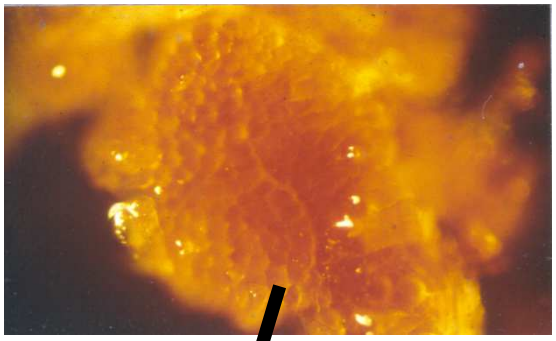
Delayed increase in satellite cell number in older men following a single bout of high resistance exercise





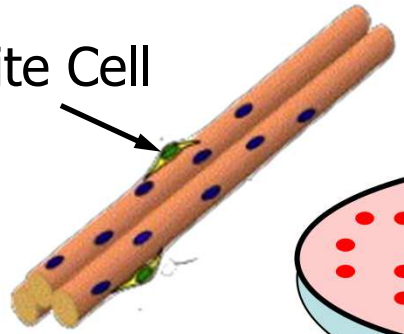
Snijdjers et al (2014)
Age

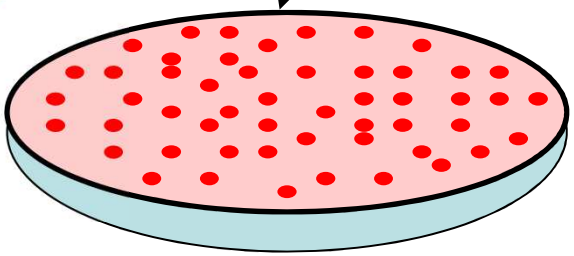
Primary human muscle cell culture



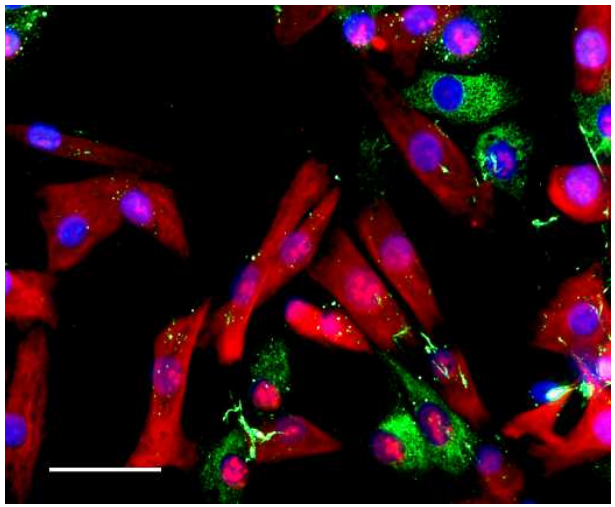
Enzyme Digest

Satellite Cell





Mixed cell population

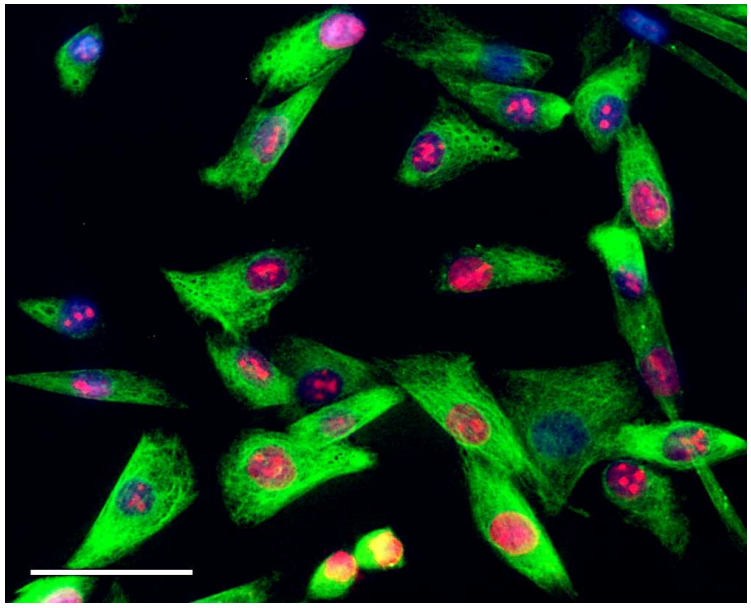


DESMIN/HOECHST/TE-7/KI67

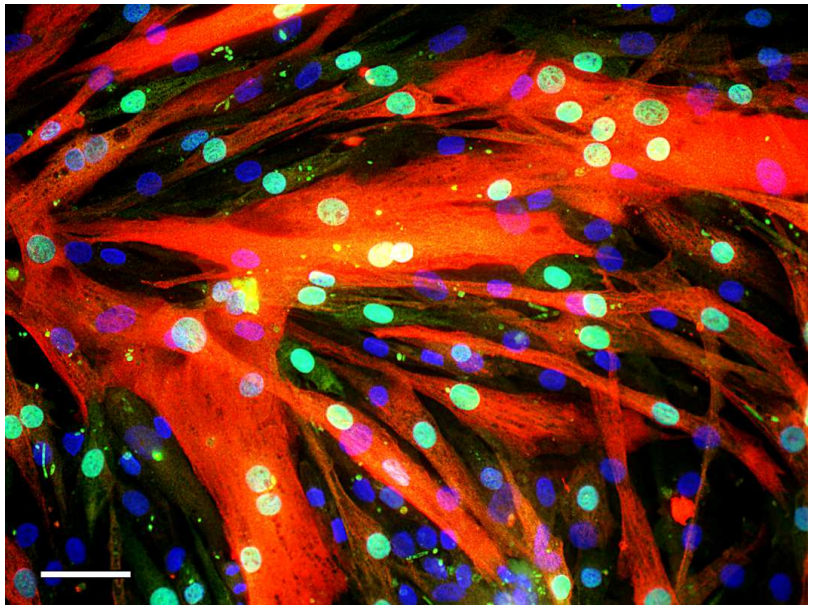
Myoblasts Fibroblasts

MPCs → myotubes

Proliferation → differentiation

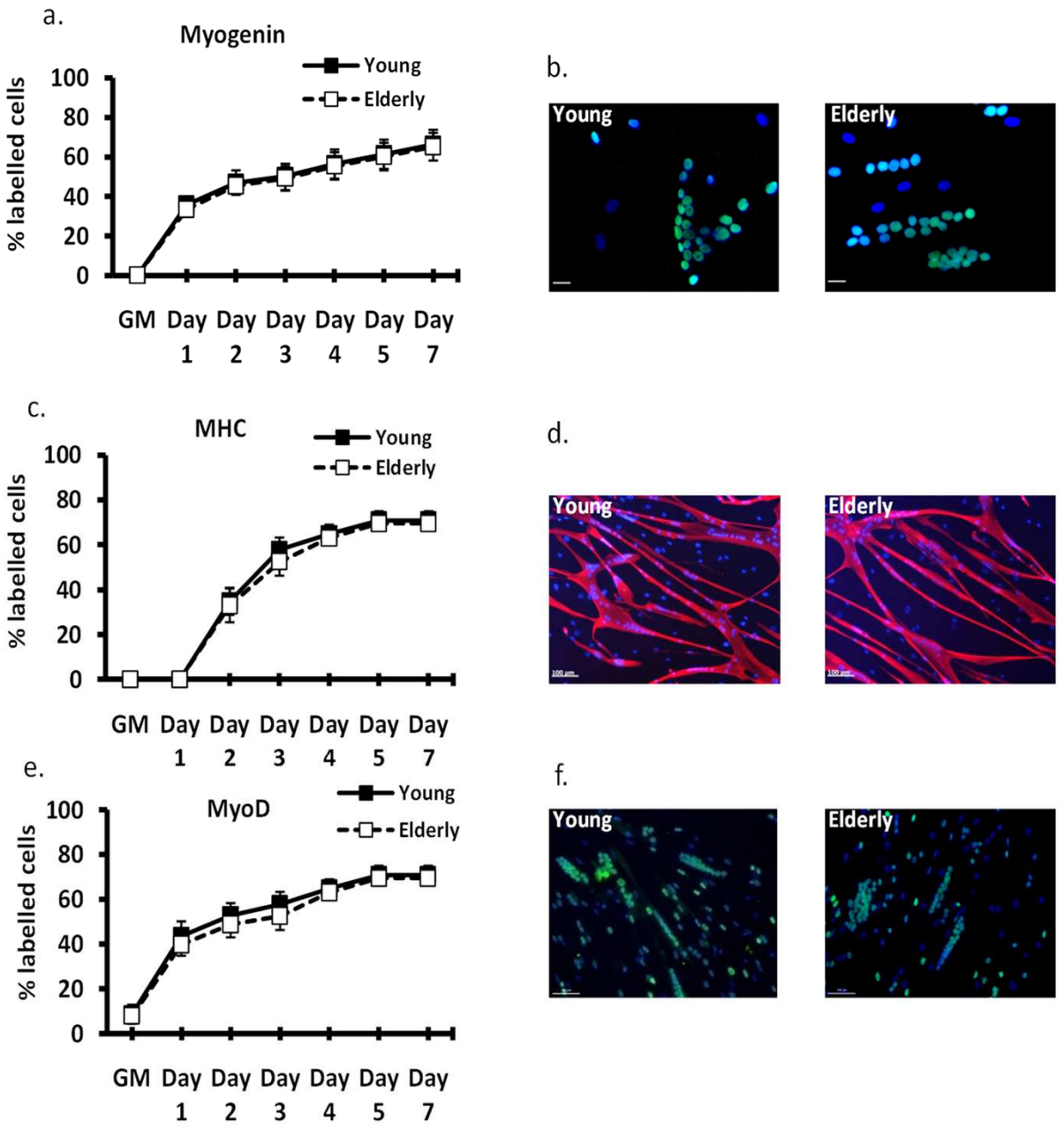


Desmin Ki67 Hoechst



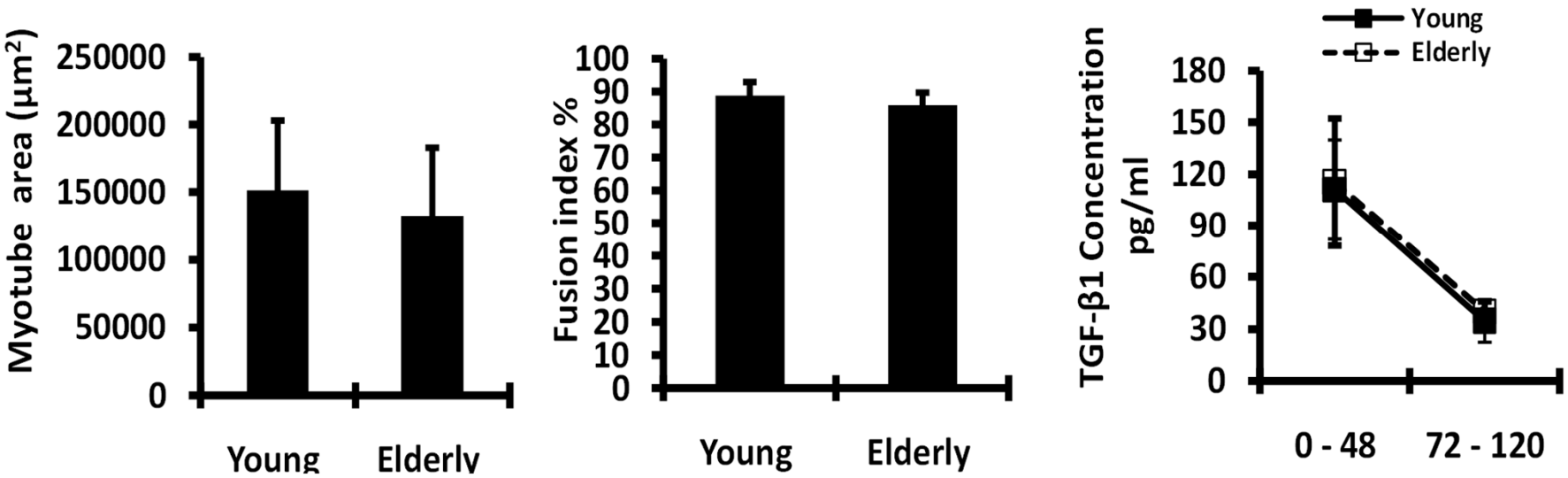
MHC Myogenin Hoechst

Differentiation: Similar responses in cells obtained from young and old people



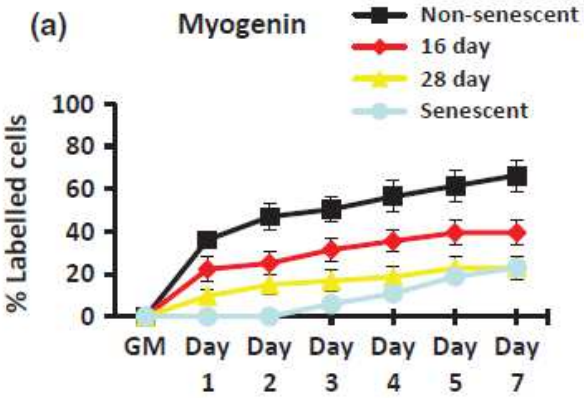
Alsharidah et al. *(2013)* *Aging Cell*

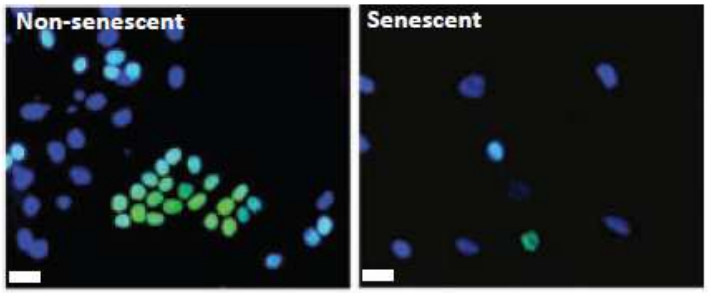
Differentiation:
Similar responses in cells obtained from young and old people

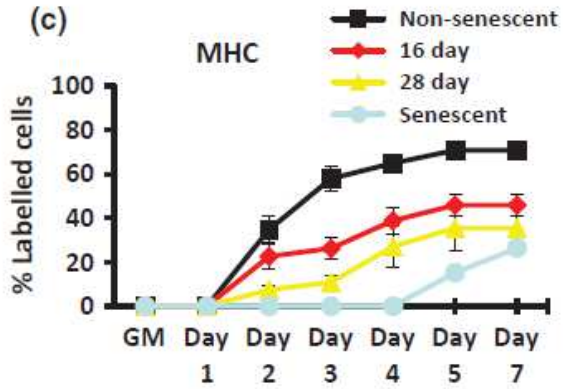


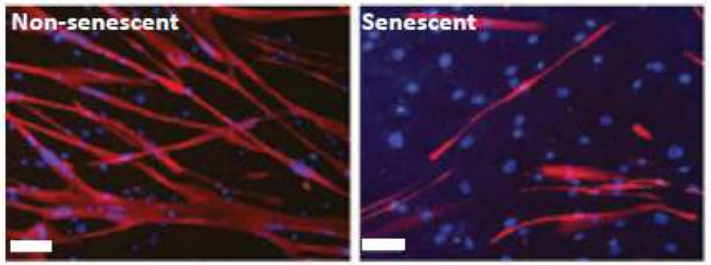
Alsharidah et al. *(2013)*
Aging Cell

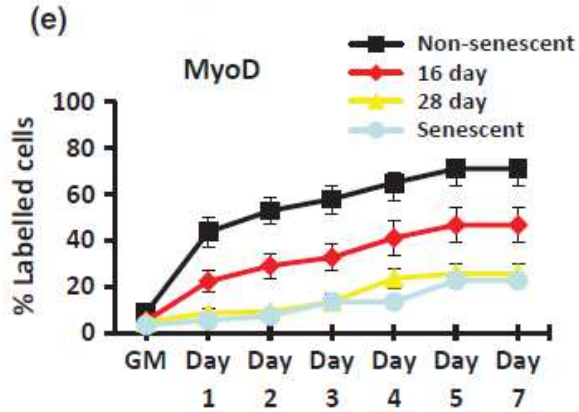
Differentiation
Senescent cells have impaired ability to differentiate

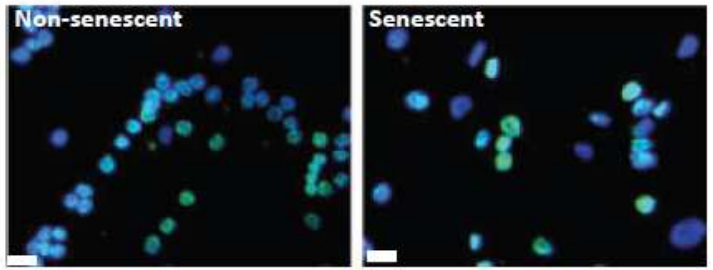






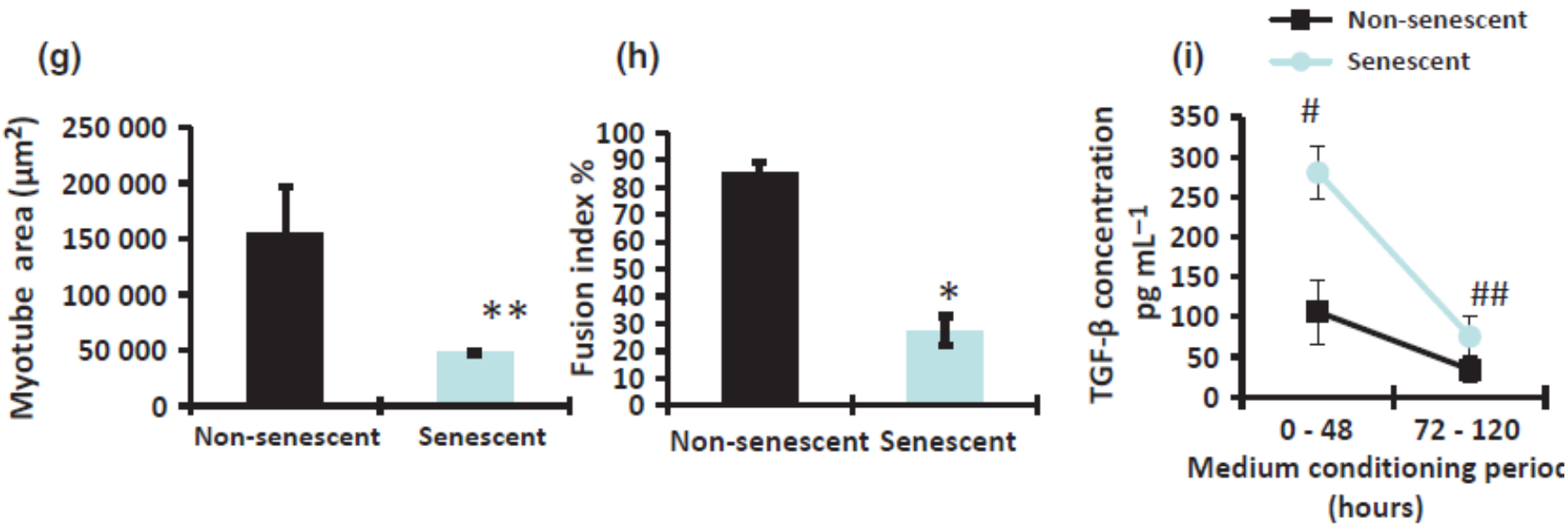






Alsharidah et al. *(2013)*
Aging Cell

Differentiation:
Impaired responses
in senescent cells



Alsharidah et al. *(2013)*
Aging Cell

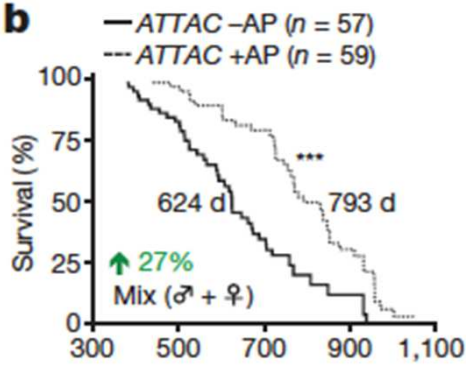
ARTICLE

doi:10.1038/nature16932

Naturally occurring p16^{Ink4a}-positive cells shorten healthy lifespan

Darren J. Baker[1], Bennett G. Childs[2], Matej Durik[1], Melinde E. Wijers[1], Cynthia J. Sieben[2], Jian Zhong[1], Rachel A. Saltness[1], Karthik B. Jeganathan[1], Grace Casaclang Verzosa[3], Abdulmohammad Pezeshki[4], Khashayarsha Khazaie[4], Jordan D. Miller[3] & Jan M. van Deursen[1,2]

Induction of apoptosis in p16^{Ink4a} (senescent cells) improves lifespan and healthspan

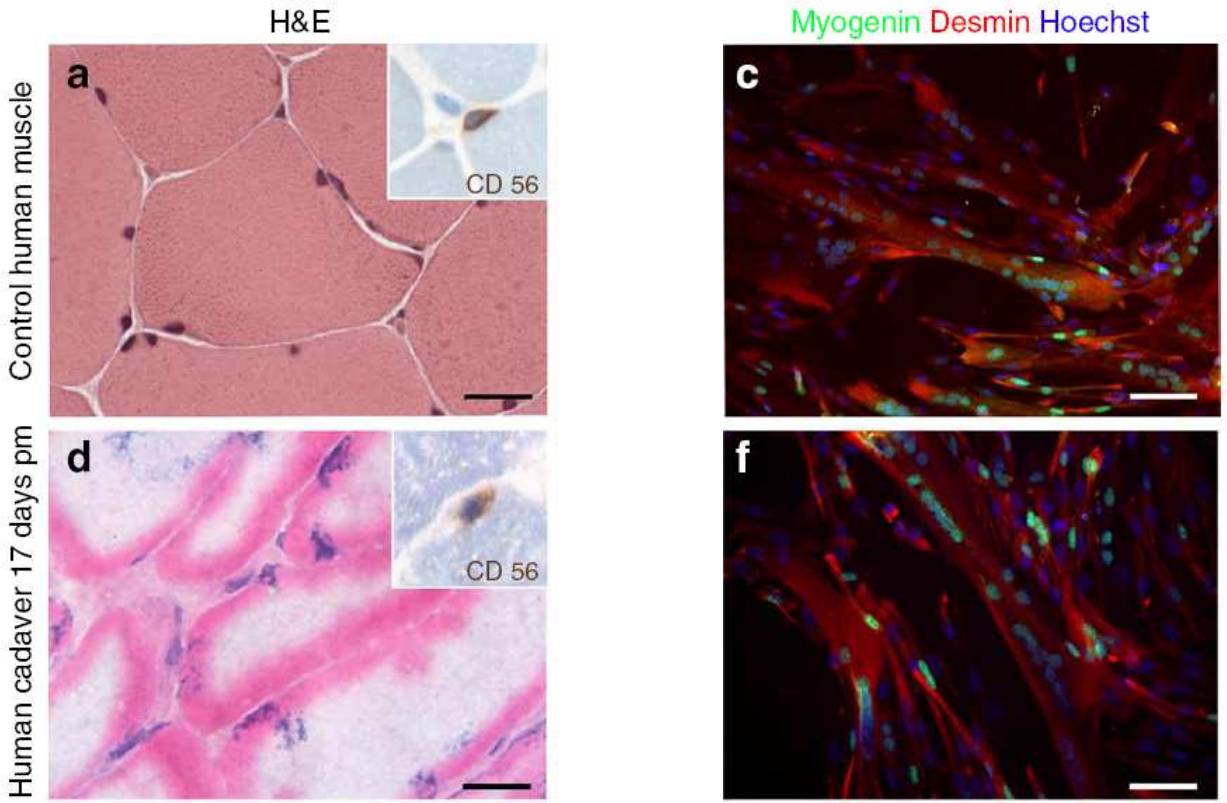


Baker et al., 2016

Ageing & human muscle precursor cells

1) **Is there an inherent ageing effect on the myogenic properties of human MPCs?**
   - ***No, but senescence in culture creates a distinct and impaired phenotype***

Functioning satellite cells obtained from a 96 yr old - 17 days after death...

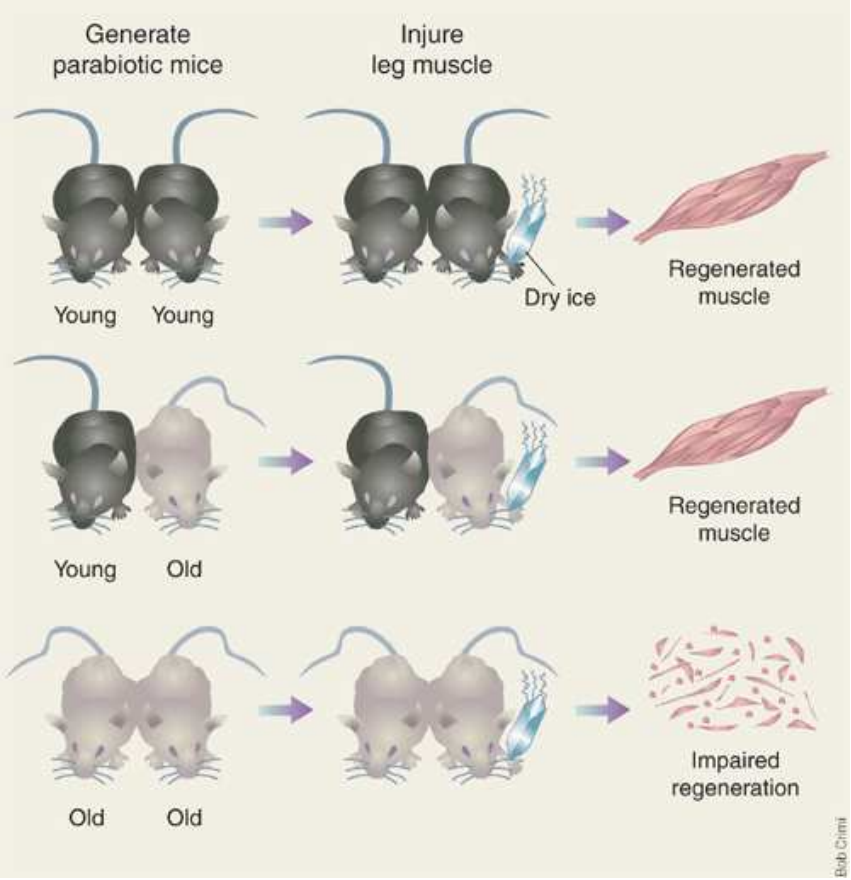


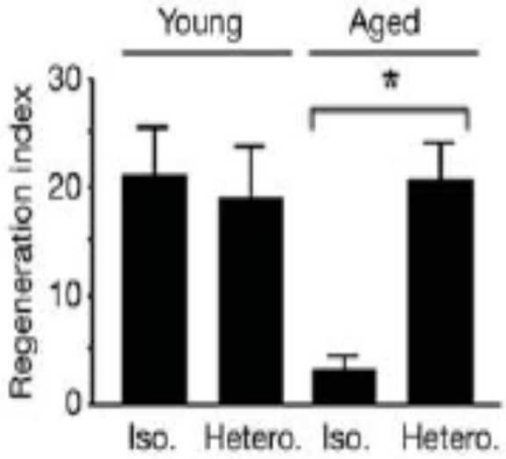
Latil *et al.* (2012)
Nature Communications

Ageing & human muscle precursor cells

1) **Is there an inherent ageing effect on the myogenic properties of human MPCs?**
   - ***No, but senescence in culture creates a distinct and impaired phenotype***
2) **Do environmental factors influence human MPC behaviour?**

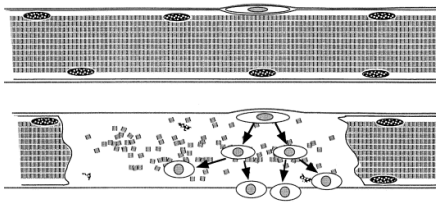
Parabiotic mice – conjoined circulation

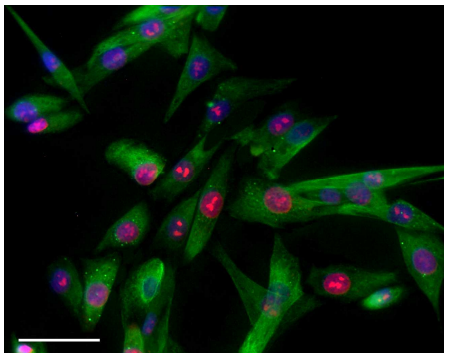




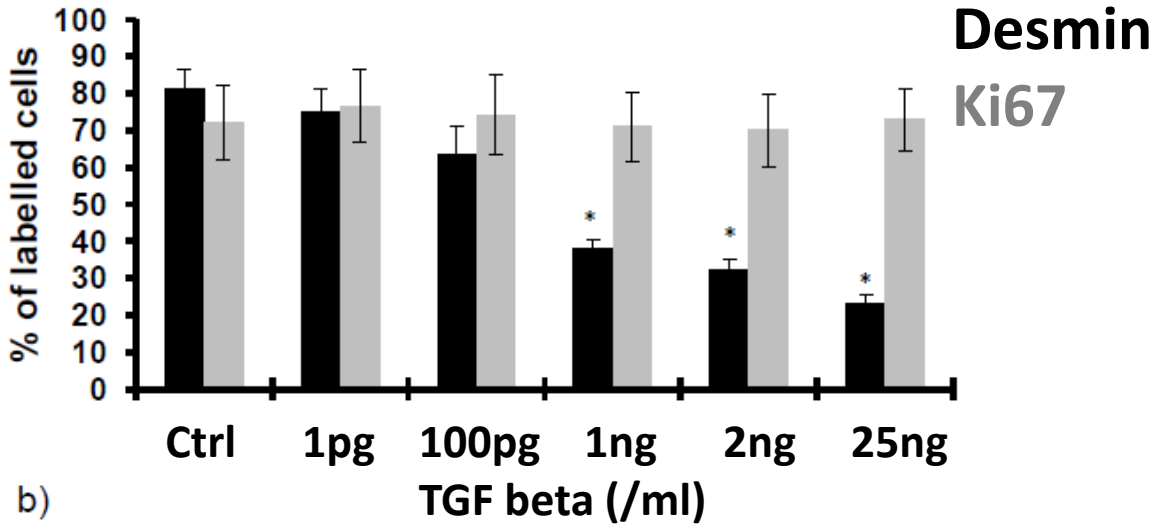
Conboy *et al.* (2005)

Inflammatory cytokines inhibit the progression of CD56^{+ve} cells to myoblasts (expressing desmin)

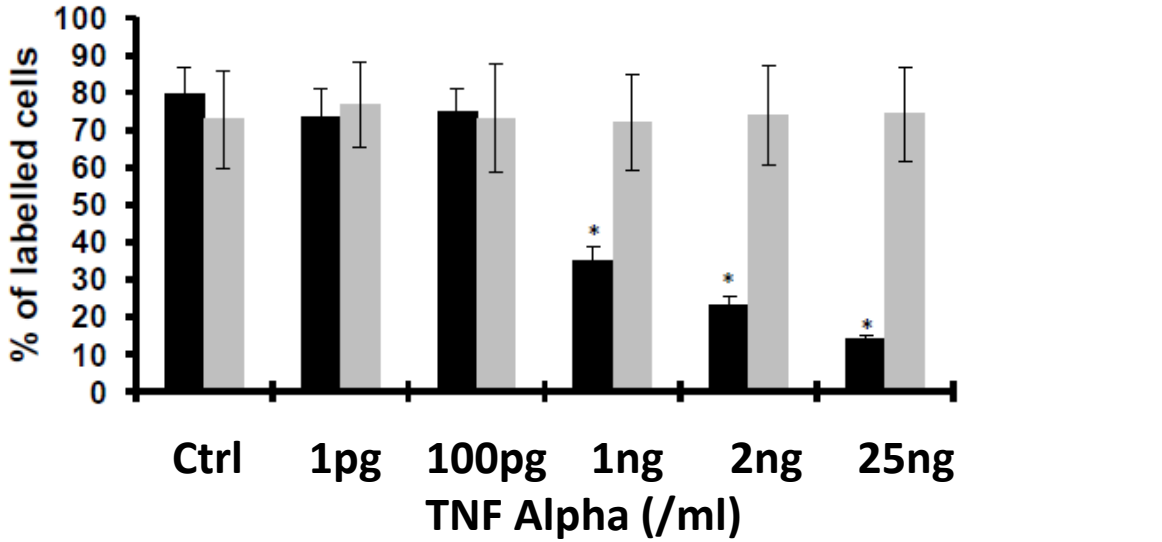




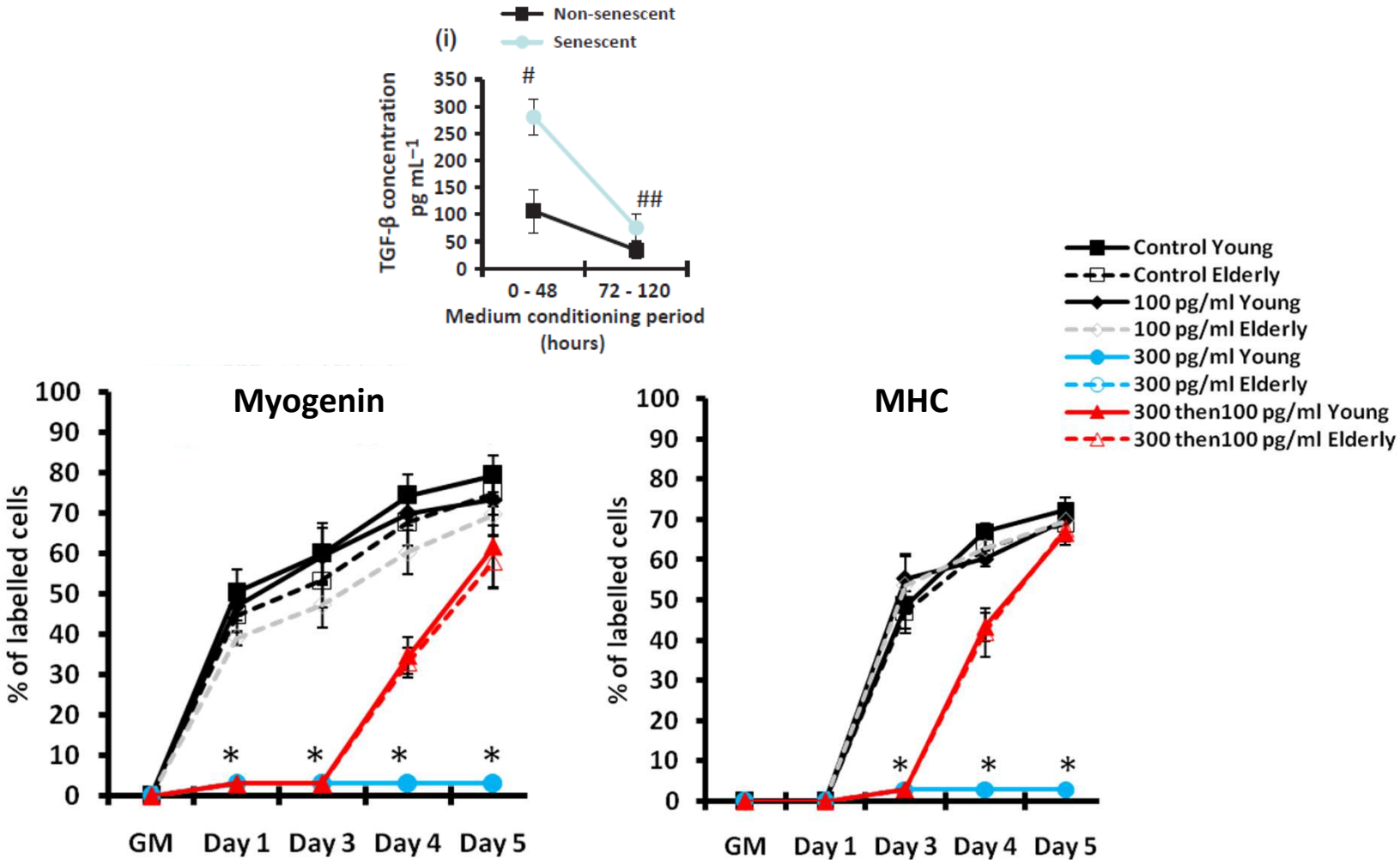
Desmin Ki67 Hoechst



b)



TGF-β1 inhibits differentiation in both young and old human cells at concentrations secreted by senescent cells



Alsharidah et al. *(2013)*
Aging Cell

Ageing & human muscle precursor cells

1) **Is there an inherent ageing effect on the myogenic properties of human MPCs?**
   - ***No, but senescence in culture creates a distinct and impaired phenotype***
2) **Do environmental factors influence human MPC behaviour?**
   - ***Yes, e.g. inflammatory cytokines (which can be released from muscle cells) impair behaviour***

Presentation Outline

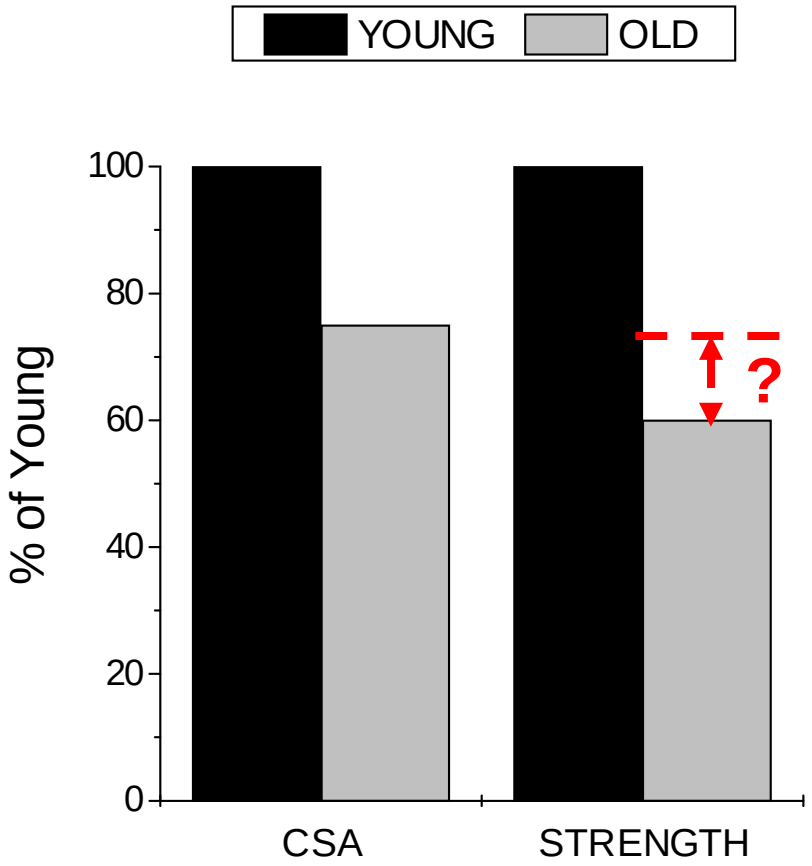
Part 1

- The “typical” older muscle, inflammation, cell senescence and the potential role of muscle-derived precursor cells in contributing to this phenotype

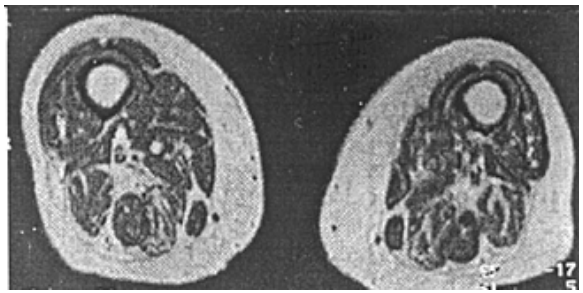
Part 2

- Heterogeneity of older phenotypes. Comparing the inactive and active older muscles. What we can learn from master athletes and life-long exercisers

Specific Force Loss (sedentary)



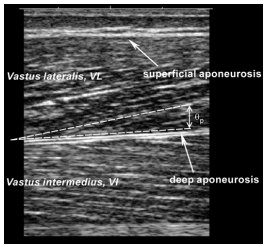
The decline in maximum voluntary isometric knee extensor force (strength) can be greater than the decline in quadriceps CSA

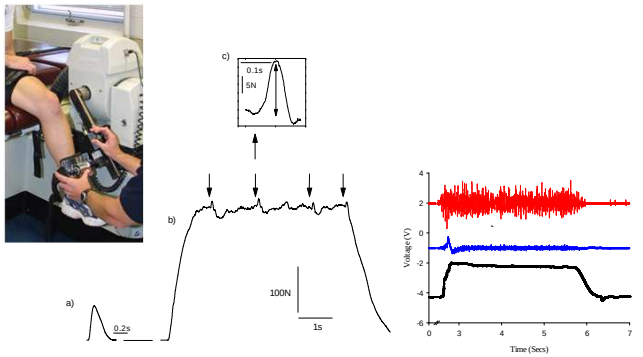


Young *et al.* (1985)

How strong is healthy human muscle *in vivo*?

- Accounting for:
  - Accurately determined muscle CSA
    - MRI for lean, anatomical cross-sectional area
    - Ultrasound for determination of muscle architecture (pennation angles) to calculate physiological CSA
  - Objective measures of function (torque)
    - Full activation of agonists (confirmed or corrected for using twitch interpolation)
    - Corrected for antagonist co-contraction
  - Calculation of force applied directly to the tendon form external torque
    - Correction applied from measurement (through MRI) of internal level systems

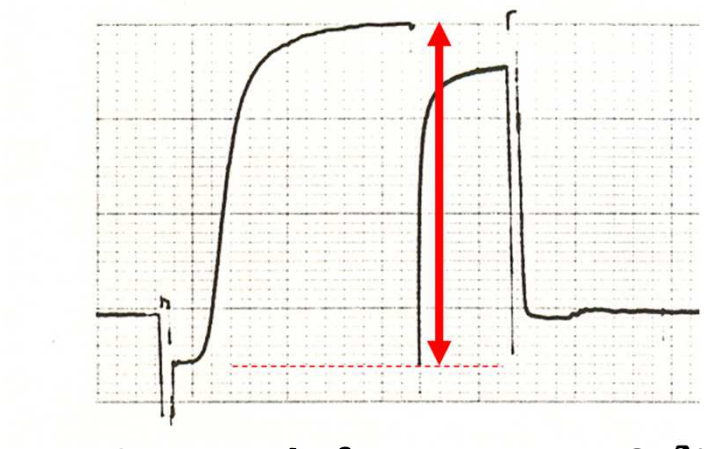




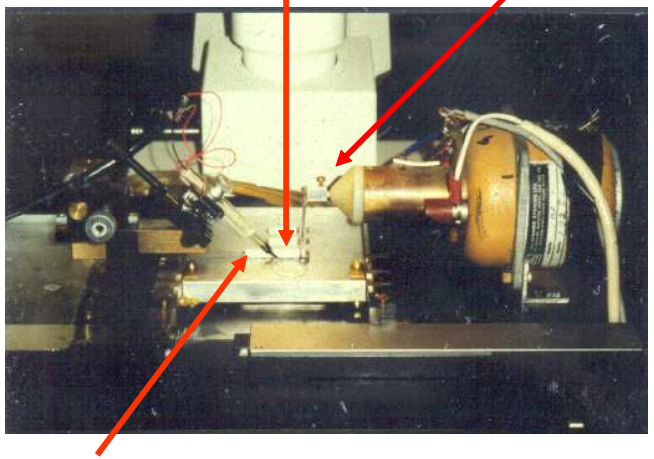
= ~55N/cm²

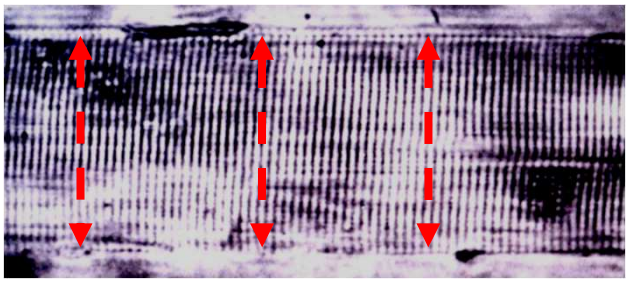
O'brien *et al.* (2010)

Isometric force in human skinned fibres



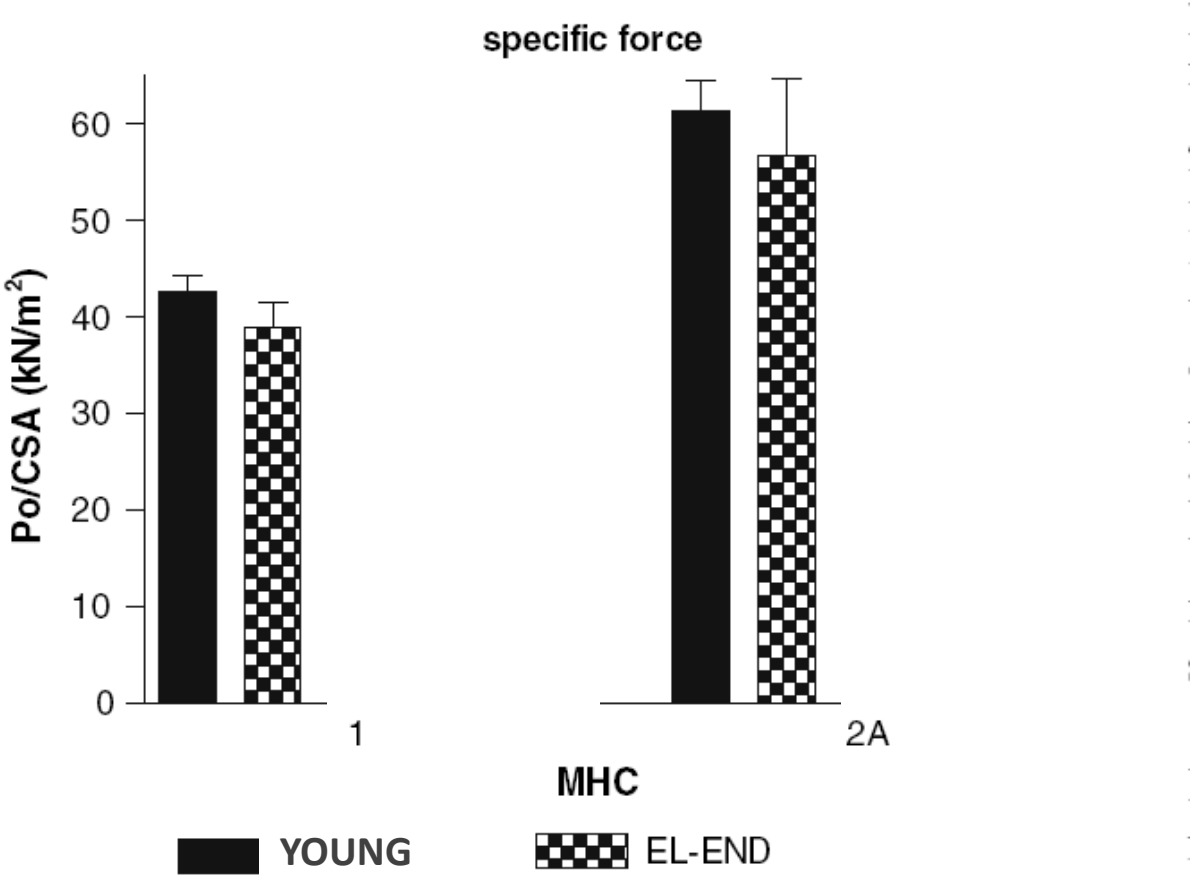
Isometric force at max Ca^{2+} activation





Measurement of fibre diameter for calculation of CSA

↓ specific force related to ↓activity not ageing *per se*



D'Antona e*t al.* (2007)

“Ageing” or the study of “older people”?

Optimal Biological Ageing

Most studies of ageing err in this direction

Exercise Continuum

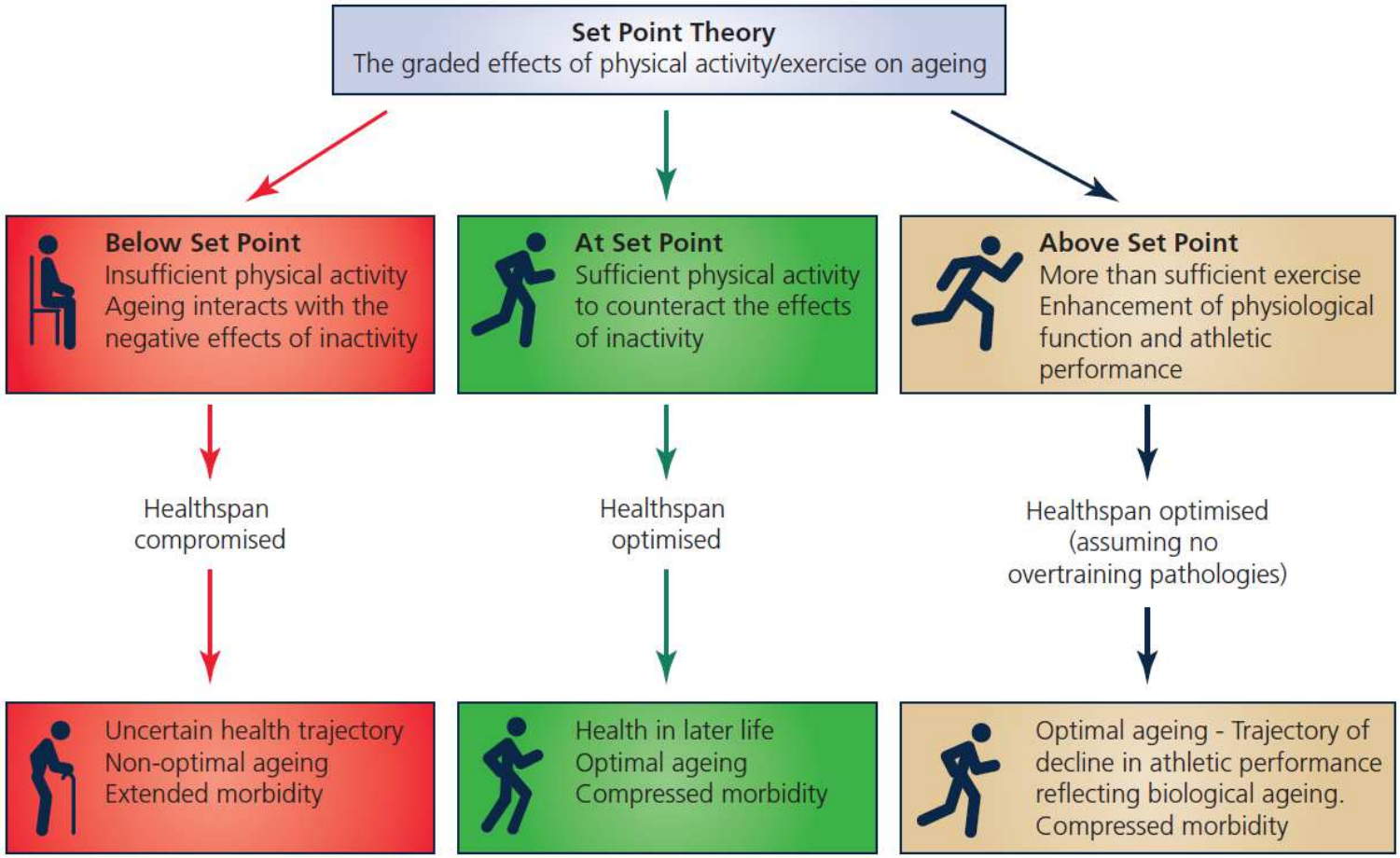
Ageing + disuse ≠ ageing

Inactivity is deleterious to health and contaminates many studies of ageing. As such it confounds our understanding of the inherent biological ageing process

Declining performance of master athletes: silhouettes of the trajectory of healthy human ageing?

Norman R. Lazarus and Stephen D. R. Harridge

Centre of Human and Aerospace Physiological Sciences, King's College London, London, UK



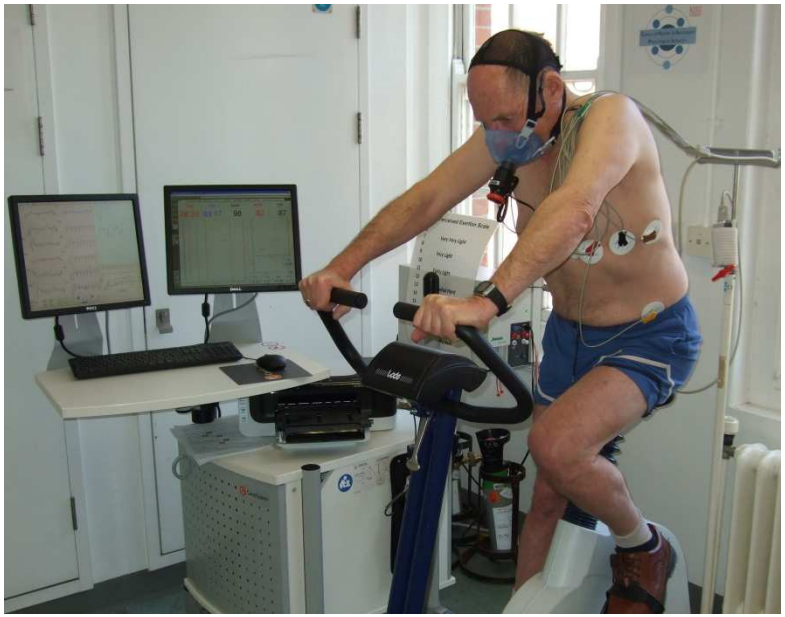
The Journal of Physiology

Optimally Ageing Phenotypes

125 amateur cyclists (aged 55-79 years)

Cyclists were:

- non-elite (amateur road cyclists primarily from Audax)
- Entry criteria
  - Males 100km < 6.5 hrs
  - Females 60km < 5.5 hrs
  - 2 rides completed 3 weeks prior to testing
- Average monthly cycling distance
  - 725km (males)
  - 600km (females)
  - No decline with age



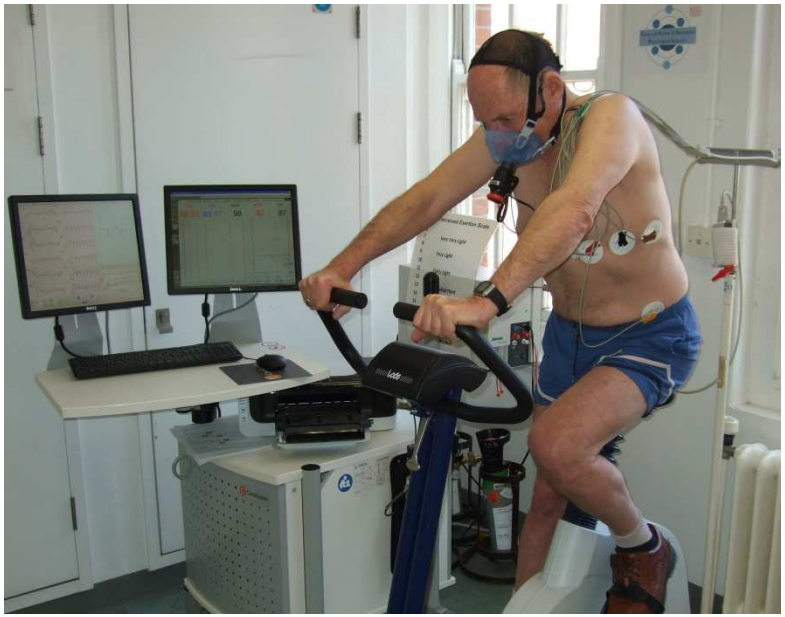
Pollock *et al.* (2015)
Journal of Physiology

A study of optimal human ageing

Detailed physiological profiling of 125 amateur cyclists (aged 55-79 years)

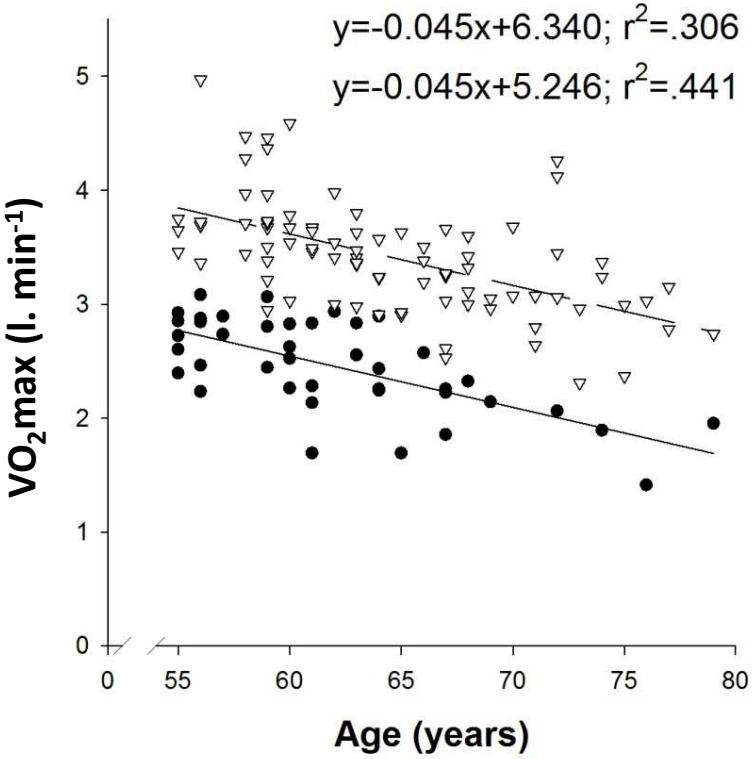
Laboratory measures:

- Exercise capacity
- Neuromuscular function
  - Muscle biopsies
- Lung function
- Endocrine, metabolic, immune function
- Cardiovascular function
- Bone (DXA)
- Cognitive function
- Quality of life….

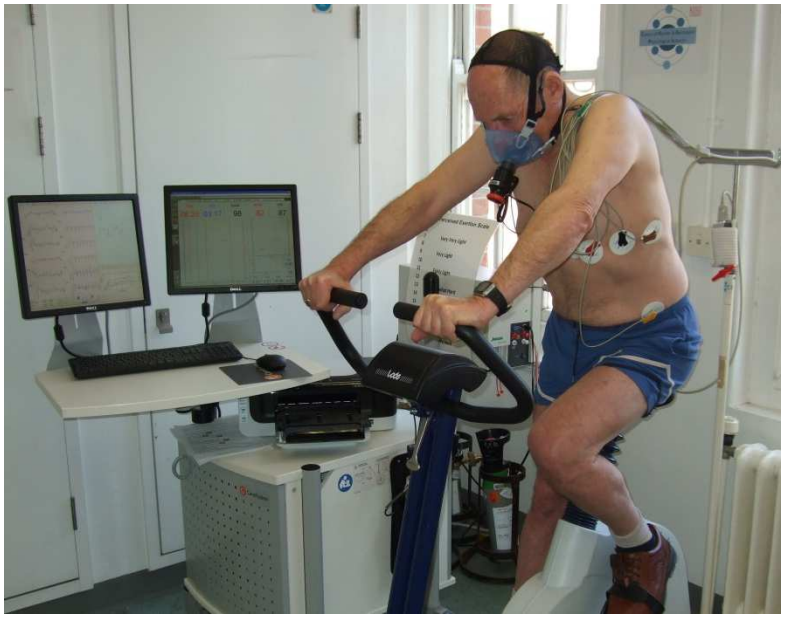


Pollock *et al.* (2015)
Journal of Physiology

Maximal Oxygen uptake ($\dot{V}O_2max$)

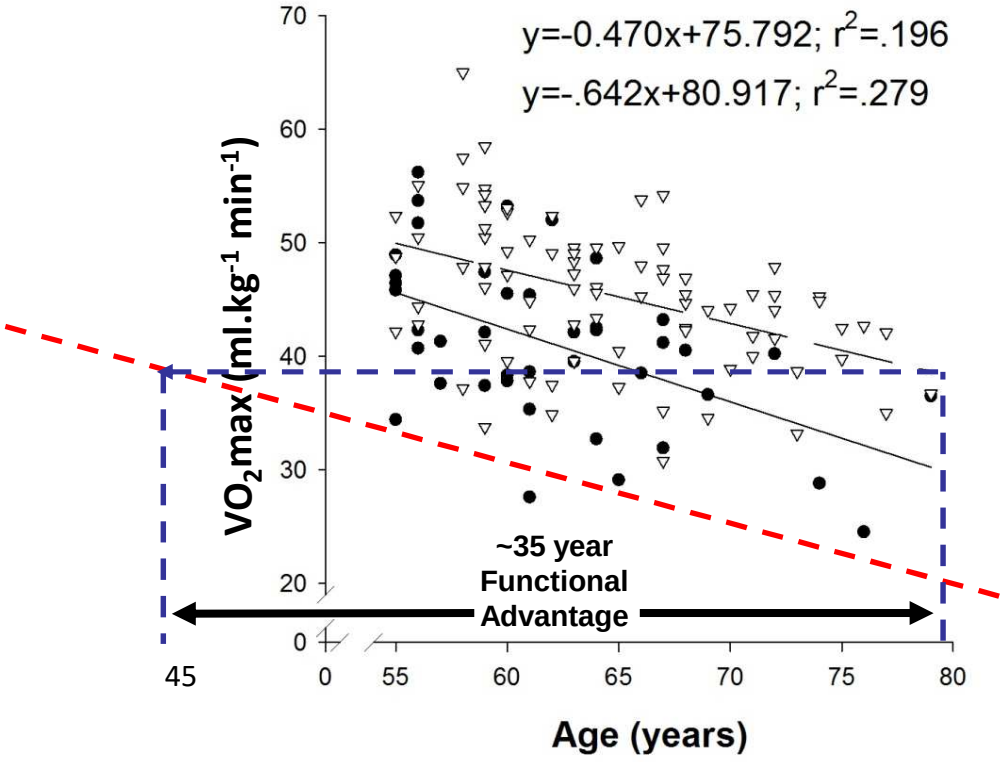


Females ● Males ∇

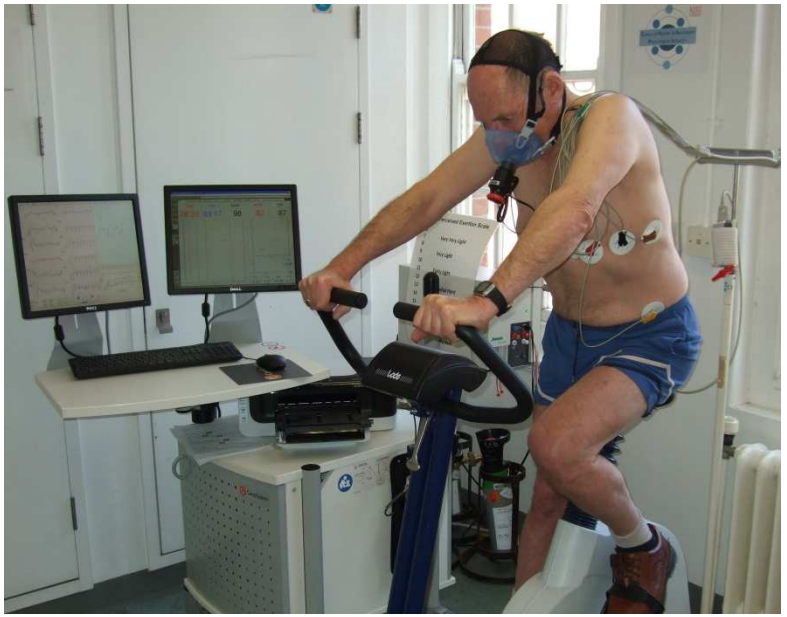


Pollock *et al.* (2015)
Journal of Physiology

Maximal Oxygen uptake ($\dot{V}O_2max$)



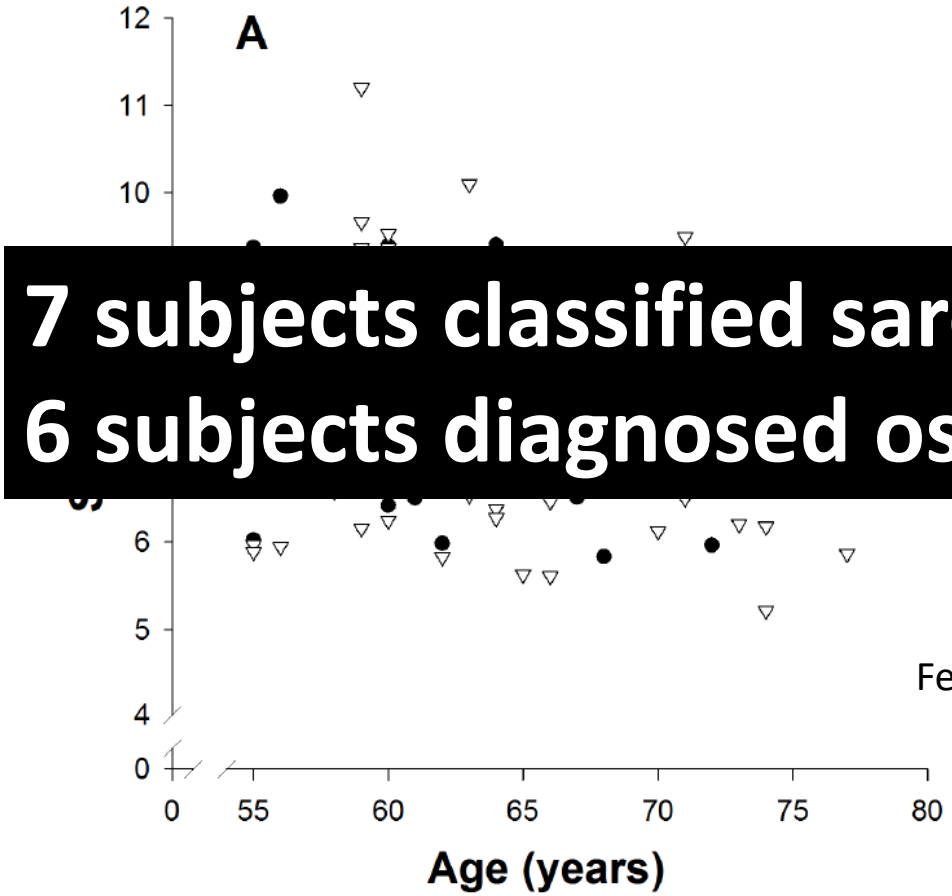
Females ● Males ∇



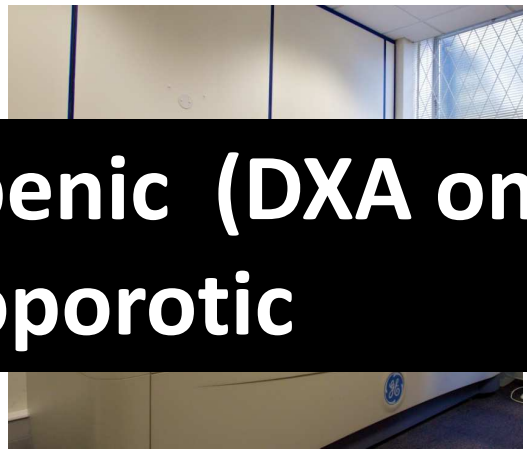
Sedentary males. From Wilson *et al.* (2000)

Pollock *et al.* (2015)
Journal of Physiology

No association between age and appendicular muscle mass (SMI)



7 subjects classified sarcopenic (DXA only)
6 subjects diagnosed osteoporotic

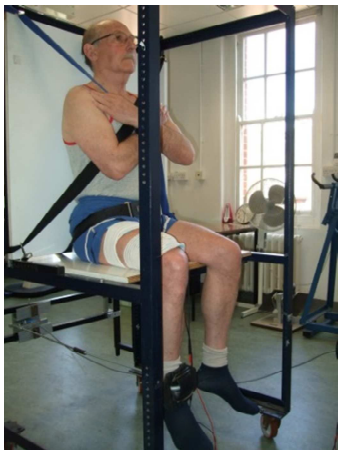


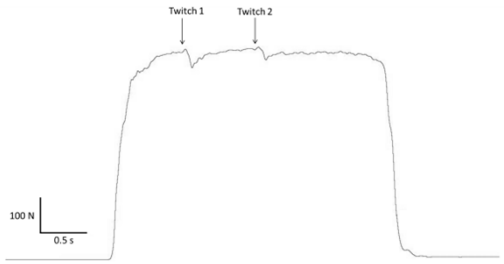
Females ● Males ∇

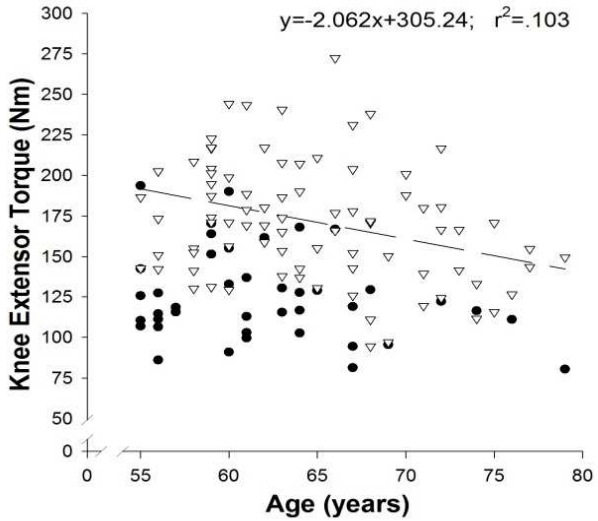
Pollock *et al.* (2015)
Journal of Physiology

Neuromuscular function

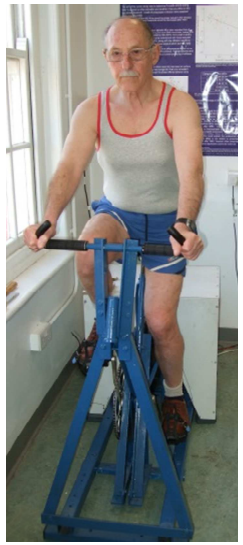
Isometric strength

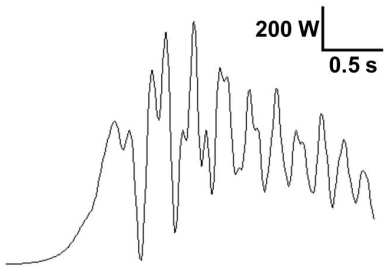


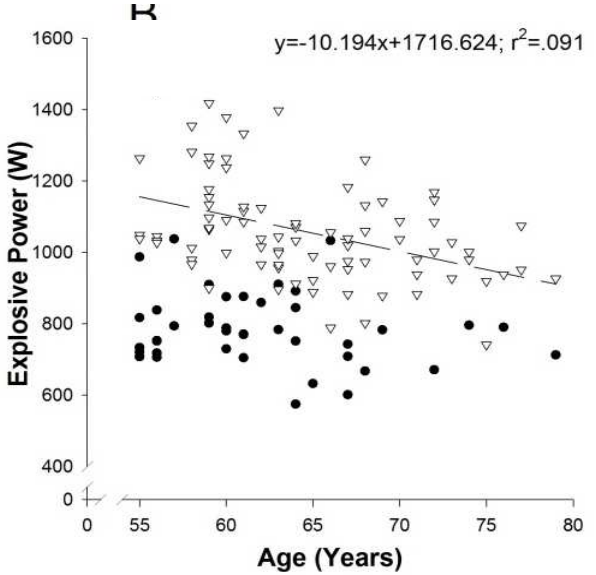




Explosive Power





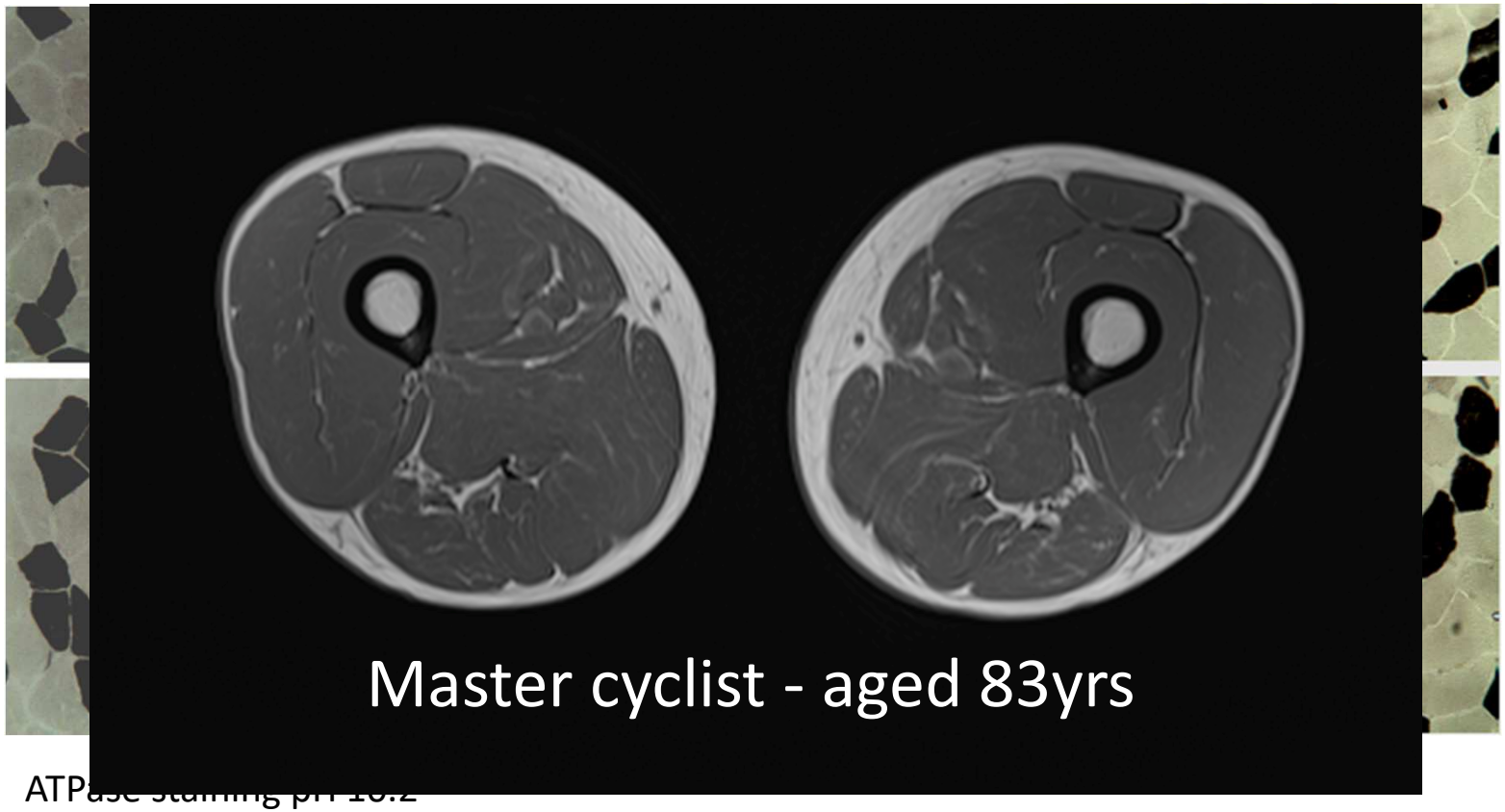


Females ● Males ∇

Pollock *et al.* (2015)
Journal of Physiology

Muscle morphology showed “young” fibres

55 Years **65 Years** **79 Years**

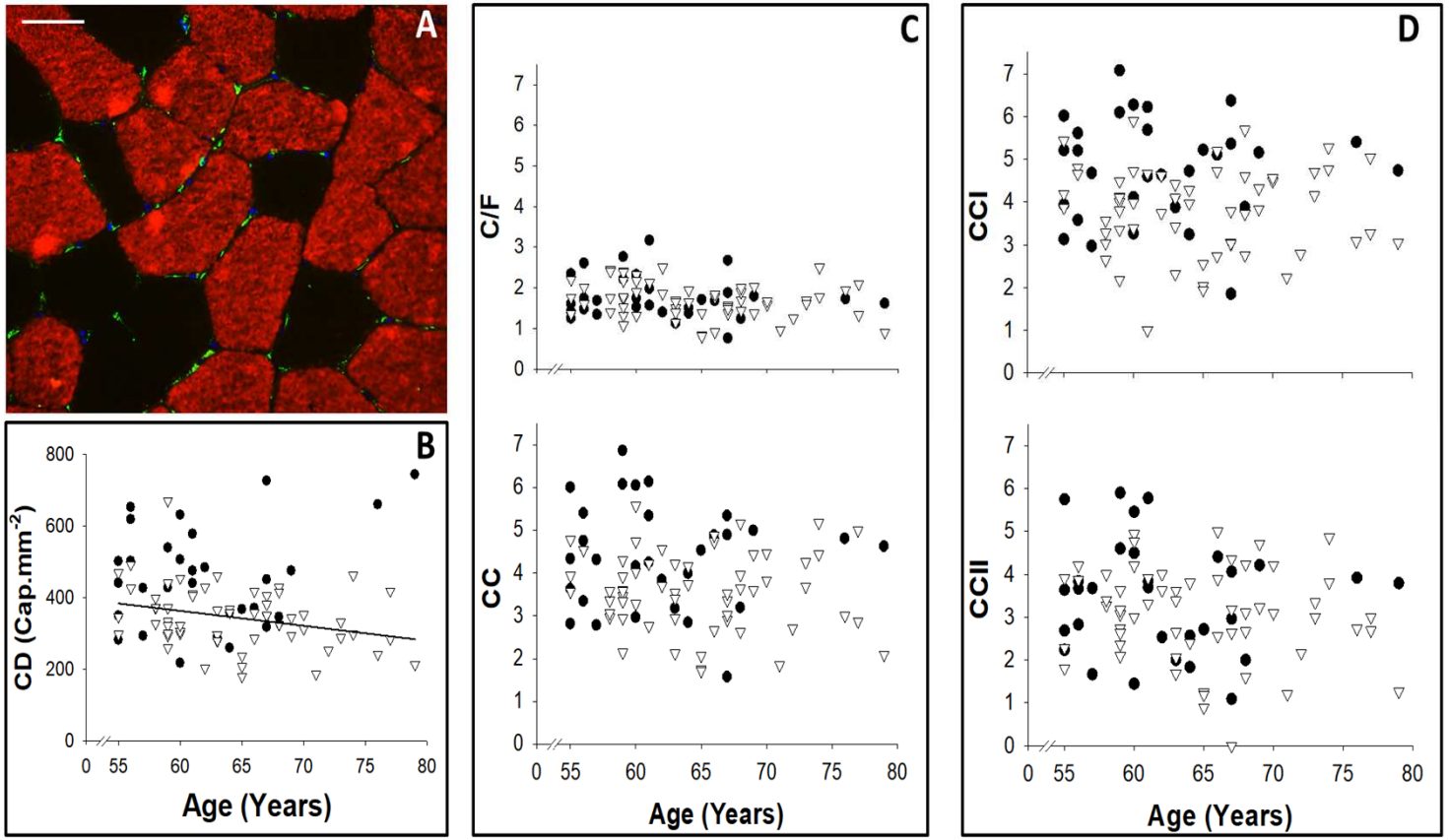


ATPase staining pH 10.2

No correlation between age and type 2 fibre area

Pollock *et al.* (2018)
Ageing Cell

No age-related ↓in capillarity*

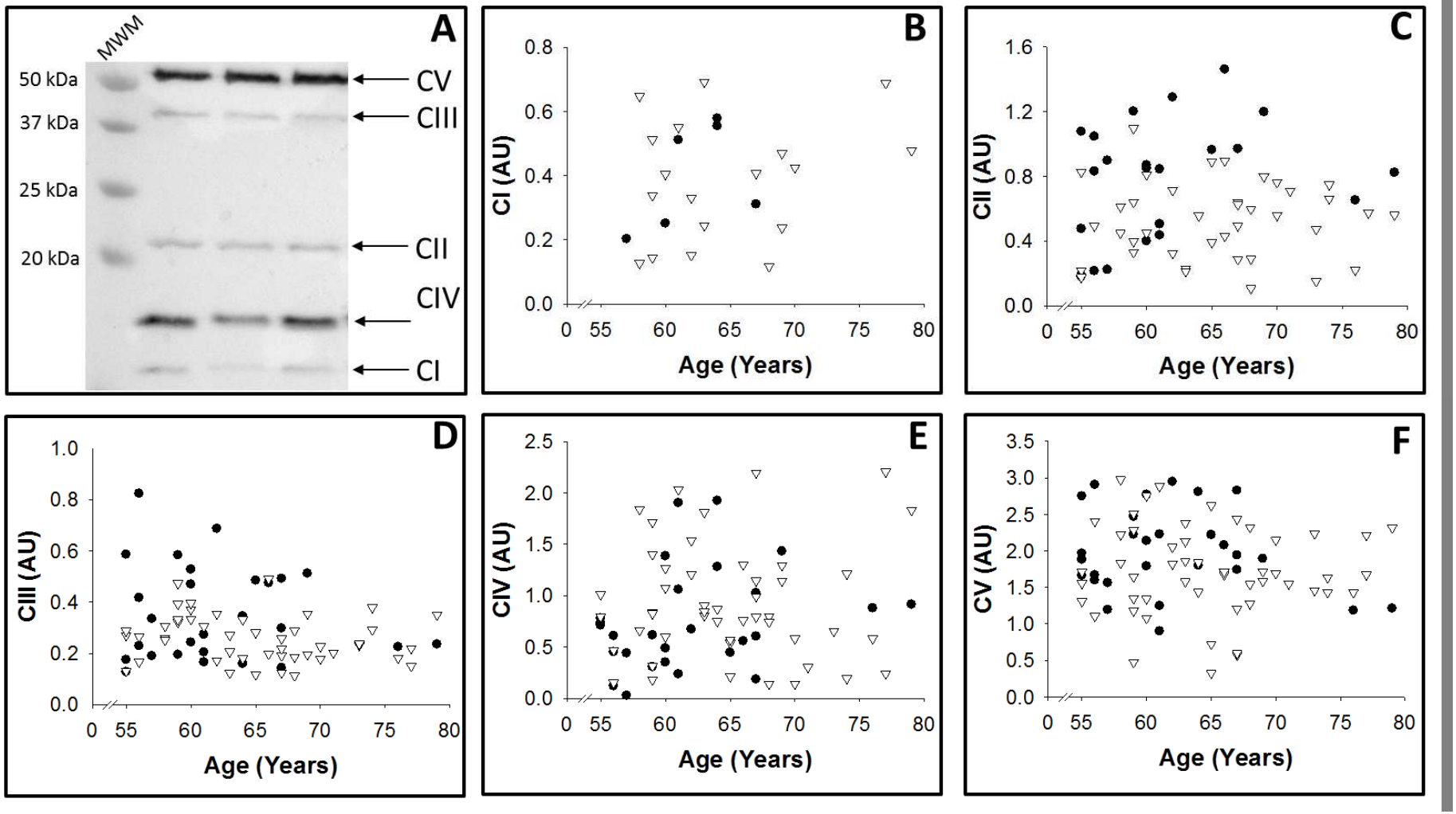


*In males (r^2 = 0.089)

Females ● Males ∇

Pollock *et al.* (2018)
Ageing Cell

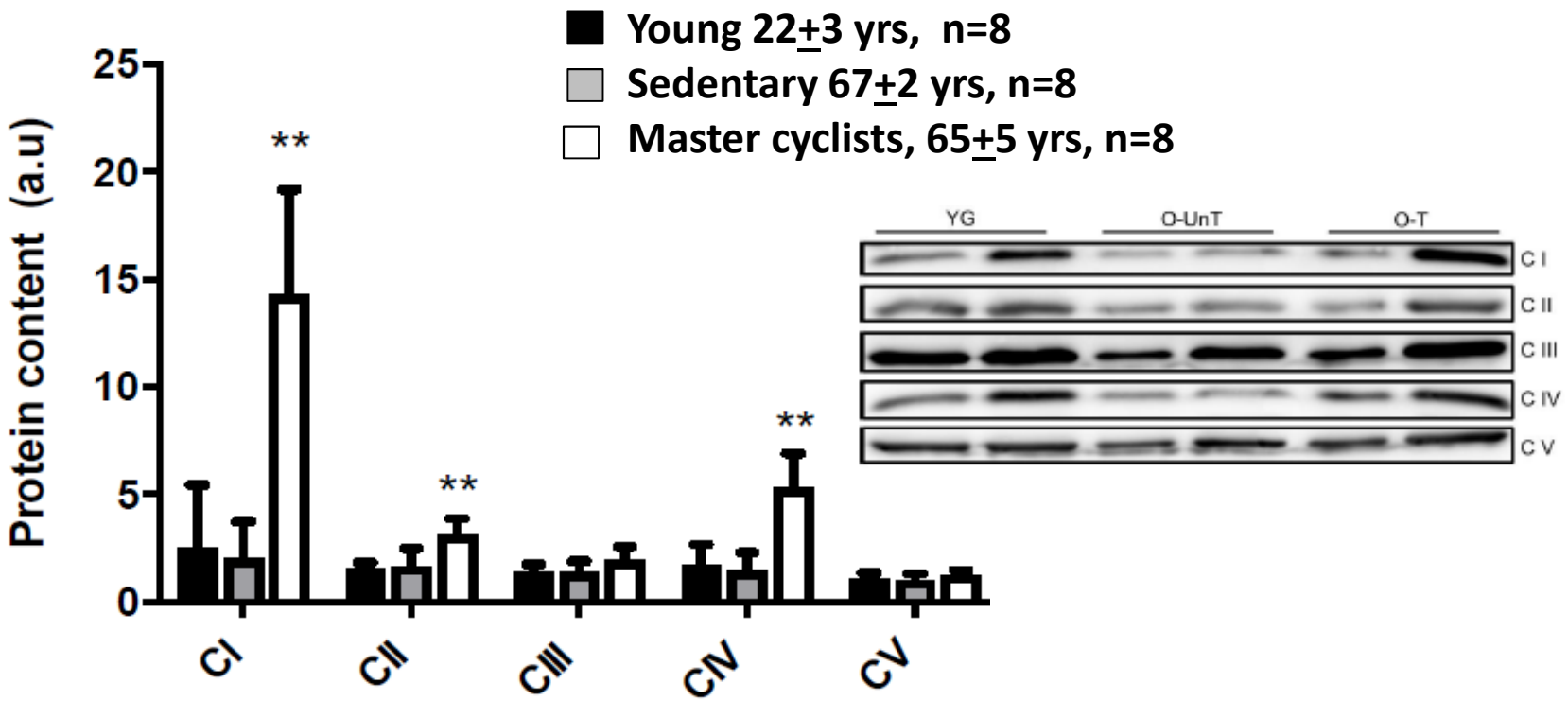
No age-related ↓in mitochondrial complex proteins



Pollock *et al.* (2018)
Ageing Cell

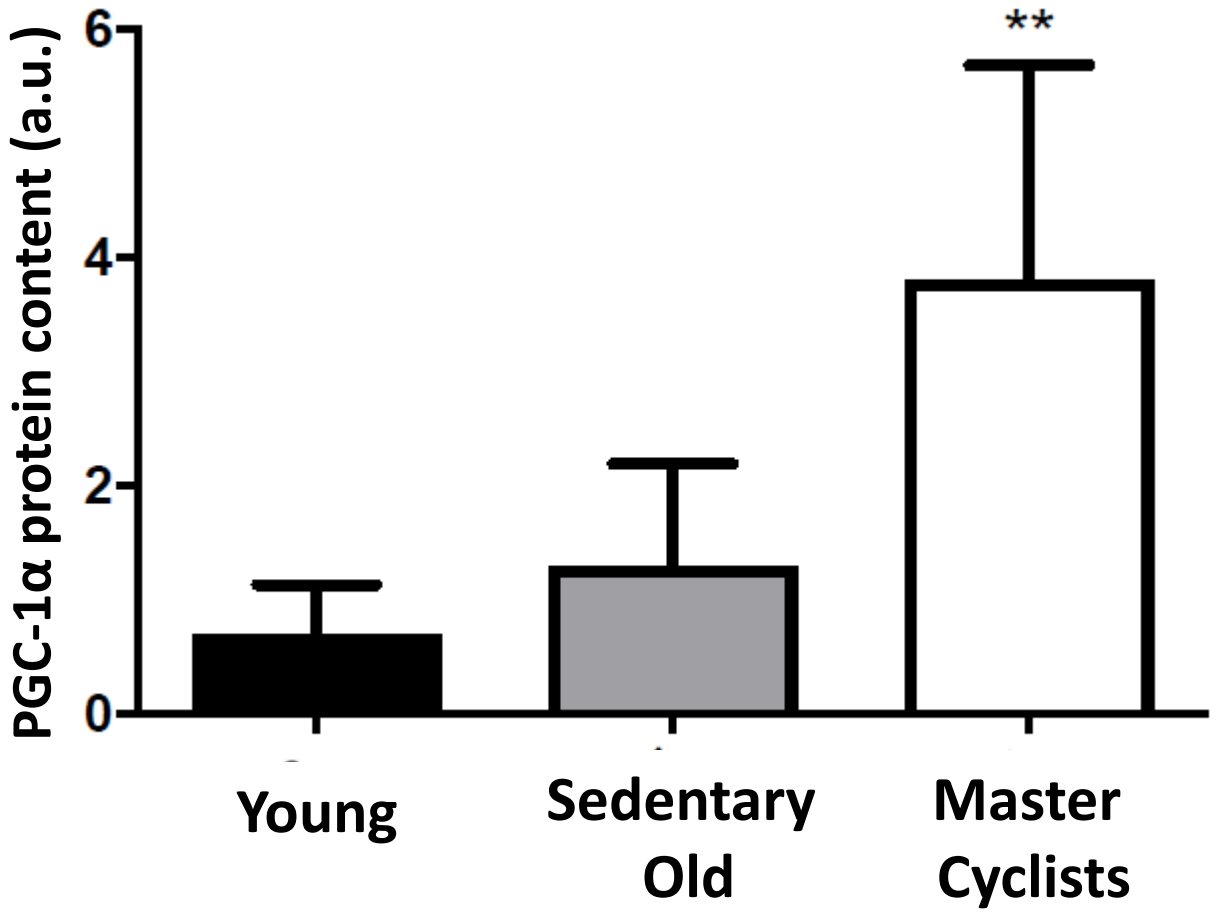
Females ● Males ∇

Higher mitochondrial complex protein content in master cyclists compared to both young and elderly sedentary



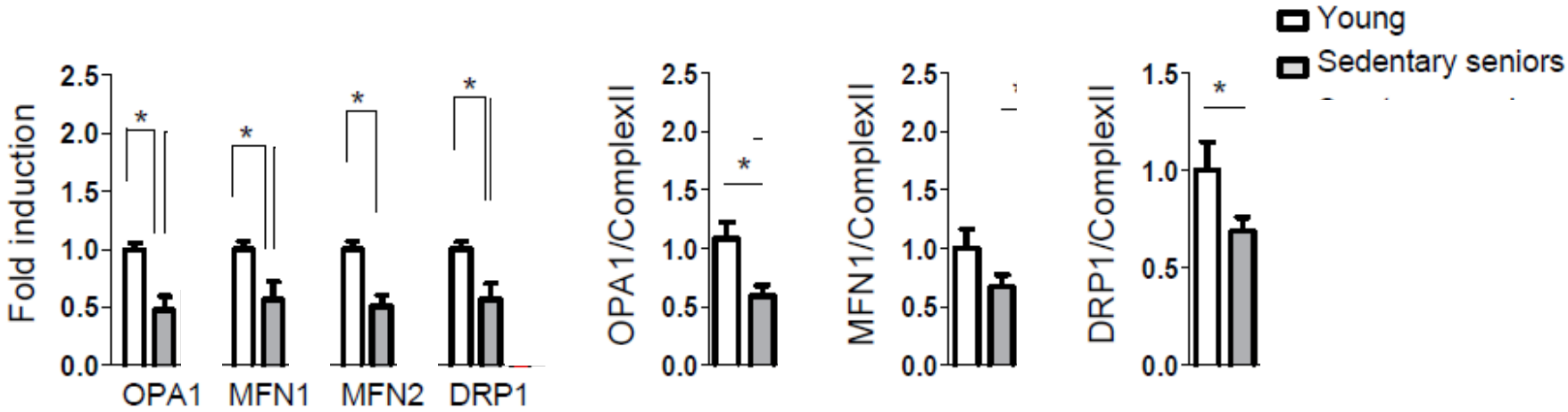
Joanisse et al.
In preparation

Higher PGC1-α protein content in master cyclists compared to both young and elderly sedentary

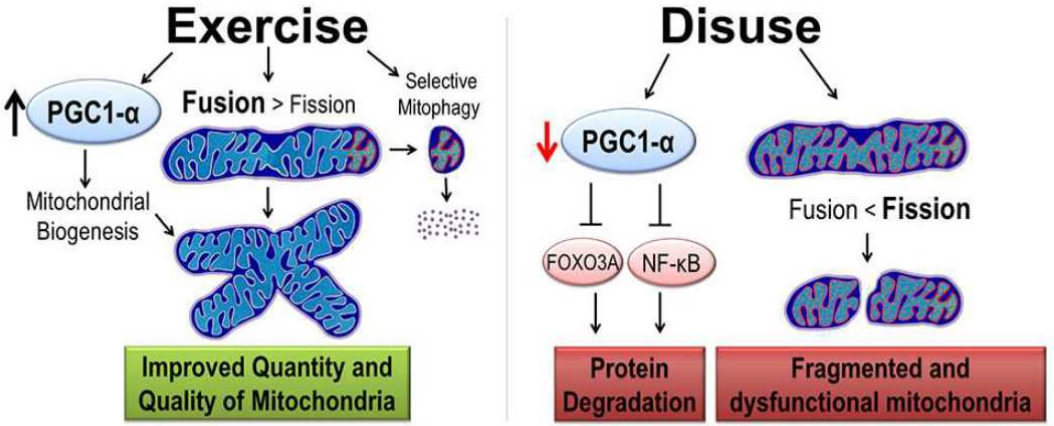


Joanisse et al.
In preparation

Mitochondrial fission / fusion – determined more by activity than by age

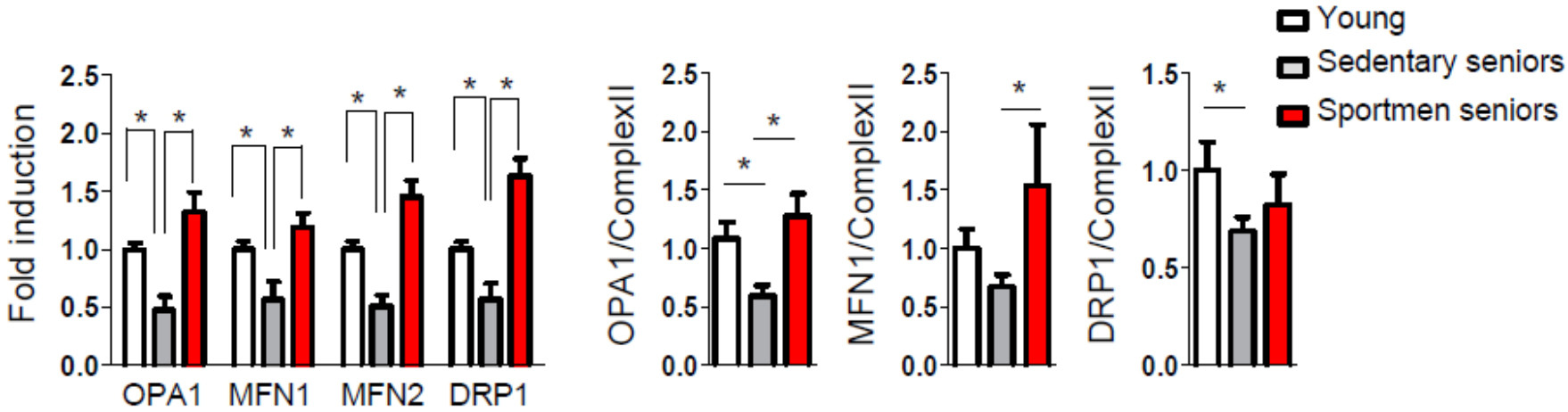


Tezze et al 2017
Cell Metabolism

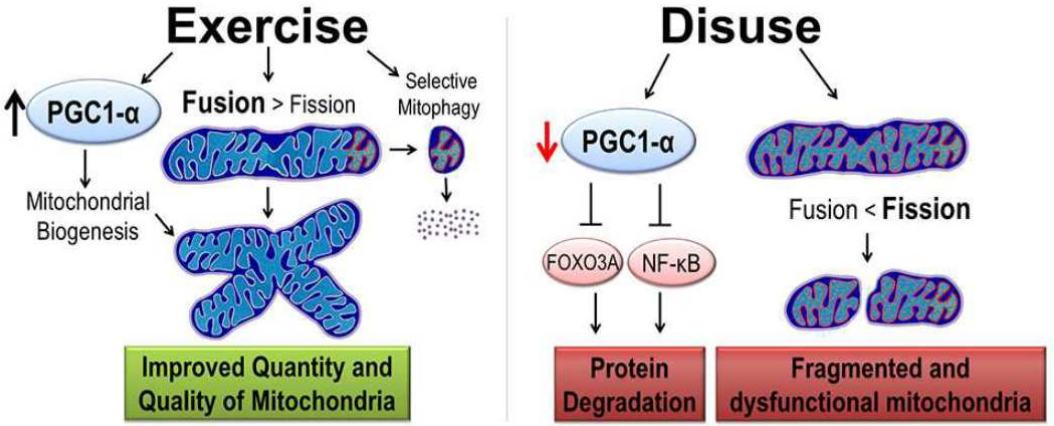


Wigg (2015)

Mitochondrial fission / fusion – determined more by activity than by age

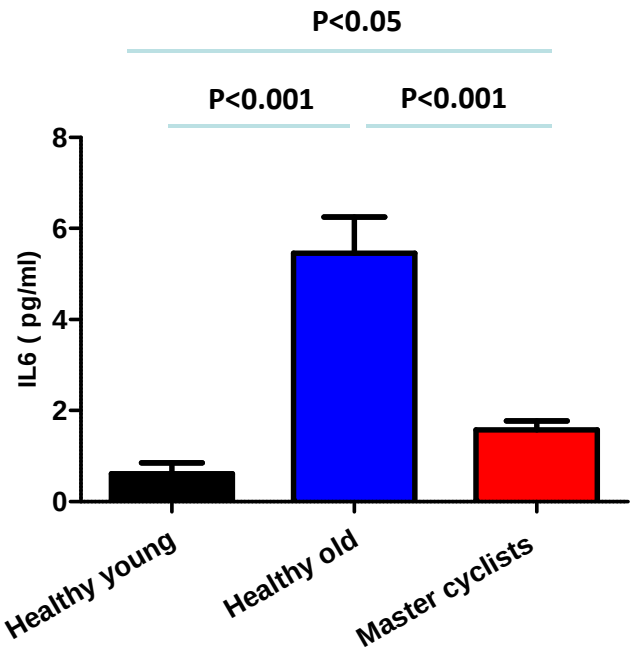


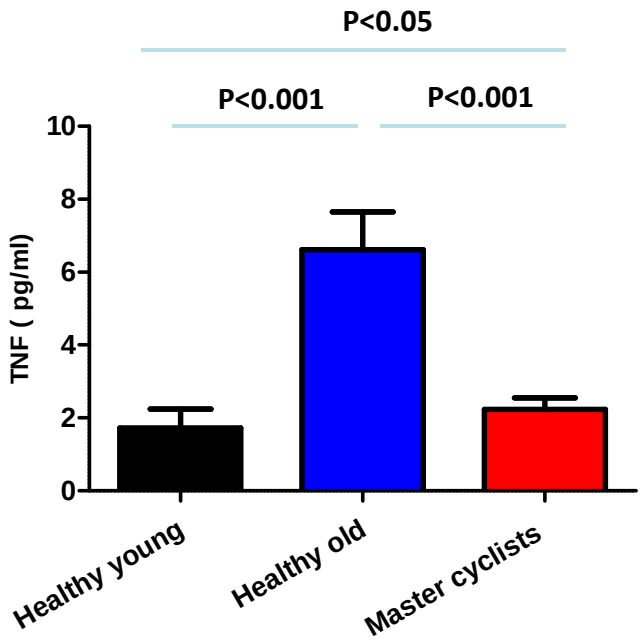
Tezze et al 2017
Cell Metabolism



Wigg (2015)

Marked amelioration of "Inflammageing" in master cyclists

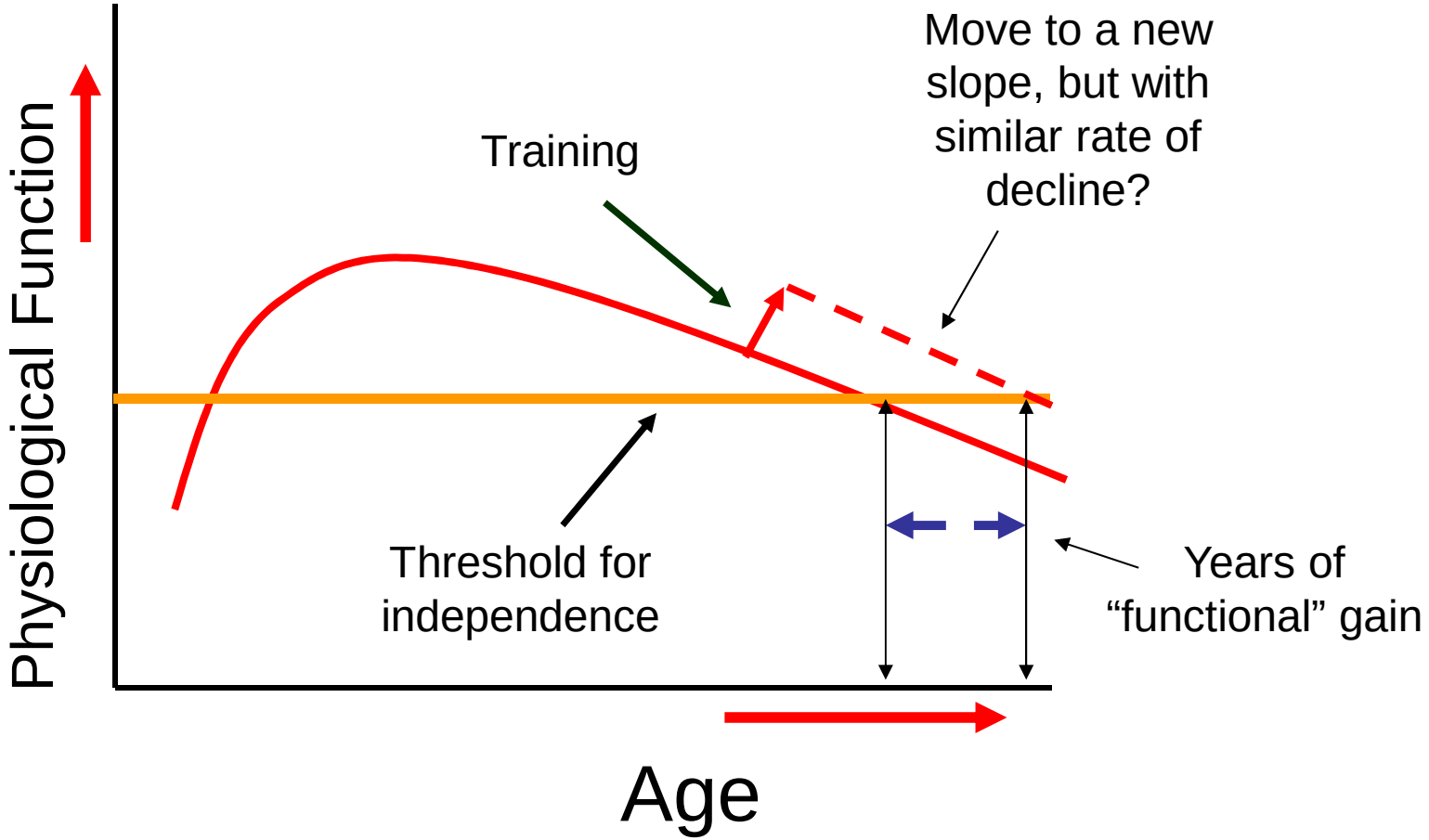




Young 20-36 yrs, n = 55 (30,m 25f)
Healthy Old (sedentary) 57-80yrs, n= 75 (43m, 31f)
Master cyclists 55-79 yrs, n=125 (84m 41f)

Duggal *et al. (2018)*
Ageing Cell

Does exercise (or being a master athlete) prevent or reverse the effects of ageing?



Harridge & Saltin (2007) – Adapted from Young (1995)

Encyclopaedia of Gerontology

General conclusions

- In most studies of ageing it is not possible to differentiate the effects of ageing from those of inactivity, disuse and pathology
- Older exercisers have generally superior levels of physiological function and global health compared to sedentary - concept of “buying back” years of function
- Physical performance declines even in the elite old (e.g. world records) - this decline accelerates in the eighth decade. Do these records reflect the change in integrative physiology due to ageing?
- However, the gap in muscle mass, contractile and metabolic function between exercisers and the sedentary demonstrates that activity pattern plays a large role in determining the phenotype of the older muscle

aptitude ACTUAR PARA LA PREVENCIÓN TRANSPIRENAICA DE LA DEPENDENCIA DE LAS PERSONAS MAYORES

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¡Gracias! / Merci! / Thank you!

Stephen Harridge

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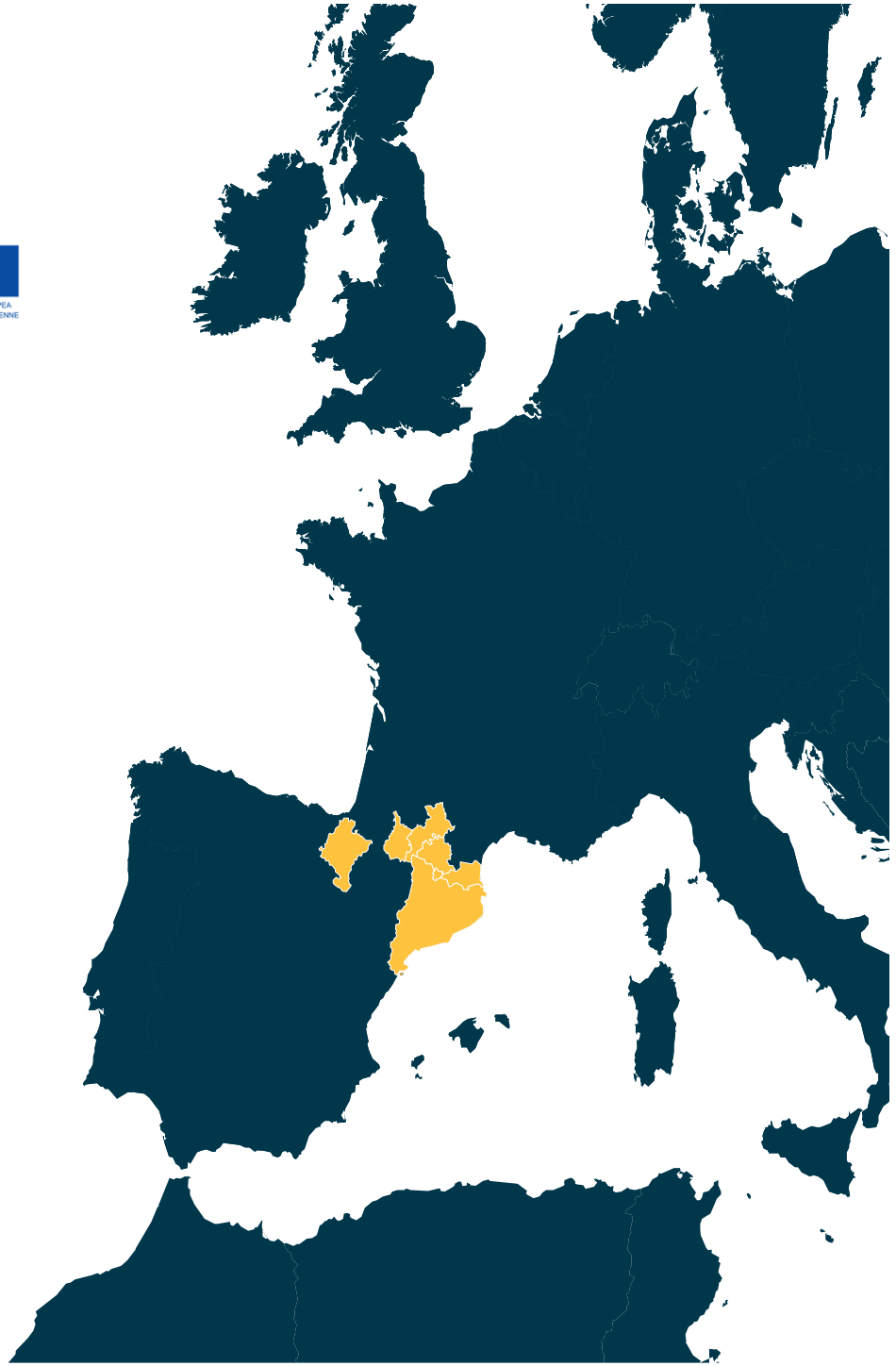

Acknowledgements

Inherent ageing

Prof. Norman Lazarus
Dr Ross Pollock
Prof Janet Lord
Dr Niharika Duggal
Dr Anthea Rowlerson
Dr Andy Philp
Dr Katie O'brien
Dr Sophie Joanisse
Prof Phil Atherton
Dr Beth Phillips

Muscle Cell Biology

Tom Francis
Dr Chibeza Agley
Dr Oihane Jaka
Dr Cristiana Velloso
Dr Mansour Alsharidah
Dr Tomasz George
Dr. Anthea Rowlerson
Dr Fiona Lewis
Dr Geena Ellison-Hughes
Dr Michaeljohn Kalakoutis
Dr Yotam Levy
Dr Julien Ochala
Prof Norman Lazarus

ITU muscle

Dr Zudin Puthucheary
Prof Nick Hart
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