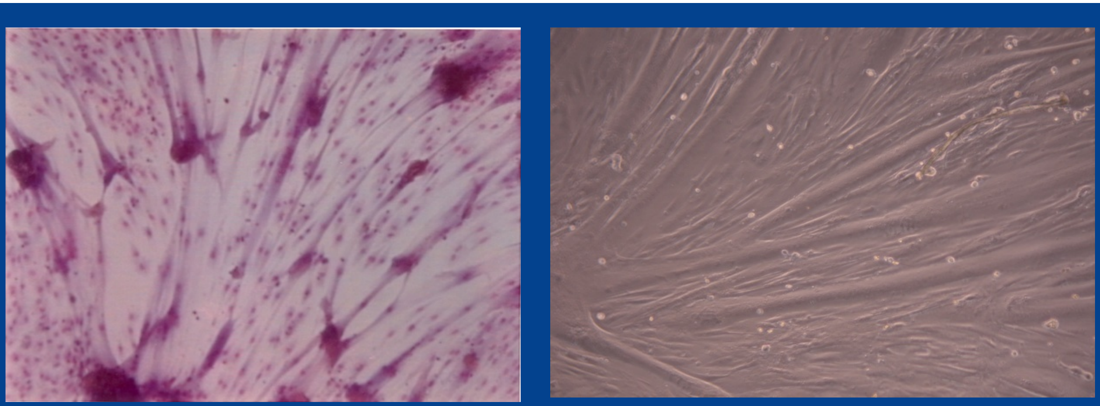
AARHUS
UNIVERSITET



The satellite cell and myonuclei: IMPORTANCE FOR GROWTH AND REGENERATION

NIELS OKSBJERG, Dept. of Food Science, Aarhus University

MUSCLE FIBRE HYPER PLASTICITY

- Muscle adapt to:
  - Feeding level
  - Age
  - Selection
  - Overload caused by ablation of synergistic muscle
  - Suspension /fixating in a cast/re-growth
  - Resistance training
  - Endurance training
  - Shivering
- It is suggested that the satellite cell is involved in these adaptations

MYOGENESIS

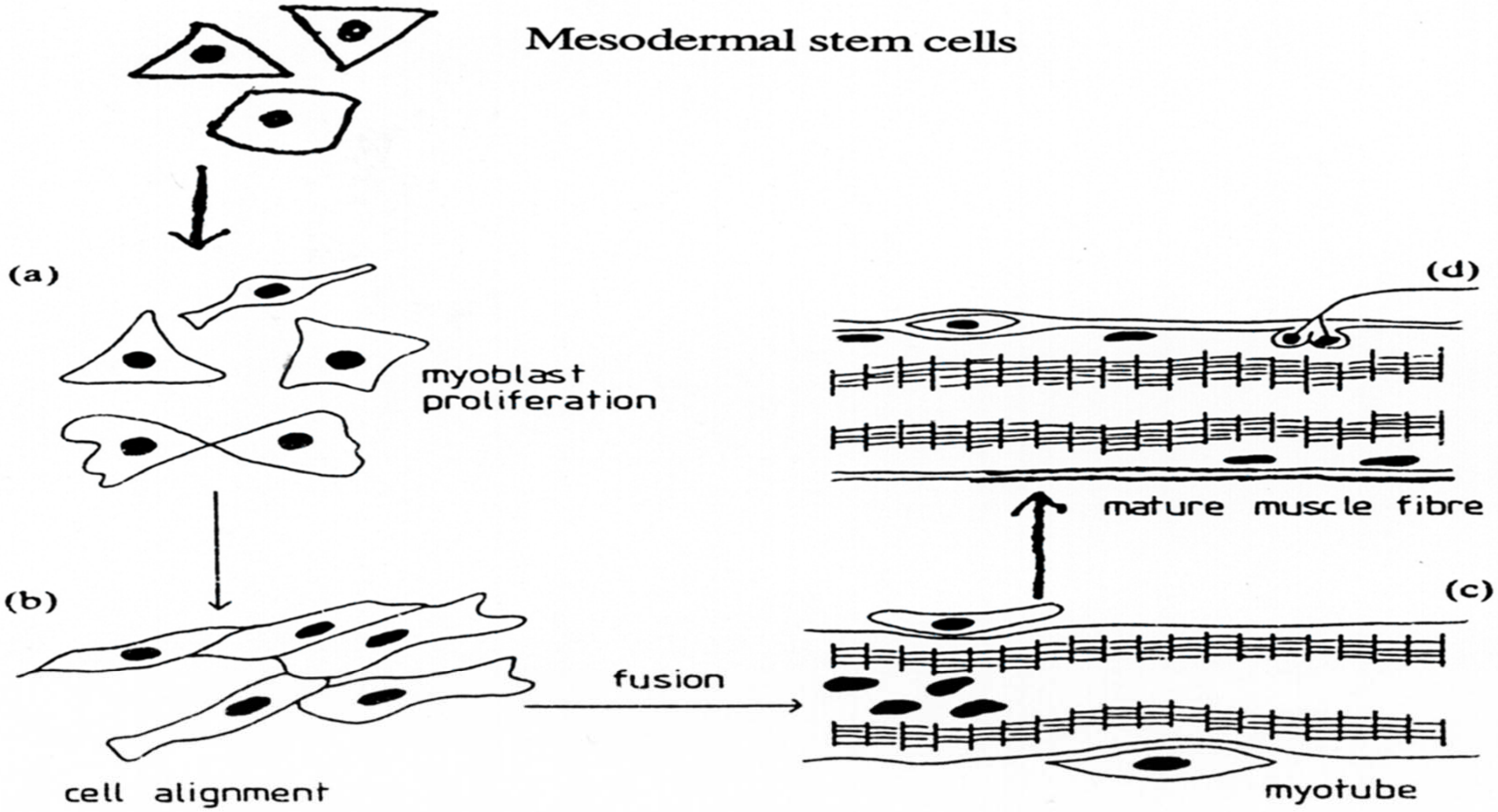
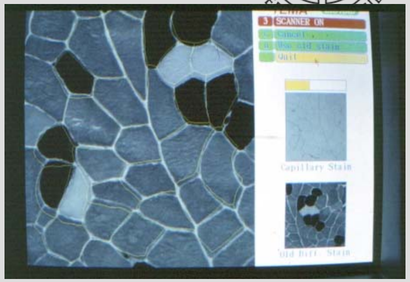
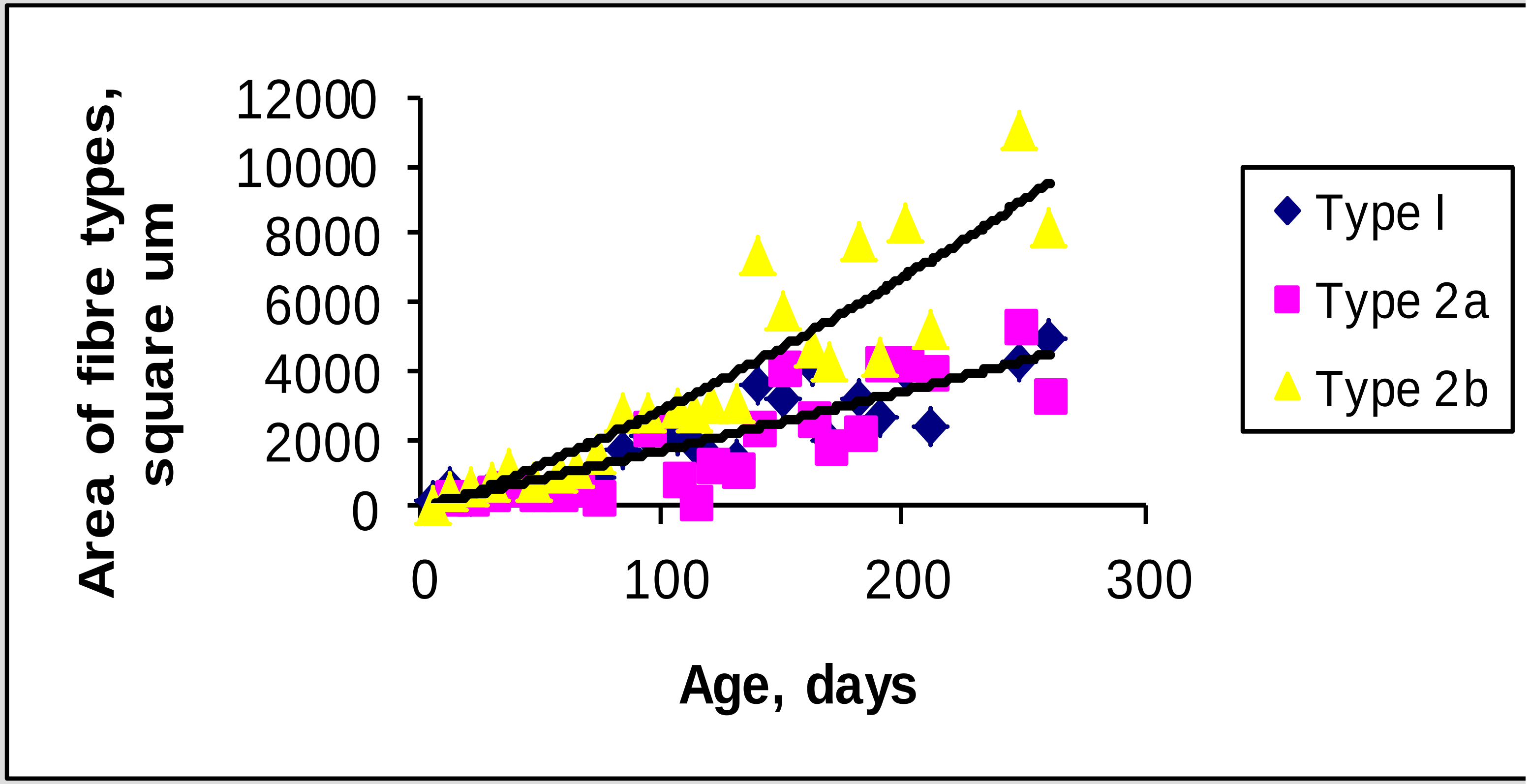


FIG. 1. The growth and differentiation of skeletal muscle.

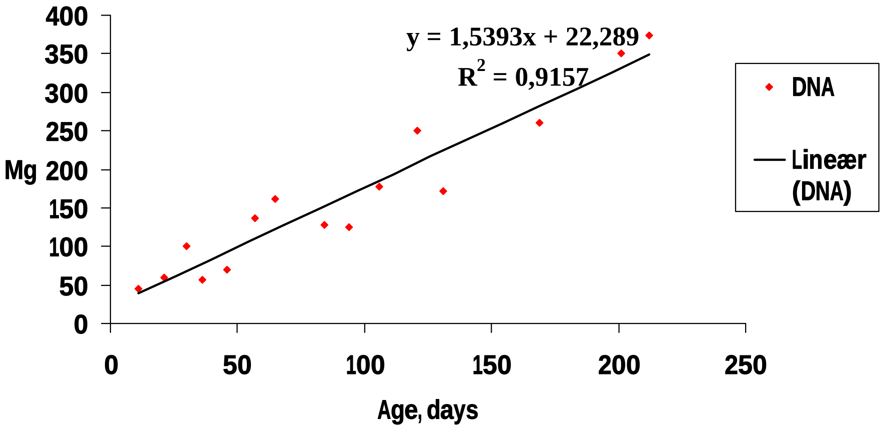
(J.M.M. Harper and P.J. Buttery, 1988)

Muscle fibre growth by age in pigs





TOTAL DNA AND MYONUCLEI CONTENT IN LONGISSIMUS MUSCLE OF PIGS



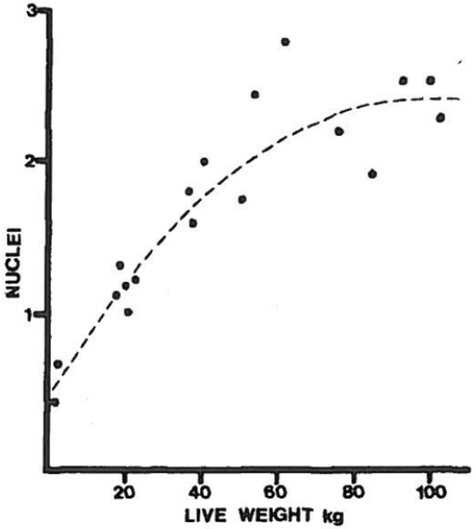


Figure 1. Increase in mean number of nuclei per sectioned myofiber with live weight growth (animals on full feed).

Unpublished,
Longissimus dorsi

Swatland et al, 1977. Journal of Animal Science, 44, 759-764.

The satellite cell: Importance for muscle growth and regeneration

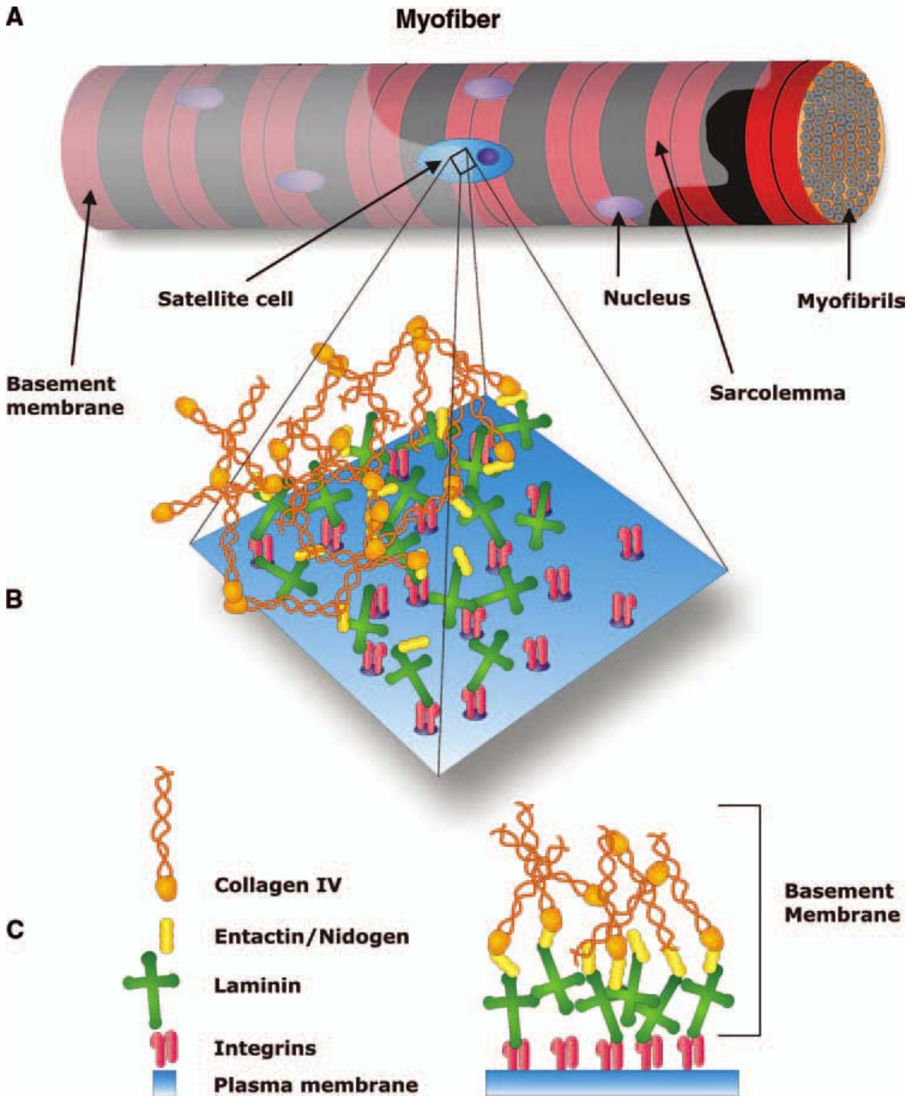
- The satellite cells: its niche
- Life cycle of satellite cells
- Are there more than one population of SR?
- Satellite cell and myonuclei by age and muscle type
- Satellite cell:
  - nutrition, selection for muscle growth rate, Light illumination
- Is SC needed for growth and regeneration?
- Sarcopenia (regeneration capacity)
- Regulation of satellite cells
- Future perspectives:
  - Increased proliferation by plant extracts?
  - Satellite cell and IMF?
  - Satellite cells for cultured meat?

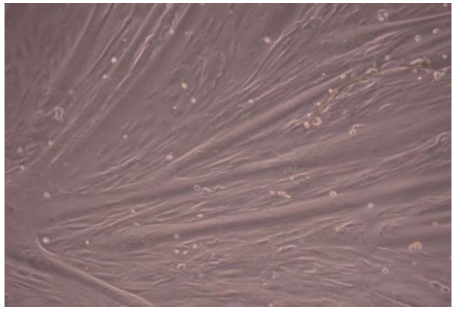
The Muscle Stem Cell Niche: Regulation of Satellite Cells During Regeneration

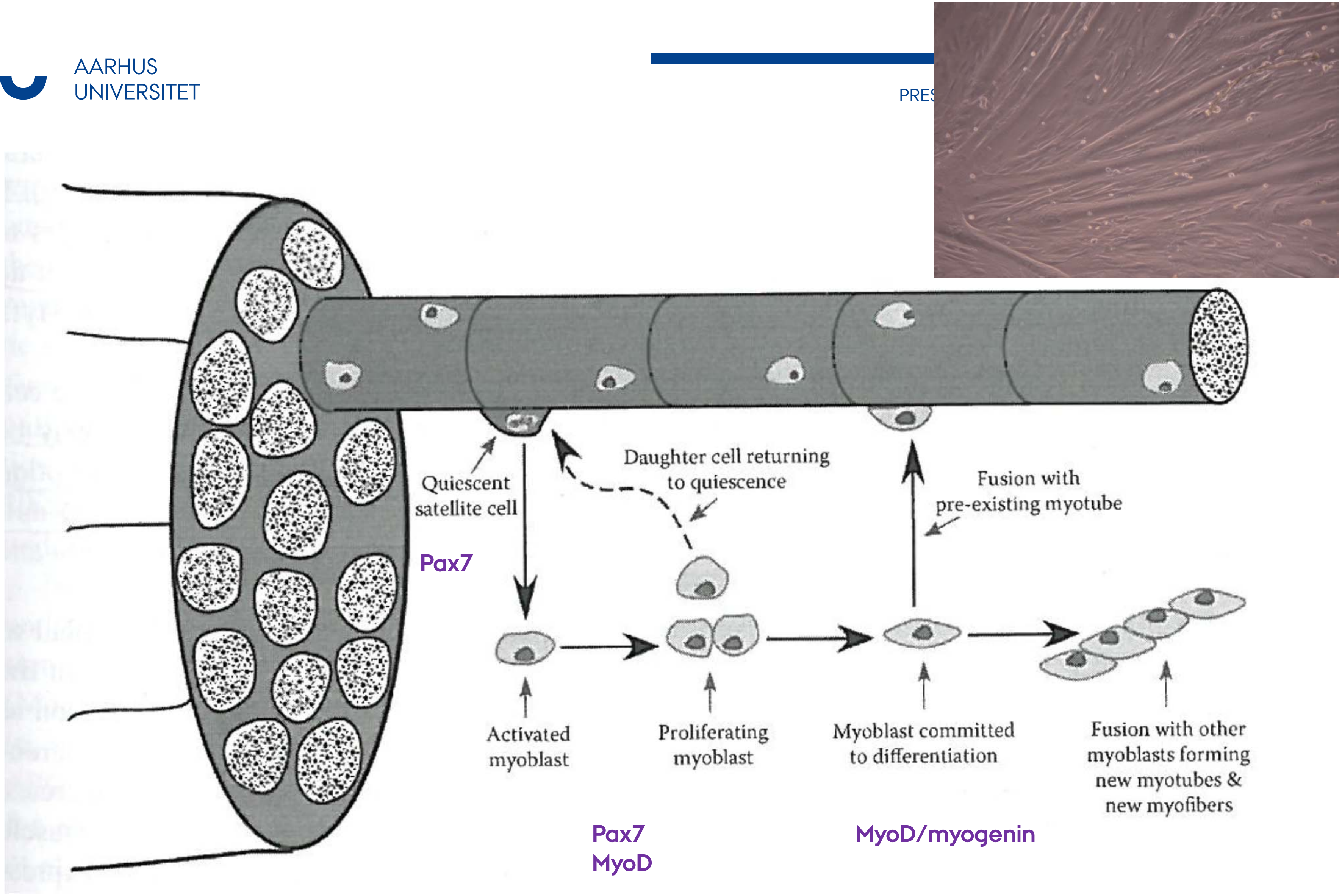
Kristel J.M. Boonen, M.Sc.,[1] and Mark J. Post, M.D., Ph.D.[1,2]

TISSUE ENGINEERING: Part B
Volume 14, Number 4, 2008

DOI: 10.1089/ten.teb.2008.0045

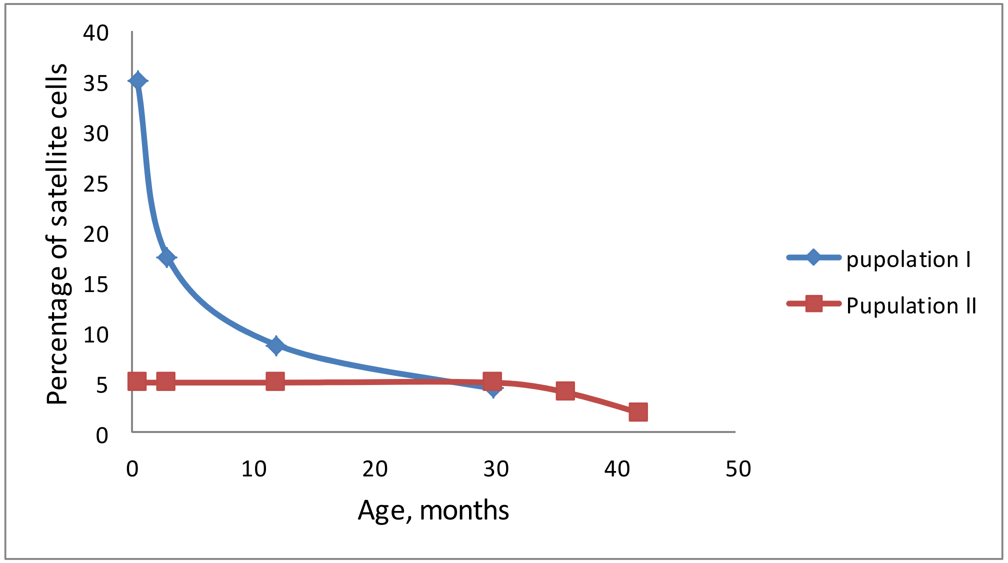






Rhoads, Rathbone, Flann. In Applied Muscle Biology and Meat Science. Pp47-67. eds. M. Du, and R.J. McCormick. CPR Press, ISBN 978-1-4200-9272.

Satellite cells populations at varying ages



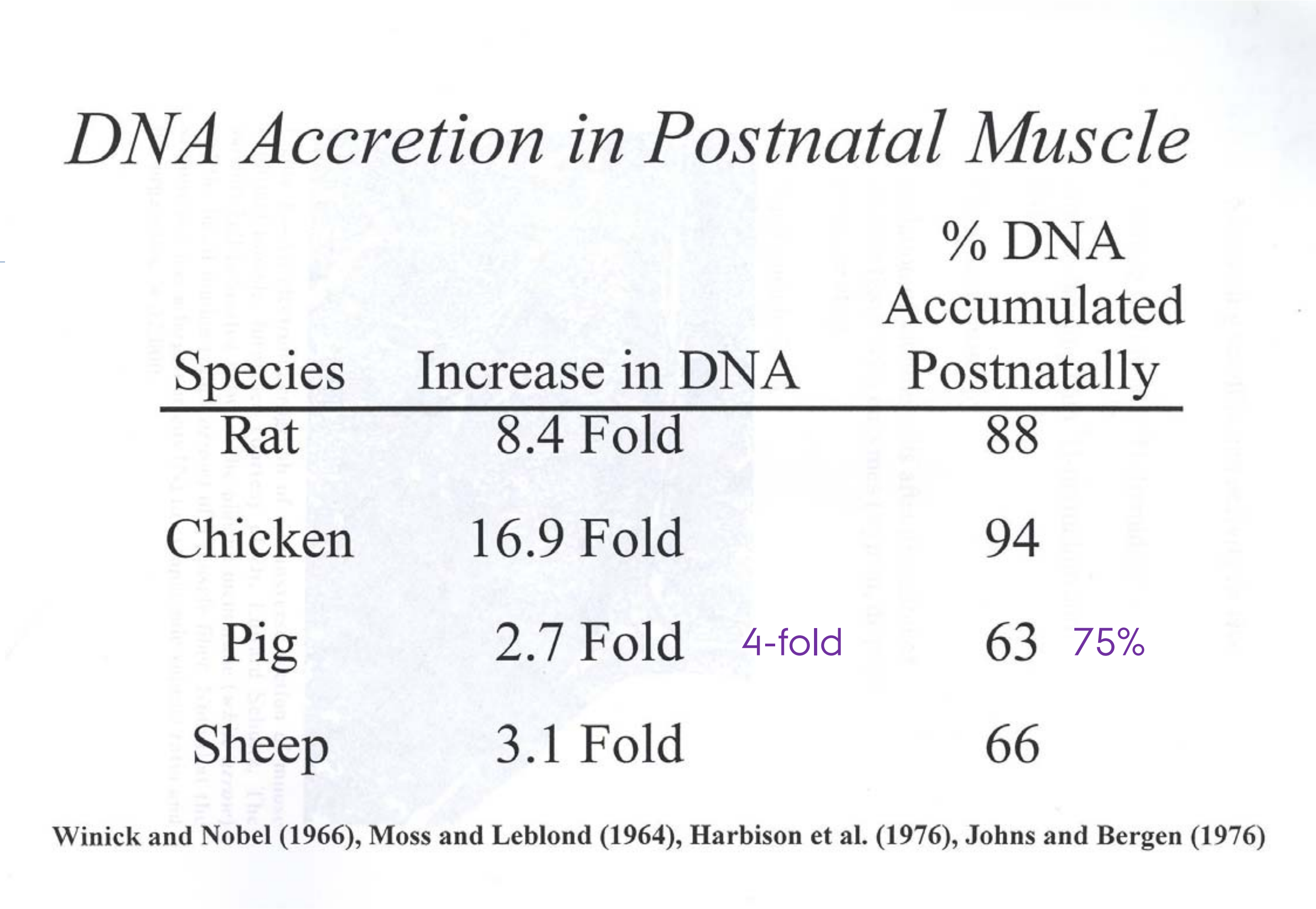
Schultz. (1996) Developmental Biology. 175, 84-94.
Rouger et al. 2004. Cell Tissue Res, 317: 319-326 (tom Turkey)
Li et al. 2011. J. Anim. Sci. 89:1751-1757. (young cattle)

For growth: fast proliferating and fusion competence and the majority of cells (80%) express Pax7/MyoD
For re-generation: slow dividing and the majority of cells express Pax7 only. Can be activated.

SATELLITE CELL CONTENT IN SLOW AND FAST MUSCLES

Muscle	Myofibre surface area mm^2	Satellite cell per fibre	Total nuclei per muscle fibre	Satellite cell/nuclei (%)
Soleus	0.64	26.23	458	5.53
EDL	0.63	7.62	273	2.79

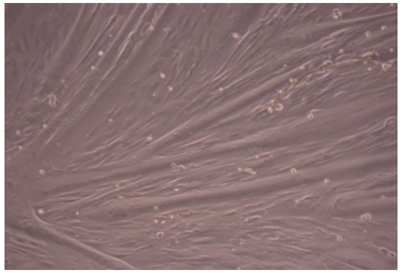
Zammit et al., 2002. Exp. Cell Res. 281:39-49



DNA Accretion in Postnatal Muscle

Species	Increase in DNA		% DNA Accumulated Postnatally	
Rat	8.4 Fold		88	
Chicken	16.9 Fold		94	
Pig	2.7 Fold	4-fold	63	75%
Sheep	3.1 Fold		66	

Winick and Nobel (1966), Moss and Leblond (1964), Harbison et al. (1976), Johns and Bergen (1976)



Assume that SCs are the major source of increased muscle DNA!

Growing period	Ad lib (A) (High DNA)		Restrictive (R) (Low DNA)	
Finishing period	AA	AR	RA	RR
Daily gain, g/d	1037	817	1101	889
Total DNA, mg	197	175	198	170

Oksbjerg, Sørensen, Vestergaard. 2002. Acta Agric. Scand., Sect. A, Animal Sci. 52:85-90

Characteristics of large (L) and small (S) fetal sheep at d 130 of gestation, and large fetal sheep from ewes fed a 30% of predicted nutrients for 1 week prior to 130 of gestation (R)

	L	R	S	Pooled SE
Fetus (g)	3701^a	3598^a	1893^b	172
Semimembranosus muscle				
Weight (g)	18.3^a	16.8^a	8.4^b	0.9
DNA (mg)	26.2^a	27.0^a	15.4^b	1.5

Muscle fibre number
unchanged

Greenwood et al. 1999. *Reprod. Fertil. Dev* 11, 281-291

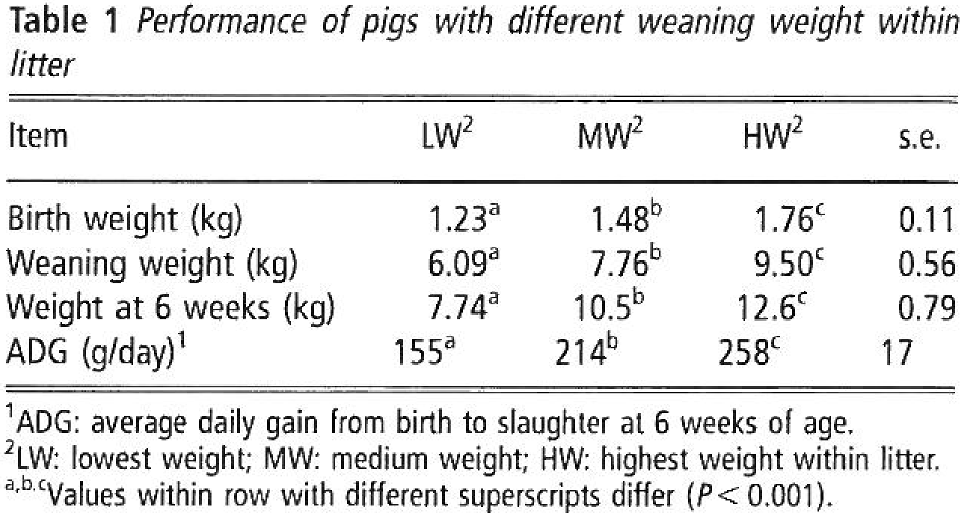


Table 1 *Performance of pigs with different weaning weight within litter*

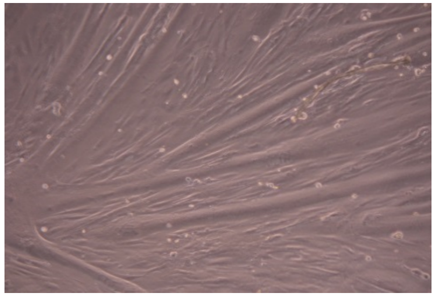
Item	LW^{2}	MW^{2}	HW^{2}	s.e.
Birth weight (kg)	1.23^{a}	1.48^{b}	1.76^{c}	0.11
Weaning weight (kg)	6.09^{a}	7.76^{b}	9.50^{c}	0.56
Weight at 6 weeks (kg)	7.74^{a}	10.5^{b}	12.6^{c}	0.79
ADG (g/day)^{1}	155^{a}	214^{b}	258^{c}	17

[1] ADG: average daily gain from birth to slaughter at 6 weeks of age.

[2] LW: lowest weight; MW: medium weight; HW: highest weight within litter.

[a,b,c] Values within row with different superscripts differ ($P < 0.001$).

Nissen and Oksbjerg, Animal 5, 703-709, 2009



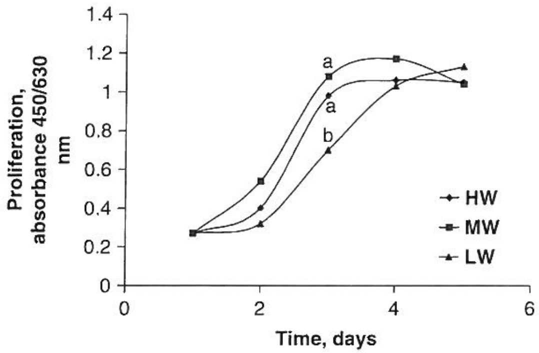


Figure 1 Satellite cell proliferation, measured as the number of viable cells at different time points, of cells isolated from the lowest weight (LW), medium weight (MW) and highest weight (HW) female pigs with eight litters. The time point 1 is the third day after seeding, but the first time the number of viable cells was measured. The time point 1 was used as a covariate in the statistical model. Each value represents the LSMeans of cultures from eight pigs. s.e. = 0.06.

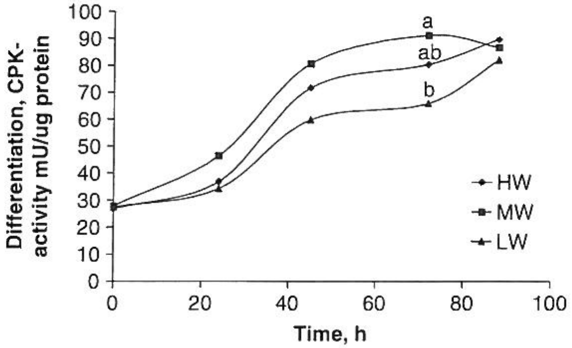
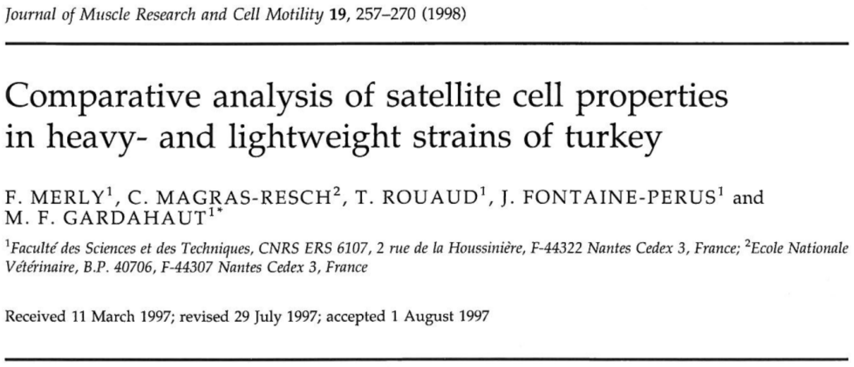


Figure 2 Satellite cell differentiation, measured as the CPK activity at different time points, of cells isolated from the lowest weight (LW), medium weight (MW) and highest weight (HW) female pigs with eight litters. At 0 h the cells were 80% confluent, and at 24 h they were 100% confluent. Each value represents the LSMeans of cultures from eight pigs. s.e. = 5.8.



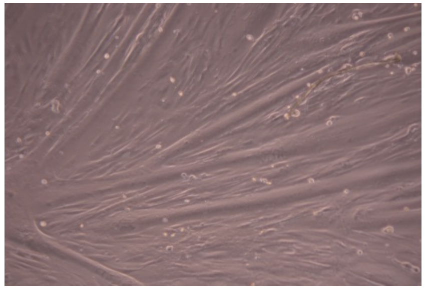
Journal of Muscle Research and Cell Motility **19**, 257–270 (1998)

Comparative analysis of satellite cell properties in heavy- and lightweight strains of turkey

F. MERLY[1], C. MAGRAS-RESCH[2], T. ROUAUD[1], J. FONTAINE-PERUS[1] and
M. F. GARDAHAUT[1]*

[1]*Faculté des Sciences et des Techniques, CNRS ERS 6107, 2 rue de la Houssinière, F-44322 Nantes Cedex 3, France;* [2]*Ecole Nationale Vétérinaire, B.P. 40706, F-44307 Nantes Cedex 3, France*

Received 11 March 1997; revised 29 July 1997; accepted 1 August 1997



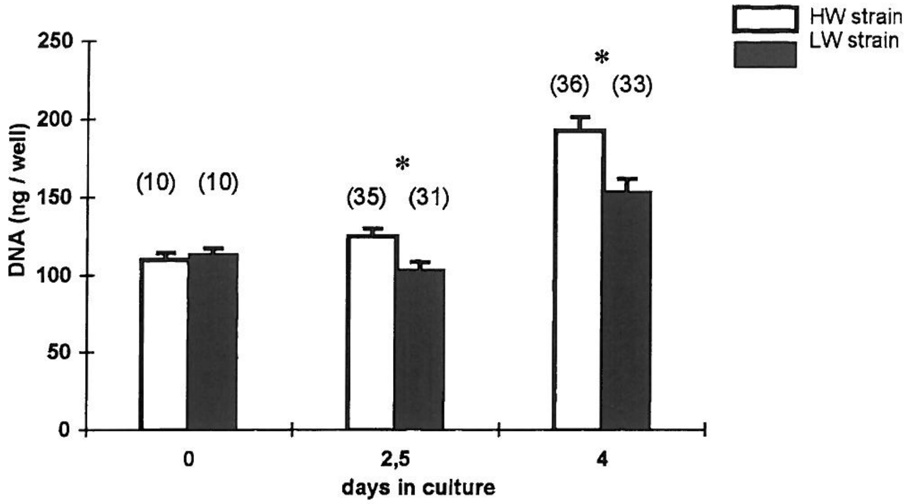
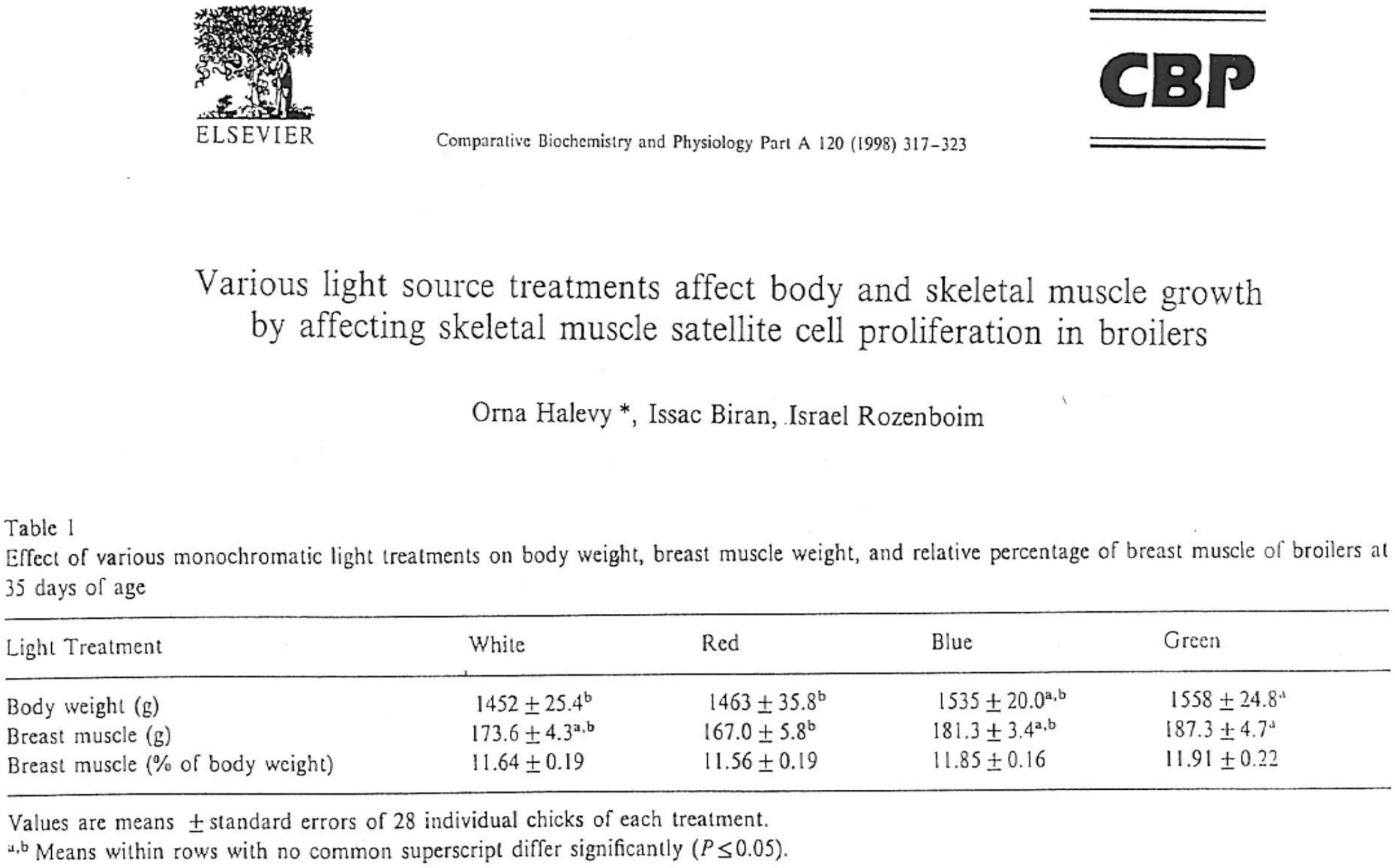


Fig. 5. DNA content of satellite cell cultures at 0, 2.5 and 4 days of incubation. Cells derived from PM of HW and LW turkeys were plated at 7.5×10^4 in DMEM supplemented with 10% FCS, and DNA content was measured as described in Materials and methods. Each bar represents the mean and standard error of DNA per well (ng) of n measurements. Statistical methods are provided in the Materials and methods section. *Significantly different from LW ($p < 0.001$).



ELSEVIER

Comparative Biochemistry and Physiology Part A 120 (1998) 317–323

CBP

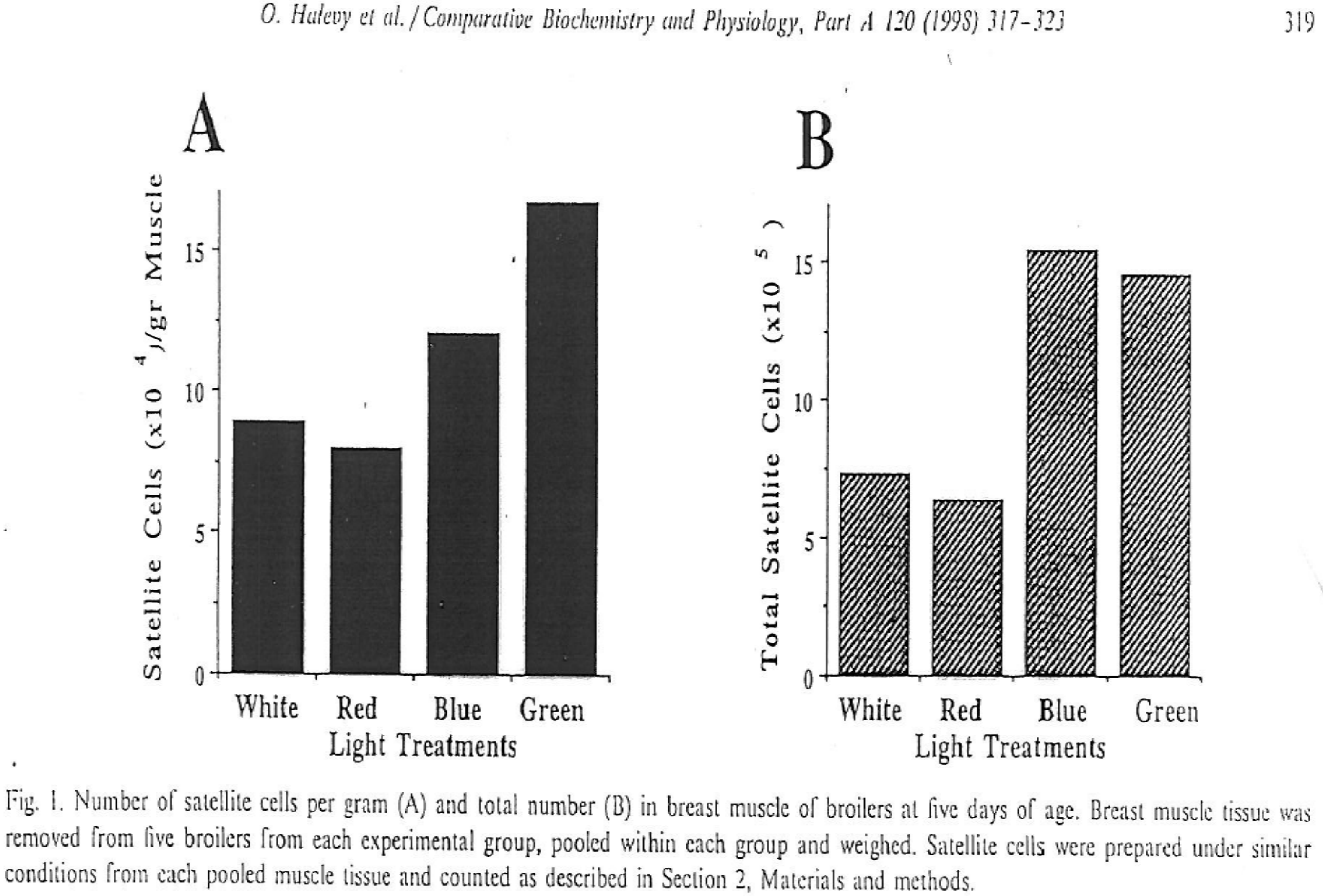
Various light source treatments affect body and skeletal muscle growth by affecting skeletal muscle satellite cell proliferation in broilers

Orna Halevy *, Issac Biran, Israel Rozenboim

Table 1
Effect of various monochromatic light treatments on body weight, breast muscle weight, and relative percentage of breast muscle of broilers at 35 days of age

Light Treatment	White	Red	Blue	Green
Body weight (g)	1452 ± 25.4^{b}	1463 ± 35.8^{b}	$1535 \pm 20.0^{a,b}$	1558 ± 24.8^{a}
Breast muscle (g)	$173.6 \pm 4.3^{a,b}$	167.0 ± 5.8^{b}	$181.3 \pm 3.4^{a,b}$	187.3 ± 4.7^{a}
Breast muscle (% of body weight)	11.64 ± 0.19	11.56 ± 0.19	11.85 ± 0.16	11.91 ± 0.22

Values are means ± standard errors of 28 individual chicks of each treatment.
[a,b] Means within rows with no common superscript differ significantly ($P \leq 0.05$).



O. Halevy et al. / Comparative Biochemistry and Physiology, Part A 120 (1998) 317–323 319

Fig. 1. Number of satellite cells per gram (A) and total number (B) in breast muscle of broilers at five days of age. Breast muscle tissue was removed from five broilers from each experimental group, pooled within each group and weighed. Satellite cells were prepared under similar conditions from each pooled muscle tissue and counted as described in Section 2, Materials and methods.

Table 1. *Body weights of tom turkeys at 1–15 wk after irradiation*

Weeks Postirradiation	*n*	Body Wt, kg
1	5	0.8 ± 0.1*
4	6	1.4 ± 0.1†
7	6	3.1 ± 0.2‡
15	4	11.5 ± 1.0§

Values are means ± SE; *n*, no. of turkeys. Values with different symbols (*, †, ‡, §) are significantly different ($P < 0.05$).

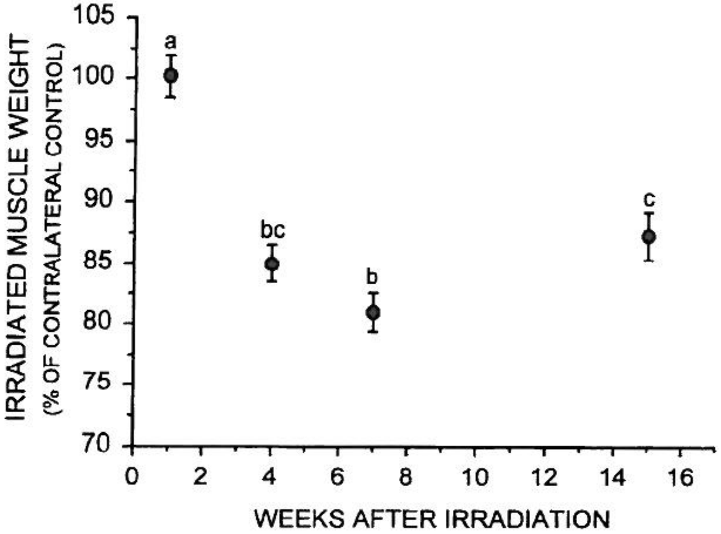


Fig. 3. Left (irradiated) pectoralis thoracicus muscle weight expressed as a percentage of right (nonirradiated) pectoralis thoracicus muscle weight of tom turkeys at 1, 4, 7, and 15 wk after irradiation. Points with different superscripts (a–c) differ significantly ($P < 0.05$).

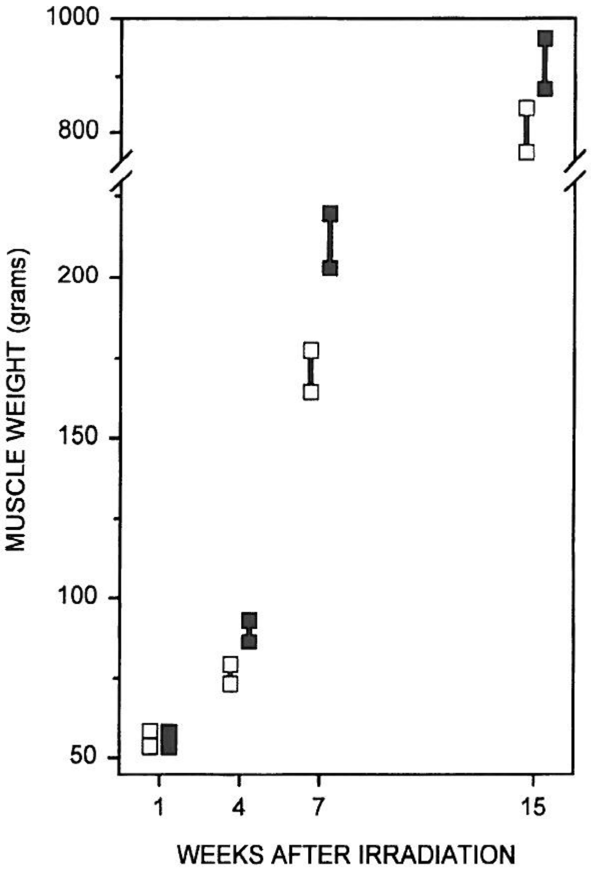


Fig. 2. Effect of irradiation on growth of tom turkey pectoralis thoracicus at 1, 4, 7, and 15 wk after irradiation. Plots represent 99% confidence intervals for each muscle at each time. □, Left (irradiated) pectoralis thoracicus; ■, right (nonirradiated) pectoralis thoracicus.

Mozdziak et al. 1997. Am. J. Physiol. 271:C565-C571.

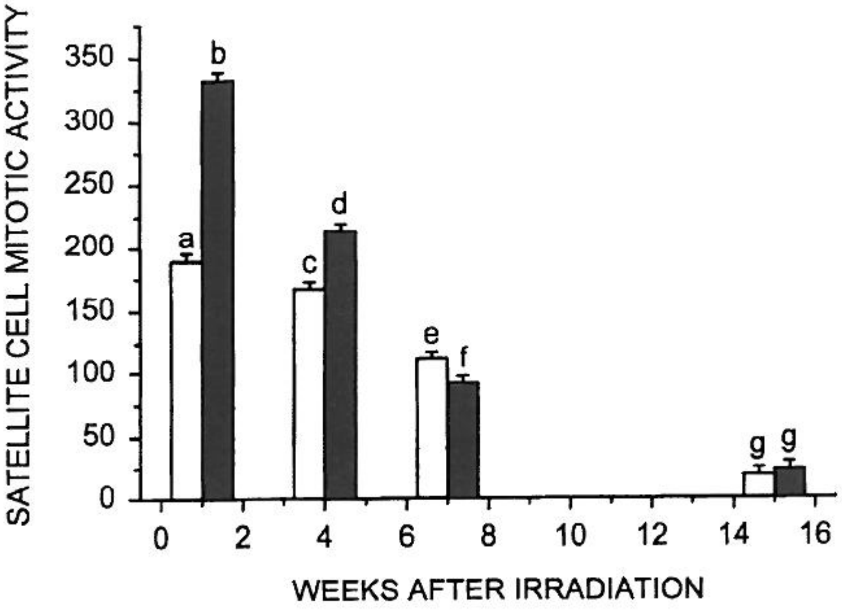
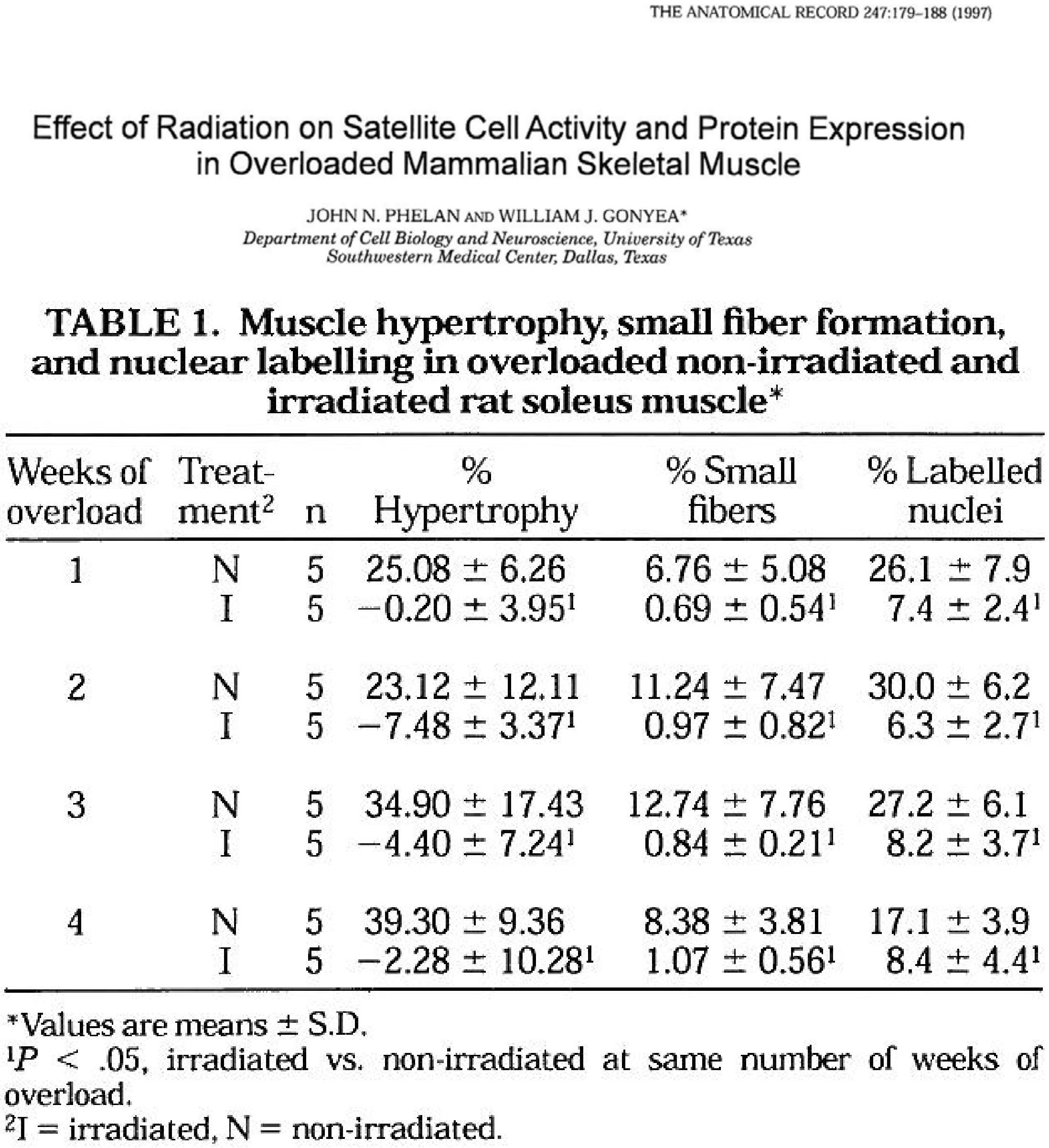


Fig. 4. Index of satellite cell mitotic activity in left (open bars, irradiated) and right (filled bars, nonirradiated) pectoralis thoracicus of tom turkeys at 1, 4, 7, and 15 wk after irradiation. Index of satellite cell mitotic activity is expressed as number of 5-bromo-2′-deoxyuridine-labeled nuclei per 1,000 total myofiber nuclei (satellite cell nuclei + myonuclei). Values are means (bars) ± SE (error bars); bars with different superscripts (a–g) differ significantly ($P < 0.05$).

Mozdziak et al. 1997. Am. J. Physiol. 271:C565-C571.



THE ANATOMICAL RECORD 247:179–188 (1997)

Effect of Radiation on Satellite Cell Activity and Protein Expression in Overloaded Mammalian Skeletal Muscle

JOHN N. PHELAN AND WILLIAM J. GONYEA*
Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas

TABLE 1. Muscle hypertrophy, small fiber formation, and nuclear labelling in overloaded non-irradiated and irradiated rat soleus muscle*

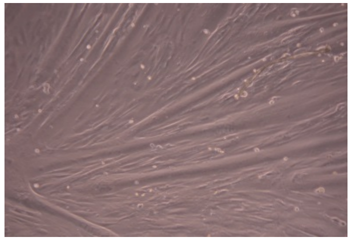
Weeks of overload	Treat-ment[2]	n	% Hypertrophy	% Small fibers	% Labelled nuclei
1	N	5	25.08 ± 6.26	6.76 ± 5.08	26.1 ± 7.9
	I	5	−0.20 ± 3.95[1]	0.69 ± 0.54[1]	7.4 ± 2.4[1]
2	N	5	23.12 ± 12.11	11.24 ± 7.47	30.0 ± 6.2
	I	5	−7.48 ± 3.37[1]	0.97 ± 0.82[1]	6.3 ± 2.7[1]
3	N	5	34.90 ± 17.43	12.74 ± 7.76	27.2 ± 6.1
	I	5	−4.40 ± 7.24[1]	0.84 ± 0.21[1]	8.2 ± 3.7[1]
4	N	5	39.30 ± 9.36	8.38 ± 3.81	17.1 ± 3.9
	I	5	−2.28 ± 10.28[1]	1.07 ± 0.56[1]	8.4 ± 4.4[1]

*Values are means ± S.D.

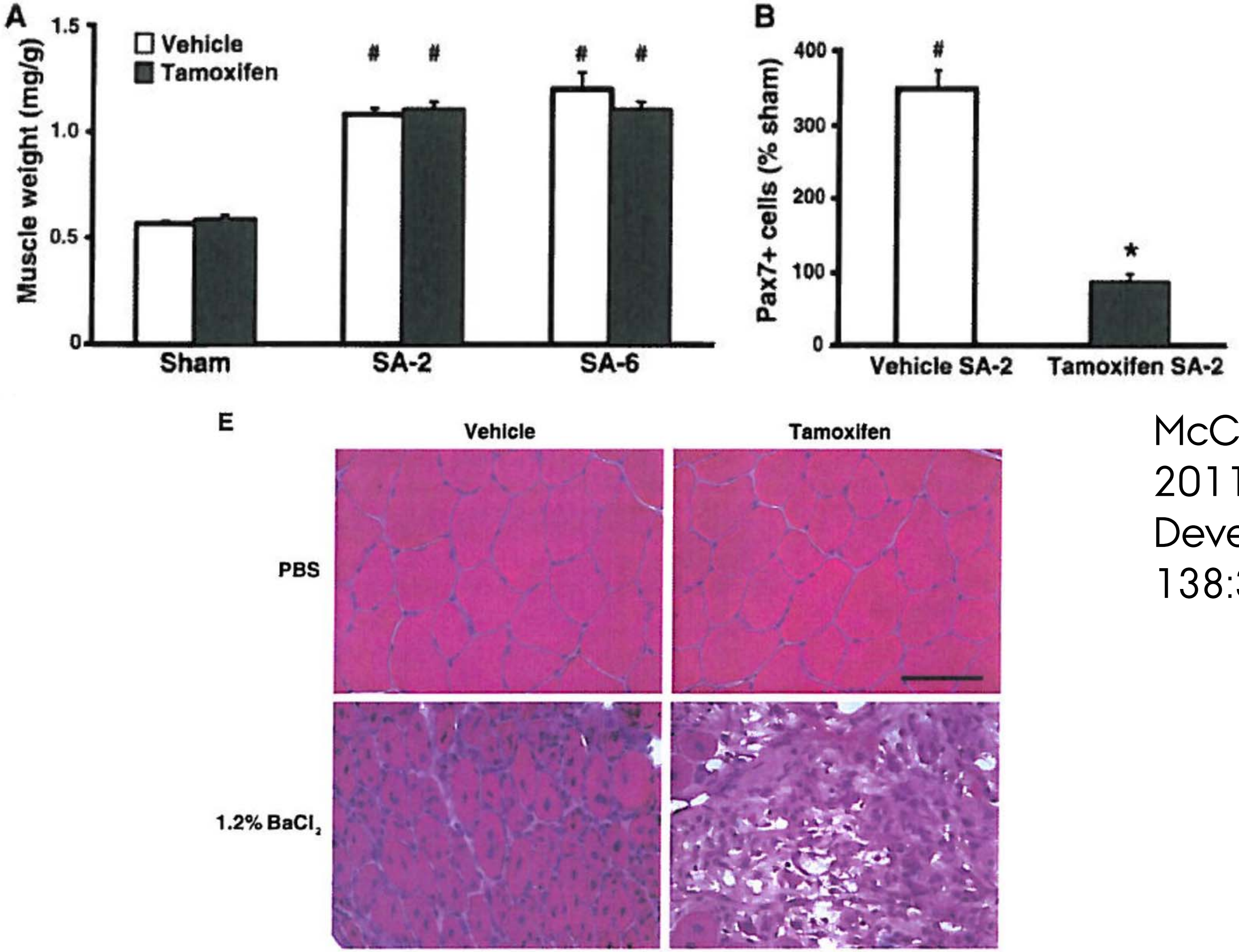
[1] $P < .05$, irradiated vs. non-irradiated at same number of weeks of overload.

[2] I = irradiated, N = non-irradiated.

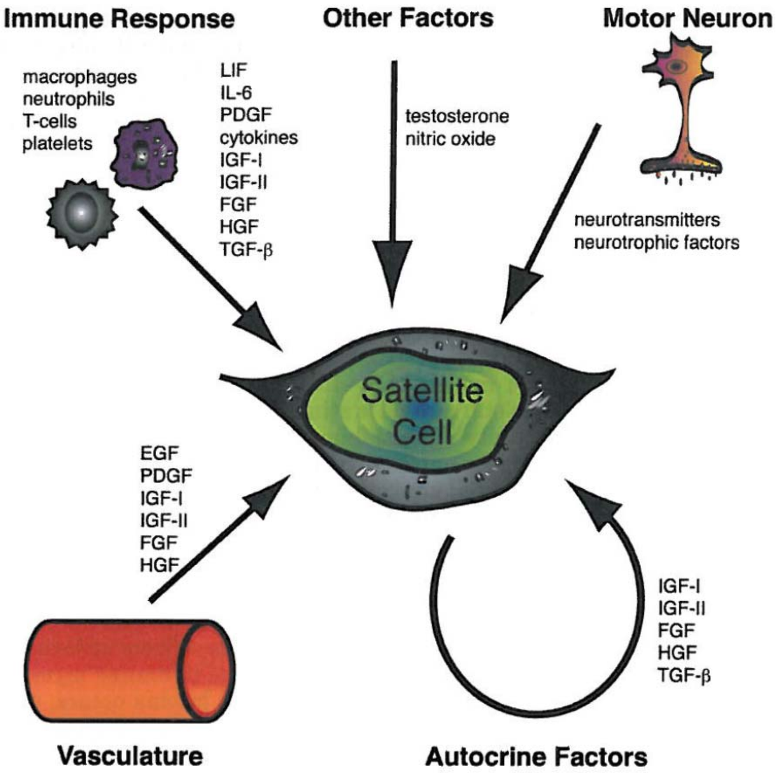
Synergistic muscle: *gastrocnemius, plantaris, and flexor digitorum profundus*.



Effective fiber hypertrophy in satellite cell-depleted skeletal muscle

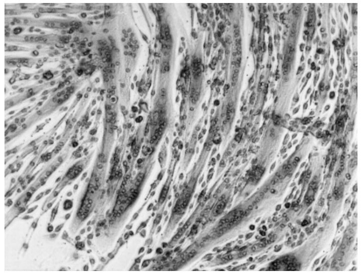


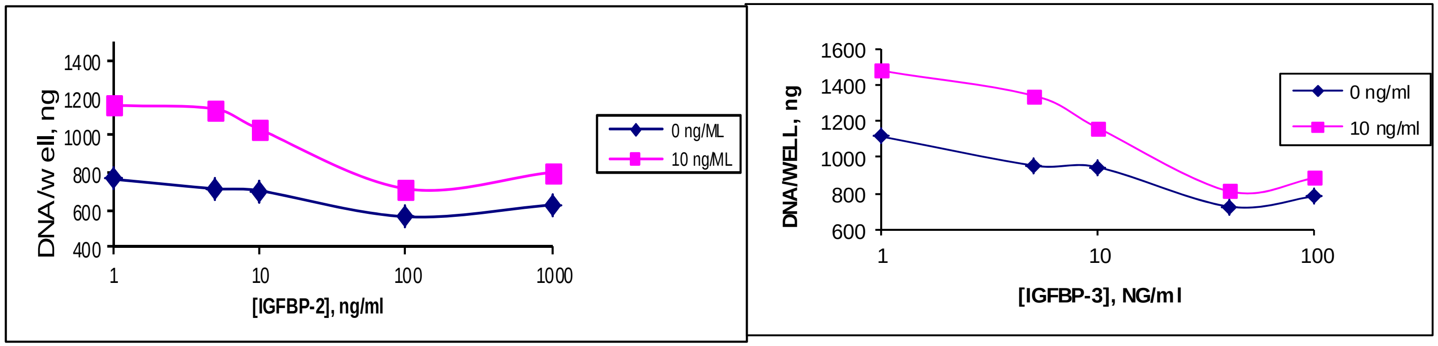
McCarthy et al.
2011.
Development
138:3657-3666



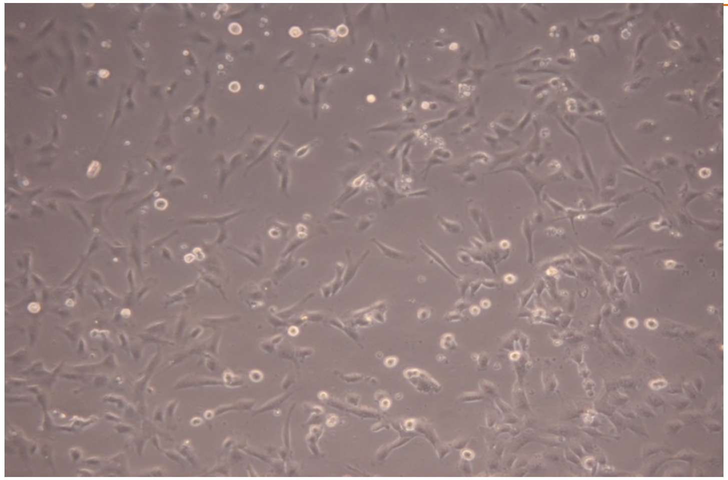
Hawke and Garry. 2001. J Appl Physiol. 91: 534-551

Effects of IGFBP-2, and IGFBP-3 on C2C12 cells at low or high level of IGF-I

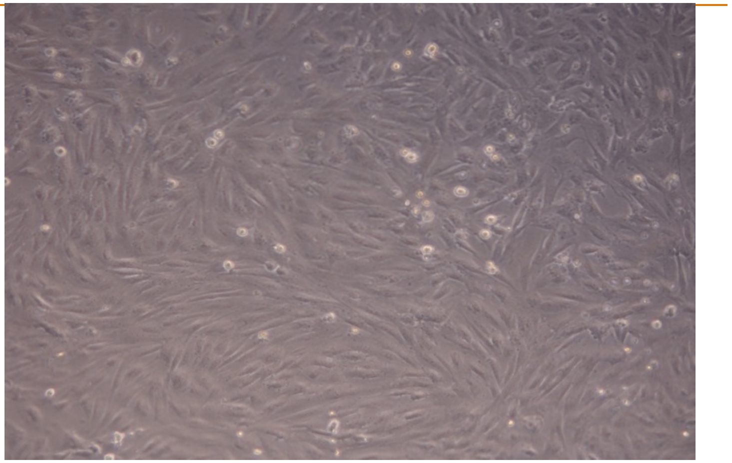




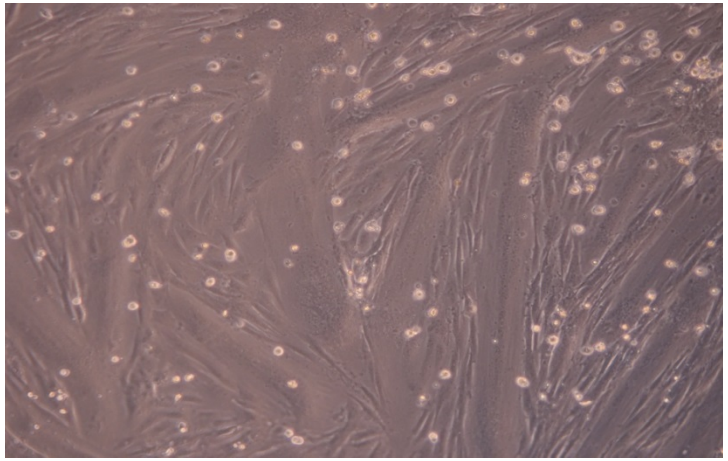
Oksbjerg, Nissen, Vestergaard.2005. J. Anim.Physiol. And Anim. Nutr. 2055



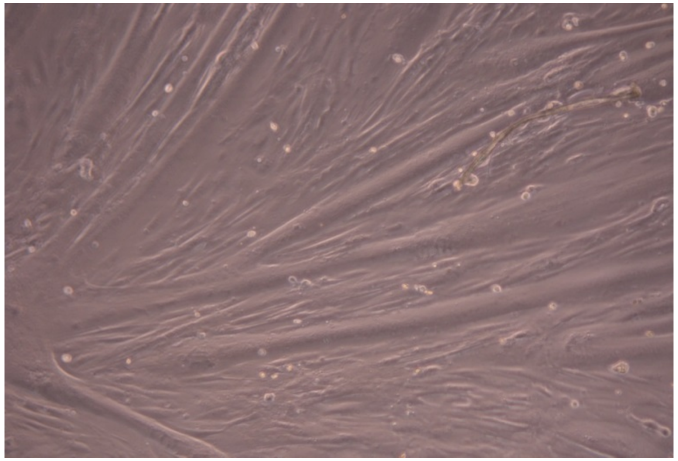
80% confluence



Day 1

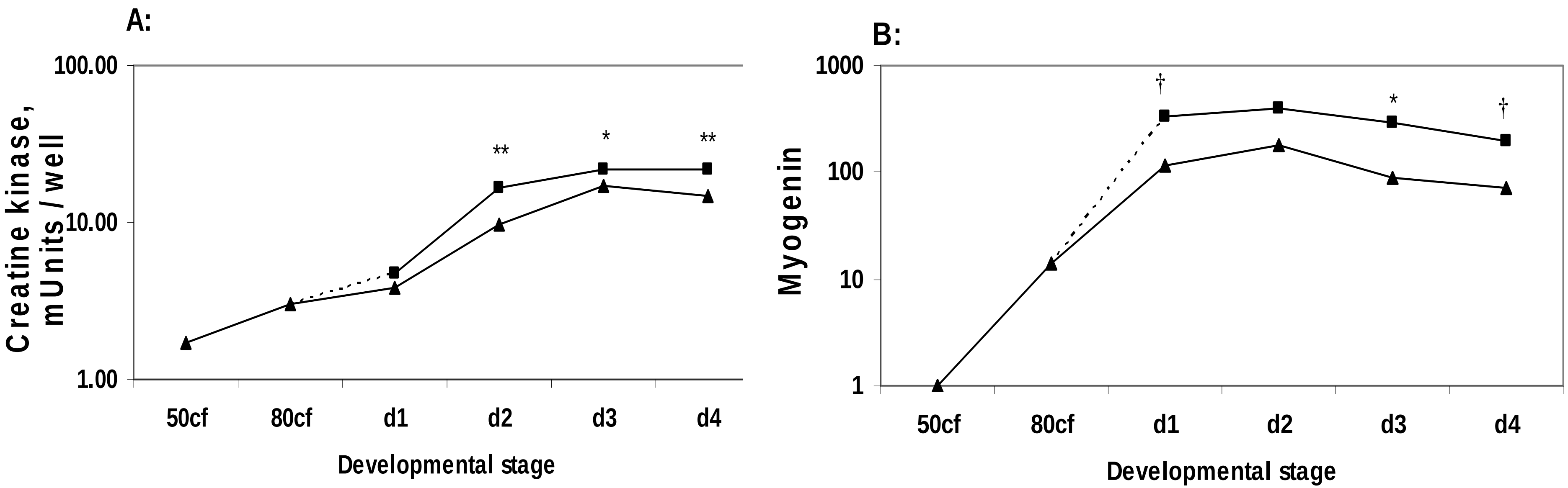


Day 2

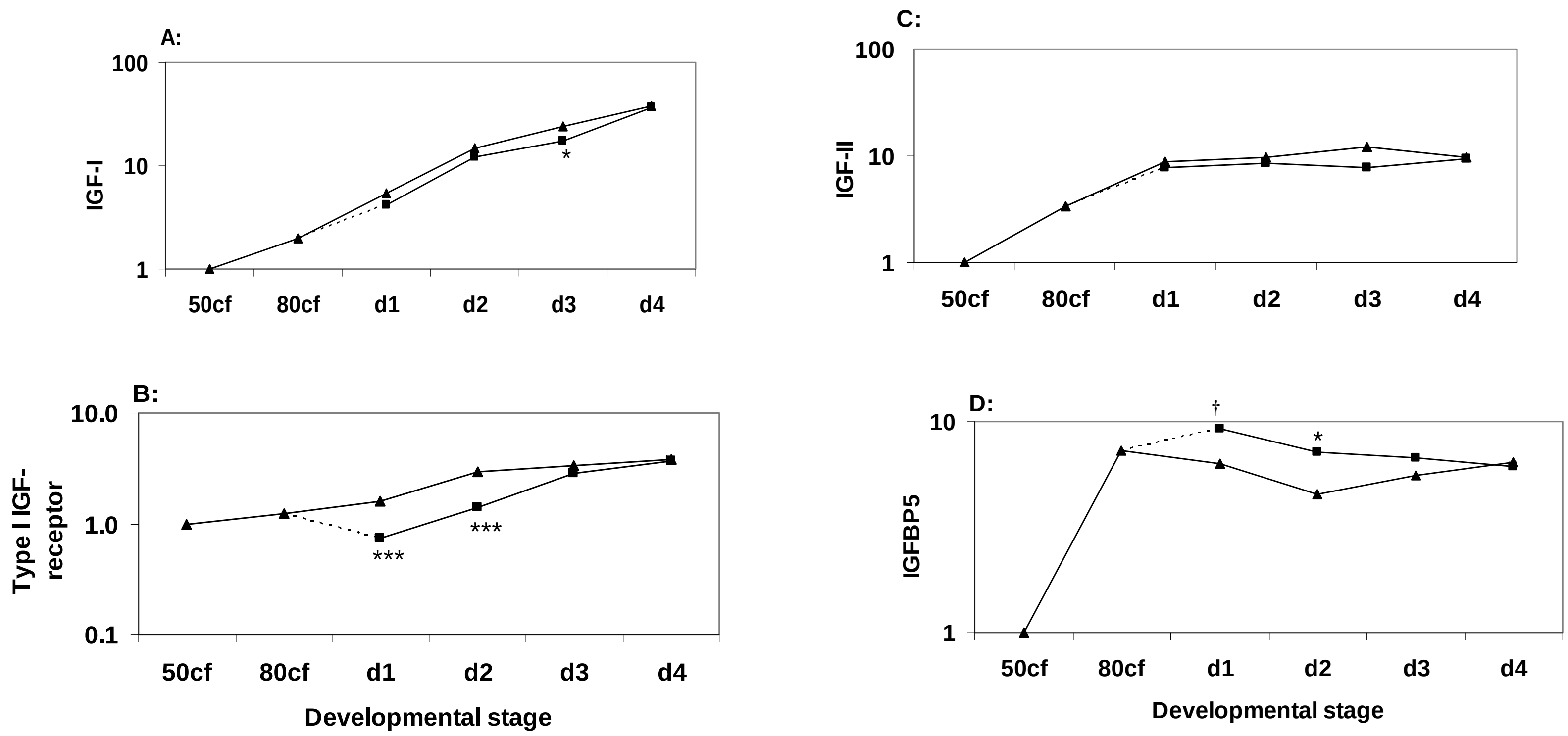


Day 3

Creatine Phosphokinase and mRNA of myogenin during myogeneses in



mRNA during myogenesis of porcine satellite cells



Theil, Sørensen, Nissen, Oksbjerg. 2006, Anim. Sci J, 77:330-337.

Summary: Changes in muscle traits by age of lower limb muscles

Muscle fibre number decline by age ↓
Type I fibres,% ↑

Muscle regenerative capacity (SC content/ activation, proliferation and differentiation) ↓

SC content of Type II ↓

Selective decrease in Type II CSA, atrophy ↓

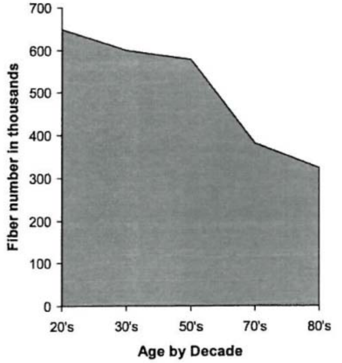


Fig. 2. Decrease in total muscle fiber number with age in whole vastus lateralis muscle. Adapted from Lexell et al. (1988).

Reduces muscle mass and muscle function (endurance and strength) leading to disability and loss of independence

SUMMARY:

- Muscle DNA increases by age and SC are the major source.
- SC reside in the niche between the basal lamina and the sarcolemma.
- In growing animals there are 2 populations of SC: One is proliferating (Pax/MyoD) and for growth, and one is quiescent (Pax7) and for re-generation through whole life.
- The number of satellite SC cell decreases by age and is higher in type I than type II fibre.
- SC number changes along with changing in muscle growth and if SC cells are killed by γ-irradiation this inhibits muscle growth and inhibit muscle repair. However, more recent studies question this.
- SCs are regulated by growth factors
- Needless to say but research is needed on satellite cells in meat producing animals (signaling) in order to optimize growth.

FUTURE PERSPECTIVES?

Plant extracts for increasing proliferation for better growth

Reprograming of satellite cell to the adipogenic lineage

Satellite cell as a donor for production of in vitro meat (cultured meat)

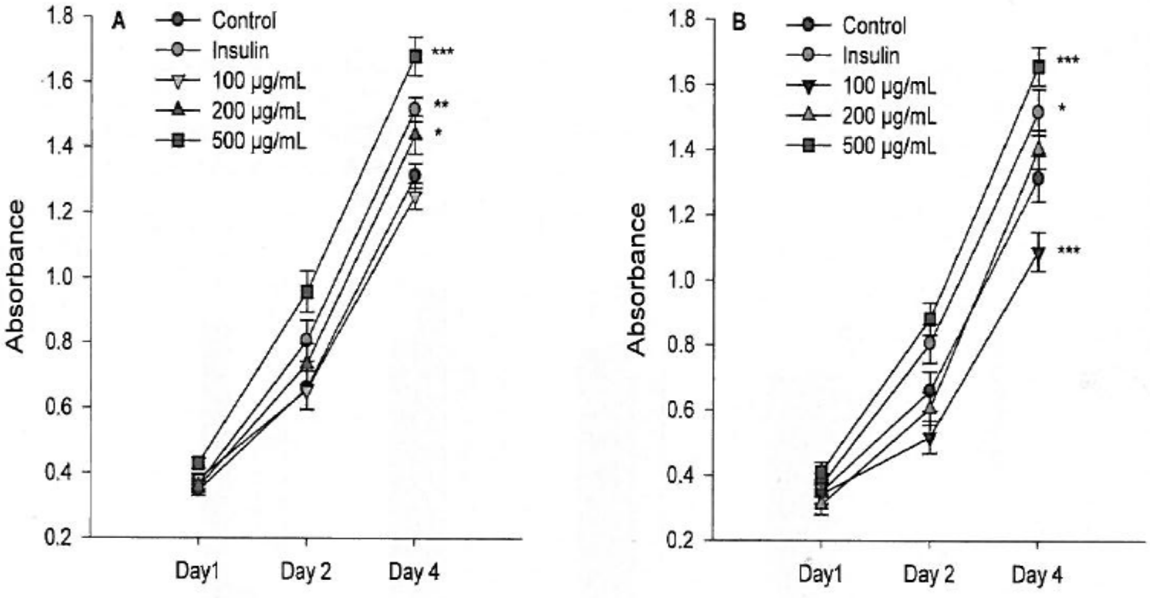


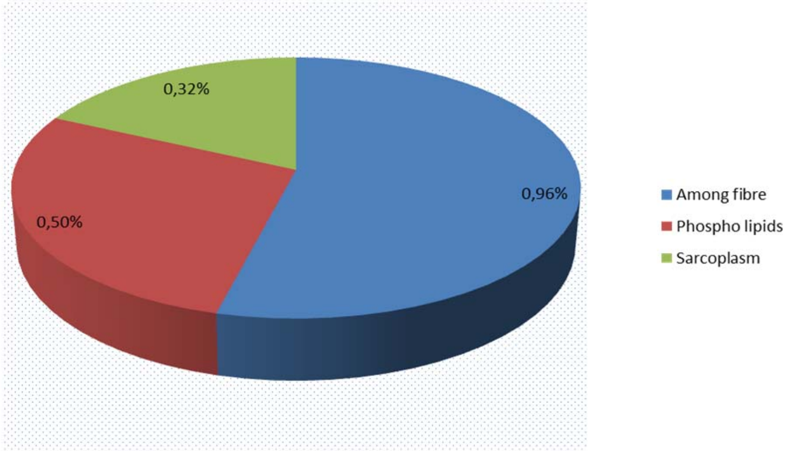
Fig. 15 Effect of selected extracts on proliferation of myoblasts

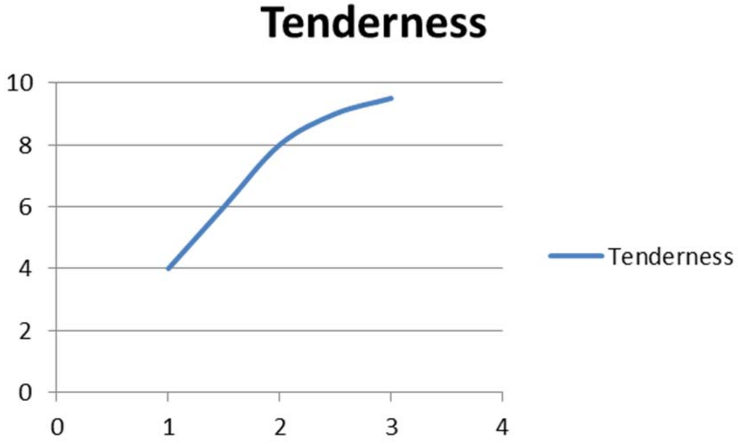
The effect of (A) *D. carota* (purple haze) MeOH extract and (B) *S. nigra* DCM extracts on satellite cells isolated from porcine skeletal muscles. The absorbance values ($A_{450\ nm}$-$A_{650\ nm}$) are proportional to the

Bhattacharya, S. 2013. Health promoting compounds in plants: targeting type 2 diabetes. PhD Thesis, Science and Technology, Aarhus University

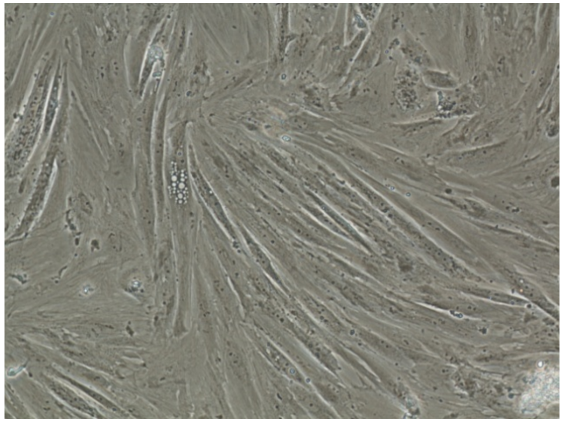
Intra-muscular fat (IMF) in pork chops of pigs (Kobe beef)

IMF=1.6%

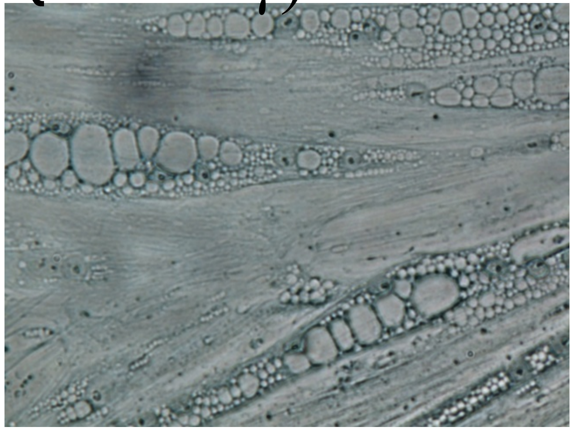




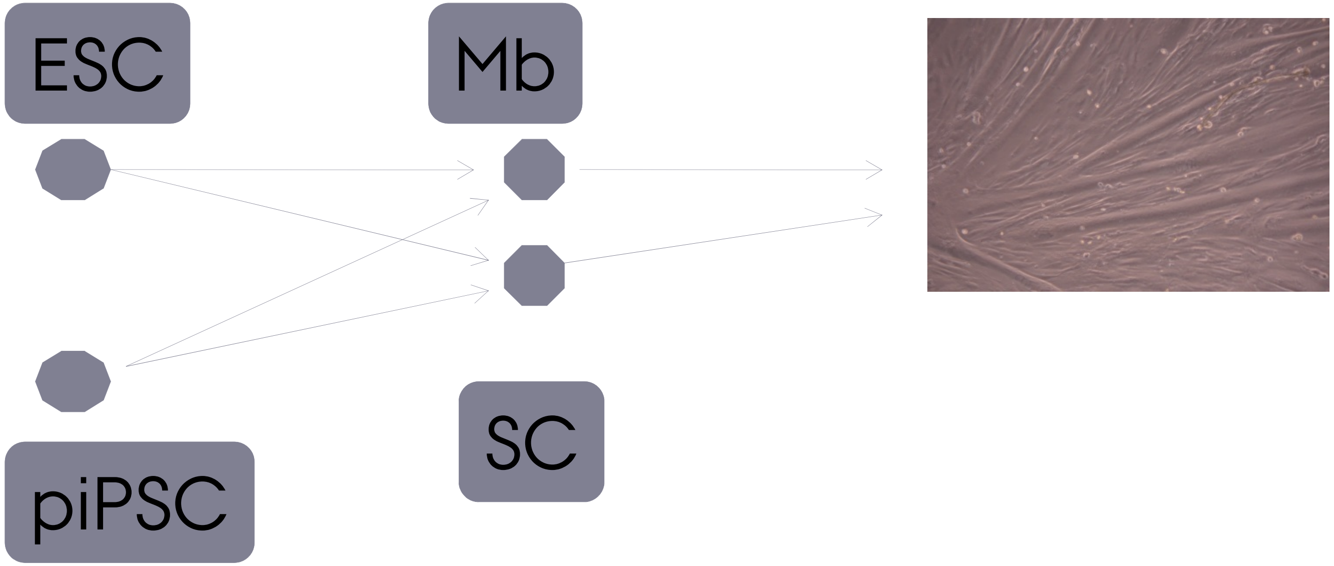
Control



50 µM Citiglitazone (PPAR-γ)



LE CREUSET



ESC

Mb

SC

piPSC

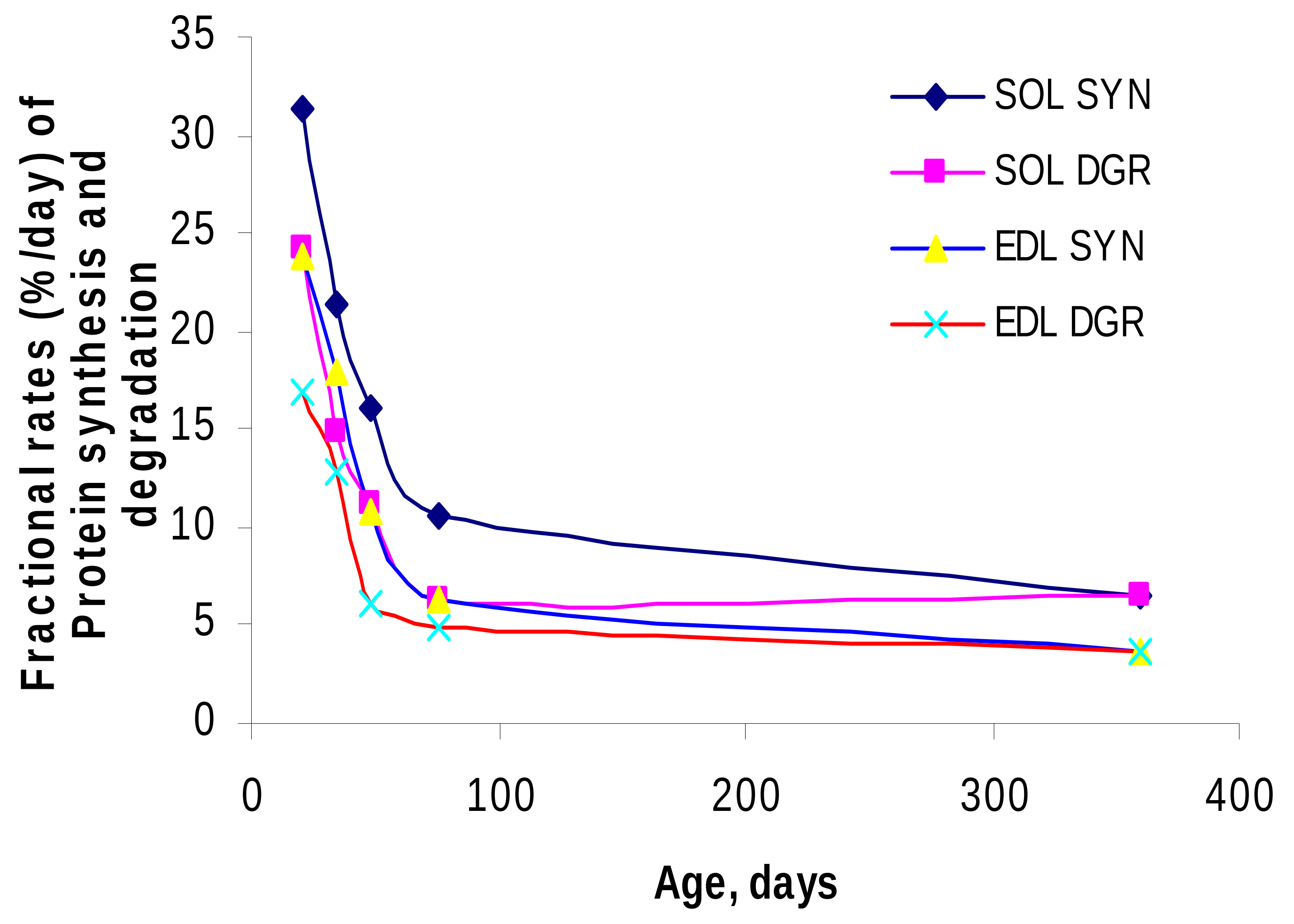
ESC = Embryonal stem cell

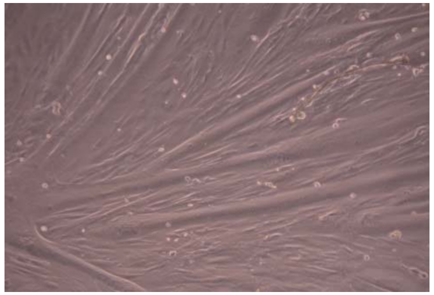
piPSC = porcine inducible pluripotent stem cell

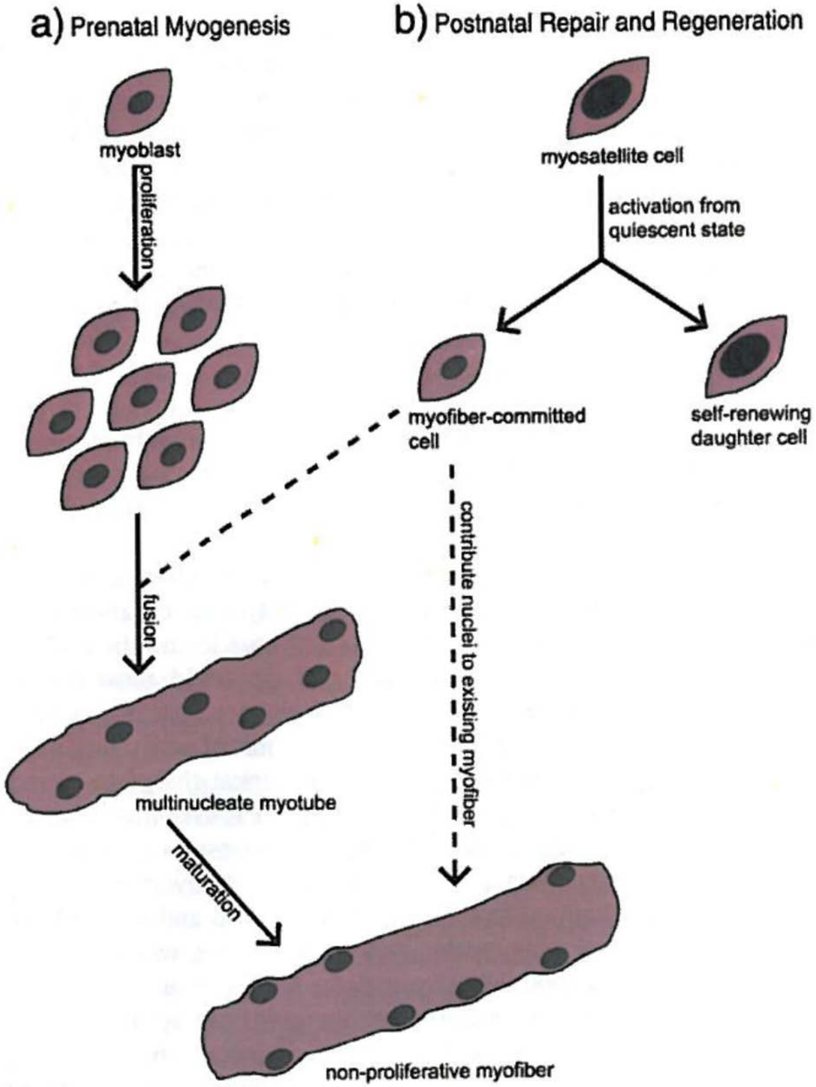
Mb = myoblast

SC = satellite cells

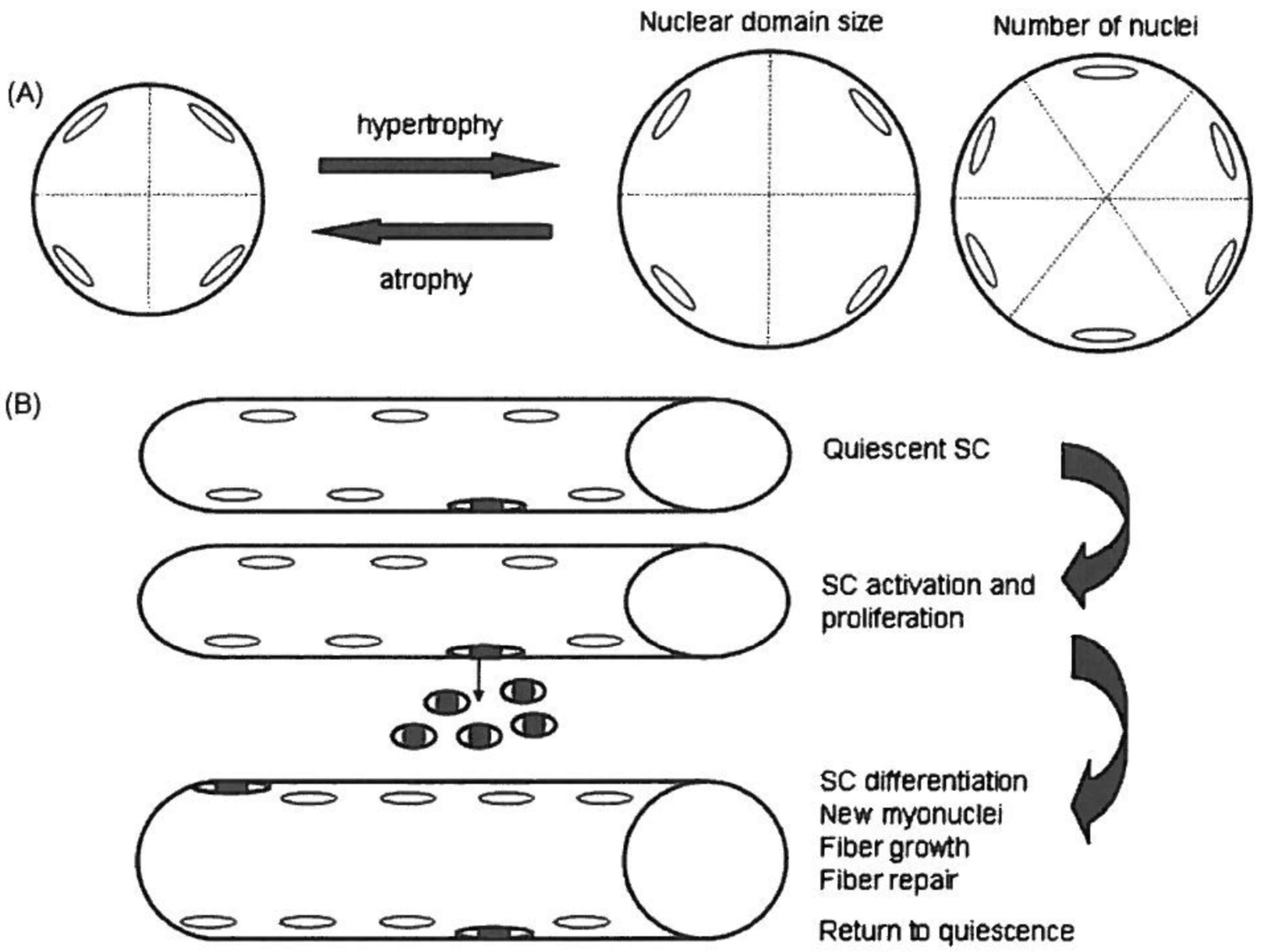
FRACTIONAL RATES OF PROTEIN SYNTHESIS AND DEGRADATION IN SLOW- AND FAST-TWITCH MUSCLE OF FEMALE RATS (AFTER *GARLICK ET AL. 1989.*)







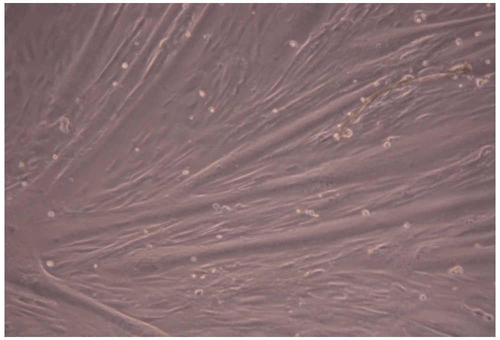
a) Prenatal Myogenesis
b) Postnatal Repair and Regeneration
myoblast
proliferation
fusion
multinucleate myotube
maturation
non-proliferative myofiber
myosatellite cell
activation from
quiescent state
myofiber-committed
cell
self-renewing
daughter cell
contribute nuclei to existing myofiber

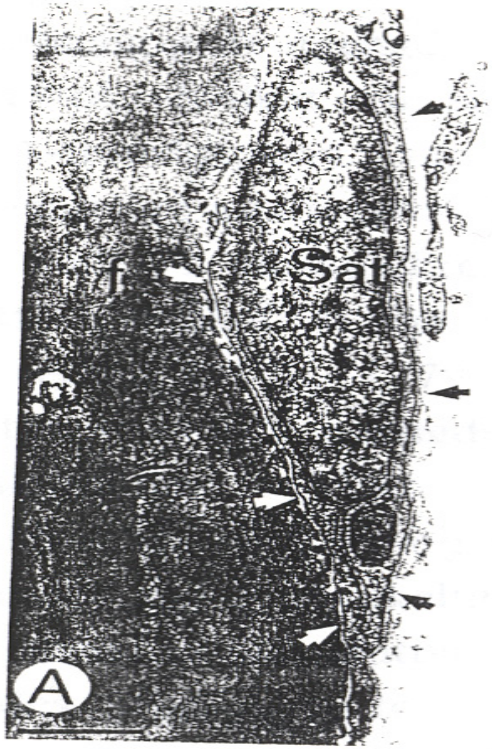


T. Snijders et al. / Ageing Research Reviews 8 (2009) 328–338

SATELLITE CELL

IMPORTANCE FOR POSTNATAL GROWTH

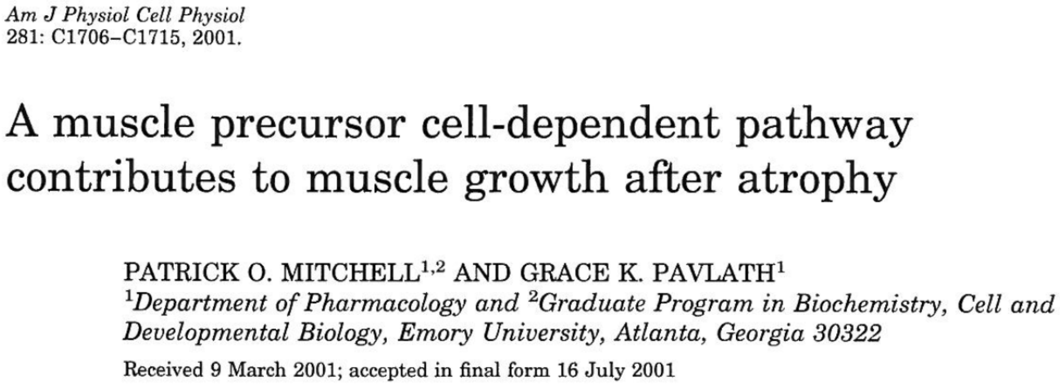




Figur 2.1. *Satellitcelle (Sat) der ligger mellem basal-membranen (sorte pile) og sarcolemme (hvide pile) (fra Yablonka-Reuveni, 1995).*

Satellite cell (SR) activator: HGF

- Conditioned medium from stretched SC stimulated SR not stretched.
- Conditioned medium from stretched SC grown with NOS-inhibitor did not accelerate activation of un-stretched control SC, and HGF was not released to the medium.
- Conditioned medium from un-stretched SR in the presence nitro Oxid donor, accelerated the activation of SR in vitro, and HGF was found in the conditioned medium.
- Assays of NOS activity were present in SR cultures revealed that NOS is stimulated when SR are stretched in vitro.
- Conclusion: It is indicated that stretch triggers an intracellular cascade of events including NO synthesis which results in HGF release and SCR activation.
- Tatsumi et al. Molecular Biology of the cell. 2002, 13:2909-2918 c-met. HGF is expressed in young but not adult animals

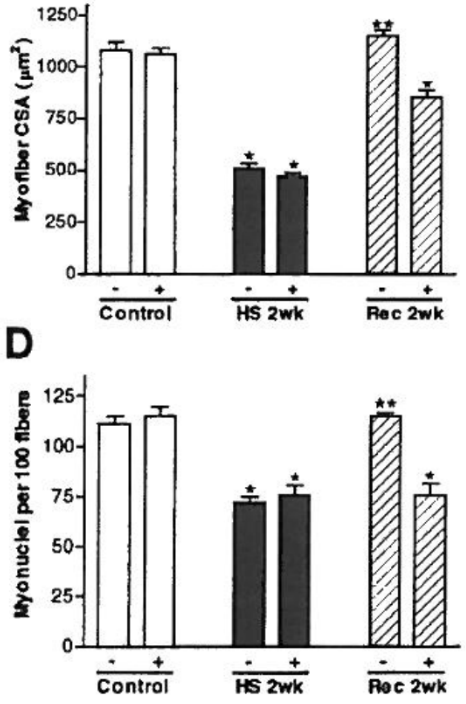


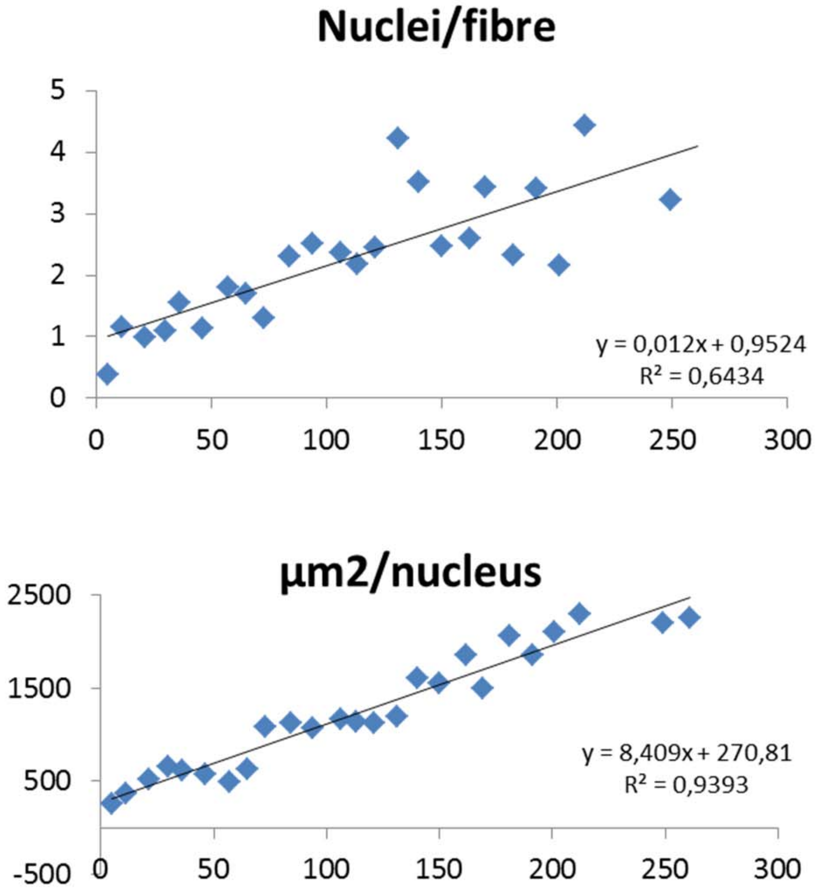
Am J Physiol Cell Physiol
281: C1706–C1715, 2001.

A muscle precursor cell-dependent pathway contributes to muscle growth after atrophy

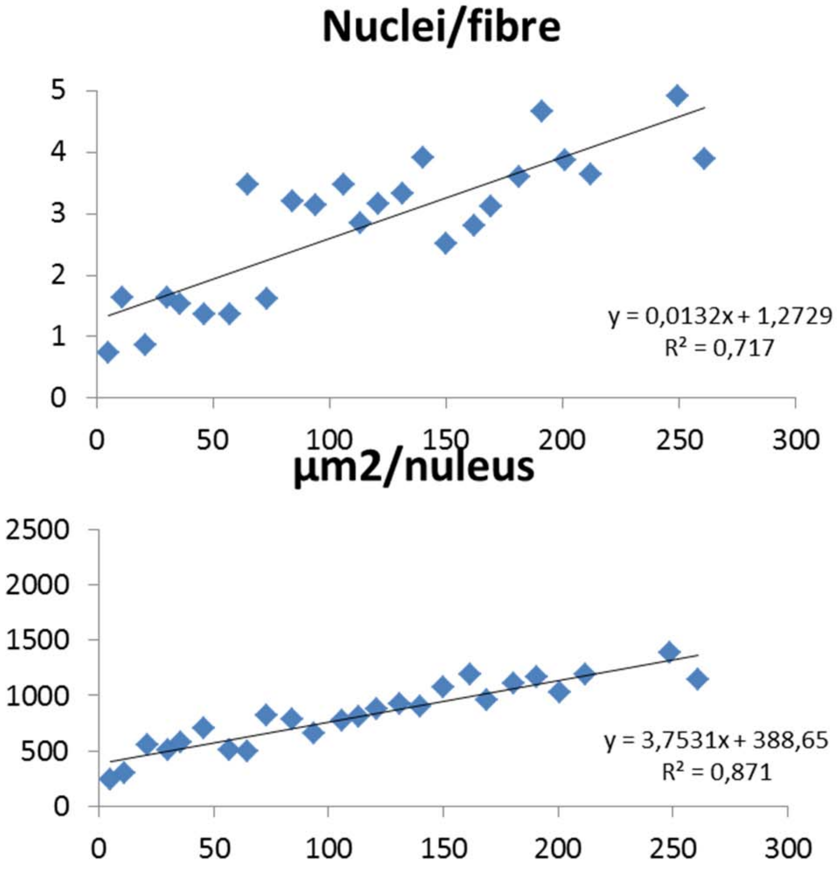
PATRICK O. MITCHELL[1,2] AND GRACE K. PAVLATH[1]
[1]*Department of Pharmacology and* [2]*Graduate Program in Biochemistry, Cell and Developmental Biology, Emory University, Atlanta, Georgia 30322*

Received 9 March 2001; accepted in final form 16 July 2001



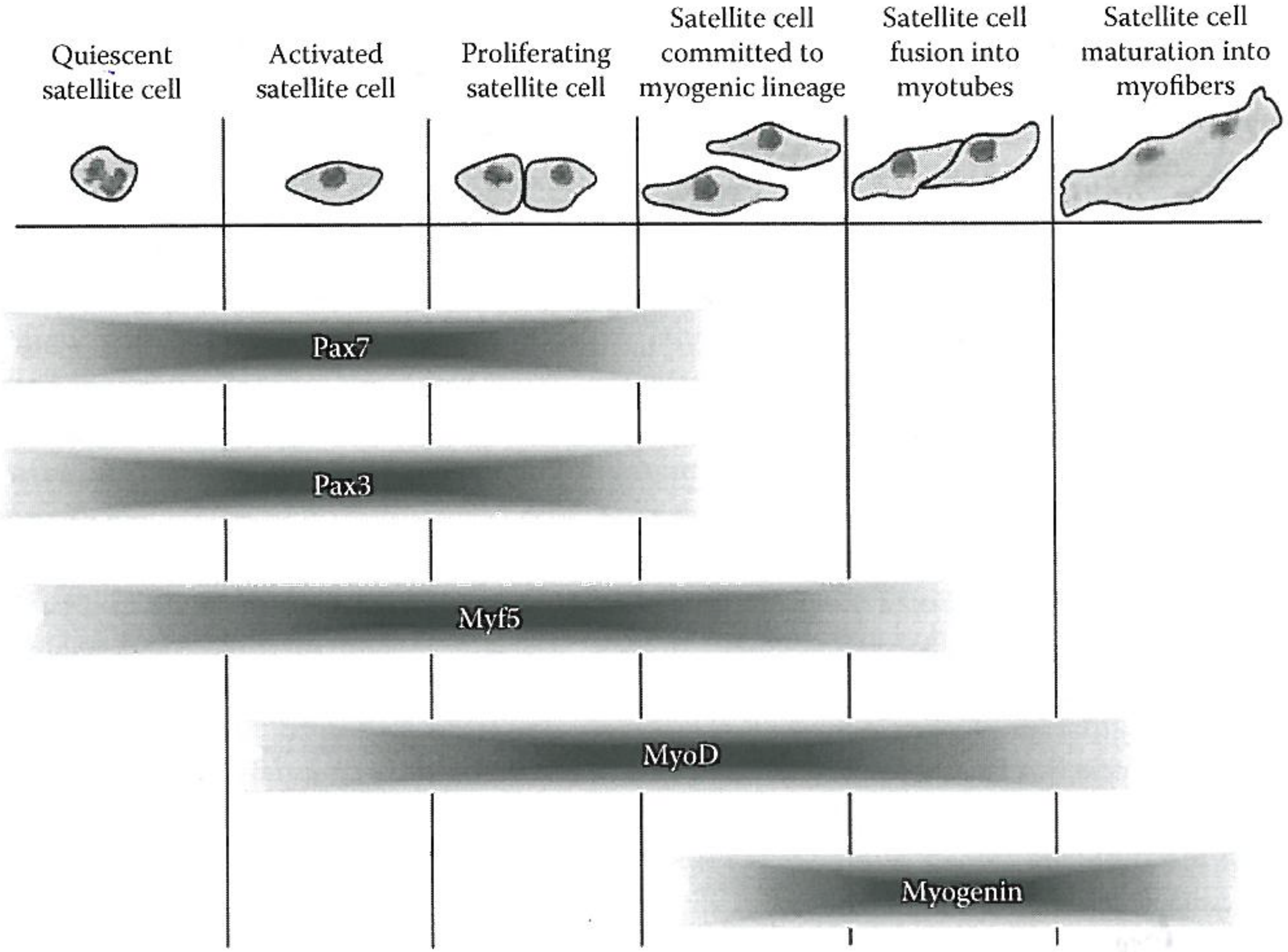


M. Longissimus dorsi



M. Vastus intermedius

(Oksbjerg et al. 2009. Trends in Comparative Biochem & Physiol)



Rhoads, Rathbone, Flann. In Applied Muscle Biology and Meat Science. Pp47-67. eds. M. Du, and R.J. McCormick. CPR Press, ISBN 978-1-4200-9272.

Duclos et al. 1996. Growth Regulation. 6:176-184

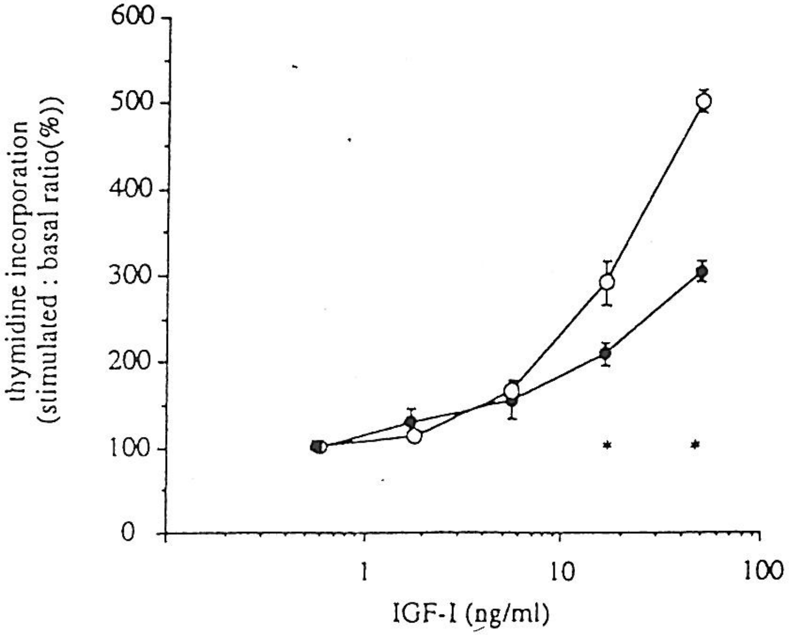
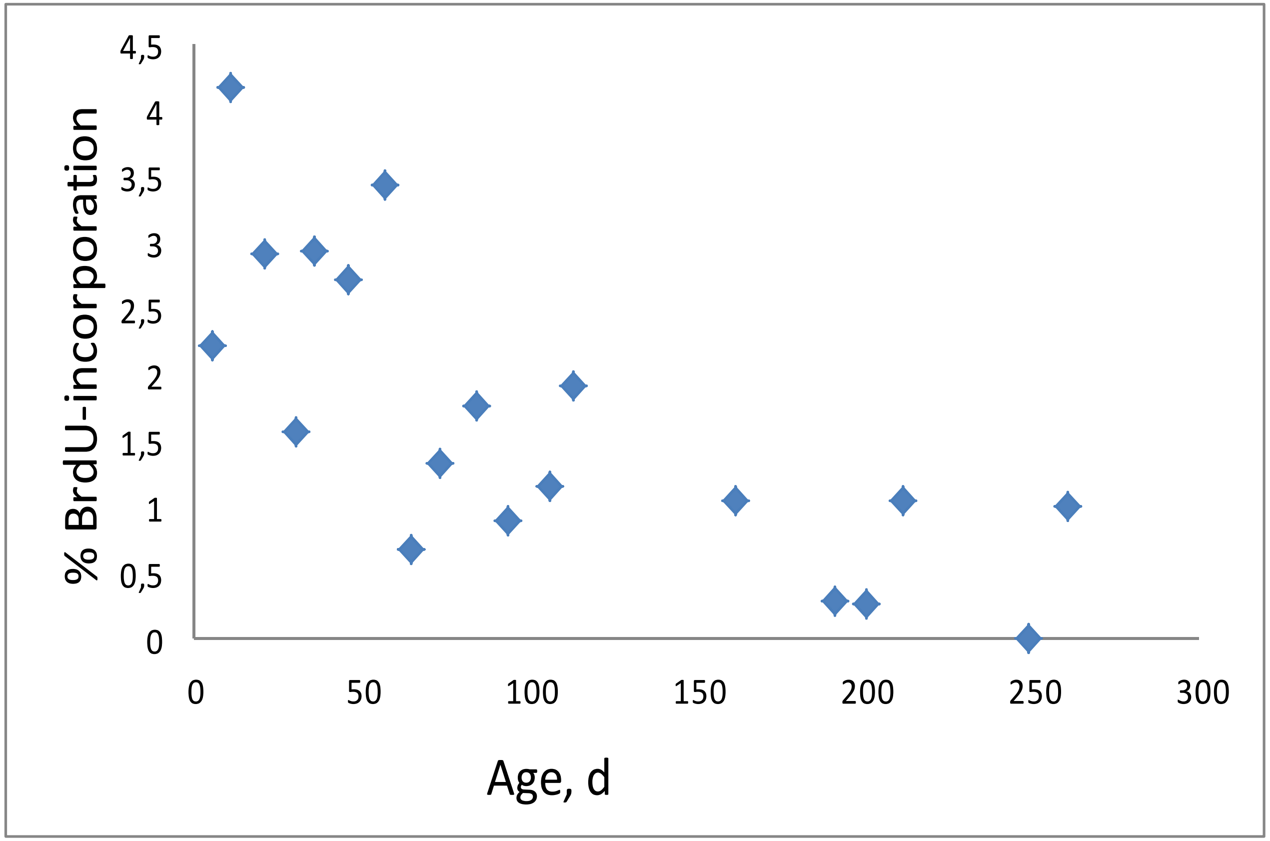


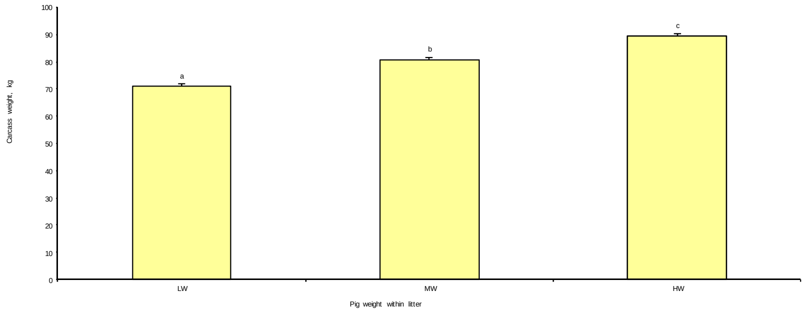
Fig. 3—Incorporation of [^{3}H]-thymidine into DNA of myoblasts prepared from pectoralis muscle of 1-day-old fast-growing (HG, ○) or slow-growing (LG, ●) chicks in response to IGF-I. Experimental details and statistical methods are provided in Materials and Methods. Data are presented as the mean ± SE of the percent of basal levels from three independent experiments. *Indicates a significant difference between lines ($P < 0.05$) at the corresponding peptide concentration.

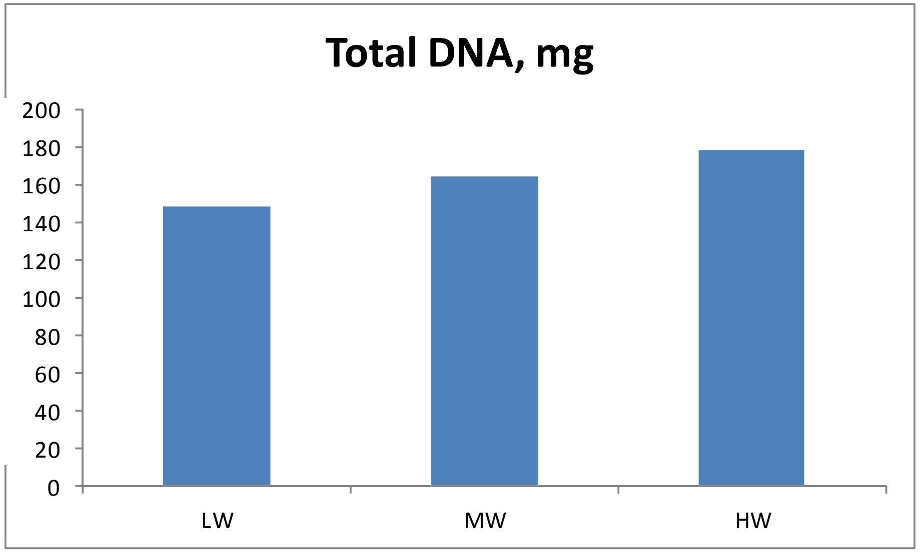
Labelling vs. age in pigs

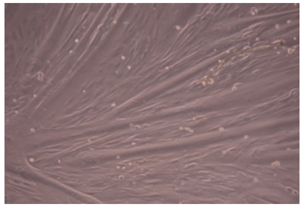
vastus intermedius



Estimated Total DNA (mg) in semitendinosus muscle of LW, MW, and HW pigs within litters)





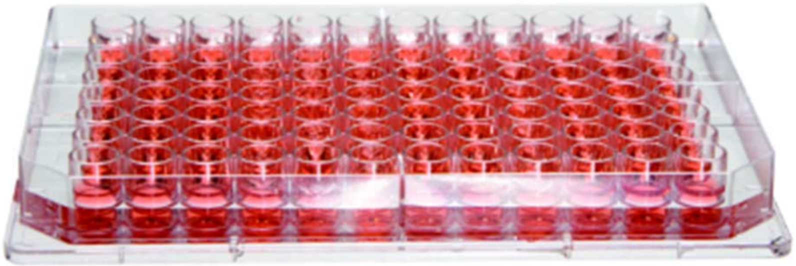


Nissen, Jorgensen, Oksbjerg 2004, JAS 82 .414-421.

Are satellite cells equal?

Clonal analysis

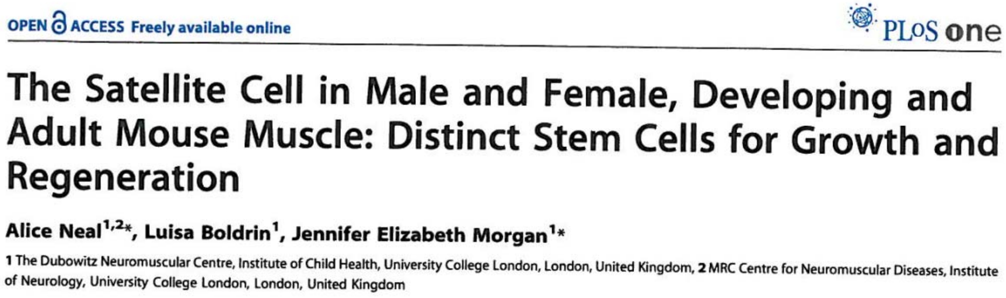
One cell/well



1) Slow dividing clones

2) Fast dividing and fusion competent clones

Schultz. (1996) Developmental Biology. 175, 84-94.
Rouger et al. 2004. Cell Tissue Res, 317: 319-326 (tom Turkey)
Li et al. 2011. J. Anim. Sci. 89:1751-1757. (young cattle)



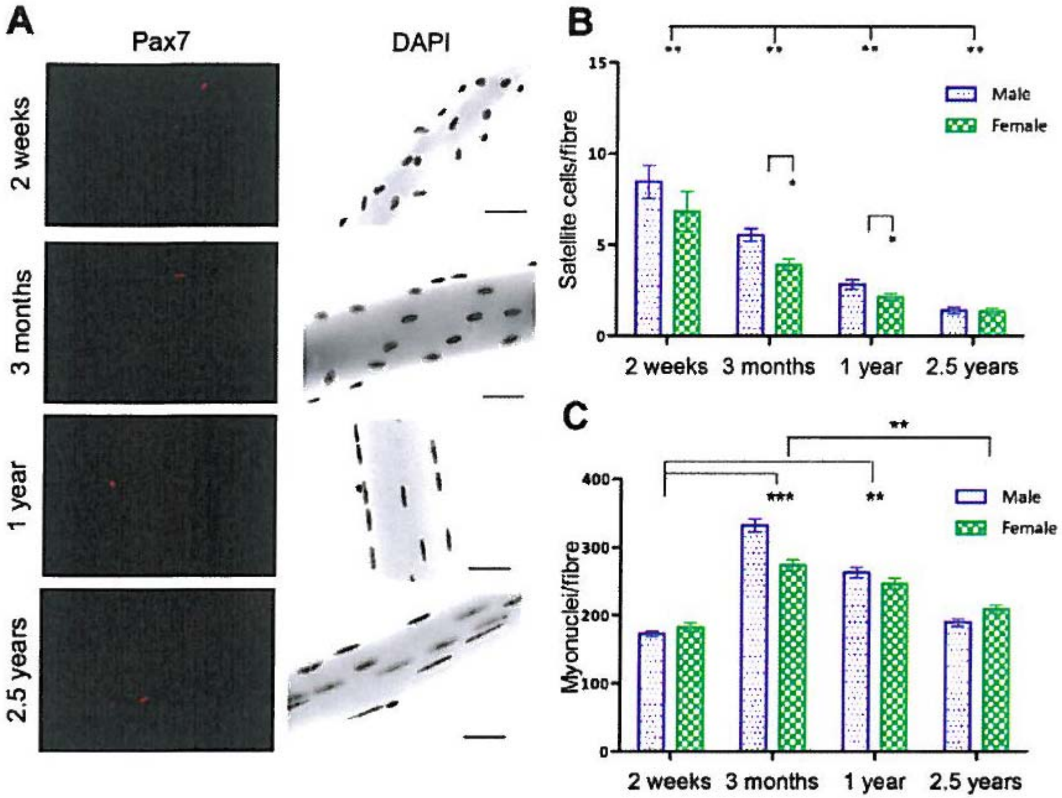
OPEN ACCESS Freely available online   PLoS one

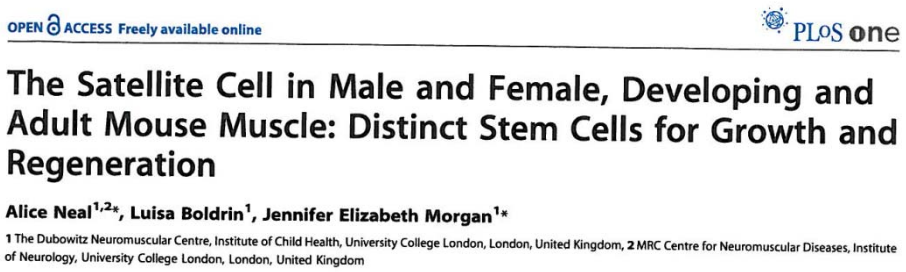
The Satellite Cell in Male and Female, Developing and Adult Mouse Muscle: Distinct Stem Cells for Growth and Regeneration

Alice Neal[1,2]*, **Luisa Boldrin**[1], **Jennifer Elizabeth Morgan**[1]*

1 The Dubowitz Neuromuscular Centre, Institute of Child Health, University College London, London, United Kingdom, 2 MRC Centre for Neuromuscular Diseases, Institute of Neurology, University College London, London, United Kingdom

PLoS One 2012. 7(5)(2012,e37950.
doi:10.1371/jounal.pone.0037950 :





OPEN ACCESS Freely available online

PLoS one

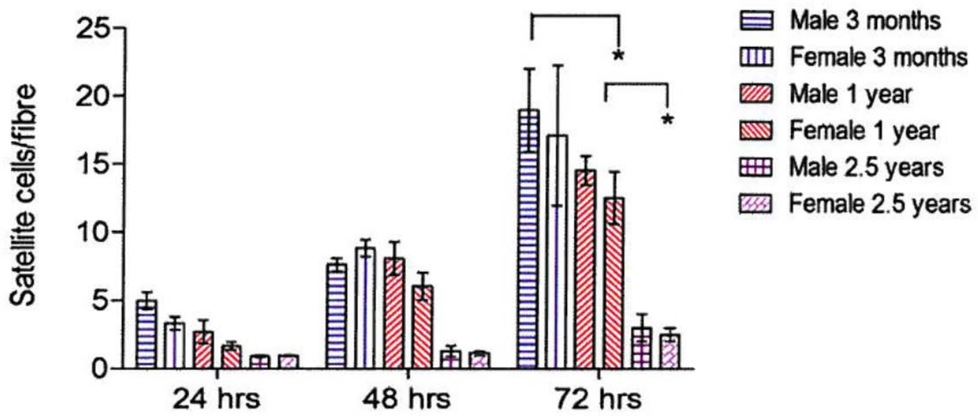
The Satellite Cell in Male and Female, Developing and Adult Mouse Muscle: Distinct Stem Cells for Growth and Regeneration

Alice Neal[1,2]*, **Luisa Boldrin**[1], **Jennifer Elizabeth Morgan**[1]*

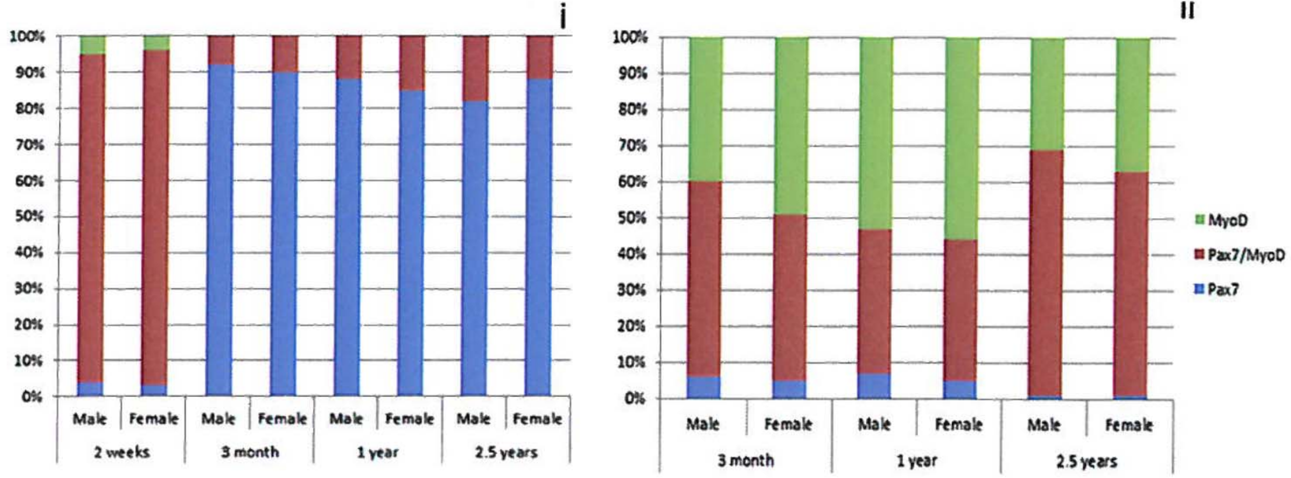
1 The Dubowitz Neuromuscular Centre, Institute of Child Health, University College London, London, United Kingdom, 2 MRC Centre for Neuromuscular Diseases, Institute of Neurology, University College London, London, United Kingdom

PLoS One 2012. 7(5)(2012,e37950.
doi:10.1371/jounal.pone.0037950 :

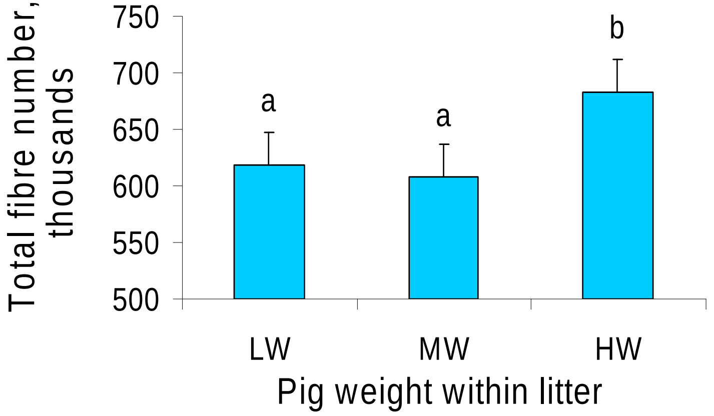
C

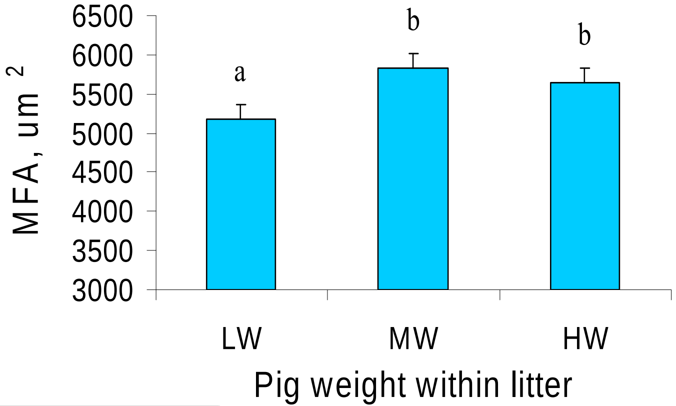


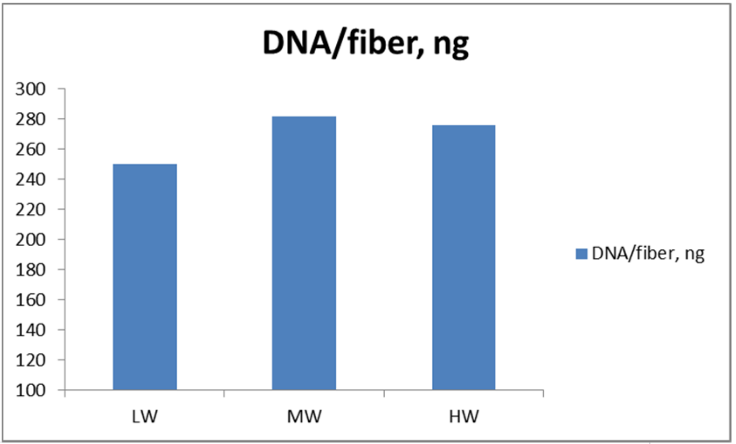
D



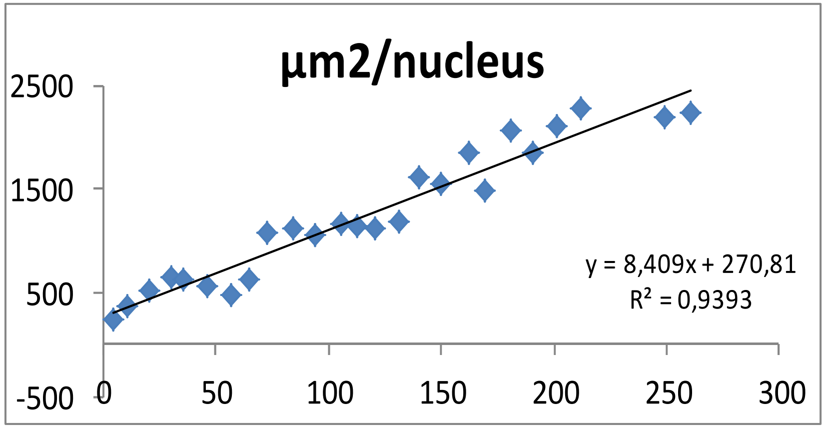
INTRA-LITTER VARIATION IN MUSCLE FIBRES

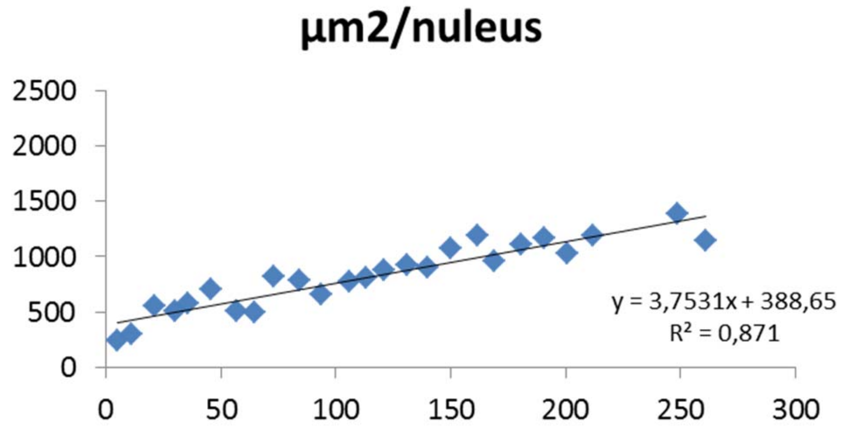






Nissen, Jorgensen, Oksbjerg 2004, JAS 82 .414-421.





M. Longissimus dorsi

M. Vastus intermedius

µm²/nucleus
µm³/nucleus

(Oksbjerg et al. 2009. Trends in Comparative Biochem & Physiol)