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Prenatal factors influencing muscle growth & development

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Division of Nutritional Sciences

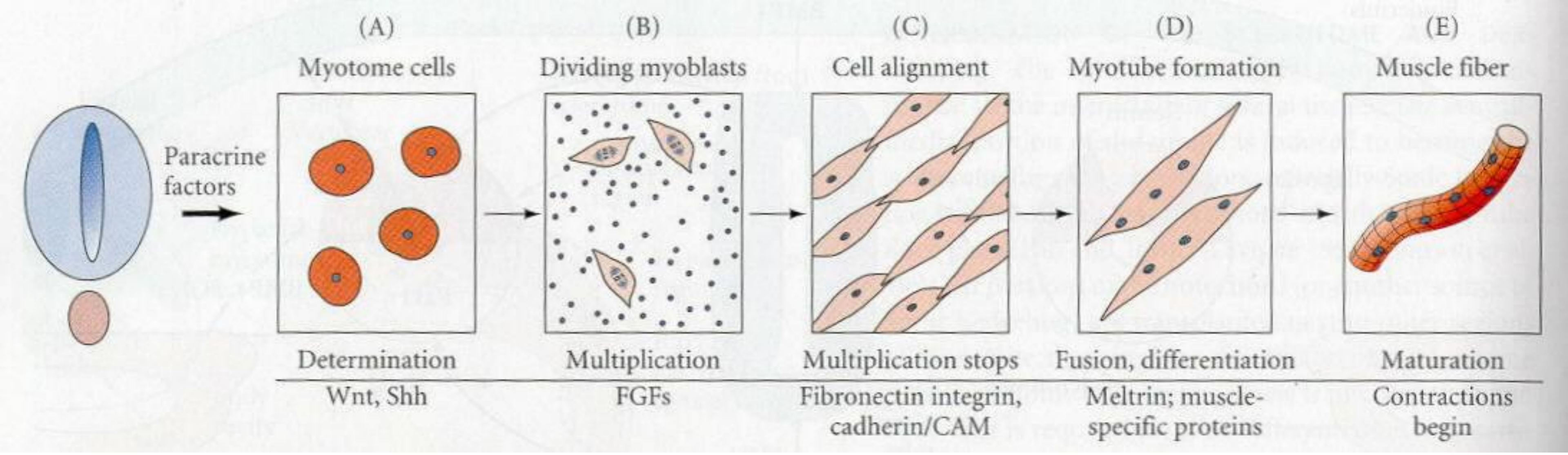
School of Biosciences

world-changing research from The University of Nottingham

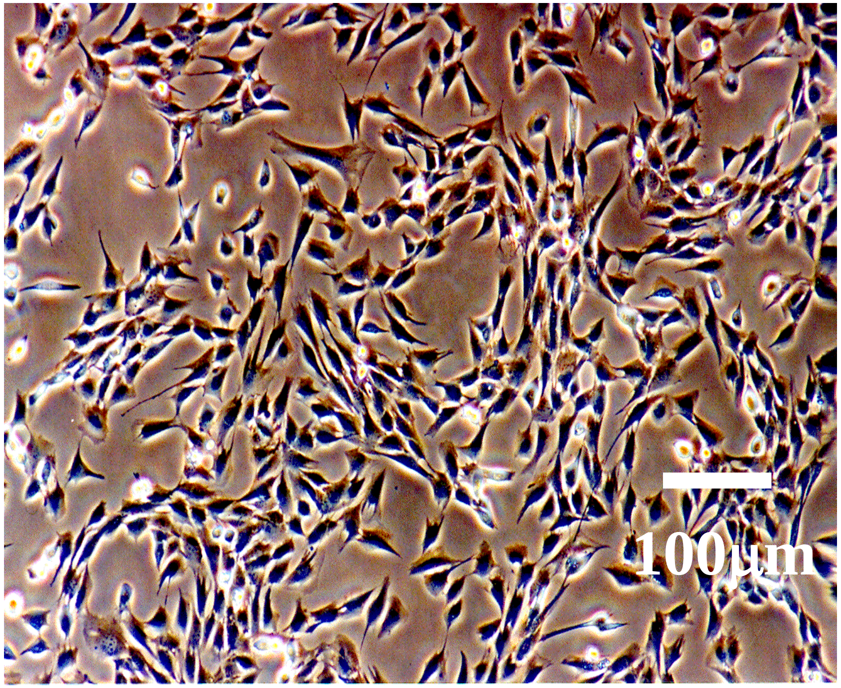
Outline

- Introduction: Myogenesis & muscle fibre development
- Genetic effects on Muscle Fibre Number
- Maternal Nutrition effects on Muscle Fibre Number
- Long-term effects on muscle wts and adiposity
- Is muscle IGF-II expression a key mechanism?

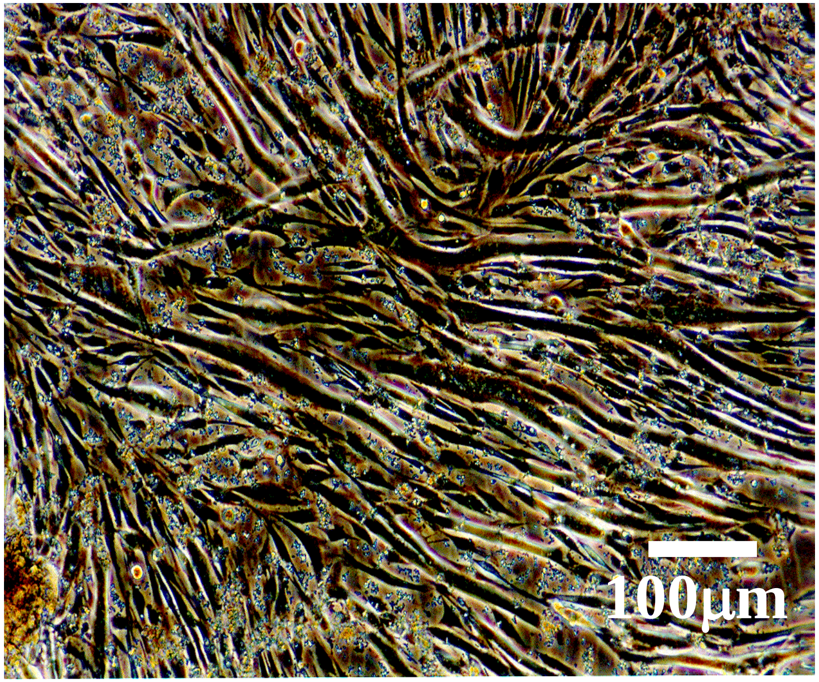
MYOGENESIS


(A)
Myotome cells
(B)
Dividing myoblasts
(C)
Cell alignment
(D)
Myotube formation
(E)
Muscle fiber
Paracrine factors
Determination
Multiplication
Multiplication stops
Fusion, differentiation
Maturation
Wnt, Shh
FGFs
Fibronectin integrin, cadherin/CAM
Meltrin; muscle-specific proteins
Contractions begin

Skeletal muscle cells in vitro

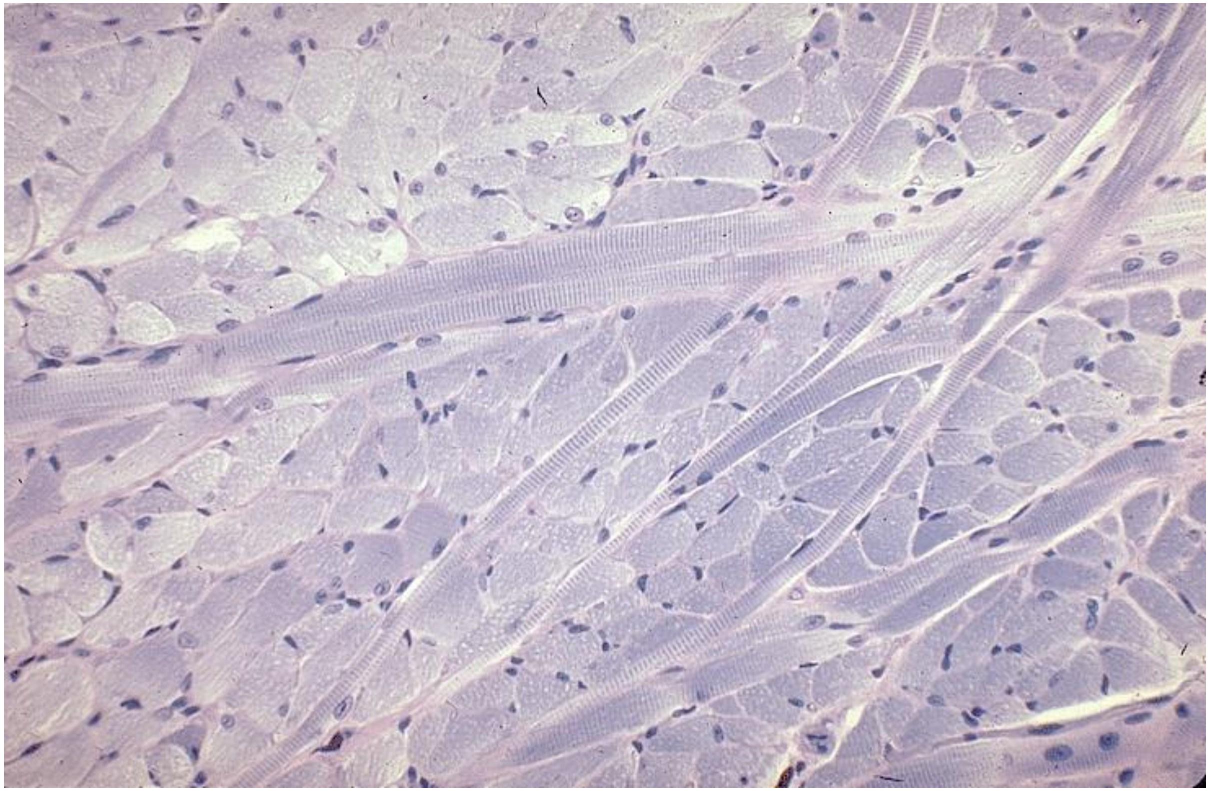


Proliferating (undifferentiated) myoblasts

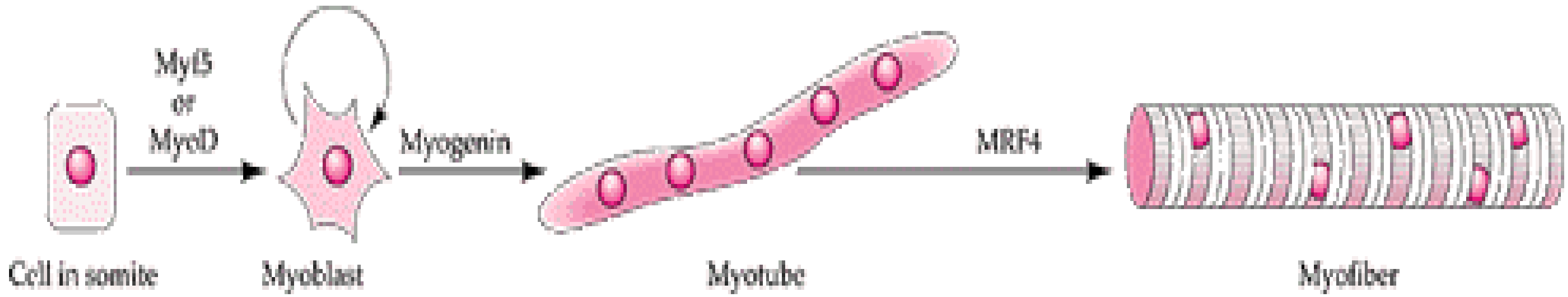


Differentiated myotubes

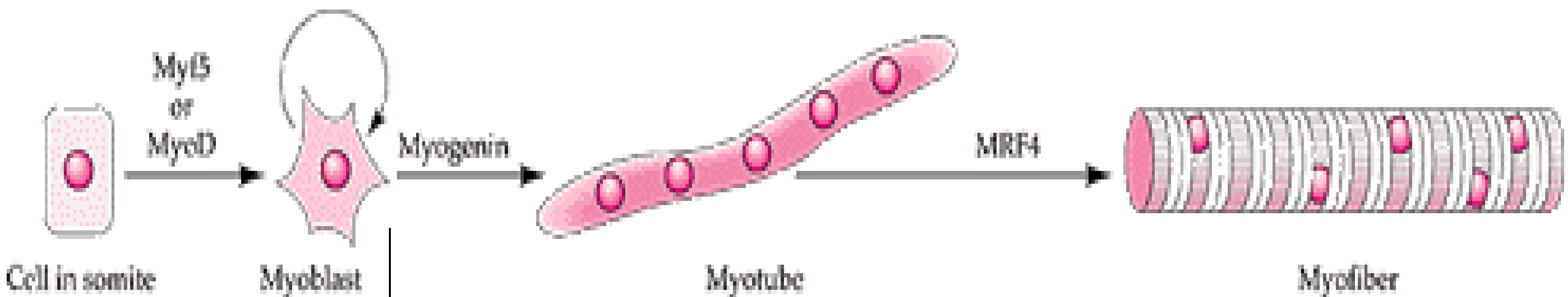
Section of skeletal muscle



Regulation of myogenesis



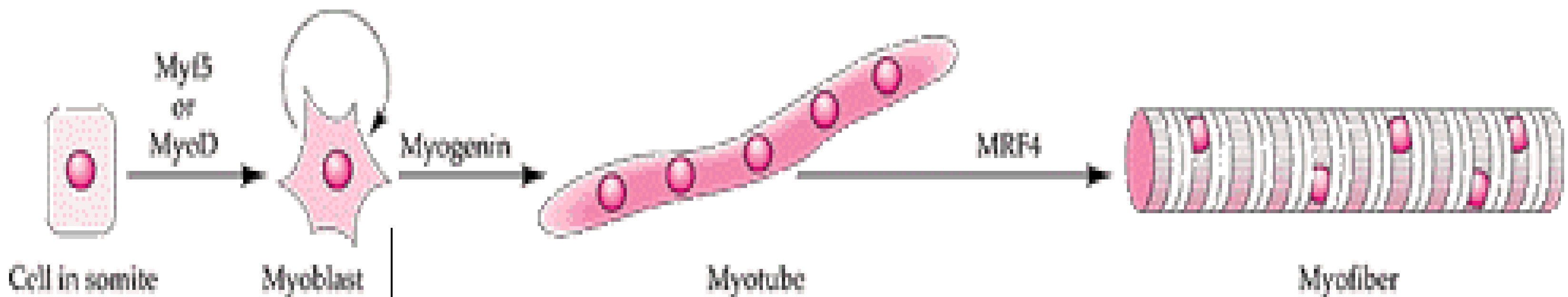
Regulation of myogenesis



Determination/
Commitment

Regulation of myogenesis

Proliferation/
cell division



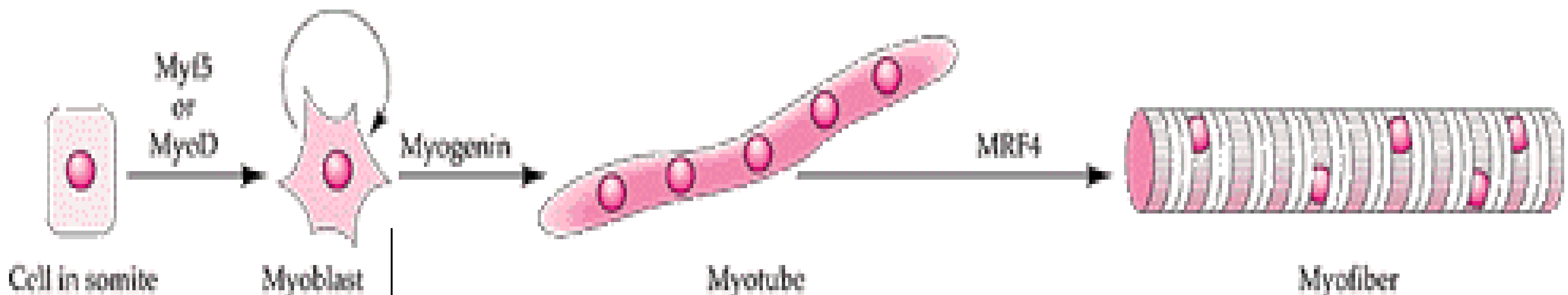
Determination/
Commitment

Regulation of myogenesis

Growth factors

+/-

Proliferation/
cell division



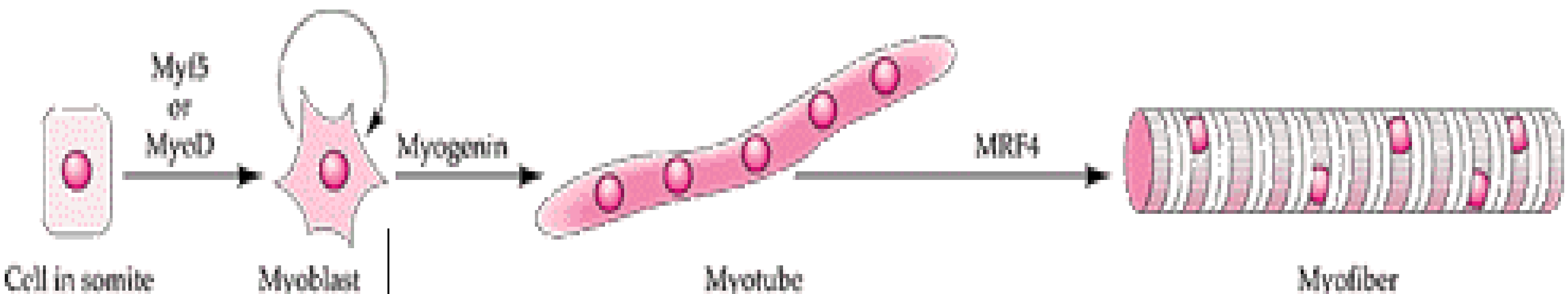
Determination/
Commitment

Regulation of myogenesis

Growth factors

+/-

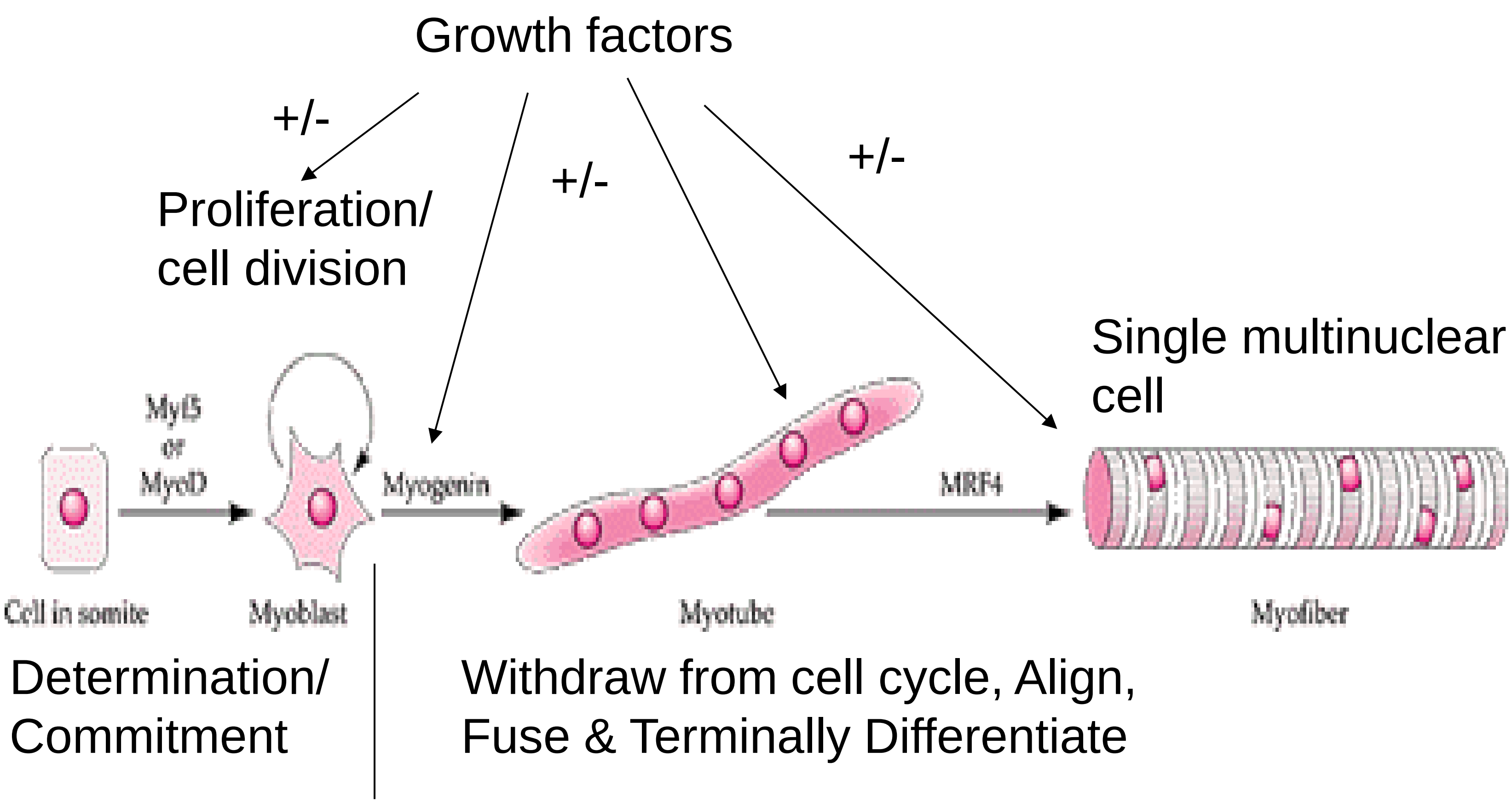
Proliferation/
cell division



Determination/
Commitment

Withdraw from cell cycle, Align,
Fuse & Terminally Differentiate

Regulation of myogenesis



Growth Factor effects on muscle cell proliferation and differentiation

Factor	Precursor cell (myoblast or satellite cell)	
	Proliferation	Differentiation
Insulin	↑→	↑
IGF	↑	↑
GH	→	↑→
FGF	↑→	↓
EGF and TGFα	↑→	↑
TGFβ	↓→↑	↓
PDGF	↑→	↓
Dex	↑→	→↓
T3	→	↑→
Test	→	↓
β-adrenergic agonist	↑→	↑→
LIF	↑	nd
IL-6	↑	nd
RA	nd	↑
LA	nd	↑
CGRP	nd	↑
TNF-α	nd	nd
PGE_2	nd	nd
$PGF_{2\alpha}$	nd	nd

IGF's and myogenesis

- Insulin-like growth factors appear to be unique in that they can act as mitogens and differentiation factors (Florini et al, 1986)
  - concentration-dependent (in cultured cells)
- IGF's shown to increase myogenin expression (cultured cells - Florini et al, 1991a)
- Knockout of IGF-II (transfected with antisense) in spontaneously differentiating muscle cell lines prevents differentiation (autocrine IGF-II effect - Florini et al 1991b)

Review – Florini et al, 1996

Skeletal muscle anatomy

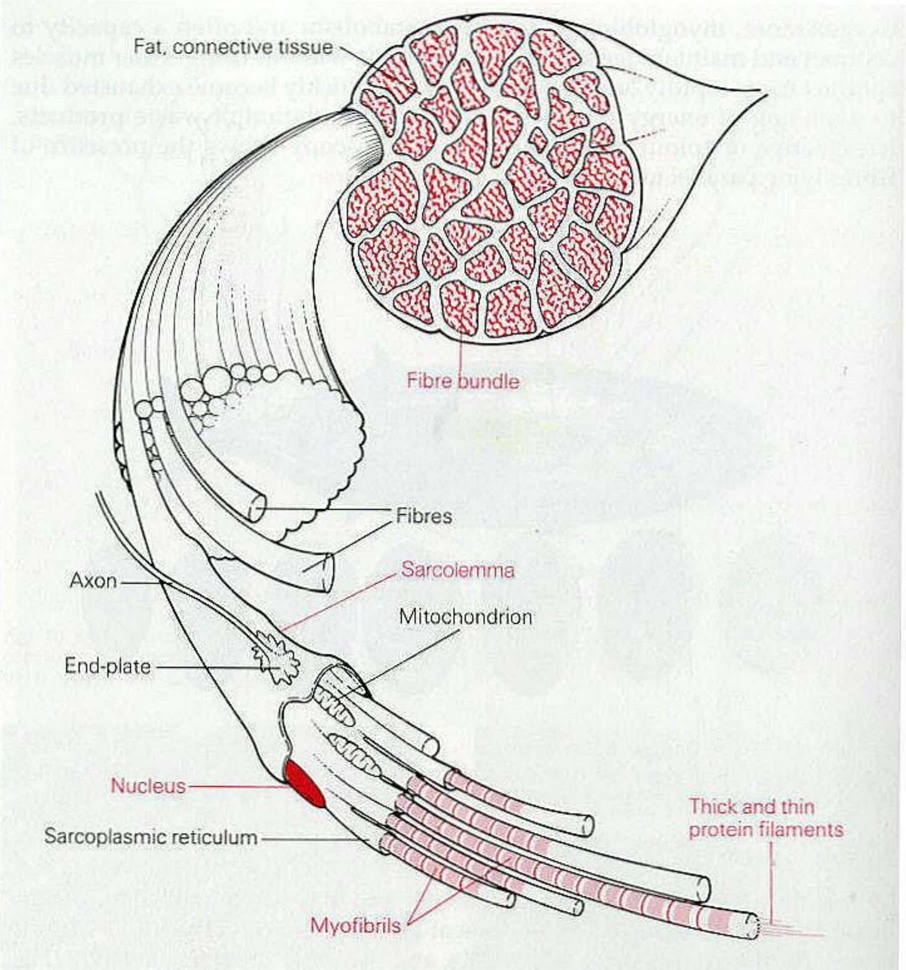
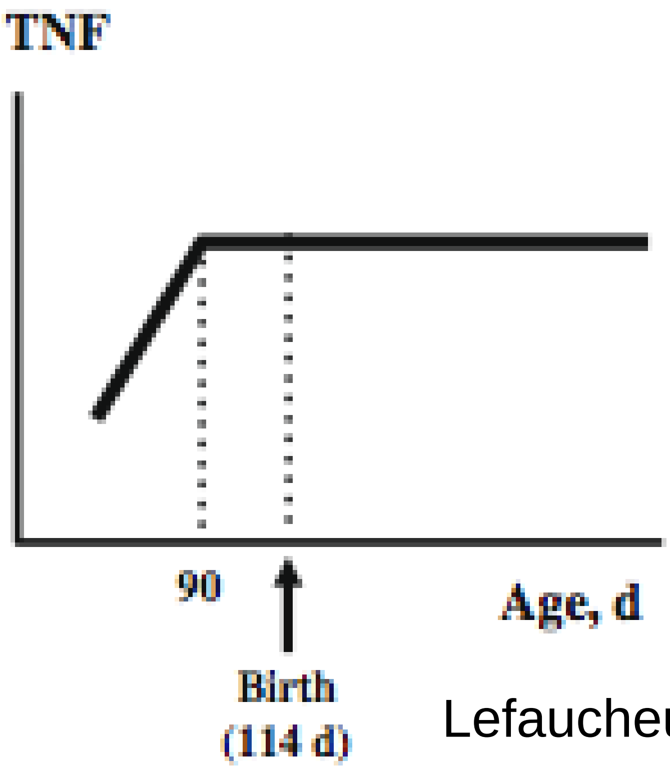


Fig. 13.4 The relationship between muscle fibres (multinucleated cells), myofibrils and protein filaments.

Muscle mass is related to……..

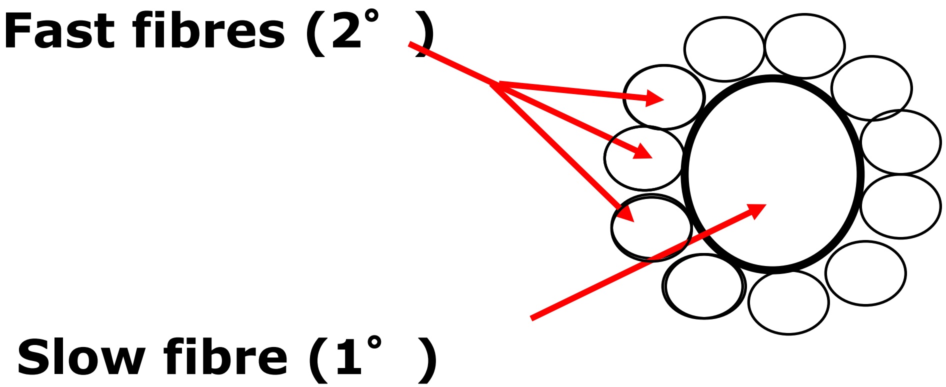
- Total number of fibres (TNF)
  - Hyperplastic growth
  - fixed at birth

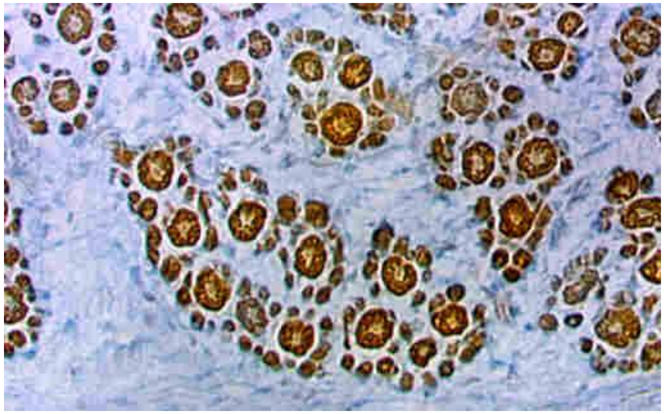
- Cross Sectional Area (CSA) and Length
  - Hypertrophic growth



Lefaucheur 2010

Primary and secondary fibre formation





Pig muscle fibre formation
(thanks to Dr C. Rehfeldt)

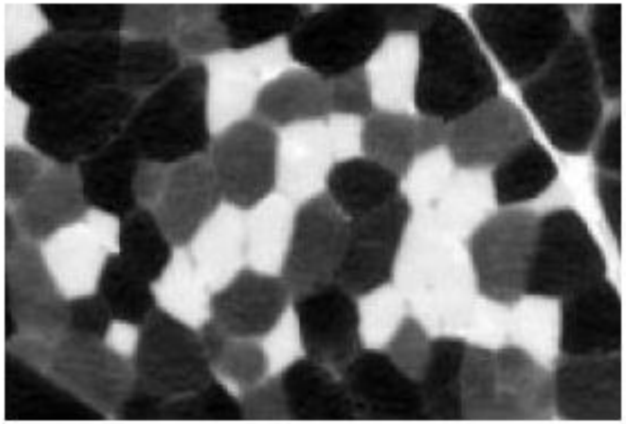
1° muscle fibres → (tend to form) → type I (slow) fibres

2° muscle fibres → (tend to form) → type II (fast) fibres

Proliferation and differentiation of myoblasts mainly takes place *in utero*

Muscle fibre type

Aberdeen Angus



Maltin et al 2001

- Classic histochemistry staining
- Type I - slow oxidative (SO)
- Type IIA - fast oxidative glycolytic (FOG)
- Type IIB (or IIX) - fast glycolytic (FG)

Timings for appearance of fibres

	Primary	Secondary	Tertiary	Length of Gestation	Reference
Poultry	3-7 df	8-16 df	-	21 days	Bandman & Rosser (2000)
Rat	14-16 df	17-19 df	-	22 days	Wilson et al (1988)
Guinea pig	30 df	30-35 df	-	68 days	Dwyer et al (1995)
Pig	35 df	55 df	0-15 dpn	114 days	Lefaucheur et al (1995)
Sheep	32 df	38 df	62-76 df	145 days	Wilson et al (1992)
Bovine	60 df	90 df	110 df	278-283 days	Gagniere et al (1999)
Human	56 df	90 df	110-120 df	280 days	Draeger et al (1997)

Adapted from Picard et al. (2002) and Brameld et al. (2003).

Genetic effects on myogenesis

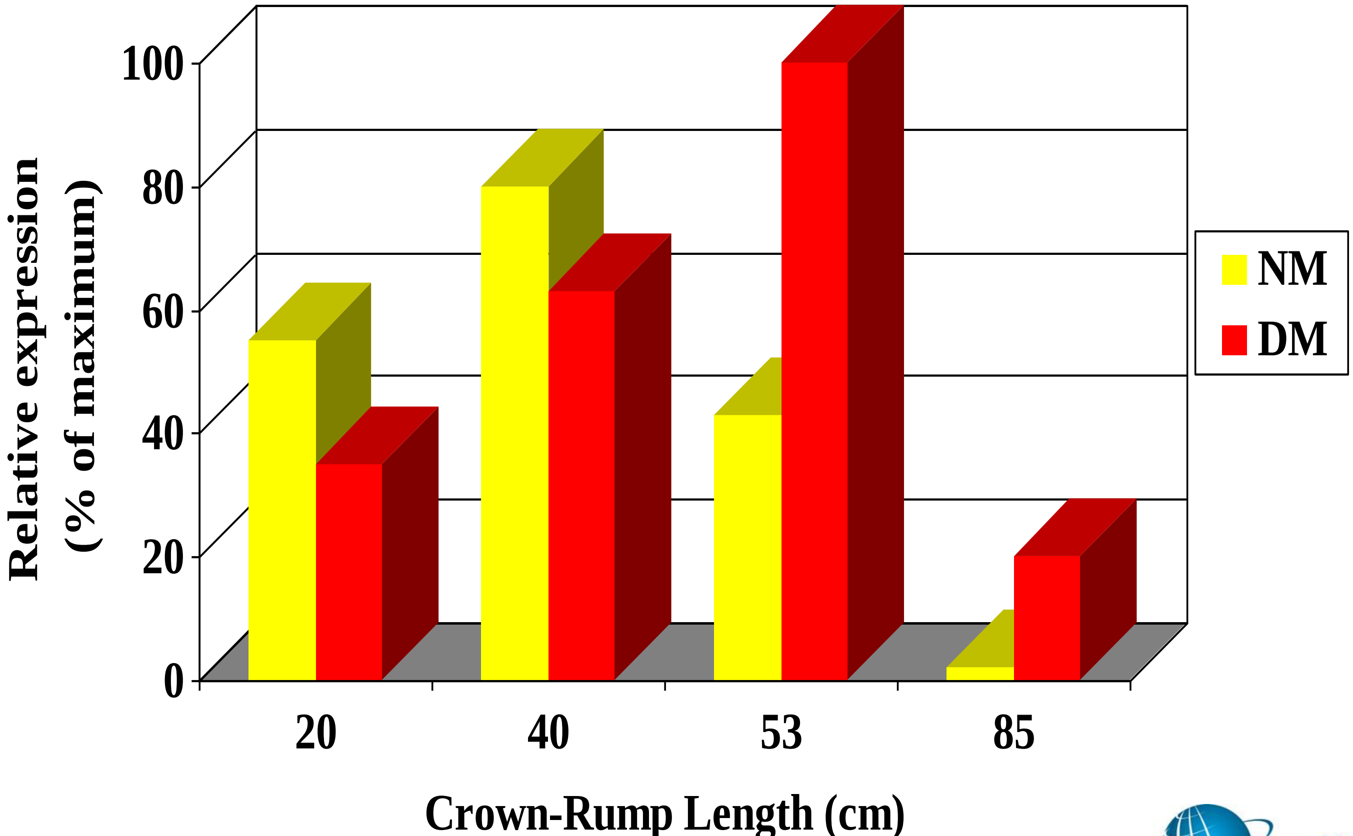
The greater TNF the greater the capacity for growth

double muscling

Double muscling

- Increased TNF at birth
- Increased mitogenic activity of fetal blood (Gerrard & Judge, 1993)
- Mutation in myostatin gene (also called GDF-8, TGFβ family)
- Results in inactive protein being produced
  - TGFβ inhibits proliferation (and differentiation) → removed an inhibitor
- More fibres due to more cell proliferation?
- BUT some cattle and pigs have similar mutations and no double muscling?
- Something else involved?

IGF-II gene expression in developing fetal ST muscle of normal (NM) and double muscled (DM) cattle



Gerrard & Grant (1994)

Double muscled Cattle

Double muscling

- Increased TNF at birth
- Increased mitogenic activity of fetal blood (Gerrard & Judge, 1993)
- Mutation in myostatin gene (also called GDF-8, TGFb family)
- Results in inactive protein being produced
  - TGFβ inhibits proliferation (and differentiation) → removed an inhibitor.
- More fibres due to more cell proliferation?
- Delay in maximal IGF-II expression
- Delay in terminal differentiation?
- More fibres due to more cell proliferation AND delayed differentiation?

Maternal nutrition & muscle fibre number

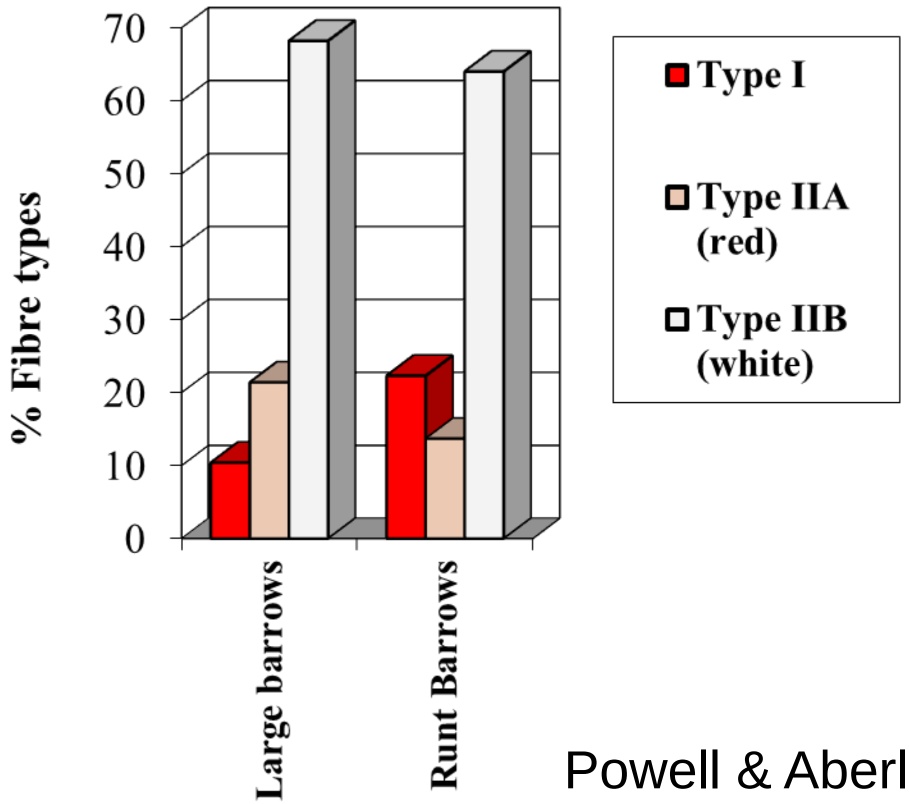
- Muscle fibre number in pigs, sheep and cattle is set at birth
- Therefore factors regulating this are active during gestation
- In pigs: runts are born with fewer muscle fibres and more intramuscular fat

Comparisons between large and runt littermate groups

Parameter	Large piglet	Runt piglet
Birth Weight (g)	1544	776
ST 2°:1° fibre ratio	25.3	22.1
Trapezius 2°:1° fibre ratio	22.9	20.7

Handel & Stickland (1987)

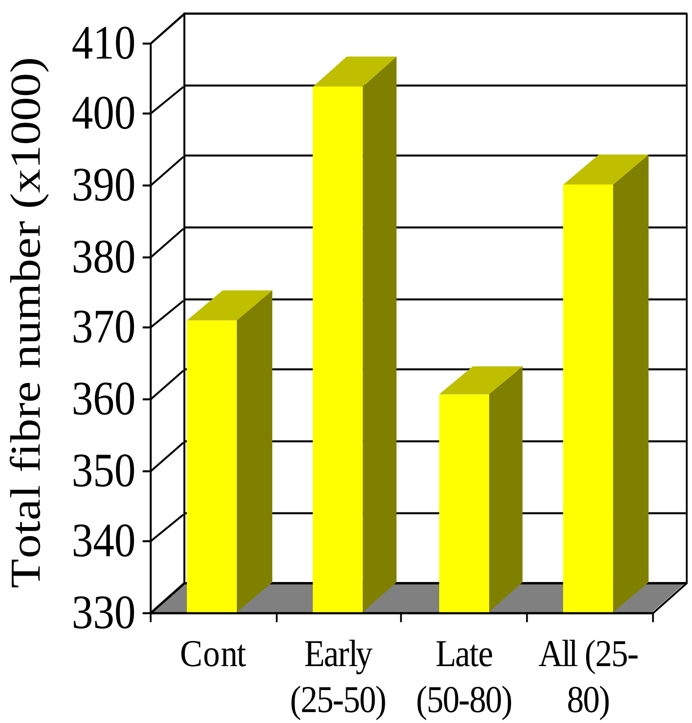
SM muscle fibre types in runt and large birth weight pigs



- Runts have a higher proportion of type I fibres and fewer type II fibres than larger birth weight pigs

Powell & Aberle (1981)

Effects of Maternal Nutrition in Pigs on ST TNF of Progeny at Birth



Dwyer et al (1994)

- Doubling maternal intake at early gestation (25-50 days) increased ST TNF
  - Mainly in smaller littermates
  - Normal practice is under-nutrition
- Increase in no. of secondary fibres, no effect on primary fibres
- May eliminate runts
  - no effect on large littermates
  - already reaching genetic potential ?

Timings for appearance of fibres

	Primary	Secondary	Tertiary	Length of Gestation	Reference
Poultry	**3-7 df**	**8-16 df**	**-**	**21 days**	**Bandman & Rosser (2000)**
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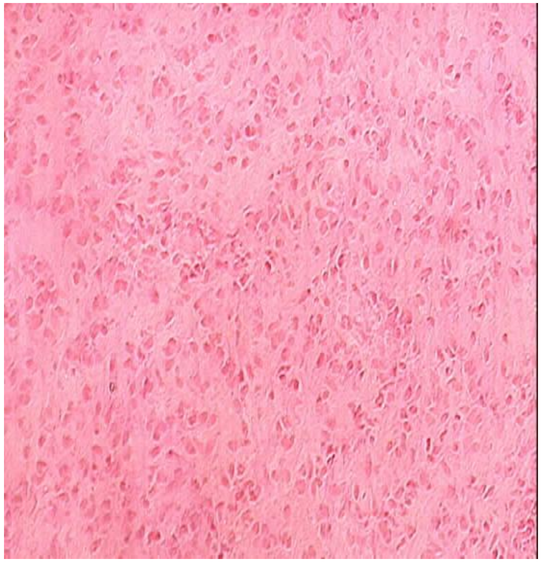
Fibre formation in fetal sheep



d70

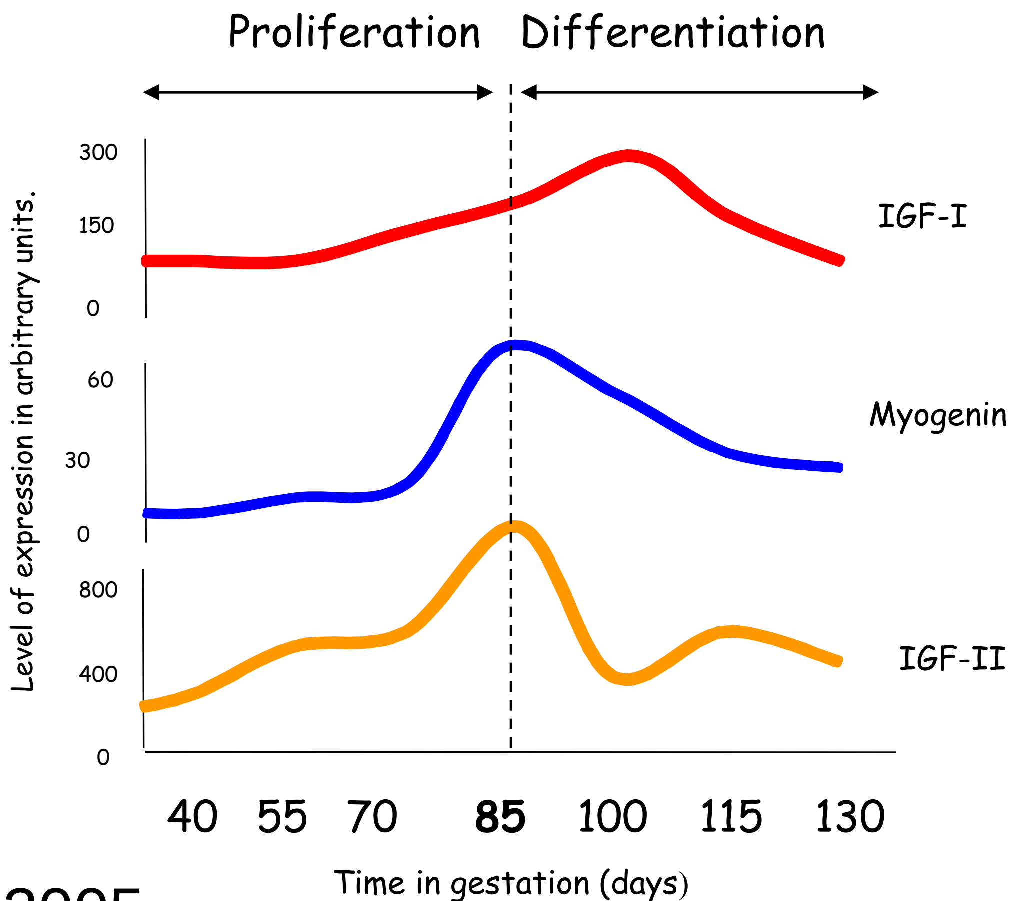
d85

d100

H&E stained muscle sections

Fahey et al, 2005

Ontogeny of factors thought to control muscle development (sheep)



Fahey et al, 2005

Timings for appearance of fibres

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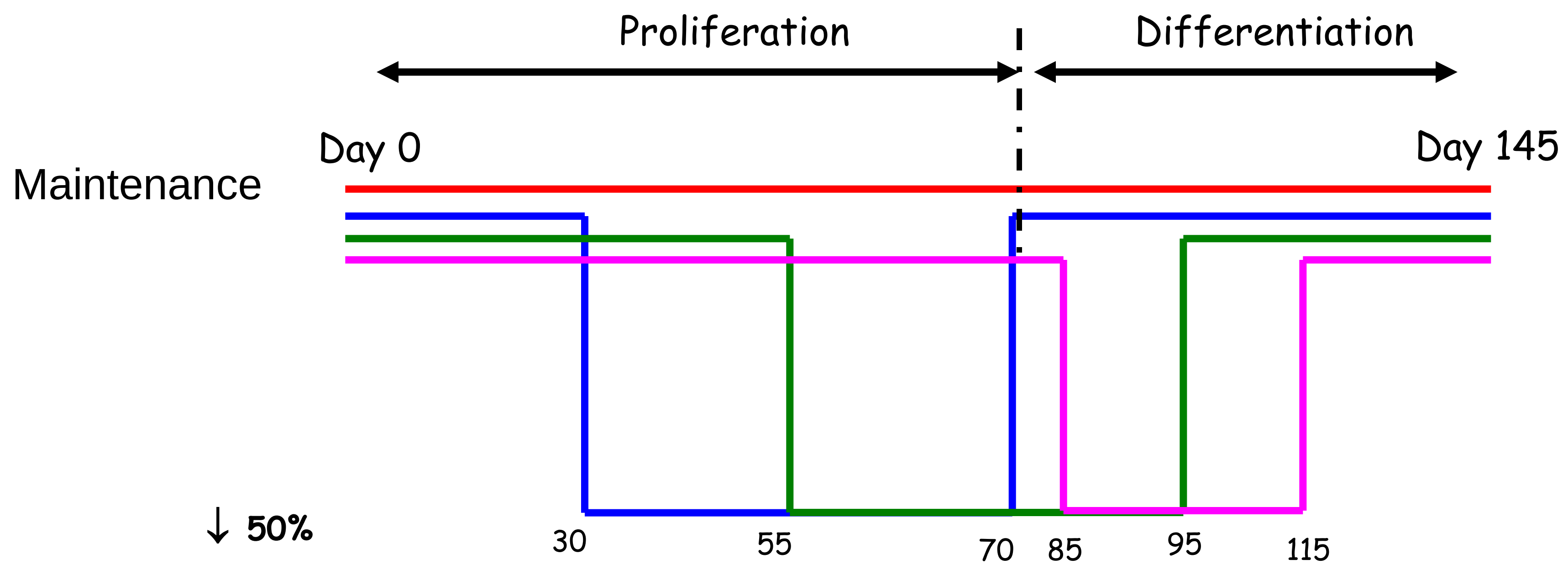
Ewe feeding trial - Fahey et al, 2005



d30-d70 during proliferation

d55-d95 during both proliferation and differentiation

d85-d115 during differentiation

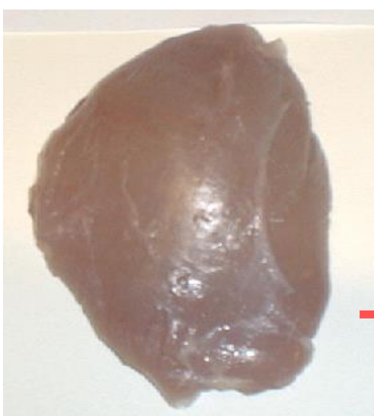
32 ewes, 8 per group

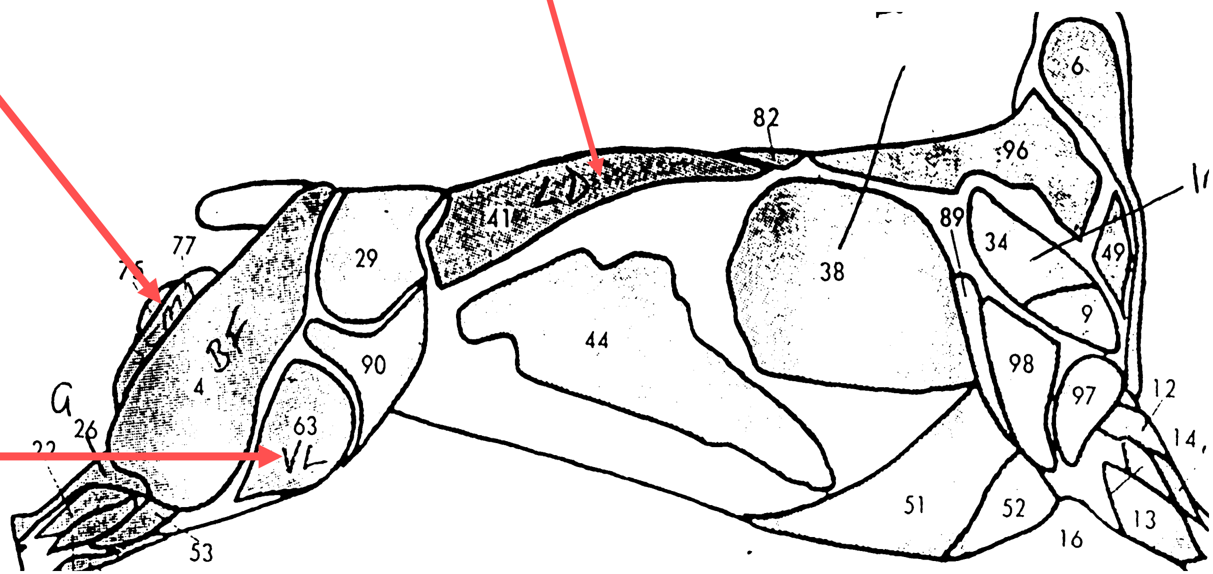
Location of muscles

ST

LD

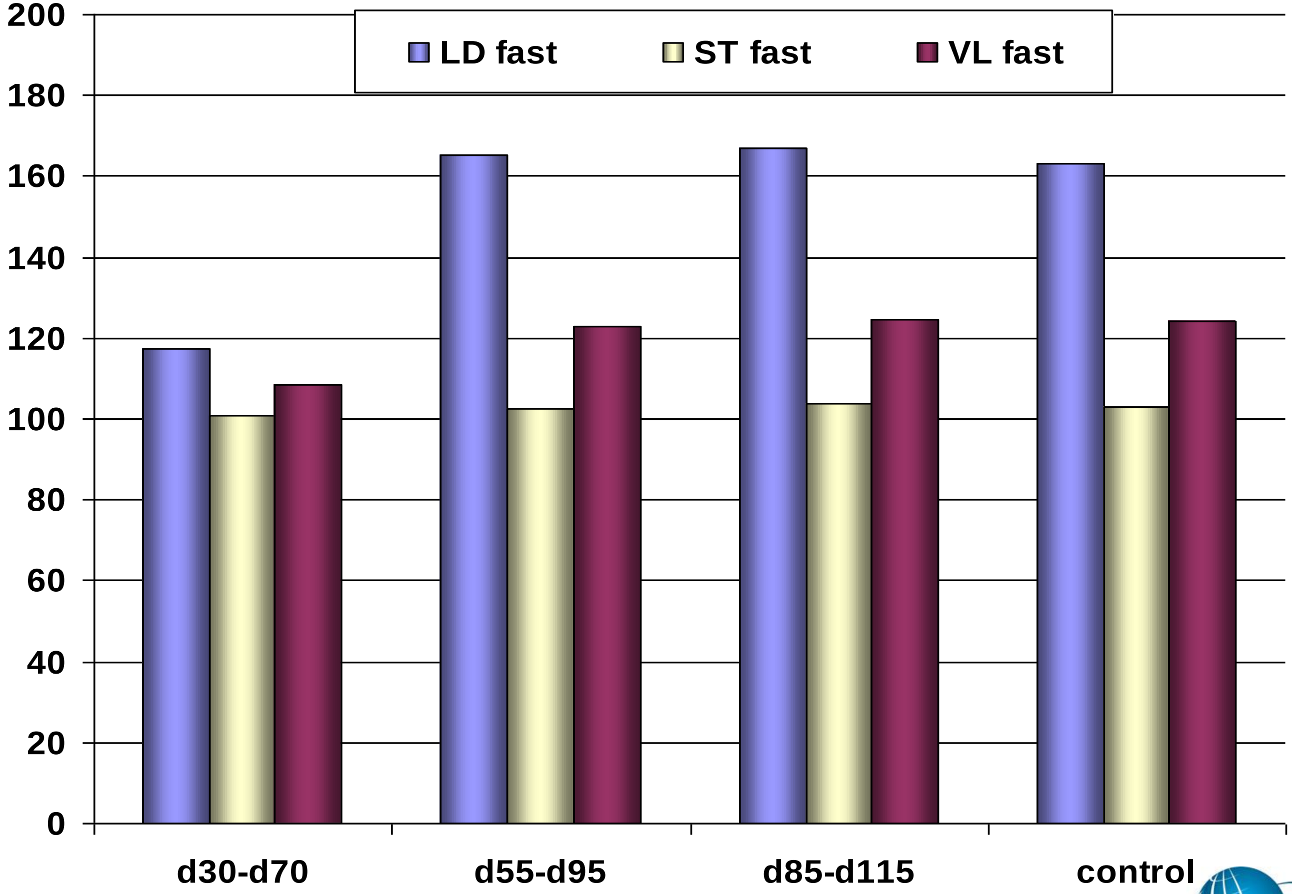
VL





Fahey et al, 2005b

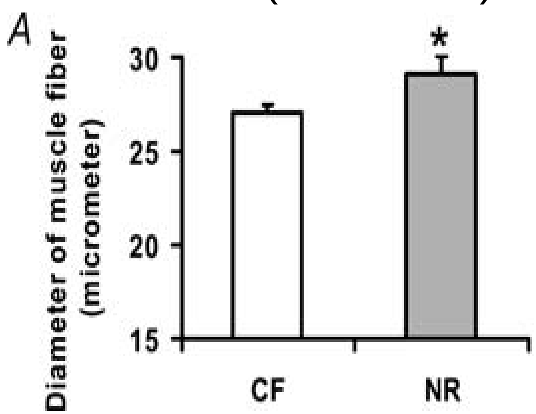
Numbers of fast fibres (14d)

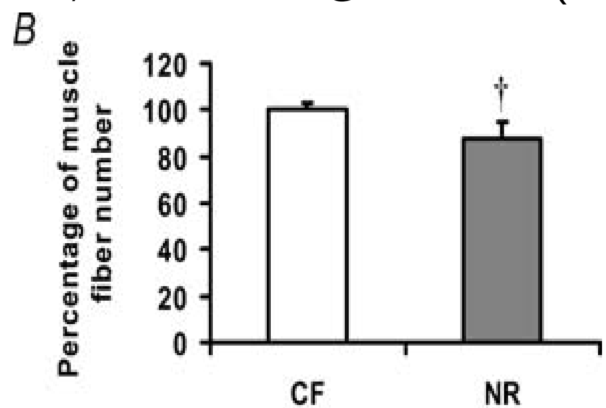


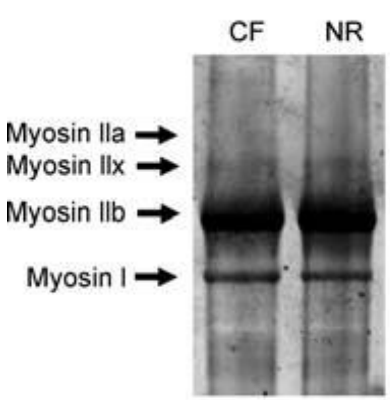
Fahey et al, 2005b

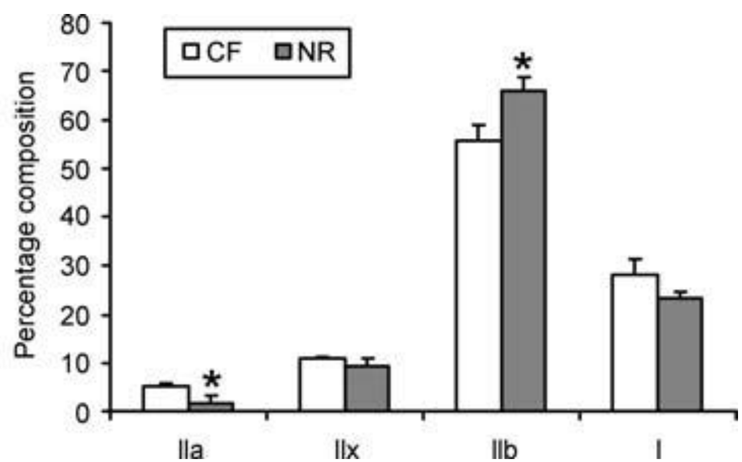
Opposite effects seen in older lambs (Zhu et al, 2006)

Restricted (50 v 100%) d28-78, male lambs @ 8months (34-35wks), LD muscle taken









We observed very similar results at younger ages (17 and 24 weeks - Daniel et al 2007)

Nutritional effects on TNF

- Effects of maternal nutrition on numbers of muscle fibres in offspring observed in various species
  - Guinea-pigs, rats, pigs, sheep

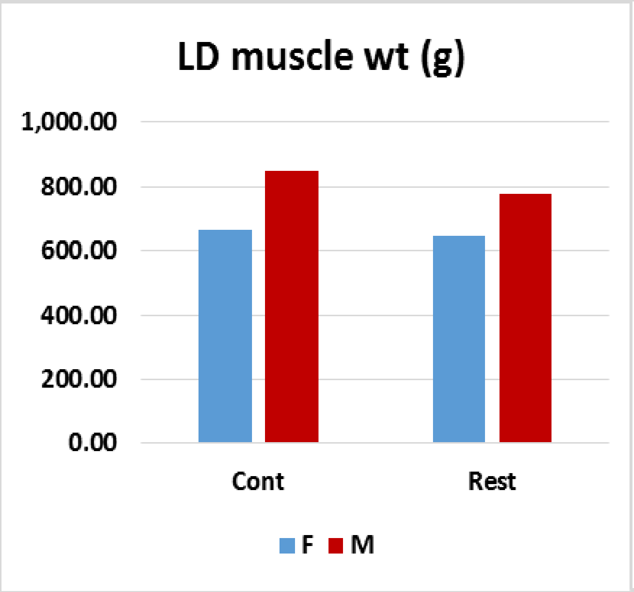
- Low(er) nutrition → ↓ no. of 2° fibres

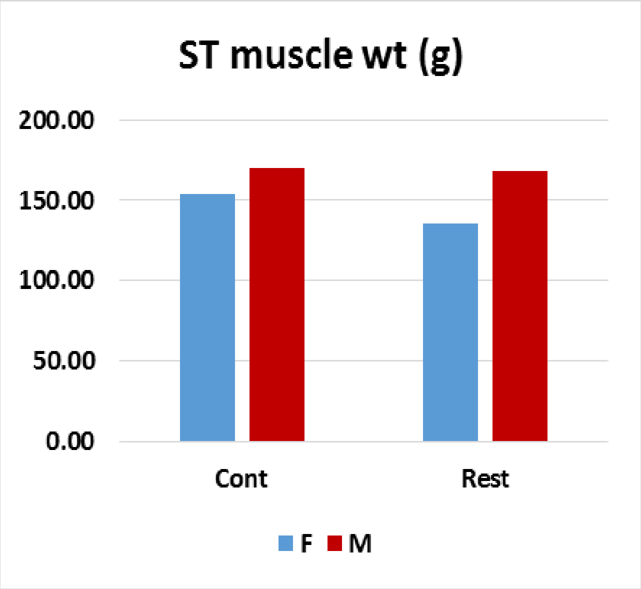
- High(er) nutrition → ↑ no. of 2° fibres

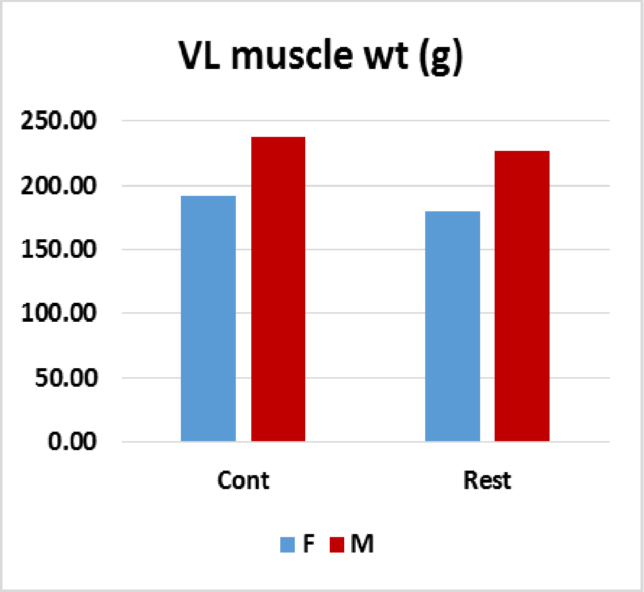
- Timing of intervention is CRITICAL
- Normally just BEFORE fibre formation
- Long-term effects are unclear!!!

Effects on muscle wts in older lambs (Daniel et al, 2007)

Restricted (50 v 100%) d30-85, male & female lambs @ 24wks







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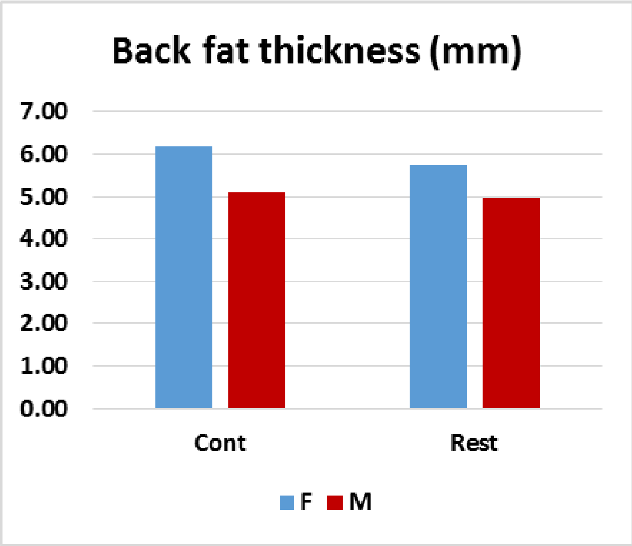
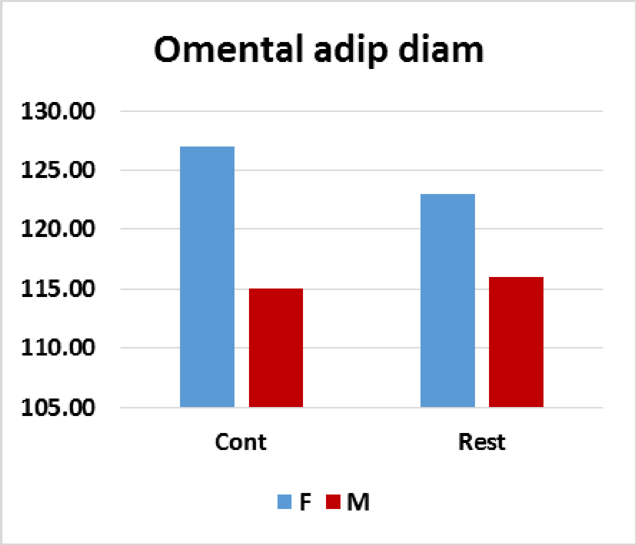
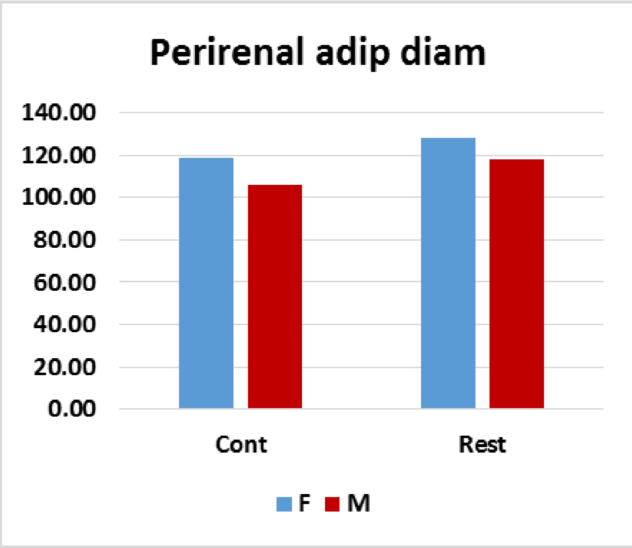
Effects on adiposity in older lambs (Daniel et al, 2007)

Restricted (50 v 100%) d30-85, male & female lambs @ 24wks







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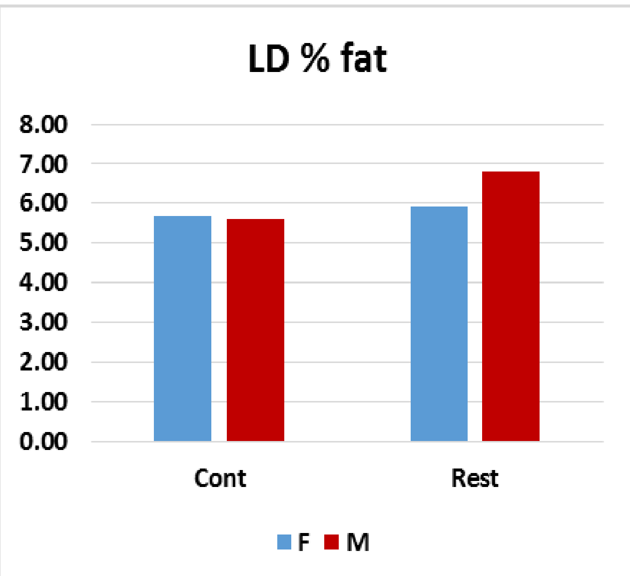
Effects on muscle % fat in older lambs (Daniel et al, 2007)

Restricted (50 v 100%) d30-85, male & female lambs @ 24wks



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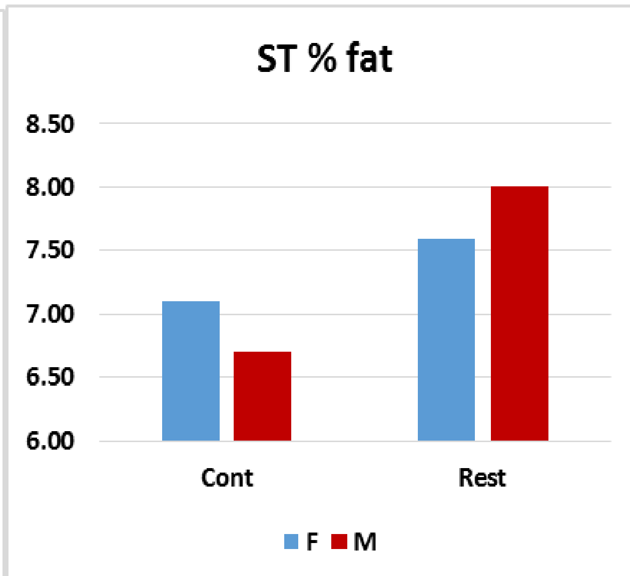
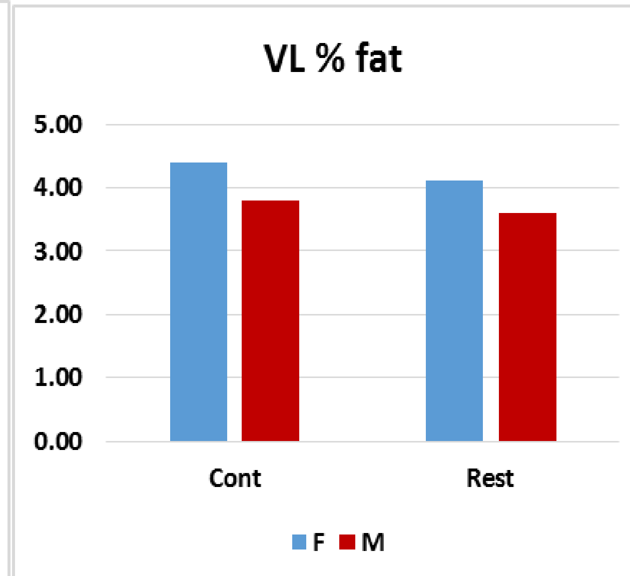


Rest>Cont



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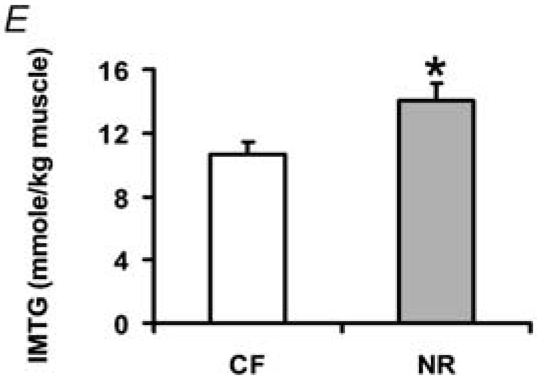
Effects on adiposity in older lambs (Zhu et al, 2006)

Restricted (50 v 100%) d28-78, male lambs @ 8months (34-35wks), LD muscle taken



Zhu et al, 2006

Restricted (50 v 100%) d28-78, male lambs 8months old (34-35wks)

Category	CF	NR
Liveweight (kg)	56.80 ± 1.23	61.72 ± 1.62*
Carcass wt (kg)	28.83 ± 0.92	31.62 ± 1.01†
Kidney + pelvic fat (kg)	0.46 ± 0.05	0.68 ± 0.06*
LD muscle (g)	776.5 ± 12.1	775.7 ± 30.0
ST muscle (g)	166.4 ± 4.4	169.3 ± 3.9
K + P fat (%CW)	1.66 ± 0.12	2.18 ± 0.20*
LD muscle (%CW)	2.71 ± 0.11	2.46 ± 0.08†
ST muscle (%CW)	0.58 ± 0.02	0.54 ± 0.02†

Nutritional effects on body composition

- Evidence that there may be long-term effects on muscle weights and some measures of adiposity

- Low(er) nutrition → ↓ muscle wts

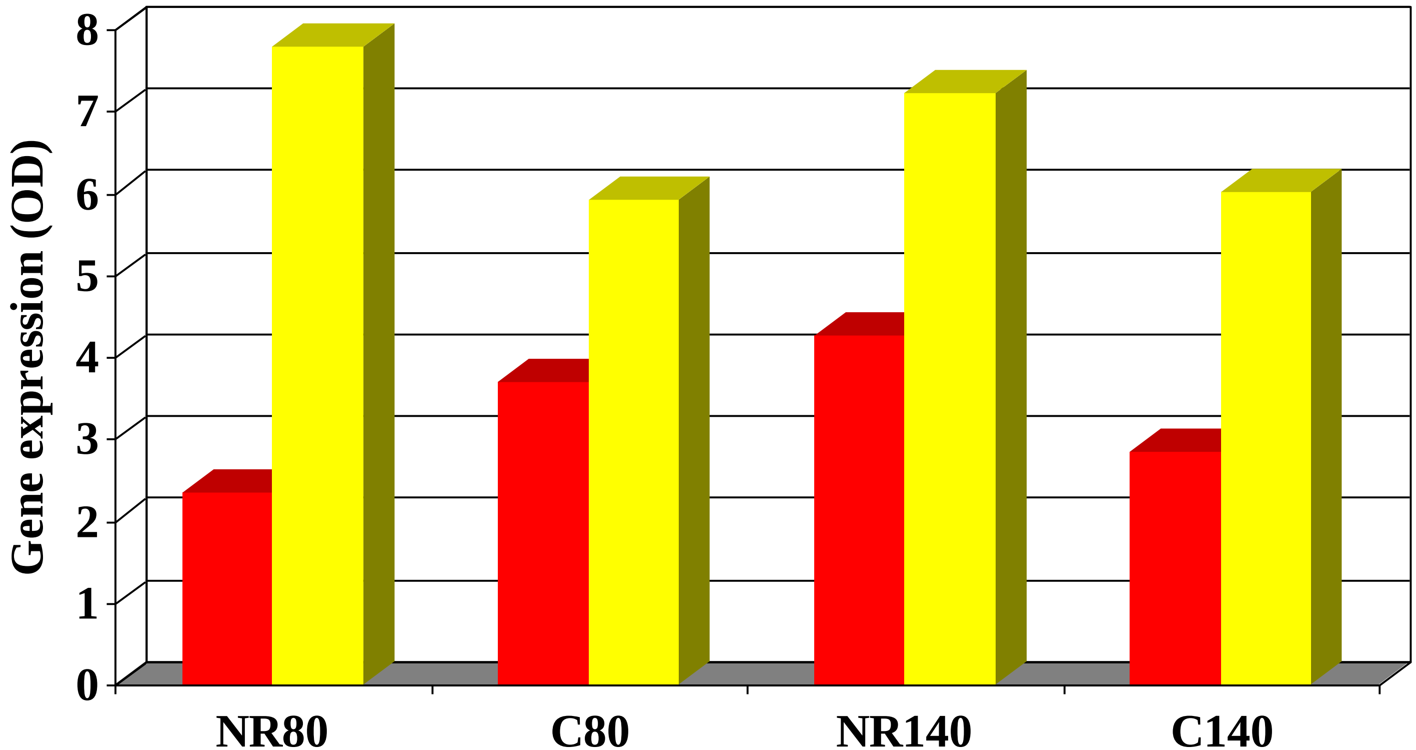
- Low(er) nutrition → ↑ adiposity

- Dependent upon the age of the lamb
  – Increased adiposity seen at 24 and 34-35wks, but not 17wks

Possible Mechanism

- Nutrition regulates the IGF axis
- High nutrition increases hepatic IGF-I production (and plasma IGF-I)
  - Does high(er) plasma IGF-I stimulate myoblast proliferation and inhibit muscle IGF-II expression and differentiation?
- Does low nutrition have opposite effect (reduced plasma IGF-I, reduced proliferation, increased IGF-II and early differentiation)?
- Is muscle IGF-II expression the KEY regulator of differentiation and fibre formation?

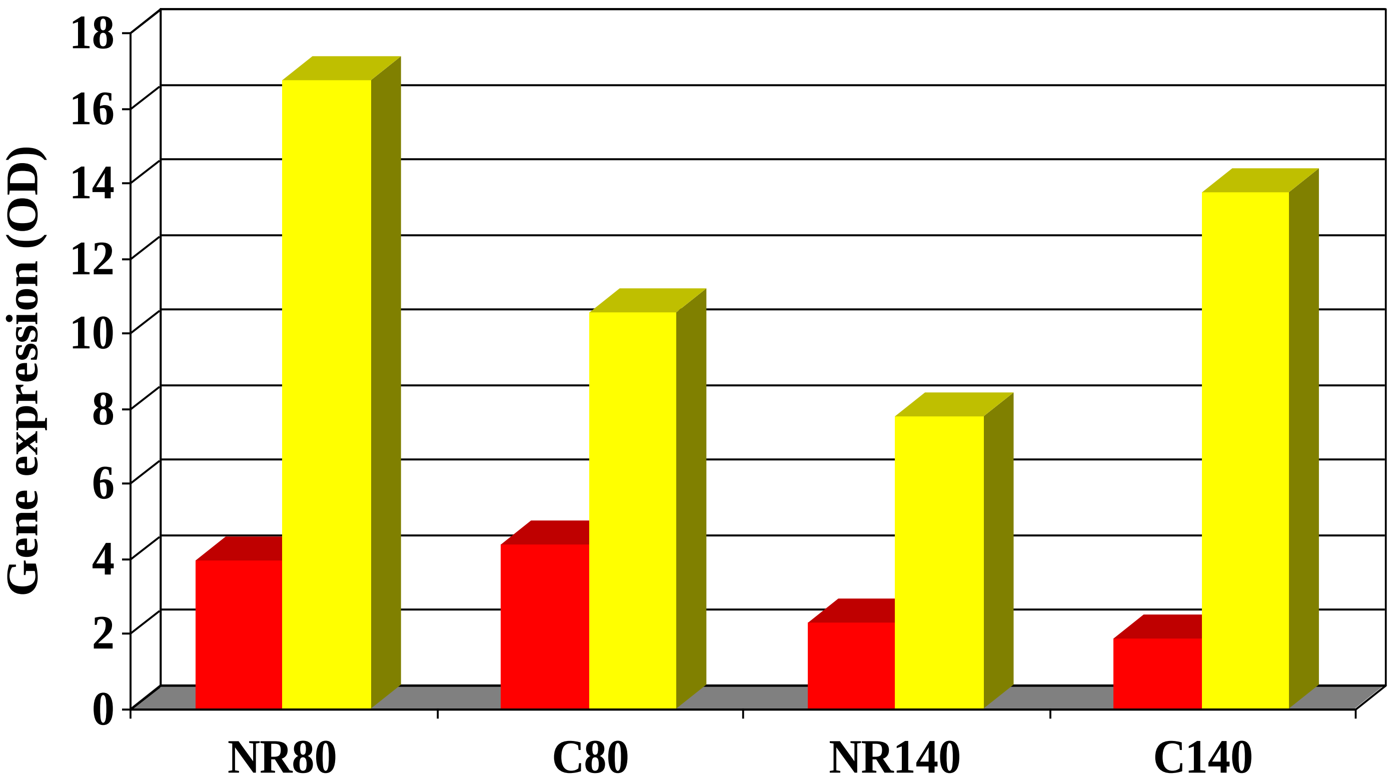
Effects of maternal nutrition on fetal sheep liver gene expression (Brameld et al, 2000)



C = 150%
NR = 60%

	Age	Diet	Age x Diet
IGF-I	NS	NS	0.061
IGF-II	NS	0.061	NS

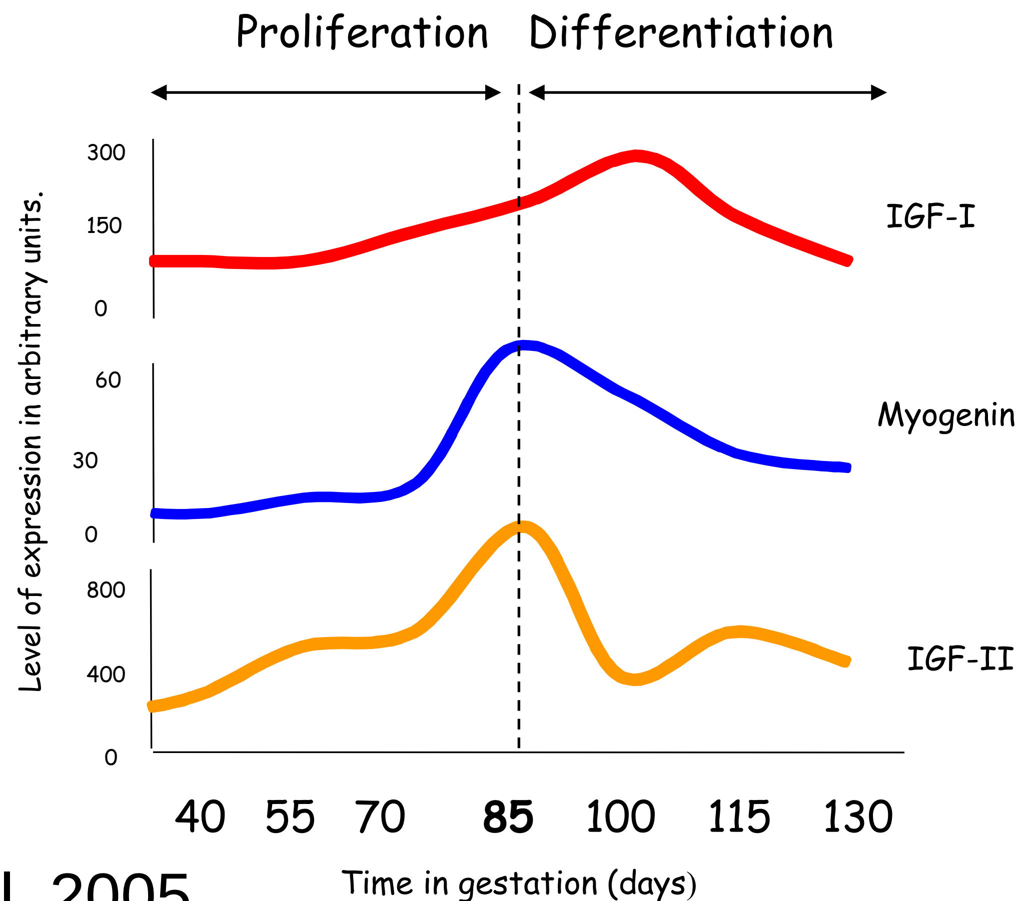
Effects of maternal nutrition on fetal sheep muscle gene expression (Brameld et al, 2000)



C = 150%
NR = 60%

	Age	Diet	Age x Diet
IGF-I	<0.001	NS	NS
IGF-II	0.056	NS	<0.001

Ontogeny of factors thought to control muscle development (sheep)



Fahey et al, 2005

Conclusions: Not all fibres are affected

- The effects of environment/nutrition are mainly on secondary fibres formed
  - no. of primary fibres genetically determined ?
  - Critical window prior to major fibre formation (species differences!!!)
  - Very few studies have grown offspring to older ages,
    - but effects may be lost/ compensated for by good postnatal nutrition (more work needed)
  - BUT TFN is difficult to measure in large muscles!!!
  - Long-term effects on muscle wts and adiposity
  - Is muscle IGF-II a key factor relating to timing of differentiation/ fibre formation?

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