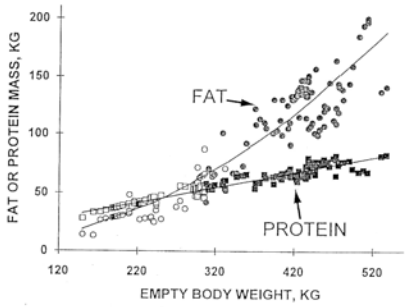
Postnatal Muscle Growth

- Postnatal muscle growth
  - Postnatal muscle growth curve ??
  - Key characteristics of muscle:
    - fiber # fixed at birth
    - increased size/wt. (length/diameter) of fibers = hypertrophy
  - example: pectoralis muscle of a chicken
    - at birth, = .7 g, at maturity, = 300 g
      → attributed to hypertrophy

Feedlot Cattle on High Concentrate Diet



Owens et al., 1995

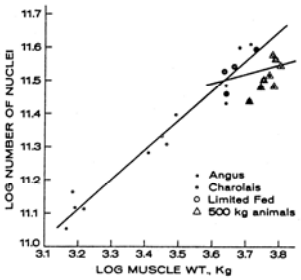
Postnatal Muscle Growth

- Increase in length (limbs of most species)
  - approximately double in length during postnatal growth
  - increase in muscle length occur via increase in length of individual fibers making up individual muscles
- Increase in diameter
  - occurs via an increase in fiber diameter
  - also due to an increase fiber length which is correlated to interdigitations
  - cross-sectional area (CSA) of fiber increases in proportion to muscle weight$^{2/3}$

Postnatal Muscle Growth

- DNA accumulation
  - multinucleated fiber → nuclei are post-mitotic but……..
    - DNA accumulation is highly related to muscle growth rate
    - also rapid periods of DNA accumulation into existing muscle fibers
    - coincides with rapid period of muscle growth
    - for individual fiber the CSA is increased in direct proportion to the DNA content

DNA and Growth



Trenkle et al., 1978

DNA Accumulation

- More rapid DNA accretion means that the DNA unit is kept smaller, so each nucleus has a smaller sarcoplasm area to dominate which will enhance efficiency of growth
- Not all DNA in skeletal muscle tissue is from muscle cells (75-80%) other 20% = adipocytes, macrophages, fibroblasts

DNA Accumulation

- **Preponderance of evidence suggests that much of muscle DNA (in fiber) was accumulated postnatally and that accretion of DNA in muscle is a key factor in limiting muscle growth**
- **60-90% of DNA in mature muscle fibers is accumulated during postnatal growth**
- **Inconsistencies**
  - **fiber # fixed at birth – muscle fibers can't divide**
  - **nuclei in muscle fiber can't divide**

Postnatal DNA Accumulation

TABLE 1. DNA ACCRETION IN POSTNATAL MUSCLE

Species, age and muscle	Increase in DNA content (X-Fold)	Percentage of total DNA accumulated postnatally	
Male rat, 21–133 days *gastrocnemius*	8.4	88%	Winick and Noble, 1966
Male rat, 16–86 days *gastrocnemius*	3.8	74%	Enesco and Puddy, 1964
Male rat, 7–56 days quadriceps	4.8	79%	Cheek *et al.*, 1965
New Hampshire female chicken, 0–28 days breast muscle	16.9	94%	Moss *et al.*, 1964
New Hampshire male chicken, 0–266 days pectoral	96	99%	
gastrocnemius	54	98%	Moss, 1968a
Pigs, 23–118 kg total muscle	2–2.7	50–63%	Harbison *et al.* 1976
Sheep, 0–120 days *gastrocnemius*	3.1	66%	Johns and Bergen, 1976

Allen et al., 1979

Satellite Cells (Alexander Mauro, 1961)

- **Mononucleated cells located between basement membrane and sarcolemma of each muscle fiber**
- **Not identifiable until embryonic myoblasts have fused into the fibers prior to birth**
- **Muscle-specific cells that have the ability to proliferate and differentiate into adjacent muscle fibers**
- **The fusion process adds satellite cell into existing muscle fiber (DNA accumulation)**
- **Once a satellite cell has differentiated and fused into the fiber, it is lost to the satellite cell population**

Cell Surface Markers for Satellite Cells

- **M-cadherin (important for fusion)**
- **CD34**
- **c-met**
- **MNF**
- **Pax-7**

- **Unique morphologic appearance**

Satellite Cells as the Source

Received for publication 28 July 1969, and in revised form 27 August 1969.

TABLE I

*Nuclear Labeling in Tibialis Anterior Muscle of 25–30 g Male Rats at Various Times after a Single Injection of Thymidine-³H, as Seen in Electron Microscope Radioautographs**

Time elapsing between thymidine-³H injection and sacrifice	No. of nuclei labeled	
	Satellite cell nuclei	True muscle nuclei
hr		
1	20	0
6	11	0
10	24	0
24	8	2
48	12	11
72	4	24

* Over 300 nuclei examined/animal.

Moss and LeBlond, 1970

Satellite Cells as the Source

TABLE 1

Labeled satellite cell nuclei and true muscle nuclei as a percentage of the total labeled nuclei in the tibialis anterior muscle of 30-g rats at various intervals after a single injection of ³H-thymidine

Time elapsing between ³H-thymidine injection and sacrifice	Total labeled nuclei classified (3 rats at each time)	Percent of labeled nuclei		
		Satellite cell nuclei	True muscle nuclei	Standard error
hr				
1	321	100	0	0
18	616	95.1	4.9	0.8
24	377	84.9	15.1	3.0
48	559	51.0	49.0	1.4
72	296	34.8	65.2	2.4

Moss and LeBlond, 1971

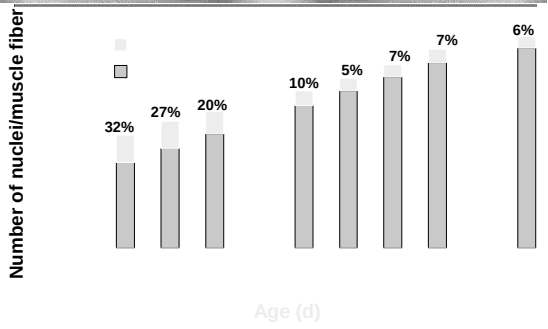
Satellite Cells

- To maintain a viable satellite cell population in growing muscle it is essential that a significant number of satellite cells continue to proliferate without differentiation and fusion with muscle fibers. Competition between proliferation and terminal differentiation occurs
- In fact recent findings clearly show that satellite cells are a heterogenous population

Satellite Cells

- Evidence for this: later stages of muscle growth (plateau of muscle growth)
  - number of satellite cells has decreased
  - →30% of muscle nuclei in newborns are satellite cells
  - →in adults, 2-10% are satellite cells
  - –this suggests a limit of DNA accretion at later stages of growth
  - as the animal grows, the satellite cells begin to withdraw from the proliferative cycle and enter G_0 (quiescent state)
- Bottom Line: Plateau results from 2 things
  - 1. decreased number of satellite cells
  - 2. satellite cells left enter G_0
- DNA is needed to support growth, it is not being supplied and so muscle growth stops (or slows)

Satellite Number Decreases with Age



Cardasis and Cooper, 1975

Growth Factors

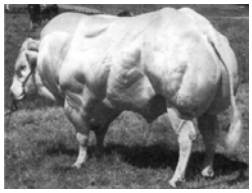
- Effects of growth factors on satellite cells proliferation/differentiation
  - IGF-I
    - considered a progression factor
    - stimulates proliferation/differentiation (unique in that it does both)
  - FGF-2 (basic fibroblast growth factor)
    - stimulates proliferation of satellite cells, gets them into the cell cycle earlier than IGF
    - inhibits differentiation
    - FGF-6 keeps cells proliferating *in vivo*, embryonic myoblasts

Growth Factors

- TGFβ and its superfamily
  - inhibits proliferation of most primary satellite cell cultures
  - inhibits differentiation
  - includes myostatin which has inhibitory effects on muscle hyperplasia
  - a mutation in myostatin actually increases hyperplasia
  - Myostatin also plays a role in postnatal growth

Myostatin Mutation

Determined Embryonically



20-25% ↑ Muscle Mass

↓ Intramuscular Fat

Dystocia Problems

http://www.champion-nutrition.com/champion/products/myostatin/research.php

Growth Factors

- IGF-I, FGF-2, and TGFβ
  - all produced by muscle cells themselves in vitro and in vivo
  - all have autocrine effect
  - none of the growth factor's will activate quiescent satellite cells
  - crushed muscle extract (CME)

ASI 902 19

Growth Factors

- Hepatocyte Growth Factor (HGF)
  - solely responsible for activating quiescent satellite cells *in vivo* and *in vitro*
  - produced by satellite cells and fibers
  - active agent in Crushed Muscle Extract (CME)

ASI 902 20

Growth Factors

- HGF receptor = c-met
  - intrinsic tyrosine-kinase activity
  - c-met is present on both quiescent and activated cells
  - autocrine/endogenous production of HGF is regulatory step of activating quiescent satellite cells

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Quiescent Satellite Cells

- Quiescent satellite cells do not express detectable levels of MRFs
- Immediately following activation, either MyoD or Myf5 are upregulated before initiation of DNA synthesis
- MyoD appears important to push some cells toward differentiation
- Pax-7 appears to control renewal and propagation of satellite cells

ASI 902 22

Quiescent Satellite Cells:New Info

- New data have challenged our previous thoughts on age-dependent depletion of satellite cells
- There may be just as many satellite cells present in mature, adult muscle as a younger animal but the mature animal has ability to respond to environmental signals
- Notch signaling pathway can activate quiescent satellite cells:ligand =Delta
- In old muscle activation by Delta is blunted by antagonist Numb
- Systemic factors appear important at regulating Notch pathway

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Growth Factors

- IGF-I stimulates satellite cell proliferation
  - over-expression of IGF-I into mouse muscle by viral-mediated gene transfer results in increased local production of IGF-I
  - IGF-I expression in young mice
    - 15% increase in muscle mass(hypertrophy)
    - 14% increase in muscle strength

ASI 902 24

IGF and Muscle Hypertrophy (Barton-Davis et al., 1999)

- **What role did satellite cells play in mediating the hypertrophic effects of IGF?**
  - **gamma irradiation was used to shut down DNA synthesis (destroying proliferating capacity)**
  - **In the IGF treatment, 50% of IGF-I effect was prevented by gamma irradiation**
  - **this suggested that the other 50% of IGF-I induced hypertrophy was due to paracrine/autocrine effects on the adult muscle fiber**
  - **for example……increased protein synthesis, decreased protein degradation → NET protein accretion enhanced**

IGF and Satellite Cell Proliferation

- **Satellite cells from IGF-I transgenic mouse (Chakravarthy et al., 2000)**
  - **Satellite cells from transgenic mouse have increased *in vitro* of replicative lifespan**
  - **cell cycle progression via PI3 kinase/Akt pathway, independent of MAPK**
  - **cell cycle enhanced due to down regulation of the inhibitor p27 KIP1**
  - **further studies showed the role of p27 KIP1 in promoting satellite cell senescence**