

ANSC/FSTC 607
Physiology and Biochemistry of Muscle as a Food
POSTMORTEM PROTEOLYSIS, IONIC STRENGTH, AND THE RESOLUTION OF RIGOR



I. Rigor and its resolution

A. The rigor bond

1. ATP consumed by non-contractile activity of A-M-ATPase and ion pumps.
2. Irreversible crosslinking of actin and myosin occurs.
3. Muscle becomes inextensible (rigor).

B. Ultrastructural changes

1. Z-Line (most obvious change).
 - a. Extensively degraded in type II.
 - b. Relatively unchanged in type I.
 - c. Both fiber types fragment at Z-disc after aging.
 - d. Loss of Z-line structure due to degradation of desmin.
2. Effects of intracellular calcium.
 - a. Increases activities of proteases.
 - b. May increase rate of glycogenolysis and thereby increase glycolysis.

II. Changes in specific myofibrillar proteins during the aging process

A. Myofilaments

1. No visible degradation of thick and thin filaments.
2. No detectable breakdown products of thick and thin filaments.

B. Desmin

1. Degraded during aging.

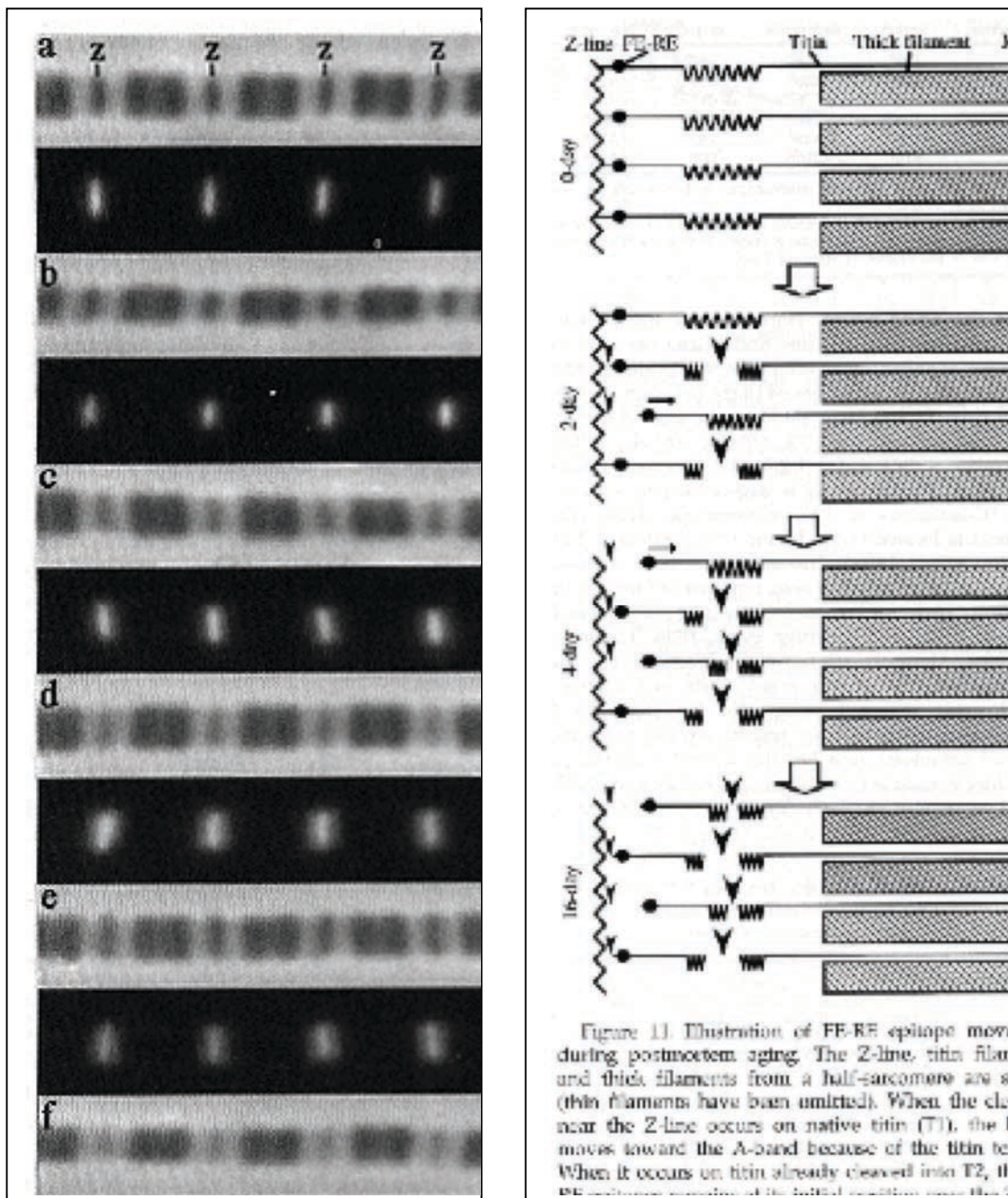
2. Probably causes fracture at Z-line.

C. Titin

1. Thought to contribute to tenderness.
2. Extensively disrupted at pH 5.5.
3. Disruption occurs early postmortem.
4. Degradation of titin at specific sites leads to a broadening of the Z-line.

D. Troponin-T

1. Degrades to "30 K subunit".
2. Extent of degradation is correlated loosely with degree of tenderness



III. Contribution of endogenous proteases to the resolution of rigor

A. Cathepsins

1. Located primarily in lysosomes, normally responsible for turnover of proteins.
3. Active at acidic pH.
4. *In vitro*, digest many proteins and structures that are not affected during normal aging.
5. Primarily involved in the degradation of sarcoplasmic proteins.

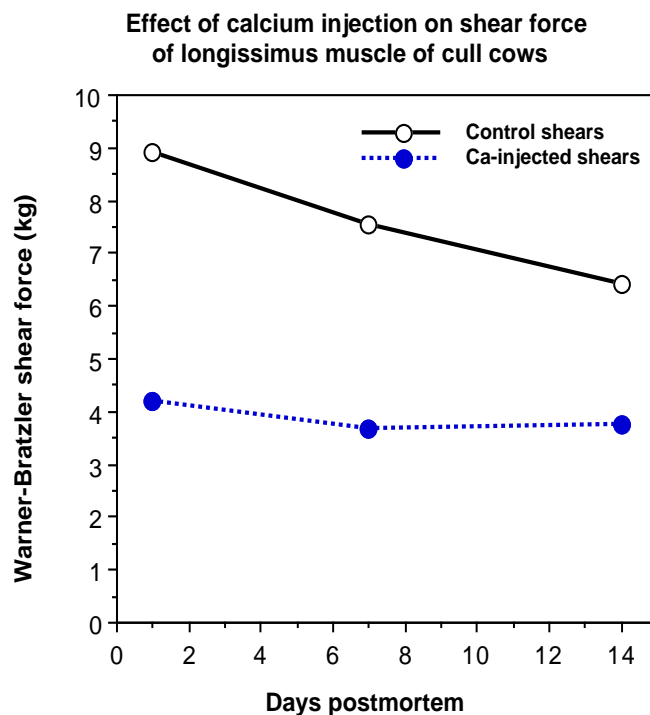
B. Calpains (calcium-activated proteases)

1. Located primarily at the Z-disc.
2. Normal function is inhibited by calpastatin.
3. Most active at alkaline pH.
4. Require calcium for activity.
 - a. Calpain system probably is involved in myofibril assembly and myogenesis.
 - b μ -calpain requires micromolar concentrations of calcium (as seen in postmortem muscle).
 - c. m-calpain requires millimolar concentrations of calcium.
 - d. Autolysis may reduce their requirements for calcium.
5. Activity is strongly depressed by increasing ionic strength.

Protease	In muscle cells?	pH optimum	Muscle protein digested
Cathepsin A	Yes	5.0-6.0	Myosin, myoglobin
Cathepsin B	Yes	3.5-6.0	Actin, myosin, intact myofibrils, collagen
Cathepsin C	Yes	5.0-6.0	Not determined
Cathepsin D	Yes	2.5-5.0	Actin, myosin, intact myofibrils
Cathepsin H	Yes	5.5-6.5	Actin, myosin, α -actinin, troponin-T, troponin-I, collagen
Cathepsin J	Yes	5.5-7.5	Unknown
Cathepsin L	Yes	3.0-6.5	Actin, myosin, α -actinin, troponin-T, troponin-I
Calpains	Yes	7.2-8.0	Tropomyosin, troponin-T (to 30 kd protein), troponin-I, C-protein, desmin, titin (?)
Multicatalytic Proteinase	Yes	9.0-10.0	Unknown

C. Evidence for calpains

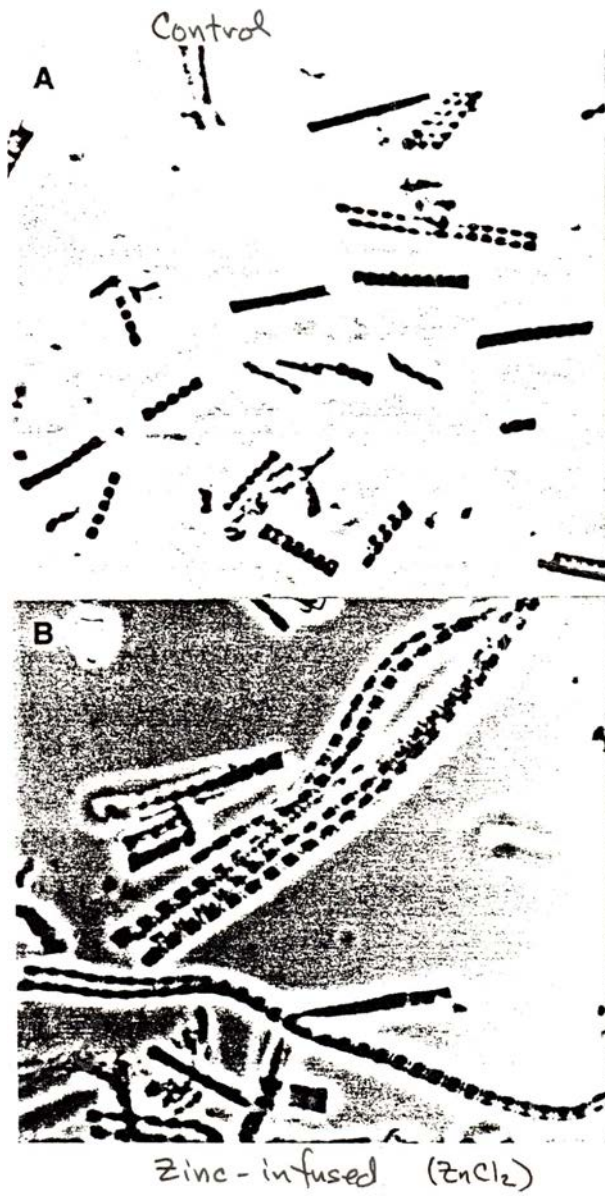
1. Calcium infusion accelerates rate of resolution of rigor.
2. Zinc infusion (which inhibits calpain activity) stops the aging process.



3. Muscle from zinc infused carcasses does not become more tender over time.

- a. Myofibrils from zinc-infused carcasses do not fragment.
- b. CDP-inhibitor (calpastatin) activity does not decline over time.
- c. Desmin and troponin-T do not fragment postmortem.

	Control		ZnCl ₂ -infused	
	d ₁	d ₁₄	d ₁	d ₁₄
Zn (μg/g)	8.25		62.48	
Shear Force ^a	11.47	5.72	11.25	10.13
MFI	43.63	75.92	39.07	39.67
CDP-I ^b	76.93	17.26	115.13	22.78
CDP-II ^c	115.19	109.30	126.01	92.88
CDP-inhibitor ^d	125.53	9.96	142.18	187.47
Cathepsin B ^e	85.17	105.82	93.88	97.57
Cathepsin B+L ^e	77.62	90.72	92.63	84.09



IV. Ionic strength and the resolution of rigor

A. The aging index

1. Increasing salt concentration (ionic strength) reduces the activity of the actomyosin ATPase.
2. Myofibrils from aged muscle are more sensitive to ionic strength than are myofibrils from fresh muscle.
3. Implies that, during aging, the normal increase in ionic strength causes dissociation of myofibrils.

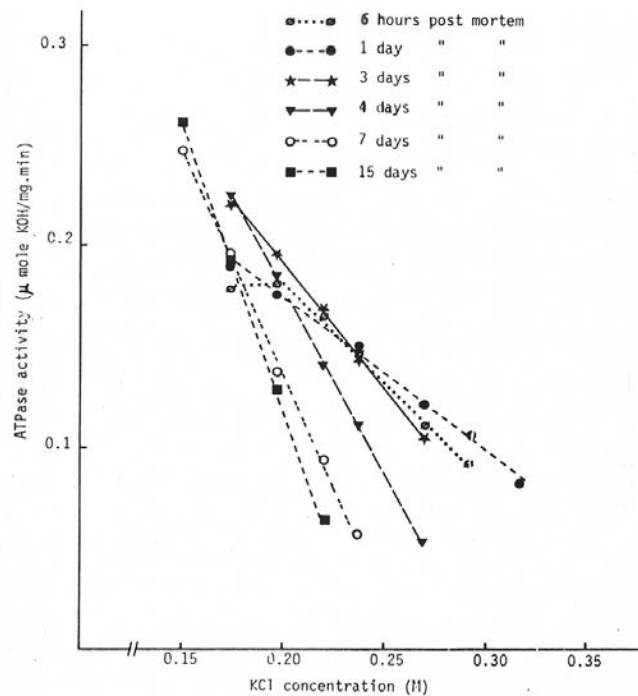
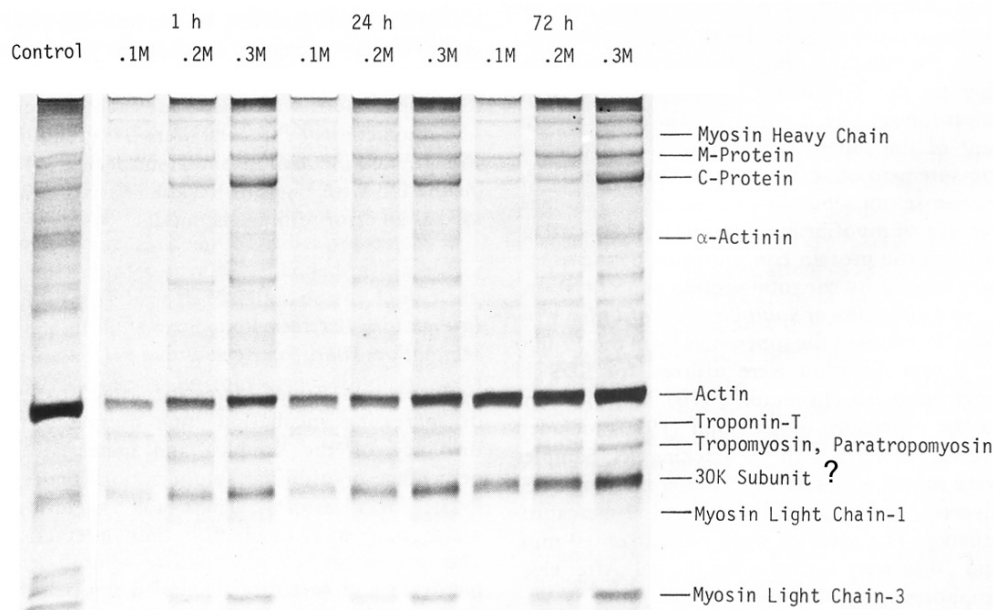


Fig. 1. Effect of conditioning of beef *Longissimus dorsi* muscle on Mg-Ca-enhanced ATPase activity and its sensitivity to ionic strength. The slope value of the straight line graph quantifies the ATPase sensitivity to ionic strength and represents the Biochemical Index of Myofibrillar Ageing (BIMA).

B. Effects of ionic strength on myofibrillar solubilization

1. Incubating myofibrils at 0.1 to 0.3 M KCl

- Many myofibrillar proteins are solubilized (myosin, actin, C-protein, M-protein).
- KCl actually inhibits protease activities.

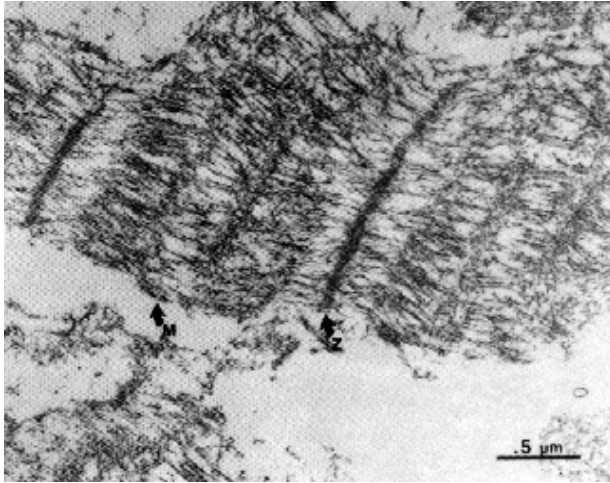


Polyacrylamide gel electrophoresis of proteins solubilized from myofibrils after incubation at different ionic strengths for 1, 24, or 72 h.

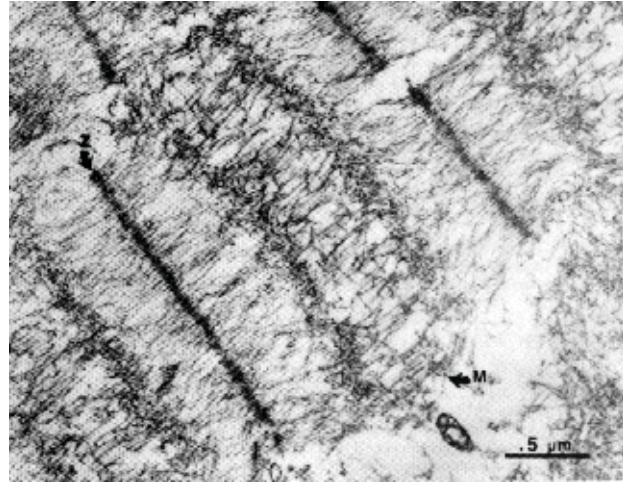
2. Interaction between trypsin and ionic strength

a. Trypsin hydrolyzes the Z-disc.

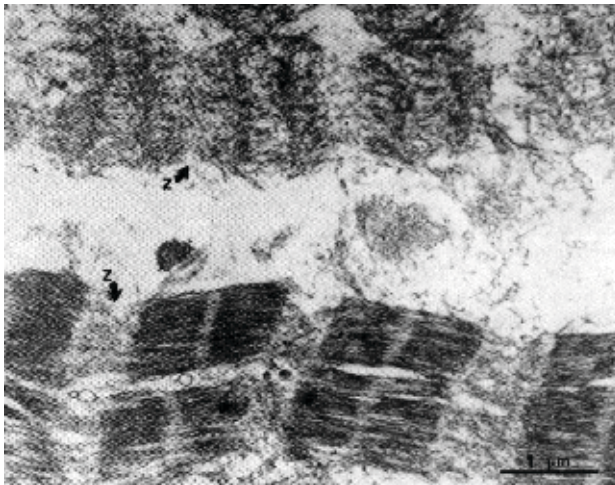
b Trypsin plus 0.3 M KCl causes complete dissolution of myofibrils.



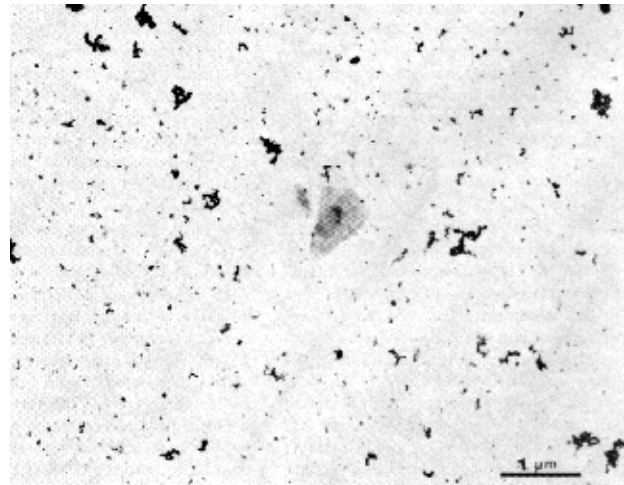
Longissimus muscle myofibrils incubated for 24 h with 0.1 M KCl.



Longissimus muscle myofibrils incubated for 24 h with 0.3 M KCl.



Longissimus muscle myofibrils incubated for 24 h with 0.1 M KCl following a 10-min preincubation with trypsin.



Longissimus muscle myofibrils incubated for 24 h with 0.3 M KCl following a 10-min preincubation with trypsin.

3. Infusing lamb carcasses with NaCl depresses shear force values.
- Rate of decline with days postmortem is *much* slower than seen with calcium infusion.
 - Ultimate shear force is the same as seen with calcium infusion.

