Influence of sarcomere length on the reduction of myofilament lattice spacing post-mortem and its implication on drip loss

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Background

It is known for cold shortened meat that short sarcomere length increases drip loss (Honikel et al. 1986). It is assumed that this is due to larger filament spacing pre-rigor leading to a stronger reduction of filament spacing during rigor and thus expelling out more water from the interfilament space (Offer and Knight 1988). Investigations on the post mortem changes in the myofilament lattice of pork meat in fixed samples and in samples in buffered solution indicated a decrease of the filament spacing (Diesbourg et al. 1988). However fixation of the sample as well as keeping it in buffered solution may have an influence on the filament spacing. Thus it would be reasonable to observe the changes directly in the muscle without any chemical interference. This can be achieved by minimising evaporation by wrapping the sample and short exposure times on the x-ray using intense synchroton radiation. How far the changes in filament spacing during rigor onset is influenced by the sarcomere length and thus by its initial spacing and reduction during rigor has yet to be shown.

Objectives

The aim of this study was to elucidate the changes in myofilament lattice spacing during rigor in relation to sarcomere length. Observations were to be made directly on the muscle without fixation or buffering solutions and correlated with measurements of driploss.

Methods

Pork *longissimus dorsi* muscle samples from 2 animals were collected at a nearby slaughterhouse 20min post mortem. Part of the muscle was cold shortened. Specimens (~4cm length, ~1mm thick) were cut out 1h post-mortem along the fibre direction and stretched to different sarcomere length. Either cold shortened or stretched specimens were wrapped in Kapton foil (DuPont), which was covered with water-saturated light paraffin oil, and fixed in clamps. X-ray diffraction patterns were taken from ~1h post mortem at ~1h intervals until 24h p.m..

X-ray diffraction was performed at station 2.1 of the Daresbury Laboratory synchroton radiation source, broadly following the method described by Wess et al. (1998). All diffraction images were converted to line profiles of diffraction intensity versus radial spacing and the spacing between thick myofilaments measured in terms of the 1,0 diffraction maximum (Diesbourg et al. 1988) with specialised software written by T.J. Wess.

The change in myofilament volume from pre-rigor to post-rigor states can be calculated as follows

$\%\ \text{volume change} = (d_I^2 - d_F^2/d_I^2) * 100$

where d_I and d_F are the initial and final 1,0 lattice spacings measured from the myofilament unit cell.

Sarcomere length was measured microscopically on the samples after fixation in 4% paraformaldehyde in 100mM MES buffer containing 0.9% NaCl pH 5.6.

Results and Discussion

The specimens investigated had a sarcomere length between 1.47 and 3.16 µm thus exhibiting a range from cold shortened to moderately stretched. These measurements were uncorrected for any shrinkage that may have occurred during fixation. The initial myofilament lattice spacing decreases with increasing sarcomere length in the way expected if the myofibrils maintain constant volume (Fig.1). In rigor the filament spacing decreased markedly at short sarcomere length, but did not change at long sarcomere length. The initial as well as the final myofilament lattice spacing correlated well with sarcomere length (initial r=0.9002, final r=0.9205). The reduction of filament spacing due to rigor is pronounced in the cold-shortened samples whereas in the stretched samples the spacing does not decrease any further on rigor onset. This lack of decrease in filament spacing implies that the 'optimum' distance between thin and thick filament for rigor has been reached just by stretching the fibres.

Based on these data, the volume change of the myofilament unit cell has been calculated (Fig.2). These calculations predict a volume change due to rigor of about 19% for short sarcomere length and about 13% for 2µm sarcomere length. Assuming a density of 1 for meat this suggests a far larger amount of water expelled from between the myofilaments during rigor than is actually lost as drip post mortem. Comparing the volume changes in myofilament unit cell to the results of Honikel et al. (1986) on drip loss in cold shortened and normal meat, the much steeper slope of our predicted curve just based on the filament lattice shrinkage suggest that the influence of sarcomere length on drip loss should be much more pronounced than it actually is. This would then suggest that not all the water that is expelled from between the myofilaments is actually lost as drip. This suggests that much of the expelled water may be in small spaces between myofibrils which would drain out only very slowly.

Conclusions

The sarcomere length has a pronounced influence on the myofilament lattice spacing and its change during rigor. Our results suggest that the shrinkage of the filament lattice is the major mechanism producing a transfer of water out of the myofibril. But it is not the only parameter which influences overall drip loss. Further investigations especially on the drip loss in stretched meat samples are needed to confirm these results.

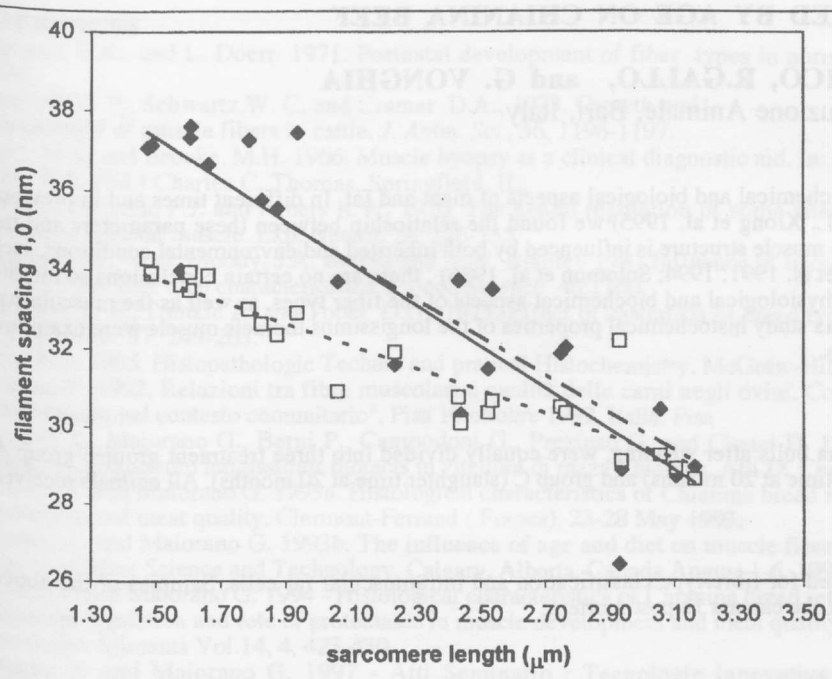

Fig.1

Initial and final 1,0 myofilament spacing determined from x-ray diffraction patterns of pork *longissimus dorsi* muscle stretched to different sarcomere lengths
◆ initial spacing [nm]; (— linear regression: y=-5.2699x+45.227; R^2=0.8104)
□ final spacing [nm]; (····· linear regression: y=-2.8998x+38.161; R^2=0.8474)
—·—· constant volume line

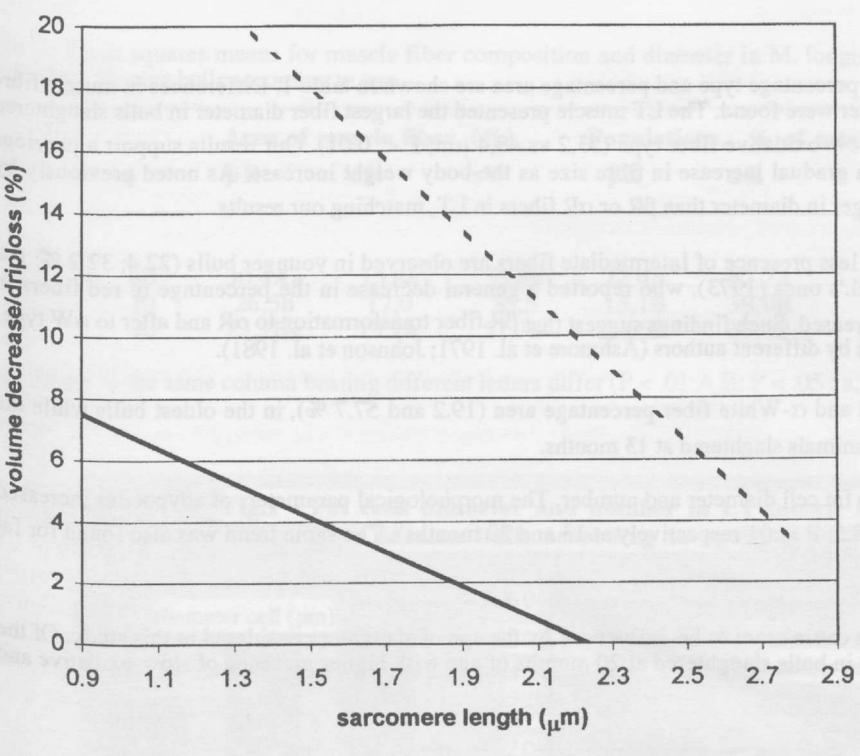

Fig.2

Calculated change in volume of the myofilament unit cell from pre-rigor to post-rigor states compared to drip loss in cold-shortened and normal meat

— drip loss (Honikel et al. 1986)
··· calculated myofibrillar volume decrease

Pertinent literature

K.O. Honikel, C.J. Kim, R. Hamm, P. Roncales, Meat Science 16 (1986), 267

G. Offer, P.J. Knight, Developments in meat science vol.4 (1988),173

Diesbourg, H.J. Swatland, B.M. Millman, Journal of Animal Science 66 (1988), 1048

T.J. Wess, P.P. Purslow, M.J. Sheratt, C.A. Shuttleworth, C.M. Kielty, J Cell Biol 141 (1998), 829