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THE RELATIONSHIP BETWEEN DISSOCIATION OF ACTOMYOSIN AND TENDERNESS

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Background:

Specific muscle proteins are degraded during post-mortem ageing of muscle (Wolfe and Samejima 1976; Uytterhaegen et al., 1994) and there is evidence that the cysteine proteases, in particular the calpains, are responsible for myofibrillar protein degradation (Uytterhaegen et al., 1994). The extent to which this degradation is responsible for changes in tenderness post-mortem is widely debated as the largest improvement in tenderness subsequent to rigor is observed within 24-48 hours, yet myofibrillar protein degradation is not considered extensive during this period (Taylor et al., 1995). It has been proposed that weakening of the bonds between actin and myosin may contribute to the increase in tenderness observed in the immediate post-rigor period (Taylor et al., 1995). Some compelling evidence that this interaction may impact on tenderness apart from its effect on contraction and fiber density was provided by Stein et al., (1979) who proposed what is called the 'modified-refractory-state model' of muscle contraction in which there are both weak and strong cross-bridge states between actin and myosin. If this is so, then actomyosin has the potential to exist in different binding states at rigor and this may affect the ageing of meat and the absolute level of tenderness achieved. Goll et al. (1995) presented a summary of other findings that suggest changes in the properties of actomyosin post-rigor. The increased ease with which actomyosin from aged rabbit muscle was dissociated by ATP compared to that from rigor muscle (Fujimaki et al., 1965) is one of the findings used to support the view that the interaction of actin and myosin impacts on tenderness. However Wolfe and Samejima (1976) found no such change in either rabbit or chicken muscle and the data of Herring et al., (1969) for beef muscle was equivocal. In none of these studies was the possible effect of proteolysis on the interaction of actin and myosin, through degradation of troponin, accounted for, nor was there any attempt to model the dissociation of actomyosin and relate this to measures of tenderness. To study the interaction between actin and myosin and the relationship of this to tenderness muscles in different states of contraction at rigor were used. The variation in contraction was achieved using tenderstretch treatment, whilst proteolysis was manipulated by injection of an inhibitor into the pre-rigor muscle.

Objective:

This study examined the interaction between the strength of bonding in the actomyosin complex and tenderness during the postmortem period using *in situ* muscle that was allowed to enter rigor in different states of contraction, whilst at the same time controlling potential confounding from proteolysis.

Methods:

Animals and slaughter procedures: Eighteen male lambs aged 10 months were slaughtered in 3 groups of 6 over a 6-week period under low stress conditions. The protease enzyme inhibitor E-64 (trans-Epoxsuccinyl-L-Leucylamindo-(4-Guandino)Butane) was injected into the *M. longmissius thoracis et lumborum (LL)* on the right side of the carcass between the $12^{th}/13^{th}$ rib and the tuber coxae within 30 minutes of death. To ensure an even distribution of inhibitor within the muscle 50 ml of inhibitor was dispensed using a bank of 10 needles 33 times. Each injection site received 0.15 ml with the delivery controlled using a multiple syringe pump, which delivered 18 mg of inhibitor per loin made up in isotonic saline (0.15 M NaCl). The left LL (control) was injected with 50 ml of isotonic saline (0.15 M NaCl). After injection carcasses were hung by either (1) Achilles' tendon, (2) The pubic symphysis or (3) The pubic symphysis with 2-kg weights attached to each hindleg through the Achilles' tendon. Carcasses were then chilled at 4.9°C for between 16 - 21 hours. The injected portion of the LL removed from both sides, divided into 3 portions, randomised and then vacuum packed for aging for 1, 3 or 7 days at 2.3°C. After ageing each portion was trimmed of epimysium to a 65g block for shear testing and frozen at -20°C. At the same time a 5-gram sample was taken for actomyosin extraction.

Meat quality measurements: The LL samples were cooked from frozen for 35 minutes at 70°C in an 80-L waterbath and peak force measured using a Lloyd with a Warner-Bratzler type head. Sarcomere length was determined by a laser diffraction technique.

Protein extraction: Actomyosin was extracted using a modified method based on salt solublization of the proteins (Briskey and Fukazawa, 1971). Briefly, the main modifications were use of a protease inhibitor cocktail during the initial homogenization of the muscle, re-homogenization of the insoluble pellet, an extraction solution with a pH of 7.4 and faster centrifugation throughout the procedure. The extracted actomyosin (in 0.6 M KCl) was stored with an equivalent amount of glycerol at -20°C. The glycerol was removed from the stored actomyosin suspensions by precipitating the actomyosin under centrifugation and the protein concentration of actomyosin suspensions determined in triplicate at 562 nm prior to dissociation using the BCA Protein assay kit (Pierce Chemical Company, Illinois, USA). The protein concentration was adjusted to 0.3 mg/ml using a buffer (0.6 M KCL, 4 mM $MgCl_2$, 0.02 M Tris, pH adjusted to 6.8 with acetic acid). Aliquots of the protein suspensions were mixed with either buffer or with $Na_2H_2P_2O_7$ (disodium pyrophosphate) at effective concentrations of either 0.025 mM, 0.05 mM or 0.5 mM. The effective protein concentration after mixing was reduced to 0.26 mg/ml. The solutions were then subjected to ultracentrifugation for 3 hours at 100,000 g (Model L8-M, Beckman Instruments, CA, USA) at 5°C. Aliquots of protein from the supernatant were taken in triplicate and the protein concentration measured. Absorption was measured at 570 nm using a microplate reader (Titertek Multiskan® Plus, Lab Systems OY, Helsinki, Finland) in accordance with the specified protocol.

Statistical analysis: Both shear force measurements (kg) and the amount of extracted actomyosin from the LL were analysed using a mixed model with main effects and their first order interactions. The main effects were hanging method, age (1, 3 and 7 days), portion (anterior, medial and posterior) and injection (inhibitor vs control), with animal within treatment as a random term. The relationship between pyrophosphate and the percentage of myosin dissociated from the actomyosin complex was modelled using an exponential function $\mathbf{Y = A - B\ exp^{-kx}}$ where **Y** = percentage of protein dissociated from the complex and x = the concentration of

pyrophosphate, **A** is the asymptote and **B** is the difference between the value of **Y** at the highest concentration of pyrophosphate and the value of **Y** when $x = 0$. k = rate of dissociation. Of the 93 samples subjected to dissociation, the model converged for 69 using a non-linear procedure (S-Plus 1997). The main effects and their interactions were tested against estimates of A, B and k. The data for 93 samples were subjected to a repeated measures analysis. For this analysis the dependent variable was protein dissociation at 0, 0.025, 0.05 and 0.5 mM of pryophosphate with all main effects and their interactions in the model.

Results:

Shear force was affected ($P < 0.05$) by inhibitor injection, hanging method and portion, but not by ageing ($P > 0.05$). Predicted means for the significant main effects are shown in Table 1. The amount of actomyosin (mg /gram of muscle sample) extracted from muscles injected with the inhibitor was found to be significantly less ($P < 0.001$) than the control and also differed among muscle portions ($P < 0.05$, Table 1). There were no other significant effects ($P > 0.05$) on the amount of actomyosin extracted. Analysis of A, B and k for the 69 samples fitted with an exponential function showed that there were no significant main effects or interactions ($P > 0.05$) on A and k. There was an effect of portion on B and also an interaction between injection and hanging method ($P < 0.05$). The repeated measures analysis also identified an effect ($P < 0.05$) of portion on the amount of dissociated protein, but no other significant effects. The portion effect showed a greater change in the amount of protein measured in the supernatant between solutions with no pryophosphate and those with 0.05-mM pryophosphate, for samples from the posterior portion. Given the effect of portion on shear force values and on the coefficient B, the correlation between shear force and B was examined but found to be zero.

Table 1: Least square means (± s.e.) of Warner-Bratzler shear values (WB; kg) and the amount of extracted actomyosin (AM; mg/gram) (n =108).

Portion	WB	AM	Hanging method	WB	Injection	WB	AM
Anterior	6.0 ± 0.19a	3.0 ± 0.26a	*Achilles tendon*	6.6 ± 0.28a	*Inhibitor*	6.6 ± 0.18a	3.6 ± 0.22a
Medial	5.4 ± 0.19b	4.5 ± 0.26b	*Tenderstretched*	5.3 ± 0.28b	*Control*	4.2 ± 0.18b	5.1 ± 0.22b
Posterior	4.6 ± 0.19c	5.5 ± 0.26c	*Tenderstretched/weighted*	4.2 ± 0.28c			

Values followed by the same letter in a column (a, b, c) are not significantly different ($P > 0.05$).

Discussion:

The muscle stretching techniques employed in this study were effective in altering sarcomere length (1.87 to 2.15 μm for the normally hung and weighted tenderstretch treatments, respectively) and presumably the degree of overlap of actin and myosin at rigor. There was however no clear evidence that stretching or ageing had an affect on the dissociation of actomyosin. Wolfe and Samejima (1976) also found no relationship between aging and actomyosin dissociation. Data presented by Herring et al., (1969) was inconclusive about the relationship and in both studies no attempt was made to quantify differences in the shape of the dissociation curve. Scrutiny of the curves published by Wolfe and Samejima (1976) suggests that a number of these would also not have conformed to an exponential function. For our data that did conform to this function, the coefficients were mostly unaffected by the treatments used in the experiment. Muscle portion and the interaction between injection and hanging method both had an effect on dissociation and shear force. There was however no apparent effect of dissociation on tenderness as measured in this experiment and therefore a causal relationship cannot be identified. The lack of change in shear force in control muscles hung by the Achilles tendon was unexpected and may have contributed to the lack of relationship between dissociation and tenderness. However myofibrillar fragmentation index data (not shown) did indicate some degradation occurred. The use of an inhibitor was effective at preventing tenderisation indicating the role of cysteine proteases in proteolytic degradation, but this also meant that any effect of the degradation of troponin on the interaction of actin and myosin was likely eliminated. In this study there was no evidence that the strength of actomyosin bonding had a major impact on post-rigor tenderness as measured by shear force, given the design of the experiment. However, portion effects may in part be explained by changes in the ease with which actomyosin could be dissociated, but this effect was not large enough to impact on shear force over and above the effects of proteolysis and fiber density. Future attention to changes in actomyosin immediately after rigor is worthy of investigation as changes 1 day after rigor and beyond appear to have minimal impact on tenderness, yet it is known for example that isometric tension decreases after rigor without alteration in sarcomere length (Devine et al., 1999).

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References:

Briskey, E.J. and Fukazawa, T. (1971). *In* "Advances in Food Research" (Ed's Chichester, C.O, Mrak, E.M. and Stewart, G.F.) Academic Press, New York, Vol 19, 346-348.
Devine, C.E., Wahlgren, N.M. and Tornberg, E. (1999). Meat Sci. 51, 61-72.
Fujimaki, M., Arakawa, N., Okitani, A. and Takagi, O. (1965). J. Food Sci. 30, 937-943.
Goll, D.E., Geesink, G.H., Taylor, R.G. and Thompson, V.F. (1995). Proc. 41st ICoMST, San Antonio, 537-544.
Herring, H.K., Cassens, R.G., Fukazwa, T. and Briskey, E.J. (1969). J. Food Sci. **34**, 571-576.
S-Plus (1997). V 4. Mathsoft, Inc. Seattle, USA.
Stein, L.A., Schwarz, Jr, R.P., Boon Chock, P. and Eisenberg, E. (1979). Biochemistry **18,** No. 18, 3895-3909.
Taylor, R.G., Geesink, G.H., Thompson, V.F., Koohmaraie, M. and Goll, D.E. (1995). J. of Anim. Sci. **73**, 1351-1367.
Uytterhaegan, L., Claeys, E. and Demeyer, D. (1994). J. of Anim. Sci. **72**, 1209-1223.
Wolfe, F.H. and Samejia, K. (1976). J. Food Sci. **41**, 244-249.