SESSION 4

MUSCLE BIOLOGY AND MEAT BIOCHEMISTRY

4-L1

THE INTRAMUSCULAR CONNECTIVE TISSUE MATRIX AND CELL/MATRIX INTERACTIONS IN RELATION TO MEAT TOUGHNESS

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ABSTRACT

Substantial variations in the composition, amount and distribution of extracellular matrix components of muscle during animal growth and development are related to variations in the eating quality of meat. The flexibility shown by the extracellular matrix of muscle tissue is hypothesised to reflect differing mechanical requirements placed on different muscles. The extreme plasticity in extracellular matrix expression within muscle should be viewed as indicating a great potential for manipulating the contribution of this component to meat quality.

Modifications of collagen crosslinking during muscle development and animal maturation have long been hypothesised to have a major influence on meat toughness. Whilst variations in crosslinking are seen with animal maturity, there has to date been no convincing demonstration of an association between mature crosslink content in intramuscular connective tissue and cooked meat toughness. Post mortem proteolysis has some effect in destabilising intramuscular connective tissue, but this effect seems to be negated by cooking.

The extracellular matrix mechanically interacts with myofibrillar structures within the muscle cells via trans-sarcolemmal and cytoskeletal links in order for muscle tissue to function in vivo, and these cell-matrix linkages continue to have significance for the physical properties of the composite structure of meat post-mortem. The relationship between degradation of these structures and tenderness development is currently questionable.

Introduction

The concept that the amount of intramuscular connective tissue (IMCT) has a role in determining meat toughness goes back over 90 years (Lehmann, 1907). For much of this century the variation in connective tissue structure and content was thought to dominate variations in muscle texture (Marsh, 1977). In the last 20-30 years it has been recognised that the total collagen content of muscles can only explain a part of the variation in cooked meat toughness (Dransfield, 1977) and attention switched to the nature ("quality ") of the IMCT, principally in terms of its covalent crosslink profile, as a more important factor (Shimokomaki et al., 1972; Bailey 1988) that explains variations in cooked meat toughness, especially with the physiological age of the animal.

More recently, attention has switched to variations within the muscle cells, and especially question of post-mortem proteolysis of myofibrillar and cytoskeletal proteins, to explain variation in meat toughness. This has increasingly led to a relegation of the role of IMCT to providing a "background toughness" (Ouali et al., 1992) which, by implication, is rather fixed.

Whilst myofibrillar proteolysis is certainly the major factor in the modification of meat texture post-mortem, there remains considerable evidence that the IMCT contribution to meat texture is by no means constant. The considerable variation of the cellular expression of IMCT between species, breed, between muscles in one animal (this latter representing a huge variation in expression from a single genome), within a single muscle, and the variations in IMCT composition and crosslinking due to animal age, nutrition and growth rate all represent mechanisms with potential for controlling meat texture. McCormick (1994) referred to the "flexibility" of the IMCT compartment of muscle. This paper sets out to highlight sources of variability in IMCT structure and properties in the animal growth and maturation phase as well as the post-mortem phase that should be viewed as unexploited potential for manipulation of meat quality. The essential interactions between IMCT and the structures within the muscle cells via trans-sarcolemmal and cytoskeletal proteins are also stressed.

Pre-slaughter factors in IMCT variability

Variations in IMCT architecture and content within and between muscles

If we look at the overall structure and composition of muscle tissue, then it is the connective tissue (extracellular matrix) components which show the greatest variation in amount, composition, architecture and post-expression modifications.

The total amount of collagen in beef muscles varies from 1.6% of dry weight to 15.1% (Bendall, 1967). The relative proportion of elastin in the intramuscular connective tissue (IMCT) varies from 0.6% in some muscles to 37% in others (Bendall, 1967). The proportion of different molecular types of collagen varies between muscles (Light et al., 1985) and between the different connective tissue structures within muscle (see Purslow & Duance (1990) for a review).

Most of the variations in IMCT content between muscles seems to be variations in the amount of perimysial connective tissue (surrounding and separating fascicles) rather than in the endomysial connective tissue (surrounding and linking together adjacent muscle fibres). Although their methods for separating endomysium and perimysium from muscle are far from perfect, Light et al. (1985) show a range of 1.4% to 7.0% in perimysial mass as percentage of muscle dry weight but only a range of 0.1% to 0.5% in endomysial mass between six beef muscles. An unpublished survey (Fisher et al., 1988) of 14 beef muscles using the same methods revealed a similar pattern of greater variability in perimysial versus endomysial content (see table 1). Variations in the molecular species of collagen, the diameter of collagen fibrils and the amount of divalent (immature) crosslinks are also seen in the perimysium (Light et al., 1985).

Muscle	Perimysial collagen as % of dry weight	Endomysial collagen as % of dry weight
Extensor carpi radialis	4.76	1.20
Infraspinatus	4.30	0.58
Sternocephalicus	3.37	0.76
Supraspinatus	2.38	0.66
Rhomboideus	2.09	0.89
Splenius	2.12	0.56
Subscapularis	1.96	0.65
Pectoralis profundus	1.62	0.89
Triceps brachii cap. long.	1.80	0.46
Complexus	1.44	0.71
Gluteus medius	1.23	0.64
Gastrocnemius	1.15	0.63
Obliquus intern. abdom.	0.54	0.55
Serratus ventralis	0.43	0.47

Table 1. Variation in perimysial and endomysial collagen content of 14 beef muscles. Data taken from unpublished work of Fisher et al. (1988). Values are means of measurements from 6 Friesian bovine animals.

Light et al. (1985) focused their studies on the perimysium because of previous mechanical and structural studies (Carrol et al., 1978; Purslow, 1985) which showed an obvious involvement of perimysial IMCT in the fracture behaviour of cooked meat. Lewis and Purslow (1990) showed that the strength of the perimysium in cooked meat was greatly in excess of the strength of the interface between the endomysium and perimysium, which is known to be easily broken during the extension of cooked meat to fracture.

The morphology of the perimysial network (i.e. the thickness of perimysial sheets and the size and shape of the primary and secondary fascicles delineated by the perimysium) also varies between different muscles. Fascicle size ("grain size") has been known to vary in beef and sheep muscles for over half a century (Hammond, 1932; Brady, 1937).
Variations of IMCT content and spatial distribution have been noted even between locations within individual muscles. Totland et al. (1988) related decreasing tenderness from superficial to deep zones of bovine semitendinosus muscle to increasing muscle fascicle size. The transverse tensile strength of meat (which is known to measure perimysial connective tissue strength; Bouton & Harris, 1972a; Purslow, 1985) has been shown to vary with position and orientation in porcine longissimus (Shäfer et al., 1999). Variations in tenderness with position within a muscle has also been documented for bovine semimembranosus (Paul & Bratzler, 1955), bovine biceps femoris, longissimus, latissimus dorsi and multifidis dorsi (Rambottom et al., 1945) and bovin percoralis (Ertbjerg et al., 1995). Bendall (1967) noted variations in the collagen and elastin content of bovine extensor carpi radialis.

Why do different muscles in one animal vary in their IMCT content and spatial distribution?

This is an important question because, if we know why variations occur we can begin to think of ways to manipulate these variations. This question begs another, more fundamental one; *what is the in-vivo role of IMCT?* Reviews (Mayne & Sanderson, 1985; Purslow & Duance 1990) highlight the necessary role of IMCT in the development of muscle, but the functions of IMCT in providing alignment templates for growing muscle cells and of controlling the site of the neuromuscular junction are arguably common to all skeletal muscles. It has commonly been thought that the variations in IMCT content, composition and spatial distribution within various muscles are adaptations to the different functions/properties of those muscles. As greatest variations are seen in perimysial content and spatial distribution, it is arguable that the perimysium plays an important role in the functioning of different muscles. So what are the functional, mechanical roles of IMCT?

The role of force transmission has been examined for both the perimysium and the endomysium. Purslow (1989) mathematically modelled the reorientation of collagen in the perimysium with changing muscle length. This model was verified against experimental measurements of collagen orientation in perimysium excised from muscle fixed at different sarcomere lengths. As shown by the data in figure 1, this model also fits the deformation behaviour of excised strips of perimysium. This reorientation explains the structural basis of the non-linear load-deformation curve for excised tissue. The results in fig. 1 therefore confirm that tensile testing of isolated strips of perimysium is a justifiable means of assessing the deformation behaviour of perimysium *in situ* within meat, as the same reorientation behaviour is observed quantitatively on excised strips as in the intact network. Lepetit (1989) independently derived the theoretical model which describes this reorientation behaviour. Purslow (1989) concluded that the tensile properties of the perimysial network were unsuitable for the transmission of contractile force produced by active muscle cells. This is quite evident from figure 1(c); near rest length the perimysium is very easily deformable.

Purslow and Trotter (1994) showed that the tensile properties of the reorienting endomysial network were similarly unsuitable for force transmission, but that shear transmission of force through the thickness of the endomysium linking two adjacent muscle cells was a possible explanation for the mechanism of force transmission in series-fibred muscle. Trotter et al. (1995) further developed analysis of this concept, showing that the shear connections between adjacent muscle fibres in a series-fibred muscle were sufficiently non-compliant to act as an efficient means of contractile force transmission.

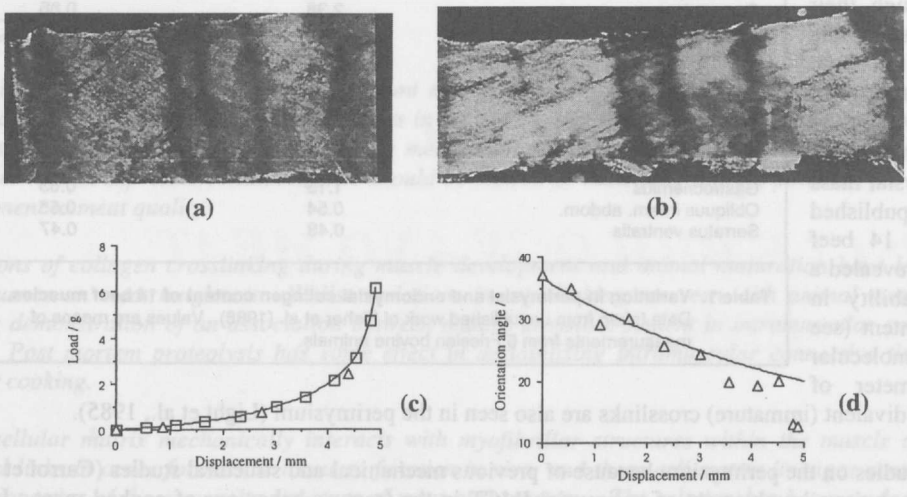

Fig.1 Excised perimysium from 24-hour post mortem bovine semitendinosus (a) unstretched, and (b) stretched transverse to muscle fibre axis. (c) shows the resulting load extension curve. (d) Measurements (triangles) of the angle θ between the collagen fibres and the muscle fibre axis agree with the model of Purslow (1989) (fitted line) for reorientation of perimysial fibres surrounding a fascicle changing length at constant volume

So, if endomysial IMCT links adjacent muscle fibres by shear, what is the functional role of perimysium? I would like to propose that the division of a muscle as a whole organ into fascicles is absolutely necessary for the mechanical functioning of muscles. (This is a separate issue from the necessity of a perimysial IMCT framework to carry essential blood vessels and nerves to fascicles - which is again a fairly standard and general requirement in muscles.)

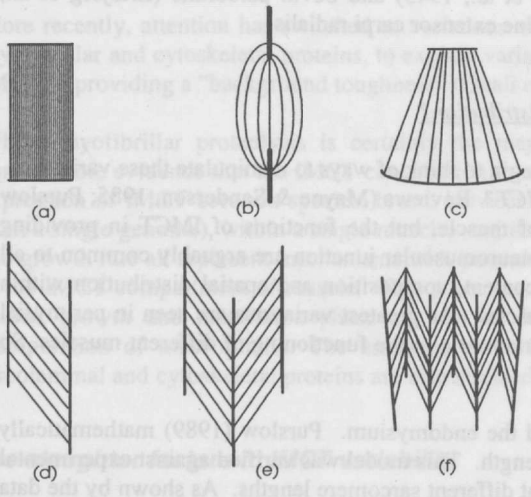

Figure 2. Six categories of gross muscle architecture, as defined by Gowitzke & Milner (1980) (a) parallel, (b) fusiform, (c) fan-shaped, (d) unipennate, (e) bipennate, and (f) multipennate.
Only in (a) can a shortening of the muscle length involve no shear displacements between elements within the muscle. Thin lines denote muscle fibre/fascicle directions. Thicker lines denote points of tendinous or aponeurotic insertion.

Figure 2 depicts the six major shapes or types of muscle at the gross (whole organ) level. Contraction in five out of six cases (b-f) necessarily involves a change in shape of the whole muscle. A straight line drawn transversely across the muscle at some point along its length must tilt or curve upon contraction in cases b-f. Mechanically, this means that some internal parts of the whole organ must slide part others - i.e. there is an absolute requirement to accommodate shear deformations within the muscle tissue. The amount and spatial distribution of shear deformation will vary considerably between muscles with different relaxed shapes and ranges of contraction. The shear deformations could generally be expected to be lowest in muscle type (b) and highest in type (f). A comprehensive study of shape changes (and therefore shear deformations) within actively contracting muscles remains to be done.

Previous analysis (Trotter et al., 1995) suggest that shear displacements between adjacent muscle fibres could be as small as 1 μm or less. If endomysium binds adjacent muscle fibres together in such a way as to prevent significant shear slippage between them, then the large shear deformations necessary for a muscle to change shape on contraction must be accommodated at another level of structure. The junctions between adjacent fascicles, as defined by the perimysial network, is the logical place where large shear deformations could be accommodated by allowing fascicles to slip past each other.

Fig. 3 shows the result of some unpublished experiments that support this hypothesis. Deformation of rigor muscle so as to shear lines drawn across the fibre direction results in movements concentrated at the junctions between fascicles. The perimysium therefore defines the "slip-planes" necessary for most muscles to change shape as they work.

The easily deformable *tensile* nature of the perimysial (and endomysial) networks easily allows substantial changes in muscle fibre length and diameter on contraction and relaxation. However, the endomysium laterally links adjacent muscle fibres within the fascicle tightly, so that contractile forces can be shared locally by *shear* and fibres kept in register, whereas the perimysium (or the perimysial/endomysial interface) permits large *shear* deformations so that the fascicles can slip past each other. Variations in perimysial network morphology within individual muscles and between muscles may thus be analysed on the basis of the amount and spatial pattern of deformations involved in normal *in vivo* working contractions. However, these analyses have yet to be performed.

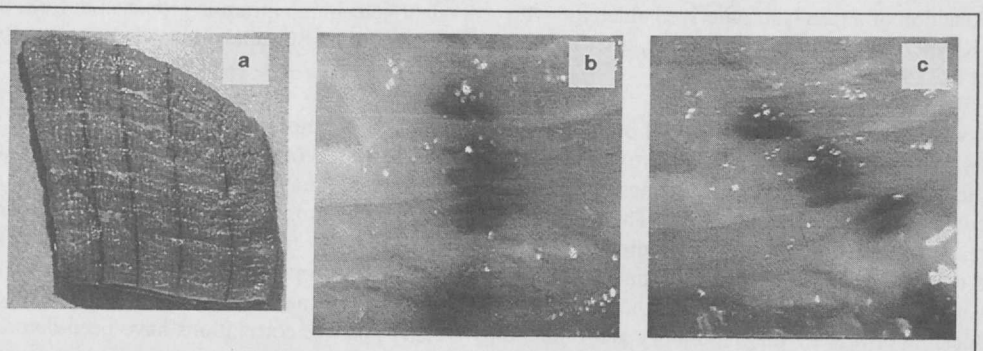

Fig. 3 Experiments demonstrating shear deformations occur at boundaries between fascicles in muscle. (a) A longitudinal slice of bovine semitendinosus (24 hours post mortem) is marked by lines of ink drawn across the fibre direction. (b) Higher magnification of an ink marks crossing three fascicles in the resting muscle. The muscle slice is then manually manipulated to change its shape, resulting in a shear of the ink mark across the three fascicles (c); note that the ink marks remain in register *within* each fascicle, but show large shear displacements *between* fascicles. Experiments on bovine sternomandibularis show similar results (not shown).

Variations in IMCT structure, orientation or distribution within a muscle are similarly explained as local adaptations to non-uniform shear deformation requirements within the whole muscle.

Animal growth, maturity and nutrition effects

The tenderness of meat on average decreases with animal age. Connective tissue-rich muscles such as biceps femoris show a greater age-related increase in toughness than muscles such as psoas major with relatively little IMCT (Shorthose & Harris, 1990). Attention therefore focuses on changes in IMCT with age as a major cause of the animal maturation effect on toughness.

The concentration of collagens in IMCT of bovine muscles clearly increases during embryonic growth (Listrat et al., 1998), as does the integrity of the IMCT network as seen by SEM (Nishimura et al., 1996). We have recently shown a difference in the chronological appearance of collagen type IV and other matrix components such as laminin in pectoralis versus quadraceps muscles in the second half of embryonic development of the chicken. (Lawson & Purslow, 1999) However, at the end of foetal development the collagen concentration of muscles broadly echoes that in adult tissues (Listrat et al., 1998). There is no consistent evidence from the literature of increasing amounts of IMCT with animal maturity. Early work (Wilson et al., 1954) found no significant increase in IMCT of bovine longissimus with physiological age and indeed another early study of bovine biceps femoris muscle (Goll et al., 1963) reported a decrease in collagen content of bovine biceps femoris muscle with age. Later studies on other muscles report contradictory findings. The concentration of collagen has been found to increase with age in a number of muscles of the rat (Alnaqueeb et al., 1984; Kovanen et al.1987; Swatland, 1975), but not in others (Zimmerman et al., 1993).

Crosslink content

There is much more consistent evidence that the composition of intramuscular collagen alters with age, particularly in terms of the covalent crosslinking that stabilises the collagen fibres. For fibrous collagens, two lysyl oxidase-initiated crosslink pathways lead to divalent (reducible, immature) forms of collagen crosslink; the heat stable oxo-imine (keto-imine) form derived from hydroxylysine & lysine residues and the heat labile aldimine form from two hydroxylysine residues. The amounts of these two forms vary between the epimysial, perimysial and endomysial components of IMCT in young animals (Light & Champion, 1984). Oxo-imine cross link concentration has been shown to correlate with meat toughness (Shimokomaki et al., 1972). However, the divalent forms are gradually converted into non-reducible mature crosslinks with increasing animal age (Shimokomaki et al., 1972; Eyre, 1987; Kielty et al., 1993) and attention has long been focussed on identifying the mature crosslinks as possible sources of explanation for the increased meat toughness in older animals. In addition, there is an increased amount of a pentosidine crosslink resulting from non-enzymatic glycation of collagen with age, but the amounts of this are thought to be too small to make a significant contribution to the physical properties of IMCT (Bailey et al., 1995).

Reaction of keto-imine crosslink with a free hydroxylysine-derived aldehyde is proposed to result in hydroxypyridinium (Pyr) (Eyre, 1987). Although the Pyr content of IMCT is low compared to some other connective tissues (Eyre, 1987), it is known to increase with age. McCormick (1994) summarises the information in the literature on the variation in Pyr concentration with age in ovine longissimus, bovine longissimus and biceps femoris, deer longissimus, rat gastrocnemius and soleus, and porcine longissimus and biceps femoris muscles. In all cases the Pyr content of the intramuscular collagen increases with age. Pyr content may also be higher in slow-twitch muscles than in fast muscles (Palongas et al., 1992).

Reaction of a free lysine-derived aldehyde with a divalent keto-imine group is proposed to form the Ehrlich Chromogen (EC) product (Kuypers et al., 1994). However, after approximately 18 months EC concentration exponentially declines in bovine IMCT (Horgan et al., 1991).

Avery et al. (1996) produced a thorough investigation of the relationships between amounts of the divalent crosslinks, pentosidine, Pyr, EC, and HHL (histidinohydroxylysinonorleucine, a mature product derived from the heat-labile aldimine divalent crosslink) in the perimysium of longissimus muscle from pigs of comparable maturity. No significant correlations were found with variations in cooked meat toughness at all. They conclude that, although crosslinks have a role in determining meat texture, they are not responsible for variations in texture between longissimus muscles from porcine animals of the same age. However, in a later study Avery et al. (1998) were also unable to show a meaningful correlation between Pyr concentration in bovine longissimus and variations in tenderness due to the chronological age of the animals over the range 400-800 days. As Avery et al. (1998) note, "despite efforts by this laboratory and others only weakly positive correlations have been demonstrated between one or more of the biochemical characteristics of IMCT and either the perceived or measured toughness of meat". The proposal that the crosslink profile of IMCT has an important effect on meat toughness now looks less clear-cut than in the past.

The degree and rate of muscle development may be thought to have an effect on the IMCT contribution to meat toughness, due to differences in animal maturity at slaughter (Avery et al., 1998). Bouton et al. (1982) showed there was a reduction in the transverse strength of perimysial networks in semitendinosus muscles from animals heterozygous for muscular dystrophy compared to homozygous animals. However, in double-muscled Belgian blue-white bulls hypertrophy results in a significant decrease in the IMCT content of the longissimus muscle, but this is accompanied by reduced protein turnover, so that meat toughness actually increases (Utterhaegen et al., 1994), or in some studies does not significantly vary from normal animals (De Smet et al., 1998). Baland and Monin (1987) found only small reductions in collagen content in ham and loin muscles of faster growing Pietrain pigs compared to Large Whites. Muscle growth rate is also a function of nutrient availability. Allingham et al. (1997) show that rapid compensatory growth after weight loss can reduce the strength of IMCT in bovine semitendinosus.

Post-mortem factors affecting the IMCT contribution to toughness

Conditioning

Whilst the major effect of post-mortem proteolysis is undoubtedly to tenderise myofibrillar structures, there are also consistent reports in the literature of decreased stability of collagen in raw muscle on conditioning. This has been demonstrated most convincingly by the studies of Mills et al. (1989 a,b) and of Stanton & Light (1987; 1988; 1990). However, it has long been known that there is no effect at all of conditioning on the transverse strength of cooked meat (Bouton & Harris, 1972b), which is due almost entirely to the strength of the perimysial network after cooking.

This question of the effects of conditioning on the structure and mechanical properties of IMCT has been the subject of a number of studies recently and deserves careful examination. In the last decade a number of studies have used sodium-hydroxide digestion of myofibrillar and cytoskeletal proteins and the basement membrane and proteoglycan components of IMCT, so as to reveal the morphology of the collagen fibre network of the endomysium and perimysium, which can then be examined by scanning electron microscopy. This digestion method was generally demonstrated on connective tissues by Ohtani et al. (1988). We have used this technique to reveal the spatial distribution of collagen fibres in the endomysial reticular layer of feline biceps femoris muscle (Trotter & Purslow, 1992) and the IMCT morphology in bovine sternomandibularis muscle (Purslow & Trotter, 1994). Nishimura et al. (1994) similarly demonstrated the three-dimensional arrangement of collagen fibres in the IMCT of bovine semitendinosus muscle using this technique. Sodium hydroxide is an effective means of removing many components of muscle and the extracellular matrix - including collagen; it must be remembered that rapid and total removal of collagen by hot sodium hydroxide was historically a means of determining the elastin content of connective tissues (Jackson & Cleary, 1967; Bendall, 1967).

In our hands the appearance of the endomysial network after NaOH digestion of muscle could be variable. Figure 4 shows the SEM appearance of the endomysial network in two preparations of bovine sternomandibularis muscle fixed 24 hours post-mortem and digested with NaOH as described by Purslow & Trotter (1994). There is a marked difference in the apparent density of the fibrous network between the two preparations. This difference does not affect the distribution of collagen fibril orientations, which was the subject of analysis by Trotter & Purslow (1994). However, it does demonstrate that variations in the efficiency of extraction are easily encountered.

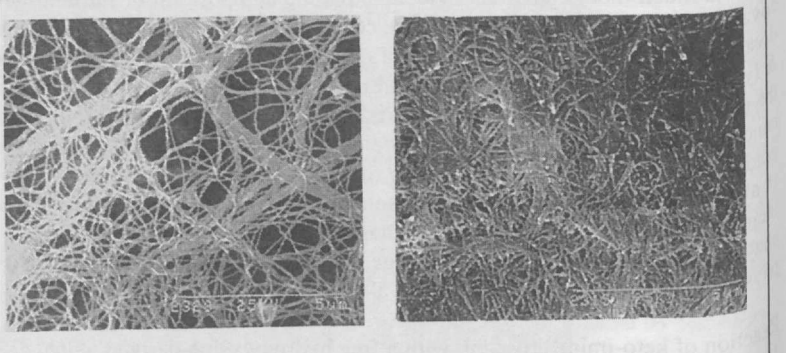

Fig. 4. Scanning electron micrographs (same magnification) of two endomysial preparations from bovine sternomandibularis muscle using the NaOH-digestion method. Small variations in the extraction time or spatial access of the NaOH resulted in variable removal of matrix components. Bar denotes 5 µm. Unpublished micrographs from the work described by Purslow & Trotter (1994).

Recent scanning electron microscopy studies of the IMCT in beef (Nishimura et al., 1994, 1995) and chicken muscle (Liu et al., 1995) aim to show decreased integrity of the IMCT networks due to conditioning by means of NaOH digestion. The authors are to be congratulated that their control of the NaOH digestion method is such that they can show consistent and reproducible differences in the apparent density of collagenous networks in the IMCT of muscle during post-mortem conditioning. Liu et al. (1994) report steadily diminishing shear force values for raw chicken semitendinosus over a period of 24 days post mortem and relate this to opening of gaps in the endomysial and perimysial IMCT networks and increased staining of proteoglycans as seen by light microscopy. Nishimura et al. (1998a) use the NaOH-extraction technique to isolate IMCT networks from bovine semitendinosus during 35 days of conditioning and then embed them in acrylamide gels for mechanical testing. The decreased shear force required to rupture these somewhat parallels the reduction in the shear force of the raw meat over this period. However, mechanical tests on extracted material re-embedded in an artificial matrix should be viewed with caution.

Proteoglycans (PGs) play an important role in linking fibrous elements of the extracellular matrix and recent studies are beginning to suggest possible effects of conditioning on PG integrity in IMCT. Nishimura et al. (1996a) identify PGs associated with the basement membrane and endomysium in bovine semitendinosus as principally containing heparan sulphates, whilst those in the perimysium were rich in chondroitin and dermatan sulphate. Nishimura et al. (1996b) study PG degradation during conditioning of the same muscle and conclude that degradation of PGs seems to be "the main factor" in weakening of IMCT during conditioning. Increased Periodic Acid-Schiffs staining of PGs in conditioned chicken semitendinosus is interpreted by Liu et al. (1994) to be consistent with "opening-up" of the IMCT structure. Nakano et al. (1997) report intense immunolabelling for decorin, together with weaker chondroitin sulphate labelling in bovine IMCT. Eggen et al. (1998) also showed a specific degradation in decorin during ageing, as well as a general reduction in PG content in beef semimembranosus muscle. Decorin is known to interact with collagen in the extracellular matrix.

These studies begin to suggest an interesting pattern of degradation events in IMCT during conditioning. However, in relation to the central issue of the effects on degradation on cooked meat texture, the original findings of Bouton and Harris (1972b) must be remembered; these show that structural and biochemical degradation of IMCT are not relevant to cooked meat texture. (The shear force measurements on IMCT and whole meat during conditioning by Liu et al. and Nishimura et al. mentioned above were all performed on raw tissue.)

Studies on the strength of perimysium excised from meat before and after post-mortem conditioning (Lewis et al. 1991) and after various degrees of cooking have previously explained this apparent contradiction, and are worth repetition in the light of the continued activity in this area, as detailed above. As shown in figure 5, conditioning does indeed reduce the strength of perimysium excised from raw meat, but after cooking to 60°C and above, no effect of conditioning on perimysial strength is seen. It is worth repeating that biochemical and structural degradations seen in raw tissue should not be assumed to have direct consequences on cooked meat texture. Subsequent studies of the strength of perimysium isolated from cooked meat (Lewis & Purslow 1991) also showed that low pH decreases the stability of raw perimysium, but that this pH-induced effect remains in cooked beef muscle, giving rise to a large reduction in the transverse strength of cooked meat after marination.

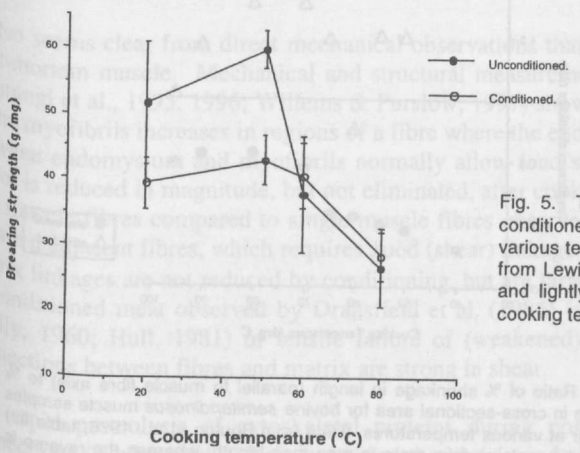

Fig. 5. The strength of perimysial IMCT isolated from unconditioned and conditioned raw bovine semitendinosus muscle (20°C) and after cooking to various temperatures for 1 hour. Vertical bars show ±SE of means. Redrawn from Lewis et al. (1991). Conditioning clearly reduces IMCT strength in raw and lightly cooked meat, but conditioning effects are eliminated at normal cooking temperatures (60°C and above).

Cooking

The process of cooking usually toughens meat, unless very long cooking times are used to solubilise connective tissue (Davey et al. (1976). As measured by the MIRINZ tenderometer, the relation between toughness and cooking temperature shows a fast rise between 42°C and 55°C, with a second rapid increase in toughness at temperatures above 65°C (Davey & Gilbert, 1974). As measured by Warner_Bratzler shear force values, there is a decrease in toughness between 50°C and 60°C, with a subsequent rising phase at higher temperatures (Bouton et al., 1972a; Harris and Shorthose, 1988). This relationship has often been explained by relating these two phases to the known thermal denaturation behaviour of myofibrillar and connective tissue proteins (Davey & Winger, 1979, Martens et al., 1982). On this basis, the first increase in toughness between 40 and 60°C is explained by myofibrillar

protein denaturation and toughening above 65°C by collagen shrinkage. However, this simple explanation does not fit with careful examination of mechanical measurements on whole meat or on IMCT excised from cooked meat. Bouton et al. (1981) show that the PF-IY (peak force minus initial yield) component of Warner-Bratzler shear force values of beef (which they, and subsequently Harris & Shorthose (1988), interpret to measure the IMCT contribution to toughness) is high at low cooking temperatures and decreases above 60°C. Lewis & Purslow (1989) excised perimysial IMCT from beef semitendinosus muscle cooked to various temperatures and measured its tensile strength. Their results substantially agree with the interpretation of Harris & Shorthose; they found an increasing perimysial strength between 20 and 50°C and a decreasing strength above 50°C. Mutungi et al. (1996) measured the strength of single muscle fibres excised from meat at various cooking temperatures and found that fibre strength continually increased up to 90°C. On this basis, it seems more rational to ascribe the rise in meat toughness between 40 and 50°C to the effects of heat on the IMCT component and the rising toughness above 60°C to the myofibrillar component, but more research is still required to clarify this.

The interaction of cooking with sarcomere length

The effect of heat can be thought of as having two effects on the IMCT contribution to meat toughness. Firstly, there are the direct effects of heat on the mechanical properties of IMCT discussed above. Secondly, the shrinkage of IMCT on heating causes shrinkage of meat as a whole, so squeezing water out of the muscle fibres and fascicles. This may cause toughening of the myofibrillar component. It is in this second, indirect mechanism, that there seems to be some effect of sarcomere length on toughness.

The great increases in toughness with shortened sarcomere length are only generated after cooking; shortened raw meat is more tender than normal length or stretched meat (Rhodes & Dransfield, 1974). It is therefore difficult to accept the argument that cold-shortening toughness may arise from a restriction of enzymatic action on myofibrillar structures in short sarcomeres (Dransfield, 1994), because proteolysis, or lack of it, would affect the toughness of the raw meat. Rowe (1974) and Purslow (1994) have put forward possible explanations of how connective tissue reorientation with changing muscle length could give rise to some of the sarcomere length-dependent effects on the tenderness of meat after cooking. Lawrie (1985) notes that the magnitude of the cold shortening effect has been observed by Bendall in unpublished work to depend on the connective tissue content of the muscle. However, the high tensile strength of single muscle fibres in shortened and cooked meat compared to normal length meat (Willems & Purslow, 1996) shows that the generation of toughness on cooking of cold-shortened meat cannot be due to direct effects on the IMCT component alone.

Figure 6 (redrawn from data by Bouton et al., 1976) shows the different shrinkage behaviour of shortened versus stretched meat on cooking. The percent shrinkage in length of the muscle, can be seen from fig. 6 to be generally greater than the % decrease in cross-sectional area of the muscle (length:diameter shrinkage ratio > 1) in stretched meat, whereas the opposite is true in shortened meat.

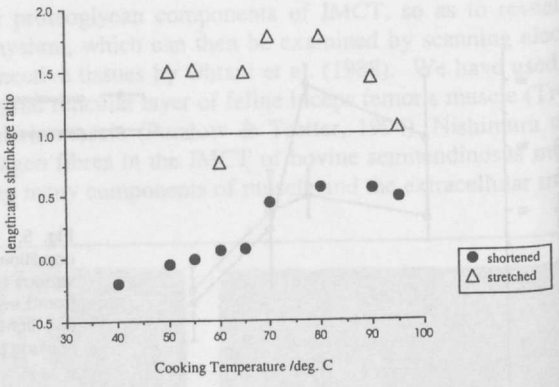

Fig. 6. Ratio of % shrinkage in length (parallel to muscle fibre axis) to % shrinkage in cross-sectional area for bovine *semitendinosus* muscle samples for 1 hour at various temperatures. (Data from Bouton et al., 1976, table III.) Shortened muscle shrinks more in area than length, whereas the reverse is true for stretched muscle.

It is tempting to suppose that these differences in length:diameter shrinkage ratio could be due to the varying geometry of IMCT networks with sarcomere length. Collagen fibres in the perimysium and in the endomysium have been shown to become progressively more transversely (or circumferentially) orientated with respect to the muscle fibre direction as the sarcomere length shortens (Purslow, 1989; Purslow & Trotter, 1994; see also fig.1). Shrinkage of more circumferentially orientated collagen fibres along their length on heating may then be expected, by intuition, to lead greater shrinkage in the cross-sectional area of the meat and to less length shrinkage than in stretched muscle. However, careful examination of this by a similar-triangles argument (see fig. 7) shows this not to be true. For any orientation of collagen fibres other than exactly along the longitudinal or circumferential direction ($0° < \theta < 90°$) the ratio of the shrinkage in the length to the circumferential direction should always be equal to unity. The shrinkage of the collagen fibre along its length may actually vary in magnitude with the angle θ because collagen fibres in IMCT of stretched or shortened meat are less wavy than in meat at normal rest length (Purslow 1989; Purslow & Trotter, 1994), but the *ratio* of length to diameter shrinkage in any muscle fibre or fascicle surrounded by these fibres still should remain at unity.

Variations in length:diameter shrinkage ratio with increasing temperature (as opposed to changing sarcomere length) have been directly observed in endomysial "tubes" produced by removing myofibrillar proteins from a suspension of muscle fibres (Champion et al. 1988). Although the "tubes" were shown by immunofluorescent labelling to be empty of actin and myosin, it is possible that a number of transverse and longitudinal cytoskeletal filaments remain in such a preparation. Differences in the amount of length and

diameter shrinkage at different heating temperatures may be due to varying amounts of thermal denaturation and shrinkage by the proteins in transverse and longitudinally oriented cytoskeletal structures within the muscle cells.

Cell-matrix interactions

The role of IMCT in determining the physical (textural) properties of meat (the "collagenous component") is often thought of as completely separate to the role of structures within the muscle cells (the "myofibrillar component"). Two previous ICoMST lectures (Purslow, 1994; Dransfield 1997) have emphasised that this view is simplistic and that the overall properties of meat as a composite structure will have a notable contribution from mechanical interactions or linkages between these components. This is a standard concept in the science of man-made composite materials (Kelly, 1970; Hull, 1981) and is equally applicable in biological materials.

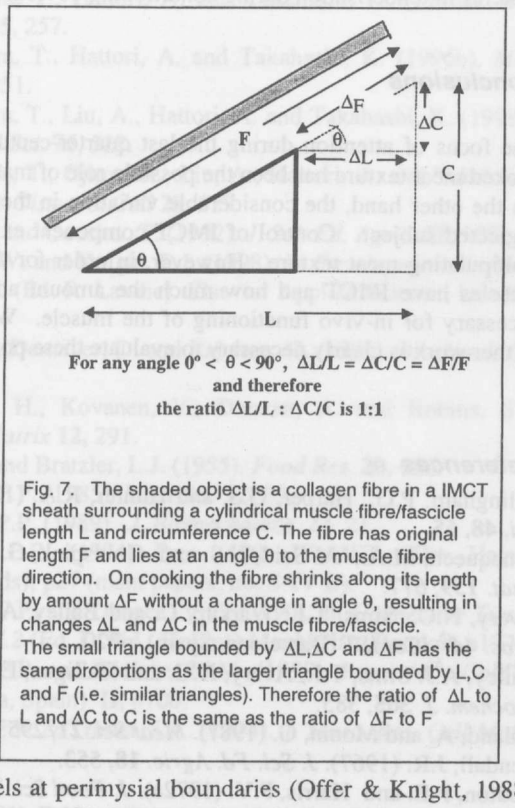

Fig. 7. The shaded object is a collagen fibre in a IMCT sheath surrounding a cylindrical muscle fibre/fascicle length L and circumference C. The fibre has original length F and lies at an angle θ to the muscle fibre direction. On cooking the fibre shrinks along its length by amount ΔF without a change in angle θ, resulting in changes ΔL and ΔC in the muscle fibre/fascicle. The small triangle bounded by ΔL,ΔC and ΔF has the same proportions as the larger triangle bounded by L,C and F (i.e. similar triangles). Therefore the ratio of ΔL to L and ΔC to C is the same as the ratio of ΔF to F

The structures that link the collagen fibrils of the extracellular matrix to the basement membrane and thence to the sarcolemma of the muscle cell (and internally the costamere and cytoskeletal protein structures that mechanically connect the myofibrils to the sarcolemma) are therefore important to our understanding of muscle properties and meat texture. Attention in the last 5-10 years has been drawn to the post-mortem proteolysis of cytoskeletal and costameric proteins (Taylor et al., 1995). It is also certain that these structures play an active role in the functioning of muscle in vivo (Trotter et al., 1995), especially in the common series-fibred muscles (Gaunt & Gans, 1992) which depend on connections between adjacent muscle cells via the endomysial matrix in order to transmit contractile force (Trotter 1991, Trotter & Purslow, 1992; Purslow & Trotter, 1994; Trotter et al., 1995), as discussed above.

Cytoskeletal and costameric linkages have a role in water holding of meat. Transverse linkages are necessary for the pH-induced shrinkage of the myofilament lattice within myofibrils post-mortem to be translated into the lateral shrinkage of whole muscle fibres and fascicles, leaving large drip channels at perimysial boundaries (Offer & Knight, 1988, section 4.1). Proteolytic cleavage of these links seems to lead to separation of the myofibrillar mass from the internal surface of the sarcolemma (Offer & Cousins, 1992), so removing the forces shrinking the fibre diameter. Reduction of desmin and vinculin proteolysis by zinc treatment of meat has been shown to increase water loss (Purslow & Mielche, 1998). Excessive shrinkage of fibres and fascicles to leave large drip channels is seen in PSE meat (evidence reviewed by Offer & Knight, 1988, section 4.6). There is some evidence to suggest that development of large drip channels and excessive water loss from RSE meat may be associated with reduced desmin proteolysis (Morrison et al., 1998).

It also seems clear from direct mechanical observations that the cell-matrix linkages do play a part in the mechanical properties of post-mortem muscle. Mechanical and structural measurements on single fibres from porcine longissimus and iliocostalis muscles (Mutungi et al., 1995, 1996; Willems & Purslow, 1997) show that in both unconditioned and conditioned raw meat the deformability of the myofibrils increases in regions of a fibre where the endomysium is broken on stretching. This implies that cytoskeletal linkages between endomysium and myofibrils normally allow load sharing by shear, so reducing the deformability of the myofibrils. This effect is reduced in magnitude, but not eliminated, after cooking (Mutungi et al., 1996). The different extensibility of small groups of raw muscle fibres compared to single muscle fibres observed by Willems & Purslow (1997) can only be explained by load sharing between adjacent fibres, which requires good (shear) linkages between the cells via the extracellular matrix. The effects of these cell-matrix linkages are not reduced by conditioning, but are largely diminished by cooking to 80°C. The regular fragmentation of fibres in conditioned meat observed by Dransfield et al. (1986) is easily explained by the standard composite materials science concept (Kelly, 1960; Hull, 1981) of tensile failure of (weakened) muscle fibres in a matrix - but this explanation again requires that connections between fibres and matrix are strong in shear.

The rapid proteolysis of cytoskeletal proteins during post-mortem conditioning and the separation of myofibril-sarcolemmal connections (Taylor et al., 1995) suggested that the degradation of intermediate filament and costameric proteins in the cytoskeleton may have some role in the changing mechanical properties of meat during conditioning. (Again, we must be cautious that comparative weakening of structures that develop in raw meat during conditioning may not necessarily be evident after cooking.) However, two pieces of recent evidence has begun to indicate against this. In porcine longissimus muscles (but perhaps not in more oxidative muscles) there is a fibre-type specific loss of immunolabelling for desmin, suggesting that this protein is preferentially degraded in type IIb fibres, but retained in the more oxidative type I and IIa fibres during conditioning (Morrison et al., 1998). In previous work relating the proportion of muscle fibre types to toughness (Henckel et al., 1997; Maltin et al., 1997) there is a tendency for muscles high in oxidative fibres to be more tender. A changing proportion of fibre types between muscles may result in confounding influences (such as differences in ultimate pH in the muscle as a whole). However, the evidence that type I & IIa - rich muscles are often tender seems to argue against a role for desmin degradation in the tenderisation process if desmin degradation is less apparent in at least some muscles within these type I and IIa fibres. The second piece of evidence is a study by Taylor &

Koohmaraie (1998) on the longissimus muscle of normal and callipyge lambs. Callipyge meat is far tougher than normal meat even after 14 days conditioning. Taylor & Koohmaraie argue that, because detachment of myofibril-sarcolemmal links occurred in both normal and callipyge muscle (albeit more slowly in the callipyge samples), then sarcolemmal detachment must be unrelated to the development of tenderness. These two contra-indications against the direct involvement of intermediate filament and costameric proteins in tenderisation during conditioning obviously stimulate further investigation of this question.

Conclusions

One focus of attention during the last quarter-century in research aimed at understanding the variations in IMCT contribution to cooked meat texture has been the possible role of mature crosslinks. The importance of these now seems more debatable than before. On the other hand, the considerable variation in the expression of collagens and other IMCT components in muscle seems to be a neglected subject. Control of IMCT component expression during muscle development and growth offers considerable scope for manipulating meat texture. However, in order for this concept to be practical, we must first understand the rules that govern why muscles have IMCT and how much the amount and architecture of this is potentially reducible, as opposed to being absolutely necessary for in-vivo functioning of the muscle. We are only just beginning to understand these in-vivo functions of IMCT, and further work is clearly necessary to evaluate these possibilities.

References

Allingham, P.G., Harper, G.S. and Hunter, R.A. (1997) *Meat Sci.* **48**, 65.

Alnaqueeb, M.A., Al Said, N.S. and Goldspink, G. (1984). *J. Anat.* **139**, 677.

Avery, N.C., Sims, T.J., Warkup, C. and Bailey, A.J. (1998). *Proc. 44th ICoMST* (Barcelona, Spain), p720.

Bailey, A.J. Sims, T.J., Avery, N.C. and Halligan, E.P. (1995). *Biochem. J.* **305**, 385.

Baland, A. and Monin, G. (1987). *Meat Sci.* **21**, 295.

Bendall, J.R. (1967). *J. Sci. Fd. Agric.* **18**, 553.

Bouton, P.E. and Harris, P.V. (1972a). *J. Food Sci.* **37**, 140.

Champion, A.E., Purslow, P.P. and Duance, V.C. (1988). *Meat Sci.* **24**, 261.

Davey, C.L. and Gilbert, K.V. (1974). *J. Sci. Fd. Agric.* **25**, 931.

Davey, C.L., Niederer, A.F. & Graafhuis, A.E. (1976). *J. Sci. Food Agric.* **27**, 251.

Davey, C.L. and Winger, R.J. (1979). In *Fibrous Proteins: Scientific, industrial & medical aspects.* (Eds. D.A.D. Parry and L.K. Creamer) Academic Press, London, p 97.

De Smet, S., Claeys, E., Buysse, G., Lenaerts, C. and Demeyer, D. (1998). *Proc 44th ICoMST* (Barcelona, Spain), p288.

Dransfield, E. (1994). *Meat Sci.* **36**, 105.

Dransfield, E. (1977). *J. Sci. Food Agric.* **28**, 833.

Dransfield, E. (1997). Proc 43rd ICoMST (Auckland, NZ) p52.

Dransfield, E., Lockyer, D.K. and Prabhakaran, P. (1986). *Meat Sci.* **16**, 127.

Eggen, K.H., Ekholdt, W.E., Host, V. and Kolset, S.O. (1998). *Basic and Applied Myology 8*, 159.

Ertbjerg, P., Larsen, L.M. and Møller, A.J. (1995). *Proc. 41st ICoMST* (San Antonio, USA), p670.

Eyre, D.R. (1987). *In Advances in Meat Research vol. 4: Collagen as a food.* (Eds. A.M. Pearson, T.R. Dutson, and A.J. Bailey) Van Nostrand Reinhold, New York, p 69

Fisher, A., Seymour, J., Light, N., Dransfield, E., Jolley, P. Avery, N, Champion, A and Purslow, P. (1988) Unpublished experiments. (MRI,Langford, UK).

Goll, D.E., Bray, R.W. and Hoekstra, W.G. (1963). *J. Food Sci.* **28**, 503.

Gowitzke, B.A. and Milner, M. (1980). *Scientific Bases of Human Movement.* Williams & Watkins, Baltimore. Ch.6: The Skeletal Muscle System.

Bouton, P.E. and Harris, P.V. (1972b). *J. Food Sci.* **37**, 539.

Bouton, P.E., Harris, P.V. and Shorthose, W.R. (1976). *J. Texture Studies* **7**, 179.

Bouton, P.E., Harris, P.V and Ratcliff, D. (1981). *J. Fd. Sci.* **46**, 1082.

Bouton, P.E., Harris, P.V., Shorthose, W.R., Ellis, R.W. and Phillips, D. (1982). *Meat Sci.* **6**, 309.

Brady, D.E. (1932). *Proc. Am. Soc. Anim. Prod.* **30**, 246.

Carroll, R.J., Rorer, F.P., Jones, S.B. and Cavanaugh, J.R. (1978). *J. Fd. Sci.* **43**,1181.

Guant, A.S. and Gans, C. (1992). *Experientia* **48**, 864

Hammond,J. (1932). *Growth and development of mutton qualities in the sheep.* Oliver & Boyd, London.

Harris, P.V. and Shorthose, W.R. (1988). In *Developments in Meat Science - 4.* (Ed. R Lawrie) Elsevier Appl. Sci., London, p 245.

Henckel, P., Oksbjerg, N, Erlandsen, E., Barton-Gade, P and Bjerholm, C. (1997). *Meat Sci.* **47**, 311.

Horgan, D.J., Jones, P.N., King, N.L., Kurth, L.B., and Kuypers, R. (1991). *Meat Sci.* **29**, 251.

Hull, D. (1981). *An introduction to composite materials.* CUP, Cambridge, UK.

Jackson, D.S. and Cleary, E.G. (1967). *Methods of Biochem. Anal.* **15**, 25.

Kielty, C.M., Hopkinson, I. and Grant, M.E. (1993). In *Connective tissue and its heritable disorders.* (Eds. P.M. Royce and B. Steinmann) Wiley-Liss, New York, p 103.

Kelly, A. (1970). *Proc. Roy Soc. Lond. A.* **319**, 95.

Kovanen, V., Suominen, H. and Peltonen, L. (1987). *Cell Tiss. Res.* **248**, 247.

Kuypers, R., Tyler, M., Kurth, L.B. and Hrgan, D.J. (1994). *Meat Sci.* **37**, 67.

Lawrie, R.A. (1985). Meat Science (4th Edn.) Pergamon Press, Oxford.

Lawson, M.A. and Purslow, P.P. (1999). *Proc Levneds-middelkongres* (Copenhagen, 1999). Ed. M Svarre. Levneds-middelcentret, Lyngby, Denmark, p 95. ISBN 87-88584-54-2

Lehmann, K.B. (1907). *Arch. Hyg.* **63**, 134.

Lewis, G.J. and Purslow, P.P. (1989). *Meat Sci.* **26**, 255.

Lewis, G.J. and Purslow, P.P. (1990). *Meat Sci.* **28**, 183.

Lewis, G.J and Purslow, P.P. (1991) *J. Muscle Foods* **2**, 177.

Lewis, G.J., Purslow, P.P. and Rice, A.E. (1991). *Meat Sci.* **30**, 1.
Light, N.D. and Champion, A.E (1984). *Biochem, J.* **219**, 1017.
Light, N.D., Champion, A.E., Voyle, C. and Bailey, A.J. (1985). *Meat Sci.***13**, 137.
Listrat, A., Picard, B. and Geay, Y. (1998). *J. Musc. Res. Cell Motil.* **19**, 1.
Liu, A., Nishimura, T. and Takahashi, K. (1994). *Meat Sci.* **38**, 315.
Liu, A., Nishimura, T. and Takahashi, K. (1995). *Meat Sci.* **39**, 135.
Maltin, C.A., Warkup, C.C., Mathhews, K.R., Grant, C.M., Porter, A.D. and Delday, M.I. (1997). *Meat Sci.* **47**, 237.
Marsh, B.B. (1977). *J. Food Sci.* **42**, 295.
Martens, H, Stabursvik, E. and Martens, M (1982). *J. Texture Studies* **13**, 291.
Mayne, R. and Sanderson, R.D. (1985). *Collagen Rel. Res.* **5**, 449.
McCormick, R.J. (1994). *Meat Sci.* **36**, 79.
Mills, E.W., Smith, S.H., Forrest, J.C., Aberle, E.D. and Judge, M.D. (1989). *Meat Sci.* **25**, 133.
Mills, E.W., Smith, S.H. and Judge, M.D. (1989). *Meat Sci.* **26**, 115.
Morrison, E.H., Mielche, M.M. and Purslow, P.P. (1998). *Meat Sci.* **50**, 91.
Mutungi, G., Purslow, P. and Warkup, C. (1995). *Meat Sci.* **40**, 217.
Mutungi, G., Purslow, P. and Warkup, C. (1996). *J.Sci. Fd. Agric.* **72**, 359.
Nakano, T, Li, X., Sunwoo, H.H. and Sim, J.S. (1997). *Can. J. Animal Sci.* **77**, 169.
Ramsbottom, J.M., Strandine, E.J. and Koonz, C.H. (1945). *Food Res.* **10**, 497.
Rhodes, D.N. and Dransfield, E. (1974). *J. Sci. Fd. Agric.* **25**, 1163.
Rowe, R.W.D (1974). *J. Fd. Technol.* **9**, 501.
Swatland, H.J. (1975). *J. Anim. Sci.* **41**,78.
Schäfer, A., Henkel, P. and Purslow, P.P. (1999). *Proc Levnedsmiddelkongres* (Copenhagen, 1999). Ed. M Svarre. Levnedsmiddelcentret, Lyngby, Denmark, p 97. ISBN 87-88584-54-2.
Shimokomaki, M., Elsden, D.F. and Bailey, A.J. (1972). *J. Food Sci.* **37**, 892.
Stanton, C. and Light, N. (1987). Meat Sci. **21**, 249.
Stanton, C. and Light, N. (1988). *Meat Sci.* **23**, 179.
Stanton, C. and Light, N. (1990). *Meat Sci.* **27**, 41.
Taylor, R.G., Geesink, G.H., Thompson, V.F., Koohmaraie, M and Goll, D.E. (1995). *J. Animal Sci.* **73**, 1351.
Taylor, R.G. and Koohmaraie, M. (1998). *J. Animal Sci.* **76**, 2811.
Totland, G.K., Kryvi, H. and Slinde, E. (1988). *Meat Sci.* **23**, 303.
Trotter, J.A. (1991) *J. Morphol.* **207**, 211.
Trotter, J.A. and Purslow, P.P. (1992). *J. Morphol.* **212**, 109.
Trotter, J.A., Richmond, F.J.R. and Purslow, P.P. (1995). *Exercise and Sports Sciences Reviews vol. 23* . (Ed. J.O. Holloszy) Van Nostrand Reinhold, NY, p167.
Utterhaegen, L., Claeys, E., Demeyer, D., Lippens, M., Fiems, L.O., Boucque, C.Y., Vandevoorde, G. and Bastians, A. (1994). *Meat Sci.* **38**, 255.
Willems, M.E.T. and Purslow, P.P. (1996). *J. Texture Studies* **5**, 355.
Willems, M.E.T. and Purslow, P.P. (1997). *Meat Sci.* **46**, 285
Nishimura, T., Hattori, A. and Takahashi, K. (1994). *Acta Anat.* **151**, 250.
Nishimura, T., Hattori, A. and Takahashi, K. (1995). *Meat Sci.* **39**, 12.
Nishimura, T., Hattori, A. and Takahashi, K. (1996a). *Acta Anat..* **155**, 257.
Nishimura, T., Hattori, A. and Takahashi, K. (1996b). *Meat Sci.* **42**, 251.
Nishimura, T., Liu, A., Hattori, A. and Takahashi, K. (1998a). *J. Animal Sci.* **76**, 528.
Nishimura, T., Ojima, A., Liu, A., Hattori, A. and Takahashi, K. (1998). *Tissue & Cell* **28**, 527.
Offer, G. and Cousins, T. (1992). *J. Sci. Fd. Agric.* **58**, 107.
Offer, G.W. and Knight, P.J. (1998). In *Developments in Meat Science 4.* (Ed. R. Lawrie) Elsevier Applied Science, London, p 173.
Ouali, A., Demeyer, D. and Raichon, C. (1992). *Biochemie* **74,** 213.
Palongas, H., Kovanen, V., Duncan, A. and Robins, S.P. (1992). *Matrix* **12,** 291.
Paul , P. and Bratzler, L.J. (1955). *Food Res.* **20**, 626.
Purslow, P.P. (1985). *Meat Sci.* **12**, 39.
Purslow, P.P. (1989). *J. Biomechanics* **22**, 21.
Purslow, P.P. (1994) Proc. 40th ICoMST (The Hague, Netherlands), p27 (main papers, no. S-IV B).
Purslow, P.P. and Duance, V.C. (1990). In *Connective Tissue Matrix vol.2* (Ed. D.W.L. Hukins) MacMillan, London, p127.
Purslow, P.P. and Mielche, M.M. (1998). *Proc. 44th ICoMST* (Barcelona, Spain) **II**, p700.
Purslow, P.P. & Trotter, J.A. (1994). *J. Musc. Res. Cell Motil.* **15**, 299.
Wilson, G.D., Bray, R.W. and Phillips, P.H. (1954). *J. Anim. Sci.* **13**, 826.
Zimmerman, S.D., McCormick, R.J., Vadlamudi, R.K. and Thomas, P.D. (1993). *J. Appl. Physiol.* **75,** 1670.

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