INTRAMUSCULAR CONNECTIVE TISSUE AND ITS ROLE IN MEAT QUALITY

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Abstract

The amount, spatial distribution and composition of the connective tissue within muscle vary with muscle position and the carcase and with animal age. This has long been recognised to influence the tenderness of cooked meat. This paper builds upon some historical perspectives with a review of some recent clarifications of the biological function of intramuscular connective tissue IMCT and of its contribution to meat texture, which is clearly multifactorial. The perimysial component of (IMCT) varies most in amount between muscles, and is also the IMCT structure most involved in defining the mechanical integrity of cooked meat. The distribution of perimysium defines muscle fascicle size (muscle "grain" size), which is also still regarded as an indicator of tenderness. Postmortem conditioning of meat has consistently been shown to reduce the strength of intramuscular connective tissue in the raw state, but equally consistently, this has been shown not to affect the toughness of cooked meat. Cooking increases IMCT strength in the range 20-50°C and decreases its contribution at higher temperatures and longer cooking times. Crosslinking of collagen in older animals is generally considered to result in tougher meat, although definitive links between mature crosslink content and cooked meat toughness have been difficult to prove. In the last quarter-century IMCT has been increasingly viewed as a "background" contributor to meat texture, which is difficult to change. However, the large variation in perimysial content of muscles in one animal represents an incredible range of expression. This appears to be firmly fixed to the functional properties of different muscles. In particular, it is hypothesised that definition of muscle fascicle size and shape by the bounding perimysium is related to the need for sub-sections of the whole muscle to slip past each other in the normal contractile function of the tissue. However, the amounts and composition of IMCT can be manipulated by animal nutrition and exercise, and factors affecting the turnover of IMCT may especially be a future target for manipulation of meat texture.

(**Note!** Figures are on Pages 15–19**)**

Introduction

The morphology, composition and amount of intramuscular connective tissue (IMCT[1]) vary tremendously between muscles, species, breeds and with animal age. We intrinsically acknowledge the large influence of IMCT on meat toughness in age-old preferences in choosing cuts of meat to purchase. The large price differential at retail between fillet steak (loin steak) and shin beef reflect our expectations of eating quality, based principally on their different IMCT content, and the preference for meat from younger, less mature, animals reflects the fact that the IMCT contribution to cooked meat toughness is higher in older animals, due to its increased mechanical and thermal stability. Although only a small component (in terms of mass fraction), the disproportionately large influence of IMCT on meat toughness ha resulted in studies trying to relate its amount, or various aspects of its composition and distribution in muscle tissue to variations in meat texture for more than three-quarters of a century (Lehmann, 1907; Hammond, 1932; Carpenter, Kauffman, Bray, Briskey, Weckel, 1963; Marsh, 1977; Light and Bailey 1989; Brooks and Savell, 2004). Modern production practices for pork, lamb and especially beef clearly aim to minimise the variations in texture due to animal maturity, so that young animals of a narrow age range at slaughter of the same breed show variation in the texture of a meat from a given muscle that is only minimally related to characteristics such as collagen content and solubility. In recent years, work to reduce unwanted meat toughness has largely been focussed on postmortem proteolysis of the cytoskeletal and myofibrillar proteins within muscle fibres, as this process can be influenced by post-mortem handling to a great extent. (This focus has led to the view that, whilst the IMCT contribution to meat texture is certainly important, it is also rather immutable and forms a "background toughness" that we can do little about in practical terms (Ouali, Demeyer, Raichon, 1992; Sentandreu, Coulis, Ouali, 2002). However, the amount, composition and distribution of IMCT is possibly the
most variable phenotypic difference between muscles within an animal, and represents a tremendous variation in protein expression and turnover. Given the large influence of IMCT on meat texture, even small manipulations of its expression and turnover may have considerable potential for reducing unwanted variations in meat tenderness.

Structure and composition of intramuscular connective tissue

The basic structure and composition of IMCT has been comprehensively reviewed by several previous authors (Borg & Caulfield, 1980; Mayne & Sanderson, 1985; Sanes, 1986; Bailey & Light, 1989; Purslow & Duance, 1990; McCormick, 1994; Greaser, 1997; Purslow, 2002; Kjær, 2004) and only a brief description will be given here as background. IMCT is principally comprised of fibres of the proteins collagen and elastin, surrounded by a proteoglycan (PG) matrix. The total collagen content in beef muscles can vary from 1-15% of dry weight; elastin is a smaller component varying from 0.6% to 3.7% (Bendall, 1967). Collagen types I, III, IV, V, VI, XII and XIV have been identified in IMCT (Listrat et al., 1999,2000). Types I and III are the major fibre-forming types in endomysium, perimysium and epimysium, and type IV collagen is the principal non-fibre-forming component of the basement membrane linking the fibrous (reticular) layer of the endomysium to the muscle cell membrane (sarcolemma). In mammalian species type V is a minor component of IMCT, although it is much more abundant in the major IMCT structure of fish muscle, the myocommata (Sato et al., 1998). As reviewed in detail elsewhere (Purslow, 2002), the relative concentration of different types of collagen varies through embryonic development in bovine (Listrat, Picard, Geay, 1999; Listrat et al., 2000) and chicken (Lawson & Purslow, 2001) muscle. There have been a number of PG components identified in IMCT, principally decorin (Eggen, Malmstrom, Kolset, 1994; Nakano, Li, Sunwoo, Sim, 1977). Heparan suphate-containing PGs are associated with the basement membrane and higher concentrations of chrondroitin sulphate and dermatan sulphate are found in the perimysium (Carrino and Caplian, 1984; Andersen, Klier, Tanguay, 1984; Nishimura, Hattori, Takahashi, 1996a). Vellman and

[1] The abbreviation IMCT has been used in the past, e.g. by Mohr and Bendall (1969) and others subsequently. In more modern biological parlance connective tissues are referred to as extracellular matrix (ECM). Although "ECM within muscle" has been used in some papers, the historical and shorter "IMCT" will be used here. Needless to say, these terms are synonymous.

coworkers (Vellman, Liu, Eggen, Nestor, 1999) reported the high levels of chondroitin sulphate-containing PGs in early developmental stages of turkey pectoralis muscles to be replaced by heparan sulphate-containing PG's later in embryonic development.

There are three main structural components of IMCT. (1) The *endomysium* is the thin connective tissue layer separating individual muscle fibres. The vast majority of its thickness is made up of a near-random feltwork of fine, wavy collagen fibres, as shown in Fig. 1. This collagen feltwork can easily reorientate with changing muscle length (Purslow & Trotter, 1994; Trotter, Richmond, Purslow, 1995). (2) The *perimysium* is the connective tissue layer that separates each muscle into muscle fibre bundles, or fascicles. There are large (primary) fascicles and smaller (secondary) fascicles, and therefore primary and secondary perimysial layers separating them. Collagen fibres in the perimysium are arranged in a crossed-ply arrangement of two sets of wavy collagen fibres (Rowe, 1981), with the fibres in each ply parallel to each other but at an angle to the muscle fibre axis (typically $\pm$54° in resting muscle, but this angle changes with changing muscle length; Purslow, 1989). Again, reorientation of this collagen network allows the perimysium to easily follow elongation or shortening of the muscle fascicles. (3). The *epimysium* is the connective tissue sheath delineating and separating individual muscles. In many muscles (e.g. bovine sternomandibularis; Fig. 2) collagen fibres in the epimysium take on the same crossed-2-ply arrangement in the perimysium. In pennate muscles, however (e.g. gastrocnemius), or in muscles where the epimysium clearly participates in transferring load to adjacent structures (e.g. bovine semitendinosus), the collagen fibres are more close-packed and longitudinally arranged, like a tendon.

The role of perimysial IMCT in meat texture

Whilst cooked meat toughness and the total collagen content of a muscle often rank similarly, precise correlations between textural measures such as Warner-Bratzler shear force (WBSF) and collagen content are poor. Dransfield (1977) showed some of the best correlations between Volodkevitch bite force on cooked meat and collagen content (measured by hydroxyproline concentration), but even so the correlation explained less than half the total variation in texture. Previous structural and mechanical studies of cooked beef (Carroll, Rorer, Jones, Cavanaugh, 1978; Purslow, 1985) have clearly demonstrated that separation of the perimysium from the endomysium of fibres on the surface of the fascicle is relatively easy in cooked meat, but that the individual perimysial layers are strong and so dominate the fracture behaviour of meat and therefore is the major contributor to toughness. The high strength of the perimysium relative to the weaker endomysial-perimysial interface in cooked beef was quantified by Lewis and Purslow (1990). Fig. 3 shows a steak of bovine semitendinosus muscle cooked to 80°C being pulled apart. In the early stages of separation (Fig. 3 top), fascicles separate from each other, by endomysial-perimysial separation, leaving intact perimysium clearly visible in the gaps between them. At later stages of rupture (Fig. 3 bottom), the perimysial layers have to be broken down to separate the whole sample into pieces. This process clearly mimics the breakdown of cooked meat in the mouth during mastication, which also involves separation of intact fascicles in the first few chew cycles (Lillford, 1991, 2000).

The collagen content in the perimysium varies much more between muscles than endomysial content (Light, Champion, Voyle, Bailey, 1985; Purslow 1999; Nakamura, Iwamoto, Ono, Shiba, Nishimura, Tabata, 2003). In a study of six bovine muscles, 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. A more extensive study of fourteen beef muscles reported by Purslow (1999) showed perimysial collagen as % of muscle dry weight ranging from 0.45% to 4.76%, whereas endomysial collagen % varied from 0.47% to 1.20%. Thus endomysial content varied by a factor of 2.5, whereas perimysial content varied by more than an order of magnitude. Although techniques of separating endomysial IMCT from perimysial IMCT are far from perfect, these studies indicate that it is the amount of perimysium that varies most between functionally different muscles. Brooks and Savell (2004) report that perimysial thickness in bovine semitendinosus muscle is on average 2.4 times thicker than in psoas major from the same animals. There are indications from the work of Swatland and coworkers. (Swatland, Gullett, Hore, Buttenham, 1985), Carpenter et al. (1963) and Cooper and coworkers (Cooper, Breidenstein, Cassens, Evans, Bray, 1968) that thicker perimysium is associated with reduced tenderness, although in some of these studies animal age is a

confounding variable. Unpublished results from the study on 14 beef muscles reported by Purslow (1999) showed the maximum thickness of primary perimysium to be 2.53 times greater than the thinnest, with variations in secondary perimysial thickness being slightly smaller (1.9). However, correlations between shear force values of muscles cooked to 80°C and perimysial thickness were low. Light et al. (1985) were able to show similarities in ranking between toughness and perimysial content of their six muscles, but correlations between WBSF and perimysial content or perimysial thickness (Brooks and Savell, 2004) remain low. Light et al (1985) demonstrated that variations in ratio of type I:III collagen, the diameter of collagen fibrils and the amount of divalent (immature) crosslinks are also seen in the perimysium of the six muscles they studied, suggesting that there are multifactorial contributions to the IMCT component of meat texture.

Muscle "grain" and meat texture

The division of muscles into fascicles by the perimysium can easily be discerned by eye, or by the ball of the thumb moving over a cut surface, as the "grain" of a muscle. The USDA grading scheme for beef has for many decades contained a reference to grain size; fine grain is a requirement for the top grades. (Large grain size is primarily mentioned in the current scheme as an indication of meat from older animals.) Grain size ("graininess") is included in the sensory parameters of note in a review of the history of sensory evaluation of meat by Szczesniak (1986). Much of the work cited in the literature on muscle grain and meat texture is 40-70 years old, and so does not appear in modern databases. For the sake of completeness, it is reviewed in some detail here. In these early works the term "texture" was specifically used to describe the visual perception of graininess of muscle cut across the fibre direction, i.e. how smooth or divided the cut surface appeared, and the size/nature of the divisions. It is assumed in the majority of the literature that grain size is synonymous with muscle fibre bundle (fascicle) diameter. Figure 4 compares fascicular architecture in cross-sections of three muscles from the same (bovine) animal: pectoralis profundus, sternocephalicus and rhomboideus cervicus. Large differences in primary fascicle size, the thickness of the perimysium, and the degree of adipose tissue associated with the perimysium can be seen in theses sections.

Hammond (1932) reviews the experimental work of his group from 1913 to 1922, together with contemporary work of others in this area in his large monograph on sheep meat quality. He notes a general belief at that time that cooked meat from fine-grained muscle is more palatable than from coarse-grained muscle, which is often described as "stringy". Thigh muscles with the highest rates of post-natal growth were felt to have the largest grain (e.g. semimembranosus, vastus externus, biceps femoris).
Hammond (1932) examined the effect of sex, age and breed differences on grain size. His comparison between 23 different leg and thigh muscles (Table CXXV, p 512) shows consistent patterns of variations in grain size between muscles between individual animals. Due to the ranking system used, the absolute variation in grain size from the biggest to the smallest cannot be quantified. However, from the examples of histological sections presented, fascicle size can easily be seen to vary by a factor of two or more. Hammond also compared the grain size of 7 muscles in rams at birth, 5 months and "adult" age, and 8 muscles in wethers at 5, 11 and 22 months. He concludes that muscle fibre bundle size increases with animal age. Rams, on average, have coarser grain sizes in the same muscles at comparable ages than wethers, and these in turn are larger than in ewes. Hammond also investigated differences in grain size between sheep breeds by ranking each of 7 muscles from 6 animals from 5 breeds. On average across the 7 muscles, grain size ranked with breed as follows:

Suffolk > Merino > Shetland > Soay > Hampshire.

Although the individual animals were all at different ages (Shetland 5yrs; Soay 2yrs; Merino 5yrs; Suffolk 4 yrs; Hampshire 11 months), the general inference drawn by Hammond is that muscle grain size is related to animal size.

In quantitative terms, this work by Hammond (1932) contains only a small amount of non-parametric data on the effect of breed on grain size. There is no correlation attempted between grain size and tenderness, but he infers that coarse grain size leads to less palatable, "stringy" meat. Hammond's conclusions (that coarse

texture is found in male animals and large-framed animals, and that this frame size effect also holds between breeds and species) are reiterated by Lawrie in his standard text on meat science (Lawrie, 1985).

Brady (1937) reports results from experiment using 6 yearling Hereford x Shorthorn steers and 7 mature Holstein cows. Measurements of the number of fibres per fascicle and fibre diameter were made on histological sections of 4 muscles (Triceps brachii, Longissimus, Adductor and Semitendinosus) in the raw and cooked state. Warner-Bratzler shear force measurements and taste panel scores for texture and tenderness were also taken. To confuse the issue, non-intuitive definitions of texture were used in the taste panel; large muscle fibre bundle size (coarse graining) was described and rated as the most desirable ("finer") texture. Brady concludes that these correlations are significant and that the data warrant the interpretation that the larger the bundle (which Brady confusingly defines as the finer the texture), the more tender the meat. Note that this conclusion is apparently in contrast to Hammond, who asserted that small grain size is associated with tenderness.

There is therefore an important difference in the use of the term "fine" to describe texture or grain size between Brady (1937) and Hammond (1932). Hammond uses "fine" to describe small grain (small bundle or fascicle size). Brady uses the term "fine" to indicate most desirable texture; in this case the "finest " texture is actually the largest grain size, i.e. the largest bundle diameter. So, although it is true that both Hammond and Brady relate "fine texture" with tenderness, they mean completely opposite things by the word "fine".
Ramsbottom, Standine and Koonz (1945) studied variations in tenderness between the muscles within the carcasses of 3 heifers of unspecified breed. They define texture as being determined by the size of the muscle fibre bundles (fascicles), but also the amount or thickness of the perimysial connective tissue separating them. They associate these rankings in grain size and definition with the same order of ranking in muscle toughness, i.e. the larger the grain size, the tougher the meat.

Cooper et al. (1968) found that the muscle bundle size increased greatly with maturity in bovine longissimus muscle. In their study, bundle size was significantly associated with visual scores of texture ($r = 0.37$, $P<0.01$), positively related to shear force ($r = 0.39$, $P<0.01$) and negatively correlated to taste panel tenderness ($r = -0.41$, $P<0.01$). They also found a significant negative correlation between the overall opinion of acceptability and bundle size ($r = -0.30$, $P<0.05$). .

Carpenter et al. (1963) studied muscle fibre bundle size and the coarseness (thickness) of perimysial connective tissue strands in 78 loins from pigs aged 4-42 months. Bundle size and CT coarseness were rated subjectively on a 5-point scale, and related to tenderometer and taste panel scores of tenderness. They give correlations of -0.39 ($P<0.01$) between muscle bundle size and tenderness, and -0.38 ($P<0.01$) between connective tissue thickness and tenderness. The trend of these relationships agrees with that found by Cooper et al. (1968) and inferred by Ramsbottom et al. (1945) and Hammond (1932), and so disagrees with Brady (1937).

Lepetit and Culioli (1994) review much of the French work on connective tissue morphology in meat. From work by Dumont (1985), they point out that if the force required to shear raw muscle is divided by the overall collagen content of the muscle, then 30-40% of the variance in shear force value remains, which they feel may be related to IMCT morphology. They cite Dumont (1983) as showing a high correlation between raw muscle shear force and the linear density of the perimysial network (i.e. the number of tracts encountered per unit length of transect). However, as discussed below, shear force measurements on raw meat are poor predictors of cooked meat texture, and so these results should be viewed with caution.

Taken together, these studies point to some consistent pattern that tenderness is partly correlated with small diameters of muscle fascicles, but as is so common in meat science, this single variable is of extremely limited value in predicting the toughness of cooked meat, due to the highly multifactorial nature of texture.

Variations in the Amount of Intramuscular Connective Tissue Relate to Muscle Function

From the forgoing two sections it is clear that IMCT varies in amount and, especially at the perimysial level, in spatial distribution between muscles. It is generally accepted that this must reflect differences in

physiological (mainly biomechanical) function between muscles, but until recently it has not been clear what the functional role of IMCT is. However, in the last decade there has been a great advance of our understanding of role of IMCT in coordinating force transmission in muscle. Evidence for load sharing via the endomysium between muscle fibres within a fascicle and via the perimysium between fascicles within a muscle is comprehensively reviewed by Trotter et al. (1985), Huijing (1999a,b) and Monti, Roy, Hodgson & Edgerton. (1999). Endomysial connective tissue facilitates load sharing within a fascicle by linking adjacent muscle cells by shear (Trotter & Purslow, 1992; Purslow & Trotter, 1994; Trotter et al, 1995). It has been shown that the easily-deformable tensile properties of the endomysial network allow it to follow necessary shape changes in contracting muscle fibres without restraining this process, but the shear properties though the thickness of the endomysium are suitable for efficient load transfer over a wide range of physiological muscle fibre lengths (Purslow, 2002). These endomysial linkages can be thought of as coordinating the deformations within a fascicle, especially in the usual physiological circumstances where not all motor units are contracting.

In terms of the very variable spatial distribution of perimysium, and via the perimysium, (Purslow, 1999) proposed that division of a muscle into fascicles by the perimysium reflects the need to accommodate shape changes as the muscle contracts, and that this is achieved by allowing fascicles to slide past each other. Ultrasonic imaging data of Fukunaga and colleagues on changes in muscle geometry during human locomotory function (e.g. Fukunaga, Ichinose, Ito, Kawakami, Fukashiro, 1997) clearly demonstrates that that these shape changes do occur in living muscle. Purslow (2002) calculated the shear strains that occur between fascicles within various muscles over their physiological range of contraction based on the data from a series of papers by Fukunaga and colleagues and showed them to be substantial, and variable between muscles. Functionally different muscles have very different requirements to accommodate the shear strains that necessarily occur as the muscle contracts and changes shape, and this begins to explain why the amounts and distribution of perimysial connective tissue varies so much between functionally different muscles. Variations in IMCT between functionally different muscles represents a great variation in expression from a given genome, and whilst genomics and proteomics approaches to describing such variation in expression may assist our understanding of this in relation to meat quality (Eggen & Hocquette, 2003), in terms of meat science a prime question is what possibilities exist to manipulate variability in IMCT expression so as to improve meat texture? Although there is clearly some possibilities, as evidenced by differences between breeds and in some hypertropic genetic conditions, these considerations of the physiological function of IMCT imply that the variations in expression of perimysial IMCT between muscles is tightly related to muscle activity, and so may not easily be manipulated without compromising muscle function in the live animal.

Postmortem effects on IMCT

Although little in the way of new concepts has come up in work on both the effects of cooking temperature and time, and on effects of postmortem storage (conditioning or ageing) of meat on IMCT since previous reviews (Purslow, 1999, 2002), it is perhaps worthwhile to highlight some salient points where there still seems to be contrary interpretations of previously established views in the last 5 years.

Cooking influences on IMCT contributions to toughness.

The toughness of meat increases with temperature of cooking. A sharp increase in toughness occurs between 40ºC and 50ºC, followed either a dip between 50º and 60ºC or a plateau in toughness depending on measurement technique, and then a second phase of increasing toughness above 65ºC. Several studies have intuitively correlated these changes with the thermal denaturation of myosin in the range 42-65ºC and collagen above 65ºC (Davey & Gilbert, 1974; Davey & Winger, 1979; Martens, Stabussvik, Martens, 1982). Although this interpretation has been proved incorrect, it still is used by some to infer that collagen and shrinkage above 65ºC is primarily related to toughness development at higher temperatures (e.g. McCormick, 1999, 2001).

The evidence for IMCT actually contributing to the first phase of toughening, at 40-60°C, goes back to Bouton, Harris & Ratcliff (1981), who inferred from their Warner Bratzler PF-IY measurements that the connective tissue contribution to toughness is high at low cooking temperatures and decreases above 60ºC. Lewis and Purslow (1989) showed by direct measurements on perimysium isolated from cooked beef meat that perimysial connective tissue strength increases in meat cooked up to 50ºC and decreases above this temperature. Mutungi, Purslow & Warkup (1996) showed that the strength of porcine muscle fibres continually increases up to 90ºC. Christensen, Purslow & Larsen (2000) confirmed these two previous findings by examining the strength of both perimysium and individual muscle fibres from the same muscle samples cooked to various temperatures. These studies all add confirmation to the interpretation of Bouton et al. (1981); cooking increases the IMCT contribution to toughness in the range 20ºC-50ºC, with myofibrillar contributions being more prominent above 60ºC. Because isolated IMCT forcefully shrinks above 65ºC when rapidly heated (Kopp and Bonnet, 1987), there is still a feeling that high-temperature shrinkage of collagen could cause the shrinkage of meat seen at 65º-80ºC, and that this shrinkage cause volume reduction in the muscle fibres, so increasing their toughness (Lepetit, Grajales, Favier, 2000). Powell and coworkers (Powell, Hunt, Dikeman; 2000; Powell, Dikeman, Hunt, 2000) have provided some interesting new findings in this area by relating the pronase-extractable fraction of collagen to its thermal denaturation above 55ºC. They report strong correlations between this fraction and peak shear force in experiments; salt-soluble collagen is not nearly as well correlated to toughness. Their explanation is that this insoluble but enyzmically extractable fraction is more related to changes in the structural integrity of the fibrous IMCT network on cooking. However, it has been pointed out previously (Purslow, 1999) that the ratio of transverse to longitudinal shrinkage in meat on cooking, and especially how these vary with sarcomere length, is not simply explainable on the basis of collagen network shrinkage. It is possible that other events, such as cytoskeletal protein denaturation, cause the shrinkage to toughen myofibrillar components at higher temperatures. Obuz, Dikeman, Grobbel, Stephens and Loughin (2004) conclude that variations in cooking losses between different sample sizes and different cooking methods alter the WBSF values seen and also complicate the relative contributions of IMCT softening and myofibrillar hardening.

Post-mortem proteolysis has no effect on the integrity of IMCT after cooking

During post mortem storage (conditioning) of meat there is significant proteolysis of both collagenous and PG components of IMCT, which increases the amount of collagen that can be solubilised from meat (Stanton and Light, 1987) and significantly reduced the strength of perimysium in raw meat after conditioning (Lewis, Purslow, Rice, 1991), as shown in Fig 5.

Over the last ten years there has been a large amount of work to reaffirm that conditioning increases the amount of collagen that is easily extracted from muscle, decreases their structural integrity as viewed by SEM, degrades PG components of IMCT and reduces the strength of IMCT networks, as demonstrated for example by shear measurements on raw muscle or on uncooked IMCT structures embedded in acrylamide gels or by Warner-Bratzler measures on raw meat (Nishimura, Hattori, Takahashi, 1994; Nishimura, Hattori, Takahashi, 1995;Nishimura, Hattori, Takahashi, 1996a,b; Nishimura, Liu, Hattori, Takahashi, 1998; Liu, Nishimura, Takahashi, 1994, 1995; Nakano, et al., 1997; Eggen, Ekholdt, Host, Kolset, 1998; Fang, Nishimura, Takahashi, 1999; Palka, 2003). Similarly, Torrescano and coworkers (Torrescano, Sánchez-Escalante, Giménez, Roncalés, Beltrán, 2003) recently found strong correlations between the total collagen content and insoluble collagen content of 14 beef muscles and raw Warner-Bratzler peak shear force values.

We must remember, however that the overwhelming majority of meat is cooked before it is eaten; textural measurements on raw meat miss out the interactions of proteolysis with effects of cooking, and it is this which leads to measurements on raw muscle being a poor predictor of cooked meat texture. Previous work by Bouton and Harris (1972) showed that measures of the connective tissue component of toughness were unaffected by extensive conditioning when followed by cooking. Lewis et al. (1991) demonstrated this clearly by measuring the strength of perimysium in conditioned meat both before and after cooking.; while there is a reduction in strength of IMCT with conditioning in raw meat, these effects are negated after cooking to temperatures of 60ºC and above, where both aged and unaged perimysial IMCT has the same strength. It is work reiterating this result, shown in Fig. 5, to caution against the recent trend to interpret properties of IMCT in raw meat and degradations during conditioning as if these will inevitably translate into

cooked meat toughness. They do not, as has been known from the work of Harris and colleagues for more than 30 years. Palka (2003) recently was compelled to revisit previous conclusions that the texture of raw meat is poorly related to cooked meat toughness.

Animal maturity, crosslinking and turnover of IMCT

Animal maturity is associated with increased thermal and mechanical stability of IMCT (Bailey and Light, 1989). Meat tenderness generally decreases with animal age, and collagen-rich muscles show this effect more than those with a low IMCT content (Shorthose & Harris, 1990). Increasing collagen fibre diameter and especially the development of mature crosslinks from immature divalent forms accompany this. Although an in-depth investigation of the relationships between a large number of types of collagen crosslinks and cooked meat toughness showed no conclusive correlations in pigs of similar maturity (Avery, Sims, Warkup, Bailey, 1996), or in beef animals in the age range 400-800 days (Avery, Sims, Warkup, Bailey, 1998), it is generally accepted that the development of mature crosslinks with age in IMCT cause it to have increased contributions to cooked meat toughness (Bailey& Light, 1989; McCormick, 1994, 1999). The mechanisms of crosslinking on maturation and ageing of collagen are extensively reviewed by Bailey and coworkers (Bailey, Paul, Knott, 1998). The formation of the immature, divalent crosslinks is controlled by post-translational modifications and can be assumed to be positively directed to provide the mechanical stability required for collagen to fulfil its functional roles.

Collagen in muscle is relatively slow to turnover. Rucklidge, Milne, McGaw, Milne & Robins (1992) report a half-life of 45 days, and although Laurent (1987) inferred a faster turnover, there are good reasons to suppose that the long residence time reported by Robins is more realistic. Relatively slow turnover of IMCT components gives time for slow modifications, including the conversion of divalent to trivalent crosslinks between collagen molecules, slowly increasing the thermal and mechanical stability of IMCT with age. This consequence of slow turnover may not be a functional adaptation, but simply an ageing process. Thus, if the turnover of collagen in muscle could be increased, there is a possibility that less mature, less thermally stable IMCT could result in more tender meat.

Woessner (1968) long ago pointed out that for growth to occur there must be degradation of collagenous networks; during animal growth the hypertrophy of a fixed number of muscle fibres s responsible for muscle weight gain and to accommodated bigger muscle fibres and fascicles, endomysial and perimysial networks must necessarily be remodelled, otherwise they will limit growth.

Turnover of connective tissue is principally under the control of matrix metalloproteinases (MMPs) and their specific inhibitors, TIMPs. Fifteen out of sixteen known MMPs and 3 out of 4 TIMPs have been found in either bovine skeletal muscle, or IMCT, fibroblasts and myoblasts isolated from it (Balacerzak, Querengesser, Dixon, Baracos, 2001). Both muscle cells and fibroblasts within muscle are known to secrete MMPs and TIMPs. MMPs are secreted in inactive forms and activated in a cascade involving plasmin, tissue kallikrein and other factors (Parsons, Watson, Brown, Collins, Steele, 1997). Injury of (cardiac) muscle stimulates MMP expression by fibroblasts in response to the cytokines IL-β and TNF-α (Siwik, Chang, Colucci, 2000). Both TNF-α and other growth factors such as bFGF stimulate increased MMP activity in skeletal myoblasts (Allen, Teitelbaum, Kurachi, 2002). Mechanical stimulus (e.g. exercise) has long been known to be a factor controlling muscle growth and turnover (Goldspink, 1999, 2003). Turnover of IMCT is especially evident in repair of injury or hypertrophic response to exercise (Koskinen et al., 2001) and down regulated during immobilization (Ahtikoski, 2003). It is not known whether turnover of IMCT structures is solely mediated by the fibroblasts that sparsely populate the IMCT, or whether MMPs are expressed by muscle cells in response to mechanical signals. Interactions between muscle cells and extracellular matrix structures involved in muscle development and in cell signalling have been reviewed recently (Purslow, 2002). Muscle strain or injury leads to a rapid production (fibrosis) of endomysial and perimysial connective tissue (Stauber, Knack, Miller, Grimmet, 1996). The remodelling of muscle tissue during rapid the hypertrophy involved in compensatory growth following feed restriction necessarily involves remodelling of the IMCT structures which link and coordinate the various level of structure within the tissue. It has previously been recognised that animals slaughtered after a period of rapid growth will have less contribution to toughness from IMCT (Aberle, Reeves, Judge, Hunsley, Perry, 1981; Miller, Tatum,

Cross, Bowling, Clayton, 1983). Allingham, Harper & Hunter (1998) showed that rapid compensatory growth after weight loss can reduce the strength of IMCT in bovine semitendinosus. Sylvestre, Balcerzak, Feidt, Baracos and Bellut (2002) have demonstrated that dietary manipulation of growth rate in lambs does alter the amounts, activation and activity of MMPs.

Conclusion

The expression of connective tissue within muscle is amazingly variable, depending on developmental stage, muscle position/function, animal breed, nutrition, exercise and injury. Much of the work on IMCT in relation to meat texture in the last century has bee devoted to documenting and understanding the variations between muscle and breed and brought on by animal age. Investigations of post-mortem treatment of meat to reduce the IMCT contribution to meat toughness has met with extremely limited success outside the sphere of long, slow cooking to dissolve extracellular matrix components in tough muscles, and has led to the view that the IMCT contribution to texture is a "background" feature that little can be done about. In the post-genomic era it is possible to return attention on how expression of IMCT components may be manipulated, so as to improve tenderness. The amounts and distribution of connective tissue in muscles seem to vary for very good reasons of muscle development, growth, and especially function. This may limit the scope for manipulation of its expression. Manipulation of the state of IMCT maturity may, however, be a possible means of reducing unwanted variability in meat tenderness.

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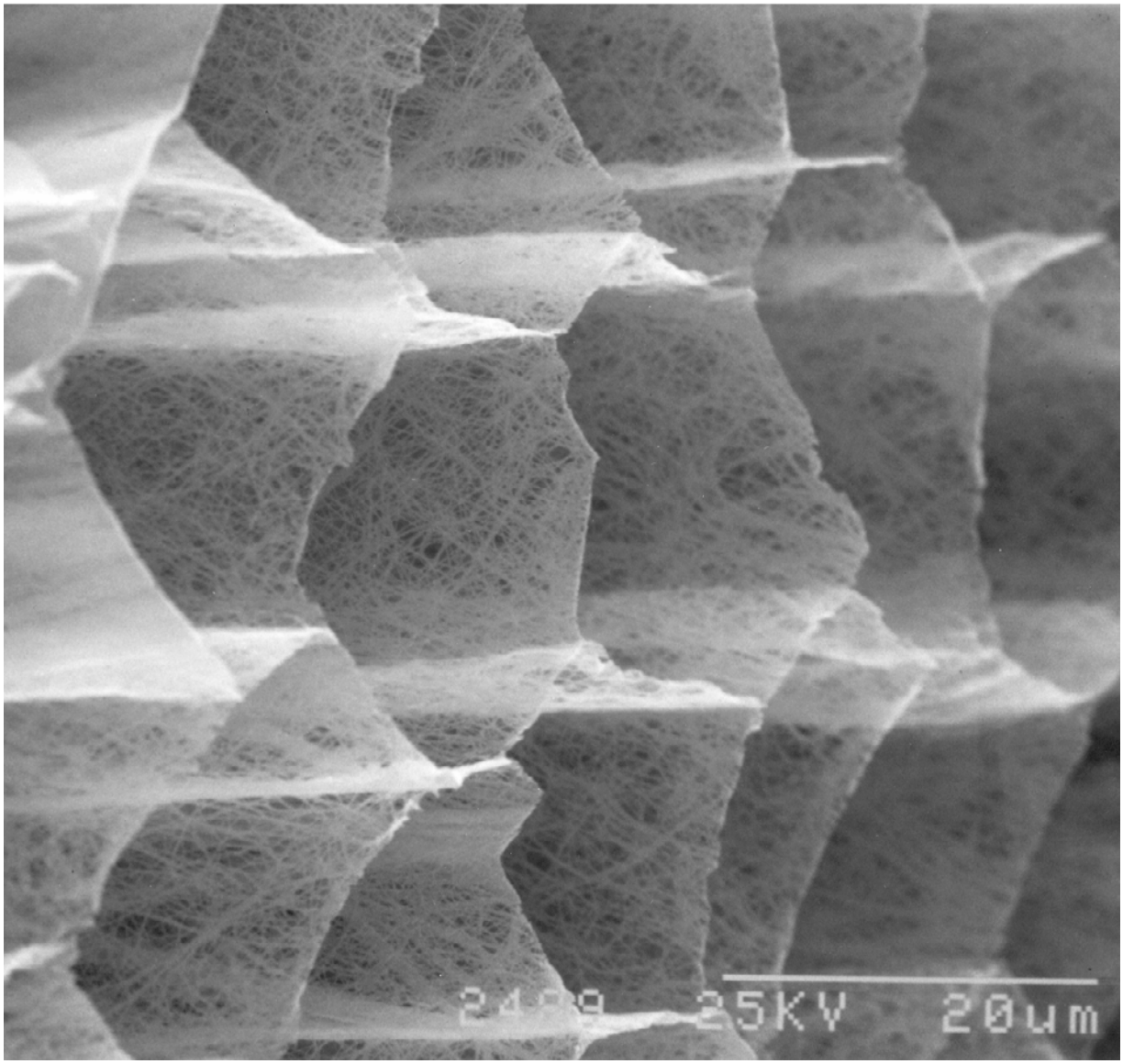

Fig. 1. Scanning electron micrograph of endomysial network in bovine sternomandibularis muscle after extraction of myofibrillar components by NaOH. The feltwork of fine wavy collagen fibres in the endomysial structures separating individual muscle fibres is clearly seen. From Purslow and Trotter (1994).

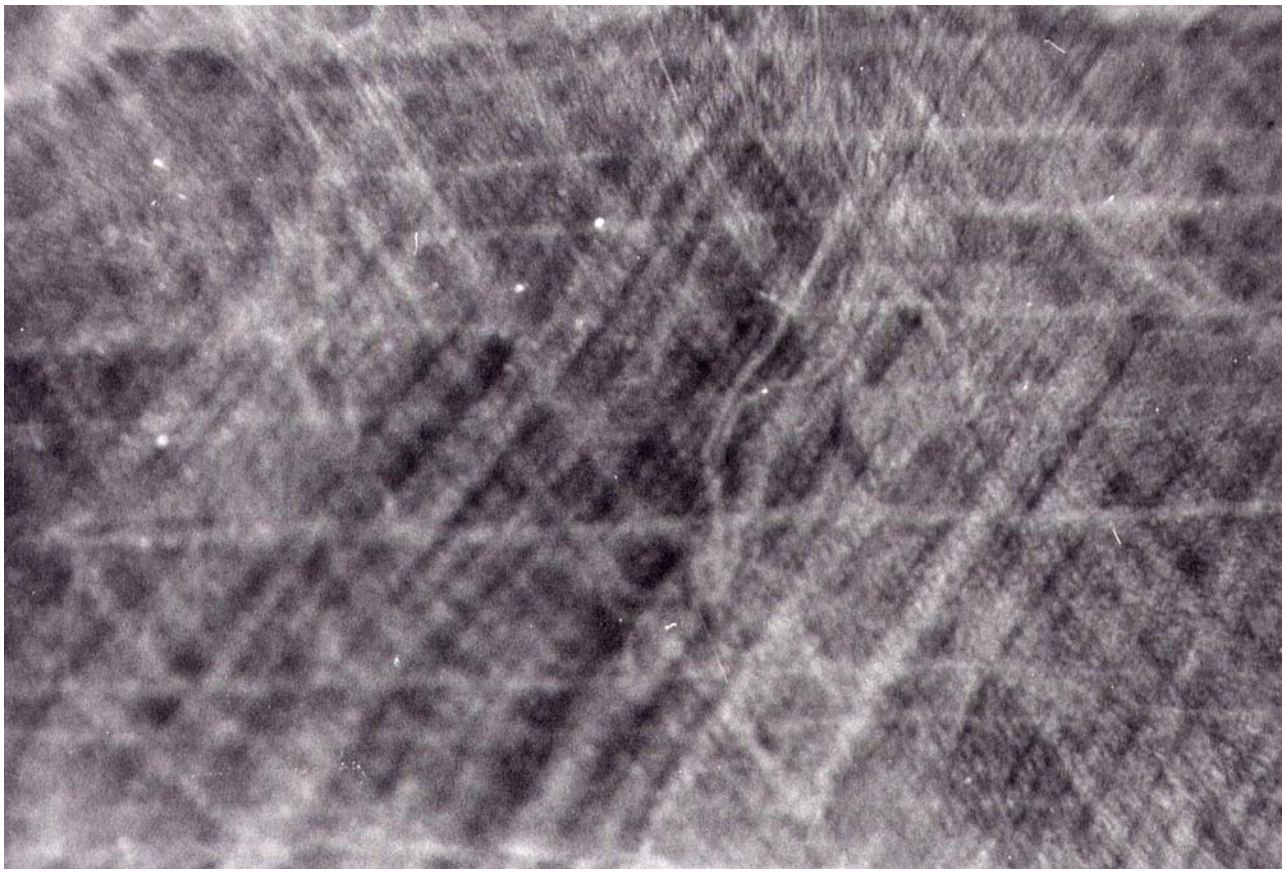

Fig.2. Light micrograph showing the arrangement of collagen fibres in the epimysium of bovine sternomandibularis muscle. Horizontal lines show the edges of individual muscle fibres in the surface of the muscle. The collagen fibres of the overlying epimysium lie in two plies of parallel fibres, at approx. $\pm$ 54° to the muscle fibre direction.

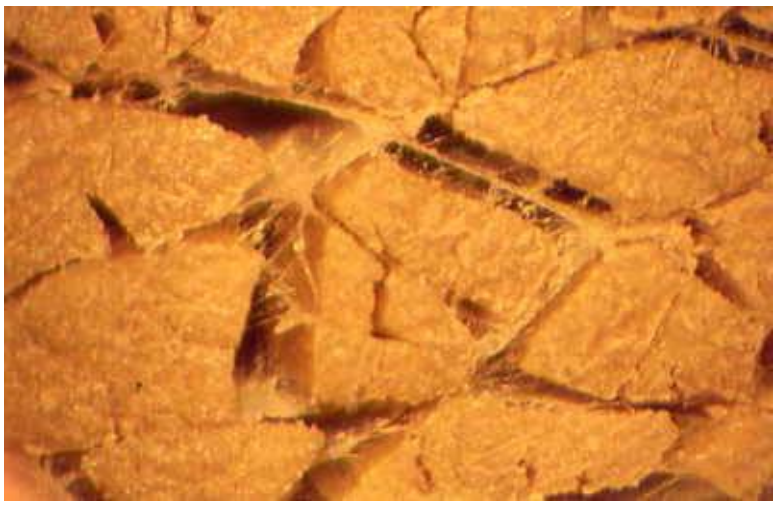

Fig.3. Bovine semitendinosus muscle cooked to 80°C and pulled perpendicular to the muscle fibre direction. For full details of preparation see Purslow (1985). Top: initial stages of separation, showing intact fascicles separating from each other, leaving perimysial sheets in the gaps between them. Bottom: subsequent pulling apart of the sample causes complete rupture after the separated perimysial sheets are broken.

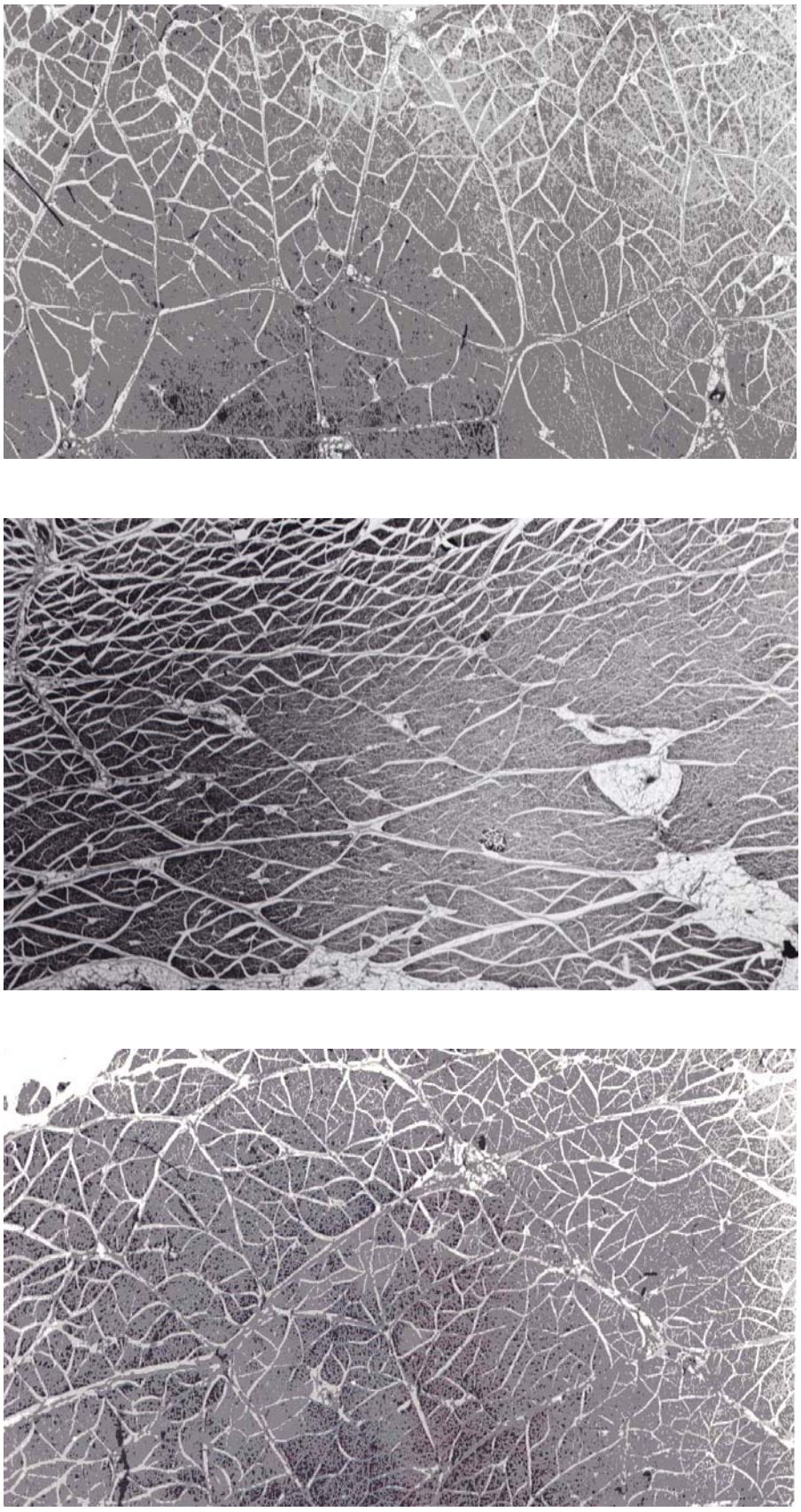

Fig 4. Comparison of the fascicular architecture in cross-sections of three muscles from the same (bovine) animal: pectoralis profundus (top), strenocephalicus (mid) rhomboideus cervicus (bottom). Clear differences in fascicle size, shape, and perimysial thickness can be seen between them.

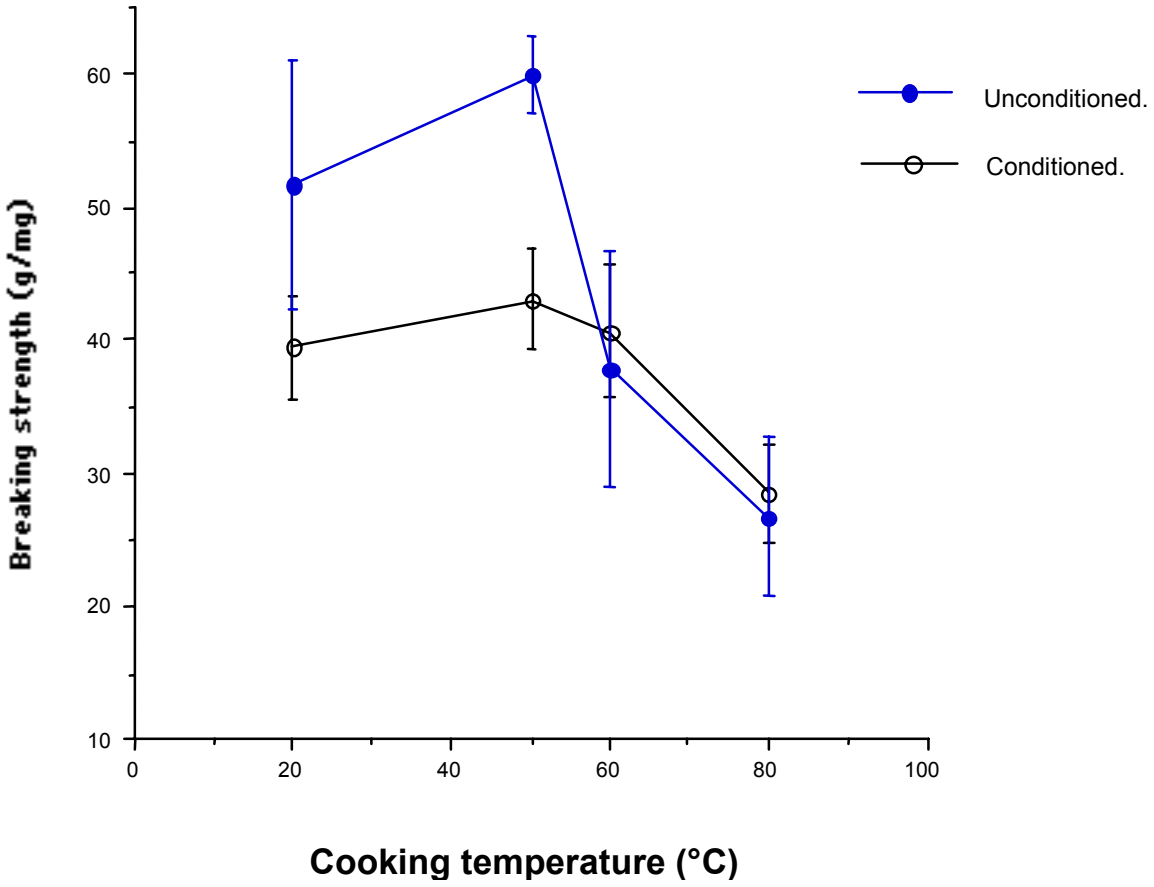

Fig. 5. Strength of perimysium isolated from meat after cooking to the temperatures shown; comparison of perimysium from one day post mortem muscle (unconditioned) or muscle stored for 14 days (conditioned). Vertical bars represent mean ± SE. From Purslow, 1999; redrawn from Lewis et al., 1991.