Physiology and Biochemistry of Collagen

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Introduction

Variations in the amounts and composition of intramuscular connective tissue (IMCT) have long been a strong element in studies on meat texture (Marsh 1977; Bailey and Light, 1989). Although focus in the last twenty years has firmly switched to postmortem proteolysis of myofibrillar and cytoskeletal components (Koohmaraie *et al.*, 1991; Taylor et al, 1995; Sentandreu et al, 2002), the link between IMCT content of a muscle and cooked meat texture is inextricably bound up in retail pricing of various cuts of meat; variations in price between shin beef and fillet steak principally reflect expectations of tenderness, based largely on variations of IMCT content. Likewise, the increase in toughness of meat from older animals in all species used for meat results from increased thermal and mechanical stability of IMCT with age, and is reflected in a preference for meat from less mature animals. Modern production practices clearly aim to minimise variations in texture due to IMCT maturity, and within young animals of a narrow age range and of the same breed, variations in texture of a given muscle after aging (post mortem conditioning) are only minimally related to collagen content and solubility (e.g. Renand *et al.*, 2001). This has led to a view that IMCT provides a "background toughness" (Ouali *et al.*, 1992), which is somewhat fixed. In addition to reviewing major elements of the role of IMCT in cooked meat toughness, my aim is also to point out sources of variation in expression of IMCT within muscle and its turnover, as potential targets for manipulation so as to improve meat texture.

Structure, Composition and Variations of Connective Tissue Within Skeletal Muscle

IMCT is comprised of three main structures. The epimysium is the connective tissue sheath separating individual muscles. With each muscle, fascicles, or bundles of muscle fibers, are separated by the perimysial connective tissue layer. Individual muscle fibers within the fascicles are separated by the endomysium. As reviewed by several authors (Borg & Caulfield, 1980; Mayne & Sanderson, 1985; Purslow & Duance, 1990; McCormick, 1994; Purslow, 2002), the structure and composition of these three distinct IMCT components differ. IMCT is principally comprised of the proteins collagen and elastin within a proteoglycan (PG) matrix. Seven molecular types of collagen have been identified in IMCT: collagens I, III, IV, V, VI, XII and XIV (Listrat *et al.*, 1999, 2000). Types I and III are the major fibrous collagen species in epimysium, perimyium and endomysium, with collagen IV making up the basement membrane linking the fibrous endomysium to the muscle cell membrane or sarcolemma. Variations in the ratio of type I to Type III collagen show conflicting relationships to variations in cooked meat toughness, although Listrat & Hocquette (2004) point out that the reproducibility of techniques to measure this ratio is generally poor.

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

Purslow (2002) has recently reviewed this aspect; only a few salient points will be revisited here.

There are considerable (temporal) variations in the expression of IMCT within muscle during development, and these differ between functionally different muscles (Lawson & Purslow, 2001). The concentration of collagen types I & III in bovine psoas and triceps brachii muscles peak at one-half to two-thirds way through gestation, coincident with the second generation of muscle cell differentiation, at which time adult myosin forms begin to be expressed. Thus a change in the IMCT collagen content coincides with a change in the contractile proteins of the muscle cells. Certainly interactions between muscle cells and IMCT components are key signals in muscle development.

Muscles from different anatomical positions on a carcass show great variation in IMCT content. In beef muscles, the total collagen content can vary from 1.6-15.1% of dry weight, and the elastin content varies from 0.6% to 3.7% (Bendall 1967). Previous structural and mechanical studies of cooked beef (Carrol *et al.*, 1978; Purslow, 1985) have clearly shown that the perimysial layer dominates the fracture behavior of meat, and therefore is the major contributor to toughness. Endomysial collagen content varies much less between muscles than perimysial content and perimysial spatial distribution (Light *et al.*, 1985; Purslow 1999; Na-

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kamura *et al.*, 2003). Brooks and Savell (2004) report that perimysial thickness in beef semitendinosus muscle is on average 2.4 times thicker than in psoas major. Although similarities of ranking exist between muscle toughness and perimysial content (Light *et al.*, 1985) and toughness and perimysial thickness (Brooks and Savell, 2004), the correlation between Warner Bratzler shear force values and perimysial thickness generally show lower R^2 values than those reported by Dransfield (1977) for correlation of variations in total collagen content with toughness of meat cooked to temperatures above 70ºC.

Variations in IMCT composition and distribution 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), a prime question should be: is there a possibility to manipulate this variability in IMCT expression so as to improve meat texture? This begs the question of why IMCT characteristics vary between muscles in the first place, and it is generally accepted that this must reflect differences in physiological (mainly biomechanical) function between muscles. In the last decade there has been a great advance of our understanding of the role of IMCT in coordinating force transmission between muscle fibers within a fascicle and between fascicles within a muscle.. This is comprehensively reviewed by Trotter *et al.* (1985), Huijing (1999a,b) and Monti *et al.* (1999). Endomysial connective tissue is now understood to facilitate load sharing within a fascicle by linking adjacent muscle cells by shear (Trotter & Purslow, 1992; Purslow & Trotter, 1994; Trotter *et al.*, 1995). Based largely on the ultrasonic imaging data of Fukunaga and colleagues on changes in muscle geometry during human locomotory function (e.g. Fukunaga *et al.*, 1997), Purslow (2002) calculated the shear strains that occur between fascicles within various muscles over their physiological range of contraction. This work supported the proposal (Purslow, 1999) 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. Functionally different muscles have very different requirements to accommodate the shear strains which necessarily occur as the muscle contracts and changes shape, and this explains why the amounts and distribution of perimysial connective tissue varies so much between functionally different muscles. In terms of meat science, however, these considerations 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 locomotory function in the live animal.

Cooking Strongly Influences the IMCT Contribution to Meat Toughness

The toughness of meat increases with temperature of cooking; there is a rapid increase between 40ºC and 50ºC, then either a dip between 50º and 60ºC or a plateau in toughness depending on measurement technique, followed by a second phase of increasing toughness above 65ºC. This has been simply correlated with the thermal denaturation of myosin in the range 42-65ºC and collagen above 65ºC (Davey & Gilbert, 1974; Davey & Winger, 1979; Martens *et al.*, 1982). This simple correlation is still 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). However, Bouton *et al.* (1981) 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) definitively showed that perimysial connective tissue strength increases in meat cooked up to 50ºC and decreases above this temperature. Mutungi *et al.* (1966) showed that the strength of muscle fibers continually increases up to 90ºC. Christensen *et al.* (2000) confirmed these findings by examining the strength of both perimysium and individual muscle fibers from the same muscle samples cooked to various temperatures. These studies all confirm the interpretation of Bouton *et al.* (1981) that cooking increases the IMCT contribution to toughness in the range 20ºC-50ºC, with myofibrillar contributions being more important above 60ºC. Because isolated IMCT forcefully shrinks above 65ºC when rapidly heated, 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 causes volume reduction in the muscle fibers, so increasing their toughness (Lepetit *et al.*, 2000). However, 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 (Purslow, 1999), and so it is possible that other events, such as cytoskeletal protein denaturation at higher temperatures, cause the shrinkage to toughen myofibrillar components. Powell *et al.* (2000a,b) have provided interesting findings in this area. They relate the pronase-extractable fraction of collagen to its thermal denaturation above 55ºC and report strong correlations between this fraction and peak shear force in experiments using multistage cooking to core endpoint temperatures of 60ºC. Salt-soluble collagen is not nearly as well correlated to toughness. Their explanation of this insoluble but enzymically extractable fraction being more related to changes in the structural integrity of the fibrous IMCT network on cooking deserves further investigation.

Degradation of IMCT During Ageing of Meat Has No Effect After Cooking

Reservations on the relevance of IMCT degradation during ageing to cooked meat texture have been highlighted

previously (Purslow, 1999). Post-mortem ageing (storage) of meat causes proteolysis in IMCT, which increases the amount of collagen that can easily be solubilised from it (Stanton & Light, 1987). This significantly reduces the strength of perimysium in raw meat (Lewis *et al.*, 1991). There have been a large number of studies in the last decade which reiterate that ageing decreases the structural integrity of IMCT networks, degrades PG components of IMCT, increases the amount of collagen that is easily extracted from muscle and reduces the strength of the networks, for example by shear measurements on raw muscle or on uncooked IMCT structures embedded in acrylamide gels (Nishimura *et al.*, 1994, 1995, 1996a,b, 1998: Liu *et al.*, 1994, 1995; Nakano, *et al.*, 1997; Eggen *et al.*, 1998; Fang *et al.*, 1999, Palka, 2003). (On this vein of relating IMCT composition to mechanical measurements of raw meat, Torrescano *et al.* (2003) found strong correlations between the total collagen content and insoluble collagen content of 14 beef muscles and raw Warner-Bratzler peak shear force values). However, there is a lack of connection between these findings and cooked meat texture. Bouton and Harris (1972) showed that measures of the connective tissue component of toughess in cooked meat were unaffected by ageing. Lewis *et al.* (1991) showed that, while there is a reduction in strength of IMCT with ageing 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. Palka (2003) also found that, despite increases in collagen solubility on ageing, differences in shear force in cooked meat were not significant. Palka (2003) revisits previous conclusions that the texture of raw meat is poorly related to cooked meat toughness.

Increasing the Turnover of Mature IMCT

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). Animal maturity is associated with increased thermal and mechanical stability of IMCT. Increasing collagen fiber diameter and especially the development of mature crosslinks from immature divalent forms accompany this. Although an investigation of the relationships between a number of crosslinks and cooked meat toughness showed no conclusive correlations in pigs of similar maturity (Avery *et al.*, 1996), or in beef animals in the age range 400-800 days (Avery *et al.*, 1998), it is generally held 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 slow turnover rate of IMCT is one factor that allows age-dependent increases in its stability by gradual maturation of directed divalent crosslinks.

Turnover of IMCT does necessarily occur during muscle growth, and is increased by muscle injury or in response to exercise (Koskinen *et al.*, 2001), and downregulated during immobilization (Ahtikoski, 2003). This turnover is principally under the control of matrix metalloproteinase family of enzymes (MMPs). Fifteen types of MMPs and three of the four types of their specific inhibitors (TIMPs) have been found in bovine skeletal muscle (Balacerzak *et al.*, 2001). Turnover of IMCT, involving the degradation of mature, heavily crosslinked components and their replacement with newly synthesised material, may have impact on the connective tissue contribution to meat toughness. Myofibrillar components of muscle tissue are turned over by proteolytic enzymes during growth (Koohmaraie *et al.*, 2002) and can be manipulated by strategic feeding to induce compensatory growth (e.g Kristensen *et al.*, 2002). Remodelling of IMCT is implicitly required during rapid muscle hypertrophy, and Allingham *et al.* (1998) have demonstrated reduced IMCT strength in bovine muscles as a result of compensatory growth following weight loss. However, the role of MMPs and TIMPs in this has yet to be shown directly. Mechanical stimulation of muscle through exercise also affects muscle growth and turnover. Mechanical stimulation of fibroblasts is well known to lead to expression of connective tissue components (Chiquet *et al.*, 2003) as well as to increase the expression of MMPs in cells forming other connective tissues (Blain *et al.*, 2001). The expression of enzymes responsible for turning over IMCT has been poorly investigated in comparison to proteases controlling myofibrillar turnover in muscle, but it does seem that many of the factors impacting on muscle turnover also may affect IMCT turnover and hence its composition and mechanical integrity. This appears to be a means of manipulating IMCT contributions to toughness.

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