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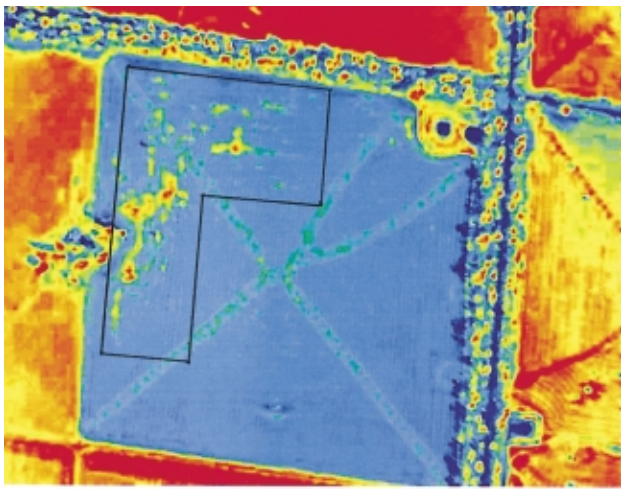

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# Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef

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*Abstract.* The intrinsic properties (those extant at the time of slaughter) of bovine skeletal muscle as they relate to the subsequent quality attributes of beef are reviewed here. Attributes of bovine skeletal muscle that ultimately affect toughness, colour, fat content, flavour, juiciness, and nutritive value of beef are discussed. The dynamic nature of muscle development, particularly with regard to structure and composition, is highlighted. Variation in development of muscle structure and composition due to inherited (genetic) factors and environment (particularly nutrient supply) are described. Examples are given of the implications of sources of variation due to animal genotype, age, nutrient supply, and hormonal environment on muscle cellularity and growth, fibre type, connective tissue composition and structure as they affect meat quality attributes.

Key intrinsic properties of muscle include muscle type, cellularity, size, myofibre type, connective tissue composition and structure, glycogen and fat content and proteolytic activity. Activity of the calpain system at slaughter is seen as an important attribute. Regulation of myofibrillar and connective tissue proteolysis *in vivo* are discussed together with implications for subsequent meat quality. Amongst the on-farm environmental factors, nutritional history and developmental pathway are identified as factors that can be responsible for significant variation in the intrinsic properties of muscle that contribute to variation in toughness, colour and fat content, and thus consumer liking of beef.

*Additional keywords*: meat, tenderness, toughness, colour, flavour, juiciness.

## Introduction

The most important assessment of beef occurs in the mouth of the consumer. In the few seconds that it takes for the consumer to chew the product, judgements are made about its toughness, flavour, and juiciness, and this adds to previous judgements made about the colour, wholesomeness and cost which influences their decision to repurchase (Issanchou 1996; Boleman *et al.* 1997). Recent advances in quality assurance systems for beef have shown that all aspects of the beef production chain need to be optimised in order to minimise variation in eating quality (Polkinghorne *et al.* 1999). For example, failure to control the post-slaughter environment can have a particularly large impact on beef quality attributes such as toughness. These issues are dealt with in detail elsewhere in this volume (Ferguson *et al.* 2001). The intrinsic properties (i.e. those extant at the time immediately before slaughter) of an animal's muscles and, to a lesser extent, fat depots influence the eating quality attributes of meat. Some of these intrinsic characteristics of muscle are changed little by processing and cooking (e.g. muscle fibre type, relative amounts of myofibre, connective tissue and fat). Other intrinsic properties of muscle (e.g. structure of connective tissue matrix and myofibres, glycogen content, proteolytic activity) interact with pre-slaughter stress, post-slaughter processing conditions and method and time of storage to subsequently influence eating quality of beef. In this review, we identify those characteristics that are established in the living animal which influence the eating quality attributes of beef, and describe how these change during development and are influenced by genotype, nutrition and pattern of growth.

## Attributes of beef quality

Beef quality is multifaceted and comprises sensory, nutritive, hygienic or toxicological and technological factors (Hofmann 1994). Factors such as colour, odour, flavour, texture and toughness are considered to be primary sensory factors. Nutritive factors in meat include the type and amount

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of protein, carbohydrate, lipid, vitamins and minerals. Hygienic and toxicological factors are outside the scope of this paper and the reader is referred to Hocking *et al.* (1997). Technological factors include the intrinsic properties of meat that govern its suitability for manufacturing. Sensory factors are the major focus of this review, with passing reference to nutritive and technological factors.

The sensory experience of tenderness (lack of toughness) of meat is critical for consumer acceptance of high quality meat products (Monin and Ouali 1991). Sensory components of toughness arise from interactions between structural characteristics of meat and the forces required to masticate it in the mouth of the consumer. It is suggested that the minimum size of muscle components that contribute to the sensation of toughness is about 20 μm (Dransfield 1997) or, in terms of muscle structure, from 10–15 sarcomere units. This is certainly well within the span of a single myofibre that may be many centimetres long and up to 100 μm in diameter. Accordingly, it is appropriate when describing the structures that ultimately contribute to meat toughness to consider animal specific factors that influence development and structure of the myofibre and its encapsulating matrix. In this regard, we have chosen to refer to those factors that contribute to the intrinsic toughness of the product, rather than its intrinsic tenderness, because it is conceptually easier to account for factors that contribute additively to toughness.

## Growth and development

### *Carcass attributes*

An animal's potential for growth, its pattern of development and, to some extent, its ultimate carcass composition in terms of the proportions of muscle, bone and fat are genetically predetermined. However, interactions with environmental factors during development influence the pattern of growth and mature size (the weight at which lean body mass ceases to grow). In Australia, beef cattle are primarily grown on pasture, and growth is dependent on the seasonal availability of forage and its nutritive value. Hence, growth and yield of meat from these animals are variable.

Overall rate and pattern of growth of a beef animal affects the relative proportions of bone, muscle and fat in the carcass (Byers 1982), and these in turn may affect eating quality. In particular, the thickness and distribution of fat on the carcass affects the inter-relationships between carcass characteristics and the post-slaughter processing conditions as they affect rate of chilling and subsequent rate of pH fall, sarcomere length and aging potential (Chrystall and Devine 1999). Rate of growth in the period immediately pre-slaughter affects the glycogen content of muscle. Glycogen content of muscle is an important determinant of pH fall post mortem and the ultimate pH (and thus colour and tenderness) of muscle (Shorthose and Harris 1991). These issues are dealt with in more detail elsewhere in this volume (Ferguson *et al.* 2001).

### *Muscle*

The anatomical and microscopic structures of adult muscle fibres are well defined (Goll *et al.* 1984) (Fig. 1). The contractile proteins, actin and myosin, are organised within the sarcomere by a network of cytoskeletal proteins that locate and stabilise the contractile apparatus within each myofibril (Wang and Wright 1988; Furst *et al.* 1988; Funatsu *et al.* 1990; Robson *et al.* 1991). Muscle fibres are composed of numerous myofibrils supported within a fibrous matrix of extracellular proteins that maintain their fascicular arrangement and attach muscles to the skeleton. Longitudinal force from muscle contraction is transferred from the contractile apparatus through the muscle fibres to tendons. Lateral force is transmitted from myofilaments to costameres (Fig. 1) then in turn to the extracellular matrix (Craig and Pardo 1983; Pardo *et al.* 1983). The costameres and extracellular matrices anchor to opposite sides of the sarcolemma through groups of transmembrane proteins. Muscle structure in the living animal is inextricably linked to the texture and eating quality of muscle as a food.

In the living animal, muscle is in dynamic equilibrium between developmental stage, external nutritional environment and workload. It is the dynamic nature of muscle that allows environmental effects to be imparted on intrinsic properties that ultimately affect eating quality.

*Myogenesis.* Growth of muscle in the embryo arises from the somitic mesodermal layer (Hauschka 1994). Muscle precursor cells called myoblasts replicate and fuse end to end to form multinucleated muscle cells known as myofibres. Initially, myoblasts form primary muscle cells called myotubes because of their centrally located nuclei and hollow appearance, and these are apparent in the bovine fetus by 47 days of gestation (Russell and Oterulo 1981). Subsequently, myoblasts fuse to form secondary myofibres along the surface of the primary myotubes. This occurs from about 90 days gestation in cattle fetuses (Robelin *et al.* 1991). These newly formed myofibres move away from the surface of the primary myotubes to allow formation of more secondary muscle fibres. It is believed that new myofibres may also form along the surface of established secondary myofibres (Maier *et al.* 1992). Myofibre bundles are present at about 120 days of gestation and primary myotubes mature into myofibres and no longer exhibit myotube-like morphology by 150 days (Robelin *et al.* 1991). Secondary myogenesis results in about a 40-fold increase in muscle cell number in *M. semitendinosus*, and myogenesis is believed to be complete by 240 days gestation in cattle (Robelin *et al.* 1991).

During fetal life, myonuclei accumulate as a result of myoblast fusion during formation of myofibres, and as a result of myoblast replication and incorporation of daughter nuclei into established myofibres. Following completion of myogenesis, accumulation of myonuclei occurs exclusively as a result of replication of muscle satellite cell nuclei. In the

bovine fetus, cells with the morphology of myosatellite cells are present between the basal lamina and sarcolemma of established myofibres by 2 months post conception (Russell and Oteruelo 1981). The number of myonuclei in muscle cross-section accumulates at an increasing rate up to about 180 days post conception (Stickland 1978). The amount of DNA in fetal bovine muscle increases in a linear manner until the final 2 months of gestation (Ansay 1974*a*) when the phase of muscle hypertrophy, which continues until mature (adult) muscle mass is attained, commences. As a result, muscle DNA concentration declines as myofibre size and the ratio of protein to DNA (indicative of cell size in the multinucleated myofibre) increase (Ansay 1974*a*).

The number of myonuclei and DNA content in muscle are correlated with muscle growth (Greenwood *et al.* 2000) and are believed to be the primary determinants of muscle growth potential and mature muscle mass. In postnatal mammals, nuclei are incorporated into myofibres from satellite cells located adjacent to the sarcolemma. Satellite cells are quiescent until stimulated (e.g. by growth signals, injury) to replicate and fuse with their neighbouring myofibre. The number of myonuclei continues to increase, albeit at a declining rate, until some time approaching mature muscle mass. By this time number of nuclei, as indicated by the total amount of DNA present, is maximal (Trenkle *et al.* 1978; Solomon *et al.* 1986; DiMarco *et al.* 1987). The age at which maximal DNA content of muscle is attained depends upon nutrition and rate of growth (Trenkle *et al.* 1978). It appears that the ratio of protein to DNA continues to increase until mature weight is achieved (LaFlamme *et al.* 1973; Trenkle *et al.* 1978; Solomon *et al.* 1986), although DiMarco *et al.* (1987) found that maximal protein to DNA in muscle was attained between 270 and 350 kg body weight.

In ruminants, the influence of growth retardation on subsequent growth and development is important during late gestation (Everitt 1967; Greenwood *et al.* 1998, 1999*b*, 2000). It has been demonstrated in various species that the number of myonuclei or the DNA content in muscle can be reduced by undernutrition during early life, but this influence diminishes as the age when the nutritional restriction is

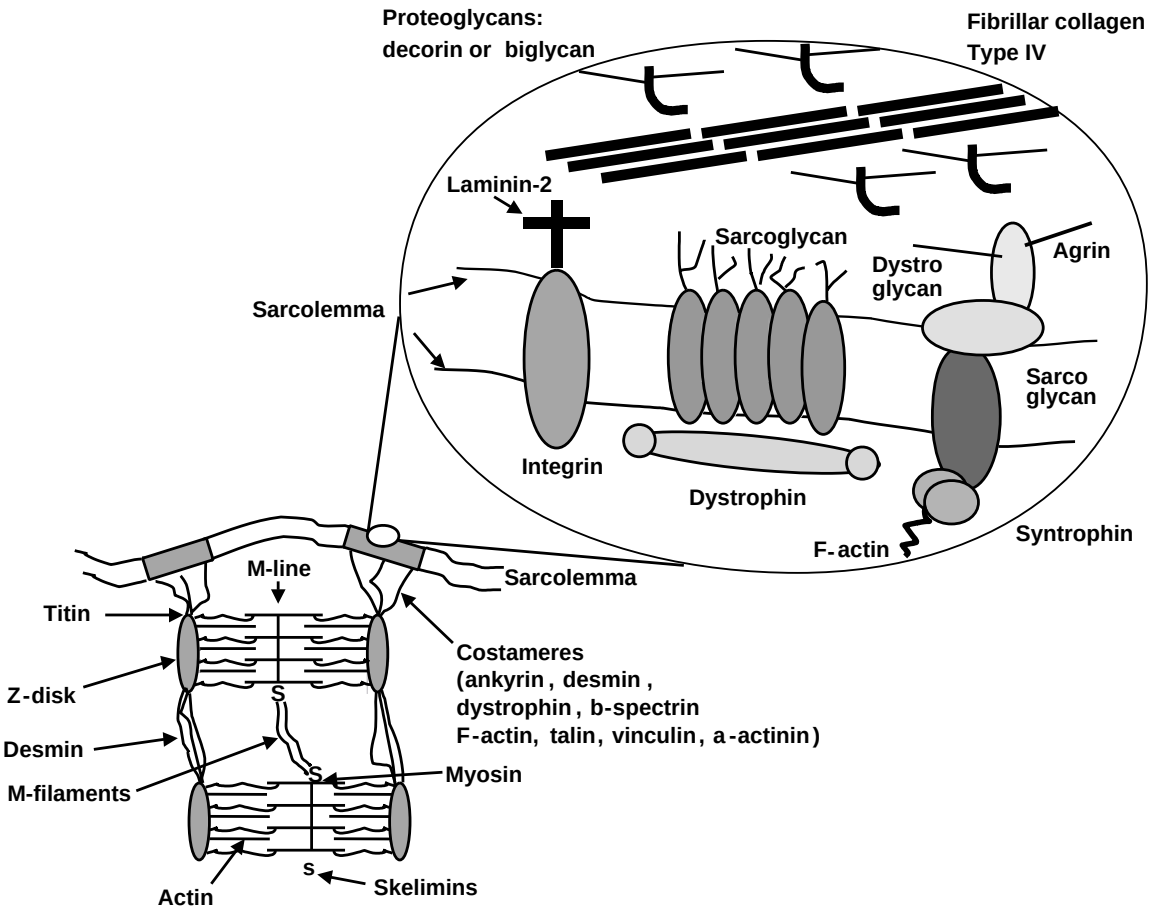


**Figure 1.** Schematic representation of structure and protein composition of costameres in striated muscle relative to Z-disks, myofibrillar lattice and endomysial constituents. The representation has been adapted from Harper (1999). Nebulin filaments have been omitted to simplify the diagram. The inset represents an expanded view of submembrane, transmembrane and extracellular matrix structures.

imposed increases (e.g. Winick and Noble 1966; Robinson 1969; Greenwood *et al.* 2000). In cattle, fetal growth restriction to about half normal growth during the period 232–271 days of gestation resulted in a 7.8% increase in muscle DNA content, compared with a 99.5% increase during normal fetal growth (Ferrell 1991). In this regard, it has been demonstrated that the number of satellite cells (Beermann *et al.* 1983), replication of satellite cell nuclei (Greenwood *et al.* 1999*b*) and subsequent accumulation of muscle DNA (Robinson 1969; Beermann 1983; Greenwood *et al.* 2000) can be reduced by prenatal and/or early postnatal growth retardation with potential longer-term consequences for growth, body composition and body size (Widdowson 1971; Greenwood *et al.* 1998).

Muscle fibre diameter attains typical adult dimensions by 12 months of age (Dreyer *et al.* 1977). Muscle is structurally stable into adult life, but retains the capacity to restructure in response to stimuli such as neural input, stretching (such as that induced by bone growth and work load (Goldspink *et al.* 1991), local hormonal environment and injury (Furuno and Goldberg 1986; Bischoff 1994). Restructuring is associated with satellite cell cycle activity and nuclei replication and hence with changes in the DNA (nuclei) content of the muscle fibres, and changes in expression of proteins (Turner 1998; Hurwitz *et al.* 1998).

The effect of marked differences in muscle fibre number on the intrinsic properties of beef is demonstrated by the double muscled (*mh*) phenotype in cattle (Ménissier 1982; Arthur 1995). The double muscle phenotype arises from failure to produce functional myostatin (gdf-8) — a member of the transforming growth factor superfamily. Functional mutations in the 3' UTR region of the myostatin gene are responsible for disrupting the expression of functional myostatin protein (Grobet *et al.* 1997; McPherron and Lee 1997; Grobet *et al.* 1998). The effects of the *mh* gene mutation are not expressed equally in all muscles of the body (Holmes and Ashmore 1972; Arthur 1995; Martyn *et al.* 1997) but are particularly evident in muscles of the proximal fore- and hind-limbs (Arthur 1995). This is consistent with the observation that muscle development proceeds as a developmental front from the cranial to caudal and the distal to proximal regions of the fetus (Palsson 1955). Myostatin regulates muscle mass through partial inhibition of myogenesis (Kambadur *et al.* 1997; McPherron *et al.* 1997) and may increasingly limit satellite cell proliferation as mature muscle mass is attained, as discussed by Greenwood *et al.* (1999*a*). Myostatin is expressed in a time and tissue dependent manner in pigs and appears to be nutritionally regulated (Ji *et al.* 1998).

Increased muscle weight in the *mh* phenotype results from a marked increase in the number of muscle fibres and there is an associated increase in the proportion of the muscle cross-sectional area comprising white compared with red myofibres (Holmes and Ashmore 1972). In some muscles, this shift is also associated with marked hypertrophy (up to 40% increase in cross-sectional area) of type IIb myofibres that becomes increasingly evident with greater postnatal age (Holmes and Ashmore 1972).

The amount of connective tissue surrounding each muscle fibre bundle (as assessed by collagen concentration of muscle) is either not changed or reduced (Uytterhaegen *et al.* 1994). In terms of eating quality, most investigators have suggested that beef from double muscled animals is less tough than that from normal animals, though this is not a unanimous conclusion (e.g. Bouton *et al.* 1978; Bailey *et al.* 1982).

The observation that the amount of connective tissue per myofibre bundle is not increased in double muscled animals (Uytterhaegen *et al.* 1994) coupled with localisation of myostatin gene expression within developing muscle fascicles and not in connective tissue (Ji *et al.* 1998) suggests that myostatin is not involved in the development of connective tissue, and that the influence of myofibres on development of the connective tissue matrix is secondary.

*Differential development of muscle fibre types*. Muscles consist of distinct fibre types that can be differentiated on the basis of their contractile and metabolic properties. They are: (i) slow-twitch oxidative fibres (type I); (ii) fast-twitch oxidative-glycolytic fibres (type IIa); and (iii) fast-twitch glycolytic fibres (type IIb) (Peter *et al*. 1972). Oxidative fibres have a high concentration of myoglobin and are rich in mitochondria. In the fresh state, muscles with predominantly oxidative fibres appear redder than those with predominantly glycolytic fibres, which contain fewer mitochondria and appear whiter. White muscles derive energy predominantly through the anaerobic glycolytic pathway. The differences in colour of muscles reflect the biochemical composition of constituent muscle fibres as well as concentration of mitochondria (see below). Muscles in different anatomical locations perform different physiological functions and the fibre type profile reflects these different functions (Briand *et al*. 1981*a*, 1981*b*; Talmant and Monin 1986). As well as varying between muscles, muscle fibre types also vary with topographical location within a muscle. For example, the deeper regions of the *M. semitendinosus* are more oxidative than superficial regions (Dreyer *et al*. 1977; Totland *et al*. 1988) that, in part, reflect differences in blood supply within a muscle.

At the molecular level, there are many other differences between muscle fibre types. For example, although myosin comprises about 43% of skeletal myofibrils (Yates and Greaser 1983; Khalili and Zarkadas 1988), at least 9 different myosin heavy chain and 6 light chain isoforms with distinct biochemical or electrophoretic properties have been identified (Sarkar *et al.* 1971; Pette and Staron 1990). Different isoforms of myosin occur in fibres of different contraction speeds (Young and Davey 1981) and are associated with fibre-distinct myosin ATPase activities

(Young 1984). Myosin consists of 2 heavy peptide chains and 2 light chains (Offer and Knight 1988). Myosin ATPase activity is located in the globular head of the heavy chain. Fast-twitch fibres contain higher ATPase activity than do slow-twitch fibres (Barany 1967). Fast and slow-type myosin heavy chains can occur in the same muscle fibre, and these fibres exhibit intermediate contraction speeds (Staron and Pette 1986).

The dynamic nature of muscle extends to variation in fibre type. Fibres change during development, respond differently to chemical and hormonal signals and neural inputs, and adapt to altered workload. Karlsson *et al.* (1999) suggest that the application of fibre type nomenclature is an over-simplification that does not adequately account for the plasticity of expression of fibre type phenotype. Nonetheless, classification by fibre type is an accepted manner of delineation of differences in muscle morphology and biochemical function that also has associations with eating quality of meat (Geay and Picard 1995; Klont *et al.* 1998; Karlsson *et al.* 1999). Accordingly it is appropriate to briefly review systematic affectors of muscle fibre type.

Primary myofibres formed by embryonic myoblasts are predominantly type I (or slow) fibres, whereas fetal myoblasts that form as secondary fibres during prenatal life may be both type I and type II (Dunglison *et al.* 1999). During the latter part of prenatal life, muscle fibre types transform (Beermann *et al.* 1978; Robelin *et al.* 1991; Maier *et al.* 1992), and the proportion of slow myofibres generally declines when myofibre number increases rapidly following commencement of secondary myogenesis. The proportion of each myofibre type within muscles begins to change towards the adult pattern as birth approaches (Sivachelvan and Davies 1981) although this change may be delayed until closer to birth in severely growth-retarded fetuses (Greenwood *et al.* 1999*a*). There is wide variation in the proportion of myofibre types within (Totland and Kryvi 1991) and between muscles in postnatal cattle (Talmant *et al.* 1986).

In addition to developmental changes in muscle fibre type, myofibre type is affected by genotype (Larzul *et al.* 1997), nutrition and the physical environment (predominantly associated with physical activity) (Donoghue and Sanes 1994). Changes in nutrition can result in at least short-term changes in fibre type (Brandstetter *et al.* 1998). The data indicate that during periods of undernutrition, growth of type II fibres is delayed but, after a period of normal nutrition, distribution of muscle fibre types returns to the pattern displayed by animals undergoing normal growth.

In double muscle (*mh* locus) cattle, there is an increase in the proportion of and, in certain muscles, the size of type IIb myofibres that becomes increasingly evident in older animals (Holmes and Ashmore 1972). In addition, there is a lower density of blood capilliaries (Stavaux *et al.* 1993), and an increase in the total number of myofibres. In the naturally derived callipyge mutation in sheep (Cockett *et al.* 1996; Freking *et al.* 1998), extreme muscle development seems to occur without an increase in the number of myofibres. In this mutation, which results in selected hind-quarter muscles being 40% larger than controls (Koohmaraie *et al.* 1995), there is hypertrophy of type IIb fibres (Carpenter *et al.* 1996). An increase in the number, size and relative area of type IIb fibres in muscle of *Bos indicus* cattle (Brahman) compared with *Bos taurus* has recently been observed (B. Gurzansky and P. L. Greenwood unpublished data).

*Connective tissue*

The physical structure and biochemical content of connective tissue changes as a function of animal age and development (reviewed by Ouali *et al*. 1988; Ouali and Talmant 1990; Harper 1999). Physical structure changes in terms of the frequency and thickness of connective tissue seams (tendon and endomysium) running through the muscle (Swatland 1994). Biochemical content changes in terms of the concentrations of the constituent macromolecules (collagens for example) as well as the chemical linkages between them. Harper (1999) has recently reviewed a large number of studies that have sought to define various aspects of development of connective tissue. Some of the key developmental changes in regard to the intrinsic properties of beef are identified here.

Muscles differ greatly in the distribution of connective tissue seams within them. In part this is due to differences in the number of muscle fibre bundles per unit volume of muscle, and in the width of the individual connective tissue seams. For example, Nishimura *et al.* (1999) have elegantly described the physical characteristics of connective tissue matrix in *M. longissimus* and *M. semitendonosus* of beef cattle. In practical terms differences in amount and physical structure of connective tissue leads to the consumer of meat encountering different amounts of connective tissue with each bite. These ideas have been the basis for development of new meat toughness measurement systems (Swatland 1995).

Morphological changes are evident in both collagen and fibroblasts with increased animal age, fibroblasts decrease in number, while collagen fibril size increases (Nakagawa *et al*. 1994). The number of crosslinks within collagen increases, such that the extracellular matrix becomes a stable multi-valent network (Robins *et al.* 1973) leading to high viscoelasticity and low compliance (Rodrigues *et al.* 1996).

The amount of collagen in muscle increases steadily during prenatal life and is highly correlated with muscle mass (Ansay 1974*b*). This accretion is not uniform across the muscle, but contributes in different proportions to the dimensions of perimysial and endomysial connective tissue through gestation (Robelin *et al.* 1991). The weight, proportion and solubility of collagen in muscle declines from birth to 6–8 months of age (Bendall and Voyle 1967; Boccard *et al.* 1979). Subsequently, the collagen content

tends to decline in *M. longissimus* (Bendall and Voyle 1967; LaFlamme *et al.* 1973; Boccard *et al.* 1979) but displays no distinct pattern of change in *M. semitendinosus, M. semimembranosus, M. pectoralis profundus* and *M. triceps brachii* (Bendall and Voyle 1967; Leander *et al.* 1978; Boccard *et al.* 1979). There is believed to be a general longer-term age or weight-related decline in the solubility of collagen (Boccard *et al.* 1979; Maltin *et al.* 1998), but the pattern of change is muscle specific and, like collagen content, may be influenced by breed, sex (Boccard *et al.* 1979; Cross *et al.* 1984), nutritional regimen or growth path (Allingham *et al.* 1998).

Skeletal muscles have a highly integrated extracellular connective tissue matrix, consisting of collagens (types I, III, IV, V and VI), elastin, proteoglycans and a range of structural glycoproteins (Harper 1999). These molecules are products of synthesis and secretion from fibroblasts, adipocytes and endothelial cells within the extracellular matrix as well as from the myofibres themselves (Beach *et al*. 1982; Rao *et al*. 1985). The collagens distribute compressive and tensional forces through the muscle, and maintain structural alignment of myofibres. Pro-collagen chains are synthesised, modified in specific ways and assembled into triple helical collagen molecules principally within fibroblasts. Following directed transport to the extracellular matrix, N- and C-terminal (signal) peptides are cleaved, whereupon collagen helices self-assemble into a lattice of fibrils (Greaser 1997). Post-translational modification of nascent pro-collagen to prepare sites for subsequent cross-linking occurs before transport from the cell. Crosslinks between collagen molecules bind collagen potentially over the entire length of the muscle and distribute forces throughout the muscle.

Collagen type I is the major constituent of the perimysial connective tissue sheets (55–75%), followed by other fibrillar collagens such as type III (25–45%) and trace amounts of type V or VI (Light and Champion 1984; Bateman *et al*. 1996). In the endomysium, collagen type IV is a major constituent (Bateman *et al*. 1996). The detailed molecular structure of the collagen superfamily of proteins as well as the chemical crosslinks that form between them have been reviewed recently by Bateman *et al*. (1996).

To explain meat toughness, researchers have focussed on collagen content and the extent to which these are crosslinked with thermally stable linkages (Horgan *et al.* 1991; McCormick 1994; Berge *et al*. 1997). Results of these studies suggest that collagen content alone is of limited value as a predictor of meat toughness, accounting for less than 10% of the total variance (Dransfield 1977; Berge *et al*. 1997; but see also Harper 1999).

A great deal of research has been conducted on the propensity of collagen to be solubilised by heat, a process that is termed collagen solubilisation. The proportion of collagen that is solubilised during cooking has been found to decrease as a function of animal age (Shorthose and Harris 1991). Collagen solubility is believed to reflect the number and types of chemical crosslinks (Horgan 1991). Kuypers *et al*. (1992), Light *et al*. (1985) and McCormick (1994) have all presented extensive reports on this aspect of connective tissue structure. Briefly, 2 major pathways of collagen crosslinks have been identified (Kuypers and Kurth 1995 and references therein). One pathway leads to chemically reducible crosslinks, the other to crosslinks that are not chemically reducible. Studies of variation of muscle crosslinks suggest proportionally fewer chemically reducible crosslinks as the animal develops. This suggests that the chemically reducible crosslinks may not be of any significance to an adult animal's muscle. More complex, chemically non-reducible crosslinks, which are stable to heat, accumulate in muscle as the animal ages. It is believed that non-reducible crosslinks (pyridinoline and Ehlichs chromagen) contribute more to the biophysical properties of cooked collagen than do reducible collagen crosslinks (Kuypers and Kurth 1995), although non-reducible and thermally stable crosslinks are themselves likely to be products of chemical modification of the reducible crosslinks (Yamauchi and Mechanic 1988).

Some of the differences in toughness that exist between muscles in animals of different ages, and some of the differences between different muscles within the carcass, are related to collagen crosslinking (Horgan *et al*. 1991). However, since muscles differ in muscle fibre type profile, collagen content and crosslinking, fascicular structure, and degree of stretch, it is difficult to attribute between-muscle differences to any one variable. While it has been hypothesised that collagen crosslink density may explain between-animal variance in toughness, recent data have not supported this conclusion (Berge *et al*. 1997).

Collagen content or crosslinking may be of more value in accounting for effects of growth rate on toughness (Harper *et al*. 1999). Harper *et al.* (1997) repeatedly sampled the ST of cattle and found large individual variation in collagen crosslink content within one muscle over several months of life. Topographical variation in crosslink content is also likely to contribute to within-and between-animal variation, as implied by the structural discussions above.

**Regulation of muscle growth**

Muscle, like other tissues, is in dynamic equilibrium between synthesis and degradation of regulatory, functional and structural proteins. This equilibrium accommodates short-term changes due to specific modification of gene expression and protein function (that to some extent is genetically determined) in response to changes in nutrient supply and workload. It is of practical interest to delineate those factors involved in short-term regulation of muscle structure, and to speculate on their possible long-term consequences, because environmental effects are known to affect meat toughness.

*Protein turnover*

Differences between the relative rates of protein synthesis and degradation (protein turnover) determine the rate of protein accretion in muscle. Rates of muscle protein turnover are influenced by genotype (reviewed by Oddy 1999) and by environmental factors such as nutrient supply, muscle workload and animal age (Goldberg 1969). The mechanism and regulation of protein synthesis in muscle cells and other cells is beyond the scope of this review (see Taylor and Brameld 1999 for a recent review). Degradation of proteins is a highly specific process achieved by an integrated system of intracellular proteases and their cofactors (Attaix *et al.* 1999). These include: the cathepsin/lysosomal system, the ubiqutin/proteasome system and the $Ca^{2+}$ activated proteases (calpain/calpastatin system) within the myofibre, and the metalloproteinases in the extracellular matrix. Of the known endogenous proteolytic systems, the calpain system (E.C. 3.4.22.17) is thought to be primarily responsible for specific cleavage of intermediate filament and costamere proteins during aging of meat (Koohmaraie 1994). The principal calpain system components identified in skeletal muscle include the ubiquitous μ and m-calpains, active at micro and milli-molar $Ca^{2+}$ concentrations respectively and the calpain specific inhibitor, calpastatin. The coding sequence for a muscle-specific calpain (known as skm calpain or p94) has been identified (Sorimachi *et al.* 1989). Although mutations in p94 are known to be associated with at least 1 type of muscular dystrophy (Richard *et al.* 1995) it appears to act through nuclear signal transduction, and is not directly involved in myofibrillar protein degradation.

Protein degradation is critical to on going remodelling of muscle structure; cytoskeletal remodelling effects transport of proteins within muscle and facilitates myofibre rearrangement for repair and growth. Myofibrillar protein degradation results in the release of oligomers or small groups of myofilaments from the periphery of the myofibre (Goll *et al.* 1992). Although myofibrillar proteins have been observed within lysosomes (Gerard and Schneider 1980), lysosomal enzymes are not able to initiate the specific degradation of myofibrillar proteins (Goll *et al.* 1989), but may be involved in the later stages of degradation. The role of calpains in disassembly of the myofibre is most likely one of initiation by cleavage of specific cytoskeletal proteins at a limited number of sites (Suzuki *et al.* 1995 and Table 1) leading to subsequent destabilisation of the myofibrillar structure (Dayton *et al.* 1976*a*, 1976*b*). Under conditions of normal growth it is believed that bulk degradation of myofibrillar proteins to their constituent amino acids is principally completed by the ubiquitin/proteasome system, although this may not be the case in extreme models of muscle proteolysis (D. Attaix pers. comm.). Because calpains have been implicated with aging of meat (Koohmaraie 1994), their activity and that of the overall rate of proteolysis in the muscles of the living animal at point of slaughter can be considered an intrinsic property of muscle likely to affect subsequent eating quality.

*Sources of variation in rates of muscle proteolysis* in vivo

Rates of muscle protein degradation in ruminants are known to be affected by age of the animal (Arnal *et al.* 1976), nutrition (Oddy *et al.* 1987), genotype (Oddy *et al.* 1995, 1998) and endocrine factors such as insulin (Oddy *et al.* 1987; Early *et al.* 1988); growth hormone (Oddy *et al.* 1988), β-agonists (Beerman *et al.* 1987; Bohorov *et al.* 1987; Bardsley *et al.* 1992) and insulin-like growth factor-1 (Oddy and Owens 1996). The reader is referred to Lobley (1998) for a comprehensive review.

**Table 1. Evidence implicating calpain involvement in post-mortem degradation of myofibrillar cytoskeletal proteins**

| Protein | Structural position | Subunit size (kDa) | Aging products (kDa) | Calpain cleavage products (kDa) | Reference |
|---|---|---|---|---|---|
| Actin | Contractile apparatus | 42 | None | None | Dayton *et al.* (1975) |
| α-actinin | Z-disk backbone | 90 | None | Releases intact 90 kDa α-actinin from myofibrils | Nagainnis and Wolfe (1982) |
| C-protein | Attached to myosin | 130 | 120 | 120 | Dayton *et al.* (1975) |
| Desmin | Cytoskeleton intermediate filaments | 55 | 38, 35 | 38 and 35 | Huff-Lonergan *et al.* (1996) |
| Dystrophin | Costamere | 420 | 250–370 | 250–370 | Taylor *et al.* (1995); Cottin *et al.* (1992) |
| Filamin | Costamere | 250 | 240 | 240 | Huff-Lonergan *et al.* (1996) |
| Myosin heavy chain | Contractile apparatus | 223 | None | None | Dayton *et al.* (1975) |
| Nebulin | Anchors myosin to the Z-disk | 800 | 600–380 | Degradation products not visible | Ho *et al.* (1997) |
| Titin | Anchors myosin to the Z-disk | 2400 | 1200 | 1200 | Huff-Lonergan *et al.* (1996) |
| Troponin I | Attached to myosin | 23 | 14 | 14 | Dayton *et al.* (1975) |
| Troponin T | Attached to myosin | 37 | 28 and 30 | 28 and 30 | Huff-Lonergan *et al.* (1996) |
| Vinculin | Costamere | 120 | 90 | 90 | Goll *et al.* (1983) |

A significant amount of the variation in rate of protein degradation in ruminant muscle is associated with measurable changes in calpain activity. For example, Thomson *et al.* (1997) reported that nutritionally induced differences in protein degradation in hind limb muscle of lambs were associated with differences in activity of μ-calpain. These studies, and others (McDonagh *et al.* 1999), indicate about half the variation in protein degradation measured *in vivo* can be accounted for by calpain system activity. Given the disparity between the scale of methodology used to measure muscle protein degradation *in-vivo*, and the substrate specificity of calcium activated proteases, the relationship is surprisingly good. The rate of change in activity of the calpain system in response to change in nutritional or endocrine status is rapid (Belcastro *et al.* 1996; McDonagh *et al.* 1999). Treatment with an IGF-1 analogue reduced μ-calpain activity *in vivo* by 22–32% within 6 h (McDonagh *et al.* 1999).

Studies using myotubes derived from L8 myoblasts have been able to specifically address the relationship between calpain system activity and protein degradation in living muscle. Huang and Forsberg (1998) used L8 myoblasts transfected with m-calpain and calpastatin inhibitory domain constructs to study the effect of inhibition of m-calpain and over expression of calpastatin inhibitory domain protein on proteolysis in culture. They demonstrated that inhibition of m-calpain resulted in a 30% decrease in proteolysis, and over expression of calpastatin inhibitory domain protein inhibited proteolysis by 63%. Nebulin was stabilised by inhibition of calpain activity. Fernandez and Sainz (1997) demonstrated in cultured myofibres that changes in calpain enzyme and inhibitor activity brought about by treatment with β-agonist or IGF-1 were associated with independent regulation of myofibrillar and sarcoplasmic protein turnover. These observations indicate a key role for calpains in degradation of sarcomeric proteins, and provide more compelling evidence of the relationship between proteolysis in muscle and calpain system activity than can be obtained *in vivo*.

It is unlikely that calpain proteolytic activity has direct and specific effects on the structure of the extracellular matrix because calpains are located exclusively intracellularly (McCormick 1994). However, by specifically cleaving the costamere proteins nebulin and desmin, calpains are acting on the interface between myofibre and the connective tissue structure of the endomysium, and thus may indirectly influence the relationship between myofibre and connective tissue toughness of meat.

Turnover of proteins from the extracellular matrix (ECM) is mediated by different classes of proteases than the intracellular proteases described above. These are the tissue serine proteases and the ECM metalloproteinases (MMPs). These enzymes act as broad-spectrum proteases for major ECM degradation events that occur during tissue remodelling such as in compensatory growth. The MMPs are a diverse group of proteins that function in the soluble phase of ECM such as perimysium, as well as at the myofibre surface (Streuli 1999; Nagase and Woessner 1999). MMPs are believed to be synthesised and exported into the ECM as zymogens that become activated only following cleavage of their amino-terminal pro-domains. Proteolytic activation occurs close to the cell surface. Nonetheless, overall regulation of MMPs is at the transcriptional level.

The MMPs have been implicated in the relatively slow basal turnover of collagen, fibronectin and the integrins, and in the relatively fast turnover of these proteins induced by inflammation or remodelling. In skeletal muscle, MMP-2 (gelatinase A), and MT1-MMP are candidates for this activity. Their activities are modulated in the ECM by TIMP2 (tissue inhibitor of metalloproteinase 2). Degradation of ECM rarely occurs without some modification of the cellular activity of associated tissue. This is due to flux of growth factors, both in latent and active forms, from the ECM. Degradation of the ECM generates specific growth factors and, through cross talk with receptors of associated cells, these mediate a plethora of responses (Streuli 1999). The degradation products of extracellular proteolysis are either lost into the circulation (and hence degraded or excreted at other sites in the body) or are degraded in the tissue after receptor-mediated endocytosis.

Clearly the kinetics of turnover of myofibrillar and connective tissue proteins are both interconnected and complex. The half-life of myofibrillar proteins has been estimated at 20–30 days (Waterlow *et al.* 1978), whereas the turnover of proteins of the ECM is believed to be slower (from 45 to 120 days, Rucklidge *et al.* 1992). As we have shown above, rates of turnover of protein in muscle vary under different nutritional and endocrine conditions. Accordingly, it is plausible that long-term alterations in muscle protein turnover associated with nutrition may alter gross muscle structure through variation in proteolytic capacity, however, direct evidence *in vivo* is currently lacking.

Sheep and cattle from different selection lines differ in rates of proteolysis in muscle (Oddy *et al.* 1995, 1998). In cattle, the activity of the calpain inhibitor, calpastatin, differs between lines selected for fast and slow yearling growth (McDonagh 1998; Morris and Speck 1998), high and low residual feed intake (McDonagh *et al.* 2000) and between breed types (e.g. Wheeler *et al.* 1990; Pringle *et al.* 1997). Callipyge sheep exhibit reduced rates of proteolysis in muscle and elevated calpastatin activity (Lorenzen *et al.* 1999).

The genetic and phenotypic associations between *in vivo* muscle protein degradation and calpain activity are found under such a wide range of conditions, and the studies of transfected myoblasts (Huang and Forsberg 1998) so compelling, that it is reasonable to suggest that calpain system activity is a pre-requisite for myofibre development and rearrangement. Accordingly, rates of muscle proteolysis and calpain system activity *in vivo* as affected by genotype

and environment are likely to determine the proteolytic environment, and thus potential aging rate, of meat post mortem (see Dransfield 1999). Because the intrinsic proteolytic environment has its major influence on the potential rate of aging of meat post mortem, the association between proteolytic enzyme activity and resulting toughness of meat is secondary. That is, higher rates of proteolysis *in vivo* are associated with higher rates of tenderisation post mortem, largely independent of absolute toughness (McDonagh 1998).

## Association between changes in muscle structure and turnover *in vivo* and eating quality attributes

*Connective tissue matrix*

Variation in the number and chemical form of the collagen crosslinks has been cited as the basis of the increased age-related toughness of individual muscles (Shorthose and Harris 1991). Indeed, this characteristic has been measured with successively improved techniques including collagen solubility and collagen crosslink density. However, in a recent study, Berge (1997) found that correlations between shear force and collagen crosslink densities are insignificant when measured within a muscle (*M. semitendinosus* or *M. longissimus dorsi*).

Age of the animal alone does not determine the toughness of meat, as indeed it does not determine the physical and chemical characteristics of muscle connective tissue. The pattern of liveweight gain that an animal experiences during its life may have an effect on connective tissue toughness. Recent studies that have addressed this issue have been reviewed by Harper (1999) who concluded that the literature was generally consistent with the contention that pattern of growth does influence meat toughness. One possible explanation is that remodelling of connective tissue takes place during periods of variation in weight, and that the newly developed structure arising from such remodelling has different structural properties to the connective tissue it replaced.

During periods of weight loss, connective tissue is degraded within muscle, and possibly, at a slower rate than the myofibrillar proteins. Certainly, McCormick (1994) found that connective tissue mass was spared relative to myofibrillar mass during weight loss. During a subsequent compensatory growth phase, newly synthesised collagen adds to the existing collagen in such a way that the overall toughness attributes of the connective tissue are lower than before weight loss. This mechanism was first proposed by Etherington (1987), who concluded that during tissue remodelling, newly synthesised collagen is less crosslinked than pre-existing, crosslinked collagen. It is possible that newly synthesised matrix is less condensed than mature connective tissue. The exact mechanism of remodelling is unknown but it is likely that MMPs are primarily involved in the process (Streuli 1999). Moreover, during *de novo* collagen synthesis, pre-existing collagen becomes a lower proportion of total collagen than it was before the weight-loss/compensatory growth cycle, and during synthesis the rate of cross-linking lags behind collagen synthesis *per se*.

While there is no direct experimental proof for the above, recent studies are at least consistent with the broad details of the Etherington (1987) proposal. In a recent study, Brahman-cross steers were fed to grow in different growth patterns: uninterrupted, or two paths that incorporated a weight-loss/weight-gain cycle (Allingham *et al.* 1998; Harper *et al.* 1999). The animals that had lost and then regained weight on a grain diet had significantly lower connective tissue toughness than the animals following the uninterrupted growth pattern (Allingham *et al.* 1998). In subsequent statistical analyses, measures of collagen cross-links (ratio of pyridinoline to total collagen) were found to account for a significant proportion of the variance in connective tissue toughness. However, parameters that reflected the myofibrillar component of muscle (lactate dehydrogenase and isocitrate dehydrogenase) also accounted for significant proportions of variance (Harper *et al.* 1999). The conclusion reached by this study was that remodelling of the connective tissue cannot be viewed in isolation from the metabolic changes and changes in fibre type that are likely to be induced by different patterns of growth.

*Muscle fibre type*

Muscles with different proportions of oxidative and glycolytic fibres differ in eating quality (Monin and Ouali 1991). Totland *et al.* (1988) demonstrated an association between increased toughness and increasing proportions of slow-twitch oxidative fibres with muscle depth in beef *M. semitendinosus*. The proportion of fast-twitch oxidative-glycolytic fibres (intermediate fibres) has significant effects on toughness in swine *M. longissimus dorsi* (Henckel *et al.* 1997; Maltin *et al.* 1997). The toughness of meat varies topographically within and between muscles within the same carcass (Shackelford *et al.* 1997), and between meat of animals of different age or maturity (Picard *et al.* 1994), in accord with differences in fibre type and collagen content and solubility.

Muscle fibres of different metabolic and contractile activities also show differences in proteolytic activity. Ouali and Talmant (1990) showed in lamb, that the activity of calpastatin differs between muscle fibre type, with calpastatin levels being highest in slow-twitch oxidative, intermediate in fast-twitch oxidative-glycolytic, and lowest in fast-twitch glycolytic muscles. Although Geesink *et al.* (1992) found no difference in μ-calpain activity between 6 bovine muscles, this may be because major bovine muscles are heterogenous and do not differ greatly in pattern of fibre type (Totland and Kryvi 1991). Nonetheless, because fibre type does vary systematically with genotype, age, muscle

type and location within muscle, and may be changed, at least in the short term, by nutrient supply, there are likely to be associated changes in proteolytic potential and subsequent aging rate through variation in calpain activity. Jones *et al.* (1998) also suggest that there may be muscle-specific differences in the calpain-system that are unrelated to fibre type composition. The toughness characteristics of muscle fibres are a reflection of their biophysical structure and the biochemical processes that occur within them. Hence, variation in toughness is derived from variation in the inextricable linkage between muscle fibre type, connective tissue content and cross-linking, proteolytic activity, glycogen content and the potential rate of glycogenolysis (Harper 1999). Given the differences in fibre type and enzyme activity within individual muscles, and the effects of muscle fibre composition on both the absolute meat toughness and the rate of tenderisation, some variation in toughness within muscles is inevitable.

Differences in nutrition, which result in differences in growth rate, lead to at least transient changes in fibre type (Brandstetter *et al.* 1998). During undernutrition, the proportion of type I fibres increases, but on recovery, fibre type proportion returns to control values. Fibre size is also influenced by nutritional status, increasing with increased nutrition (Yambayamba and Price 1991).

Hypertrophy of type IIb fibres relative to type IIa and type I has been a consistent observation in muscle of animals treated with β-agonists (Hamby *et al.* 1986; Maltin *et al.* 1987) without an increase in DNA content (Beermann *et al.* 1987; Kim *et al.* 1987). These changes are associated with reduced myofibrillar proteolysis through reduced calpain activity *in vivo* and carry over into reduced rates of myofibre fragmentation in post mortem muscle (McDonagh *et al.* 1999). Muscle hypertrophy in callipyge sheep displays similar morphological characteristics to animals treated with β-agonists, with marked hypertrophy of type II fibres (Carpenter *et al.* 1996), in particular type IIb fibres. However, DNA content is elevated in the callipyge phenotype (Koohmaraie *et al.* 1995). Nevertheless, this hypertrophy is associated with increased calpastatin activity, reduced rates of proteolysis *in vivo* and increased toughness post mortem.

*Proteolysis and aging rate of meat*

The toughness of cooked meat reflects the mechanical properties of the 2 fibrous components of muscle, the contractile muscle fibres themselves (myofibres) and the extracellular connective tissue matrix that supports the myofibres (Fig. 1, Shorthose *et al.* 2001). As discussed above, the structural properties of these 2 components are determined in the growing animal. However, the structural integrity of meat, and hence its toughness, is determined by the resulting structure at rigor and changes thereafter during storage (tenderisation, Goll *et al.* 1964).

The rate and extent of tenderisation is influenced by post mortem treatments such as electrical stimulation and chilling regime (reviewed by Tornberg 1996 and Ferguson *et al.* 2001; see also Hwang *et al.* 1999), and by *in vivo* determinants of proteolysis. Tenderisation results primarily from loss of myofibrillar integrity due to the enzymatic degradation of myofibrillar, costameric and cytoskeletal proteins (Taylor *et al.* 1995). Evidence for involvement of the calpains in the process of tenderisation has accumulated since the original observation that addition of purified calpain to meat replicated the Z-disk degradation observed in aged meat (Penny *et al.* 1974). Several lines of evidence suggest that the action of the μ-calpain and m-calpain proteinases and the interaction with their inhibitor protein, calpastatin regulate the process of post mortem myofibrillar degradation (Penny 1980; Goll *et al.* 1992; Koohmaraie 1994). Perhaps the strongest evidence is the ability of purified calpains to reproduce many of the proteolytic cleavages of cytoskeletal proteins *in vitro*, that are seen during post-mortem tenderisation in intact muscle (Table 1) (see also Dransfield 1999).

Evidence of an association between *in vivo* proteolysis and calpain system activity and tenderisation of meat from sheep subject to a range of nutritional and endocrine treatments *in vivo*, and from cattle from lines selected for different traits, has recently been obtained in our laboratory. In sheep, McDonagh *et al.* (1999) reported effects of nutrition and treatment with β-agonist for 1 week, and treatment with a high affinity IGF-1 analogue on *in vivo* proteolysis. Post mortem measurements included calpain system activity and rate of aging in meat as assessed by rate of myofibrillar fragmentation, and rate of change of shear force over a period of 9 days. There was a significant relationship between calpain activity (estimated as the μ-calpain:calpastatin ratio) immediately after death, and subsequent rate of change in myofibre release and shear force over a 9-day aging period (Table 2). In a separate study, using Angus cattle from lines divergently selected on the basis of average daily gain from birth to 1 year of age (Parnell *et al.* 1997), it was found that selection for high yearling weight was associated with reduced muscle protein degradation (Oddy *et al.* 1998), increased calpastatin concentration and reduced rate of post mortem tenderisation (McDonagh 1998). These results are consistent with Morris and Speck (1998) who also observed differences in calpain activity and meat toughness in Angus cattle selected for high rates of gain. An increase in calpastatin activity and reduced proportion of myofibre release post mortem have also been observed in steers selected for high net feed efficiency (McDonagh *et al.* 2001). The above results clearly indicate that both genetic and environmental factors affecting proteolysis *in vivo* also influence rate of tenderisation and, potentially, toughness of meat.

**Table 2. Effects of nutritional, endocrine and genetic influences on protein degradation in the hind limb, and the activity of the calpain system in lambs and in cattle divergently selected on the basis of growth rate or net feed efficiency (NFE)**

Values given are percentage change relative to their control groups (lambs) or comparative low selection-line trial mates (cattle)

| | Calpain system activity (%) | | | Protein degradation | Reference |
|---|---|---|---|---|---|
| | μ-calpain | m-calpain | calpastatin | (%) | |
| *Lamb effects (relative to controls)* | | | | | |
| Nutritional restriction (60%) | +6 | +2 | –15[A] | +46[A] | McDonagh *et al.* (1999) |
| Treatment with a β-agonist (1 week) | –23[A] | 0 | +13[A] | +8 | McDonagh *et al.* (1999) |
| *Cattle genetic selection effects (relative to low selection lines)* | | | | | |
| High growth rate-line steers | –9 | +5 | +14[A] | –41[B] | Oddy *et al*. (1998); McDonagh (1998) |
| High growth rate-line heifers | +21 | +8 | +14[A] | n.a. | McDonagh (1998) |
| High NFE-line steers | +2 | +4 | +18[A] | n.a. | McDonagh *et al.* (2001) |

[A]Indicates changes that were significantly ($P$<0.10) different.
[B]Protein degradation results obtained from a prior cohort group to calpain system measurements.
n.a., data not available.

These observations are consistent with prior observations of the effects of β-agonists on calpain system activity and meat toughness (Higgins *et al.* 1988; Koohmaraie *et al.* 1991), and with observation in callipyge sheep which have both high proportions of type IIb fibres, higher calpastatin and less tender meat (Koohmaraie *et al.* 1995; Carpenter *et al.* 1996; Lorenzen *et al.* 1999).

Recent studies have found positive correlations between the ratio of μ-calpain to calpastatin and myofibrillar fragmentation index: an established indicator of the progress of post mortem tenderisation (McDonagh *et al.* 1999) (Table 3). Likewise, calpastatin alone is correlated with shear force (Koohmaraie 1994). Interestingly, similar analyses have suggested associations between the activity of the calpain/calpastatin axis and the compressive toughness of beef (McDonagh 1998). Historically, compression tests have been understood to predominantly measure the connective tissue contribution to toughness, and are influenced to a lesser extent by the integrity of myofibres (Bouton and Harris 1972). Hence these correlations may reflect an influence of the calpains on the integrity of physical connections between the myofibrillar elements of meat and the connective tissue elements, although direct biochemical evidence supporting this explanation is lacking.

**Table 3. Relationships between different estimates of tenderness in the *M. longissimus dorsi* and measurements of the post-slaughter functional activity of skeletal muscle calpain system proteins in steers and heifers divergently selected on the basis of growth rate, steers divergently selected on the basis of net feed efficiency (NFE)**

| Related traits | | Trend | Aging time | Signif. | Reference |
|---|---|---|---|---|---|
| Protease | Meat quality | direction | (days) | | |
| | | | *Wt + Wt – steers*[C] | | |
| μ-cal:calp | Shear force[B] | – | 1 | * | McDonagh (1998) |
| | | | *Wt + Wt – heifers*[C] | | |
| μ-cal:calp | MFI[A] | + | 10, 17 | ** | McDonagh (1998) |
| μ-calpain | Shear force[B] | – | 1, 3, 10 | * | |
| Calpastatin | Shear force[B] | + | 3, 10, 17 | ** | |
| Calpastatin | Compression[F] | + | 1, 3, 10, 17 | * | |
| | | | *NFE + NFE – steers*[D] | | |
| μ-cal:calp | MFI[B] | + | 7, 14 | ** | McDonagh *et al.* (2000) |
| μ-cal:calp | Shear force[C] | – | 1, 7, 14 | * | |
| μ-cal:calp | Compression[E] | – | 1, 7, 14 | † | |

†$P$<0.10; *$P$<0.05; **$P$<0.01.
[A]Myofibrillar fragmentation index estimates myofibrillar influence on meat toughness.
[B]Estimates predominantly myofibrillar influences on meat toughness.
[C]Refers to the Trangie growth rate selection line cattle.
[D]Refers to the net feed efficiency selection line cattle.
[E]Estimates predominantly connective tissue influences on meat toughness.

The calpains are known to degrade cytoskeletal structures such as costameres and intermediate filaments post mortem (Uytterhaegen *et al.* 1994; Taylor *et al.* 1995; Huff-Lonergan *et al.* 1996). The cytoskeleton maintains the integrity of muscle cells and anchors the contractile filaments to organelles and the sarcolemma (Robson 1995; Robson *et al.* 1997). Degradation of costameres will promote detachment of the cell membrane from the underlying myofibrils (Taylor *et al.* 1995; Dransfield 1997; Papa *et al.* 1997), and given that sarcolemmal proteins attach indirectly to the endomysial connective tissue matrix (Fig. 1), it is likely that their degradation will disrupt at least some physical connections between connective tissue and the myofibrils.

The above series of observations indicate that variation in connective tissue matrix (both content and cross-linking), myofibre type and turnover, and associated proteolytic systems *in vivo* are sources of variation (albeit in some cases small) in meat toughness, and in the rate of tenderisation of meat post mortem. Because each of these components of muscle are subject to systematic variation during animal development, it is reasonable to consider that in practical terms under Australian production systems, genotype and pattern of growth are likely to have significant effects on beef eating quality.

**Pattern of growth**

Growth and development are inextricably linked with physiological age in determining characteristics of muscle that influence beef quality. In circumstances of unlimited nutrition, animals grow at a rate and to final dimensions that are determined genetically (Berg and Butterfield 1976). In extensive beef production systems, periods of inadequate nutrition lead to periods of suboptimal growth and are often followed by periods when quality of feed supply is sufficient to induce compensatory growth (Hunter and Buck 1992; Allingham *et al.* 1998). Harper (1999) recently reviewed studies of the effects of pattern of growth on meat toughness, and concluded that the pattern of growth is associated with variation in meat toughness. He concluded the direction and magnitude of any change in meat toughness reflected the severity and duration of nutritional restriction. In terms of the myofibrillar component of toughness, it is difficult to determine if there are significant growth path effects because post-slaughter conditions (i.e. electrical inputs, chilling rate, aging time and conditions) are rarely identical and are therefore confounded with production variables. In terms of connective tissue toughness, Harper (1999) suggested that there is support for the view that both compensatory growth and duration of feeding a high energy diet may reduce the connective tissue contribution to toughness in at least the *M. longissimus* and *M. semitendinosus*. It is not clear if this is due to the dilution effects of accumulating intramuscular fat (Nishimura *et al.* 1999) or restructuring of the connective tissue itself.

In recent studies, we have found that the connective tissue toughness of animals that had grown consistently over a period was greater (as assessed by compression) than in animals that had undergone a weight-loss/weight-gain cycle. The same weight cycling treatment had no effect on the myofibrillar toughness (Allingham *et al.* 1998; Harper *et al.* 1999). The temporal complexity of effects of pattern of growth on meat toughness is highlighted by observations that early life growth rate may lead to no change or reduced toughness where animals are slaughtered at less than 30 months of age (Hearnshaw 1997) or to increased compression force measurements in meat from animals with poor early life growth slaughtered at over 36 months of age (J. A. Lindsay pers. comm.). Clearly the long-term sequelae to short-term changes in growth are not well understood when it comes to predicting effects on meat toughness. Nonetheless, the data clearly indicate that pattern of growth can influence meat toughness, and that high rates of growth pre-slaughter, such as may be achieved during compensatory growth or in a feedlot, generally produce the intrinsic muscle properties associated with a reduction in ultimate meat toughness.

**Colour**

Under the current system of meat marketing, colour is often the primary criterion by which consumers evaluate beef quality and acceptability at the point of sale. Australian consumers prefer to purchase bright red beef. The characteristic colours of meat predominantly result from the interaction of oxygen with myoglobin in muscle. Meat colour is not merely a reflection of the myoglobin content of the different muscle fibre types within muscle (see earlier section), but is also a function of exposure to oxygen and light at point of sale (Kropf 1980), and the *in situ* biochemical changes that occur in post mortem muscle.

Myoglobin concentration in muscle increases with animal age (Lawrie 1961; Boccard *et al.* 1979) and size (Ledward and Shorthose 1971), and is dependent on dietary availability of iron and copper (Bray *et al.* 1959). However, the greatest influence on meat colour is the ultimate pH of meat (Shorthose and Harris 1991). The pH of meat is primarily determined by glycogen content at slaughter. Low muscle glycogen at slaughter leads to reduced lactate accumulation and high ultimate pH. Under these conditions post mortem mitochondrial respiration is extended resulting in prolonged deoxygenation of myoglobin, and hence dark meat (Ashmore *et al.* 1973). High muscle glycogen at slaughter favours extended anaerobic glycolysis and increased lactate accumulation that generates a lower ultimate pH (in the range 5.4–5.5) (Newbold and Harris 1972). Under these conditions, myoglobin is oxygenated and meat colour is light (Pan and Solberg 1972). Colour in meat is therefore related to ultimate pH, which, in turn, is related to tenderness (Purchas 1990; Jeremiah *et al.* 1991). Hence,

meat colour is not only of importance for visual appraisal by the consumer, but is used as a means of grading meat to ascertain tenderness (Wulf *et al.* 1997; Wulf and Wise 1999).

Variations in muscle glycogen content at death and subsequent pH and meat colour, are a reflection of pre-mortem nutrition and activity (Howard and Lawrie 1956; Ashmore *et al.* 1973; Pethick *et al.* 1994; Pethick and Rowe 1996). Muscle glycogen is further influenced by stress levels before and at slaughter through the action of adrenaline (Ashmore *et al.* 1973) and possibly by $\beta_2$-adrenoreceptor densities (Hoey *et al.* 1995). Pre-slaughter nutrition can be managed to ensure adequate glycogen stores in muscle before death. Studies by Liu *et al.* (1996*a*, 1996*b*) have shown that vitamin E influences the visual attributes of the meat. Steers fed diets containing up to 2000 mg of α-tocopheryl acetate produced meat that had improved retention of redness, yellowness and colour saturation, even after aging for 14 days. Dosage of cattle with α-tocopherol for up to 126 days was associated with a marked increase in shelf life of meat.

**Intramuscular fat content and marbling**

Marbling is the industry term used to describe the visual appearance of fat deposited within muscle. Intramuscular fat in beef cattle predominantly consists of adipocytes (fat cells) in the connective tissue seams surrounding the muscle fibre bundles, with a small contribution as intracellular lipid droplets. Adipocytes arise from precursor adipocytes (pre-adipocytes) derived from stromovascular cells, and although development of fat depots begins *in utero* (Russell and Oteruelo 1981), the associated increase in adipocyte cell number in each depot continues throughout life (unlike muscle). There is evidence that adipocyte number continues to increase in intramuscular depots to a greater extent than in other depots (Hood and Allen 1973; Cianzio *et al.* 1985), but the proportional accumulation of intramuscular lipid relative to all other fat depots does not changes during post-natal growth (Johnson *et al.* 1972; Cianzio *et al.* 1982). Lipid deposition into all fat depots (including intramuscular depots) increases with age (liveweight) and growth rate (Zembayashi 1994). Partition of fat deposition between depots differs between sexes of the same breed and between breeds of cattle (Cianzio *et al.* 1982; Johnson 1987; Wegner *et al.* 1998). Rate of fat deposition (including the intramuscular depot) is dependent also on nutrient intake. High intake relative to maintenance and to the capacity to deposit protein results in higher rates of fat deposition (Johnson 1987; Zembayashi 1994; Owens *et al.* 1995).

There has been extensive debate as to the contribution that intramuscular fat makes to the sensory attributes of meat (e.g. Purchas and Davies 1974; Dikeman 1996). Intramuscular fat may make some contribution (between 3 and 10%) to sensory palatability (Campion *et al.* 1975; Crouse *et al.* 1978; Tatum *et al.* 1980). Measures of sensory palatability incorporate attributes such as tenderness, juiciness and flavour. Although the relationship between intramuscular fat and objective and sensory measures of tenderness is poor (Rymill *et al.* 1997), that between intramuscular fat content and flavour is stronger. Muscle type may affect the relationship between intramuscular fat and tenderness. Nishimura *et al.* (1999) reported that high levels of intramuscular fat were associated with improved tenderness in *M. longissimus* but not *M. semitendinosus* of Japanese Black cattle. However, recent work (MSA 1999) has demonstrated that increased intramuscular fat was associated with decreased failures in consumer assessment. This is thought to be related more to improved perception of juiciness and flavour rather than an effect of increased intra muscular lipid content, and to subsequent improvements in chilling rate and pH decline through temperature influences on glycolysis rates (Shorthose and Harris 1991). Intramuscular fat may also stimulate the salivary glands in the mouth of the consumer, increasing the human perception of juiciness. Nonetheless, in the extreme case of Japanese Black cattle, intramuscular fat, at least in *M. longissimus*, may physically alter connective tissue structure and thereby reduce toughness of the meat (Nishimura *et al.* 1999).

**Flavour and aroma**

Flavour and aroma are sensory attributes that significantly influence consumer acceptability of beef (Shahidi 1998). Although raw beef has little aroma and only a blood-like flavour, it contains all the compounds that will subsequently become flavours and flavour enhancers during the cooking process. The flavour precursors include free amino acids, peptides, reducing sugars, nucleotides, lipids and vitamins. Interactions between these molecules and/or their degradation products via complex chemical reactions such as the Strecker degradation and the Maillard reaction, produce a large number of intermediates and/or volatiles that create the flavour and aroma of beef (Shahidi 1998, and references therein). Most of these molecules are present in all healthy muscles and are unlikely to be the source of unique flavour characteristics.

Dietary regimen of the living animal has been shown to influence the subsequent flavour of beef (Melton 1990; Shahidi 1998), pork (Larick *et al.* 1992) and sheep meat (Rousset-Akrim *et al.* 1997; Young *et al.* 1997), and this is associated to some extent with the lipid component of the feed. Some controversy remains about the relative contribution made by lipid components of meat as opposed to the other classes of molecules, in regard to the determination of flavour and aroma. Lipids take on their greatest significance in generation of unpleasant flavours in meat during storage (Skibsted *et al.* 1998). These flavours are generated by the hydrolysis of triacylglycerols and phospholipids, as well as the oxidation of fatty acids. Oxidative decomposition of unsaturated lipids is the main

factor in off-flavour generation and deterioration of beef (Skibsted *et al.* 1998). The significance of on-farm factors to the subsequent flavour and aroma of beef is most graphically demonstrated by supplementation of animals with α-tocopherol, which acts against the *in situ* oxidation of lipids (Faustmann *et al.* 1989).

In a practical sense, the flavour and aroma of beef may be controlled by standardising the diet that animals consume, together with use of vitamin E supplementation to control the subsequent degradation of beef. Characterisation of the flavours and aromas of beef produced under particular dietary regimens may eventually become a basis on which to differentiate beef product from different environments. Marketing flavours as positive attributes of beef has already begun in the Australian food service sector (Egan *et al.* 2001).

**Human health aspects**

Without delving deeply into current controversy over appropriate quantities of beef in the human diet, it is clear that lean red meat remains an important food source for people. In the context of this review, we will briefly discuss the potential of genetic or on-farm management practices to influence the nutritive properties of beef.

Beef provides all the amino acids that are essential for the human adult. Given that this comes from digestion of the constituent proteins of muscle, variation in muscle structure induced by growth will lead to a difference (albeit small) in the quantity of amino acids available for absorption from a standard 100 g meal. In addition to differences between muscles (cuts) in terms of fat content, which affects the relative proportion of protein in the meal, beef from very young animals will contain less fat and more total protein per meal than beef from older, fattened animals. The composition of amino acids in protein will vary between cuts (muscles), and to a lesser extent between meat from animals of different ages and growth rates, principally through variation in the relative proportion of sarcoplasmic, myofibre and connective tissue proteins. Although a wide variation in the essential amino acid content has been reported for different cuts of beef (Altman and Dittmer 1968), and therefore in terms of proportions of myofibrillar and connective tissue proteins, relative to foods of plant origin the composition of beef is closer to an ideal protein for human consumption. Beef is also a good source of iron, zinc and phosphorus. Again, given the abundance of these micronutrients, individual variation between animals is not likely to have much significance in relation to the total consumption of the food.

Saturated fat content is one aspect of the intrinsic properties of beef that can impact on the health of its consumers, or at least their re-purchase decisions (Schaefer and Brousseau 1998). Publicity concerning consumption of saturated fat and the fatness of beef has resulted in decreased consumption of red meat in western countries. The fatty acid composition of subcutaneous fat is known to reflect both genetic and environmental influences (Kelly *et al.* 2001). Apart from the production of leaner carcasses by restricting total nutrient intake, it is possible to reliably manipulate composition of triglyceride fatty acids by feeding diets in which unsaturated lipids are protected from biohydrogenation in the rumen (Cook *et al.* 1970). Studies have been conducted in man, in which beef with a greater proportion of unsaturated fatty acids in intramuscular lipid (produced by feeding lipid protected from rumen breakdown) was consumed. It would be anticipated that this would have a similar effect as consuming comparably modified dairy products. It has been shown that ingestion of dairy products with a greater proportion of unsaturated fatty acids was associated with corresponding changes in fatty acid profile and reduced concentration of lipoprotein and cholesterol complexes in consumers compared with normal dairy products (Noakes *et al.* 1996). The recent observation that ruminant fat, particularly of animals consuming forage, contains relatively higher proportions of conjugated linoleic acids (CLA) than fat from other species, indicates that composition of specific fatty acids in beef may be a more important indicator of nutritive properties than total fat content. CLAs are apparently protective against cancer, at least in rodent models (Hargreaves 1985; Ha *et al.* 1987). Amongst the environmental factors, finishing regimen, background nutrition, average ambient temperature and season contribute to the variation in fat and acid composition. For example, Kelly *et al.* (2001) found that level of nutrition during the backgrounding phase of growth affected the proportion of the fatty acids C18:1c9, C16:0 and C16:1c9.

**Drip or water-holding capacity**

Water-holding capacity of meat is its ability to passively immobilise within it, all or part of its own or added fluid. It is important because several quality attributes of meat (colour, texture, firmness, juiciness and toughness) are at least indirectly affected by its water-holding capacity (Monin and Ouali 1991). Loss of fluid will ultimately impact on the value of product, as meat is sold on a weight basis.

Fluid is present in several forms within the structure of meat. About 90% of the water in muscle is held by weak surface forces such as capillary or association with protein molecules of the muscle; 4% of this water is firmly bound to the muscle proteins by direct molecular interaction (Offer and Knight 1988). The remaining 10% is associated with the extracellular space. The amount of water that is bound within these compartments depends on myofibrillar volume and the rate of pH decline and ultimate pH of meat (Bouton *et al.* 1971; Offer *et al.* 1989). As muscle contracts during *rigor mortis* and as the lateral spacing of filaments declines (Diesbourg *et al.* 1988) water is forced from the myofibrillar

structure into the extracellular space (Currie and Wolfe 1983). As pH falls towards 5.5, myofibrillar proteins also lose iso-electric charge and their ability to associate water (Guignot *et al.* 1993; Zamora *et al.* 1996). Most of this water that hydrates the extracellular space is lost from meat as drip loss.

In a practical sense, several factors influence water-holding capacity and drip loss. The largest effects are processing and handling factors, such as electrical stimulation and the rate of chilling (Honikel *et al.* 1968) and the effects of freezing and thawing (Petrovic *et al.* 1993). Factors such as carcass size and fatness which are affected by development and nutrition can interact with electrical stimulation and chilling conditions, and through subsequent effects on glycolytic rates and pH fall, also influence drip loss (George *et al.* 1980; Ferguson *et al.* 2001). The influence of nutritional history on susceptibility to pre-slaughter stress may be more significant. Long-term stress can reduce the capacity of muscle to decline in pH, whilst short-term stress can accelerate it (Tornberg 1996). As discussed above, pre-mortem stress can reduce muscle glycogen stores, limiting post mortem glycolysis and acidity. Under these conditions, meat appears dark and holds more water due to greater electrostatic interaction with myofibrillar proteins.

The importance of genetic difference in stress susceptibility on drip loss is graphically demonstrated by the Pale Soft Exudative (PSE) phenotype in pigs. This phenotype results from a mutation in the porcine ryanodine receptor (Fujii *et al.* 1991), leading to abnormal flow of calcium across the sarcoplasmic membranes of muscle cells post mortem (Mickelson and Louis 1996). This fluid accumulates outside the myofibre bundles and drips from the muscle, leaving the meat dry and unacceptable to the consumer (Offer 1991). A bovine equivalent of PSE has not yet been identified.

## Conclusion

Variations in nutritional status (over the short and long term) affect the intrinsic character of muscle that, in turn, will subsequently influence the toughness of meat. Some of these characteristics affect the structural components of meat (myofibre number, type, connective tissue content and degree of cross linking, fat content and fatty acid composition). The time scale by which variation in these characteristics is expressed is realistically in the order of weeks and perhaps months. The factors most likely to be affected in the short term include variation in glycogen content and glycogenolytic and proteolytic activity. It is the interaction between: (i) the intrinsic physical structure of muscle, (ii) the capacity of muscle components to affect the transition of the structure of living muscle into meat (initially rate of pH decline which in part reflects glycogen content and glycogenoltyic activity at the time of slaughter), (iii) subsequent structural disassembly (post mortem aging — a function of proteolytic activity), and (iv) processing conditions (see Ferguson *et al*. 2001 for review), that ultimately determine the toughness of meat available to the consumer.

The importance of adequate growth pre-slaughter and processing conditions in determining ultimate eating quality of meat is becoming clearer. With the drive towards improving quality of all consumer products (including meat) it will no longer be acceptable for meat production systems to ignore factors that influence quality anywhere within the chain from conception to consumption. In future, meat production systems that take account of whole of life growth and development of beef cattle, not only to optimise economic returns from production, but to ensure consumer satisfaction with meat as a food will become the norm. To assist the development and management of such beef production systems there is an ongoing need to elucidate the associations between muscle growth, changes in fibre biochemistry and stabilisation of the extracellular matrix, and the molecular signals responsible for these changes.

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