The Significance of Connective Tissue Structure and Content to Meat Quality: Growth Path & Nutritional History.

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ABSTRACT

The role that connective tissue plays in determination of meat toughness can be understood best with reference to connective tis biology in other mammals, including humans. The aim of this research has been to identify properties of muscle connective tissue that be studied in the living animal with a view to understanding on–farm influences on meat quality. This understanding could be used to optimise on–farm management for improved meat quality. Data is presented from three experiments, designed to characterise muscle connective tissue at the biophysical, biochemical and supra–molecular levels.

INTRODUCTION

Connective tissue is the structural material which binds together and supports the myofibre bundles which make up muscle. It transmi contractions of the muscle fibres through to the bones to produce movement. The amount and development of connective tissue in mus reflects the functions of the muscle and perhaps the animal's recent history in terms of nutrition and physical activity (Mc Cormick 199

It has been known for several decades that connective tissue is more resistent to biological turnover than other soft tissues (Lau 1987). Indeed, it was thought that connective tissues such as cartilage and bone were quite static once deposited, and the name given t these tissues, ground substance, reflected this static view. A more modern view incorporates turnover, but at a rate that is slow relative other tissues of the mammalian body.

In the context of meat toughness, both the myofibrillar and connective tissue components of meat are known to contribute to th final textural properties of the tissue after cooking (Tornberg 1996). In the living animal then, relative rates of turnover of these two components will be important in understanding the impact of on–farm practice on the eating qualities of meat. Meat toughness has bee modelled traditionally in terms of a summation of these two components though this approach ignores synergisms and antagonisms that might arise (Lepetit and Culioli 1994). The connective tissue component of toughness is considered to result from both the amount of collagen in the muscle as well as the number and type of chemical cross–links that bond individual collagen molecules together (Short and Harris 1990; Horgan *et al.* 1991; McCormick 1994). It is the properties of collagen that cause meat to shrink on heating, and the properties of the collagen cross–links that control how shrinkage occurs. The end result for the consumer is the 'chewiness' of the meat Other components of muscle connective tissue may also contribute to toughness and 'chewiness' (Nishimura *et al.* 1996).

A significant research effort has been dedicated to definition of the respective influences of connective tissue and myofibrillar components on meat toughness and several reviews have been presented (Lepetit and Culioli 1994; Purslow 1991; Harris and Shorthos 1988). This article seeks to describe on–farm influences that will affect the connective tissue component with a magnitude sufficient to of commercial significance to the beef production industries.

SOURCES OF VARIATION IN CONNECTIVE TISSUE

So what are some of the sources of variation in connective tissue toughness? Muscles vary in structure both macroscopically and molecularly, according to their location in the animal and function in locomotion, posture or more specific activities. The content and distribution of connective tissue reflects force distribution within these muscles. Muscles develop along trajectories that indicate functional requirements of the animal, eg muscles of the peritoneal wall develop post–weaning to accomodate a larger rumen (Berg and Butterfield 1967). Forequarter development in young bulls correlates with development of butting behaviour. From the context of meat the collagen content of muscles mostly eaten as steaks range from 0.5– 2% of dry weight, whilst muscles in the forequarter, which are mostly eaten as roasts or stews, have contents up to 10%. Despite little difference in collagen content, the same muscles from young animals are often more tender than those from older animals. This can be explained, at least in part, by differences in the number of he stable cross–links in connective tissue collagen (Horgan *et al.* 1991).

Within the bovine breeds, there are variations in muscle structure that reflect the outcomes of long–term genetic selection and evolution. Double muscled breeds and the Japanese Black breed illustrate the significance of selection to the structure of the meat (Ar 1996; Oyama *et al.* 1996), in that the former has markedly different ratios of myofibrils to connective tissue, and the latter has significa infiltration of connective tissue seams by fat cells. Further, *Bos indicus* content has been associated with coarser grain and perhaps, a broader range of toughness values (Shackelford *et al.* 1994).

A characteristic feature of experiments in this area is the proportion of the variation that can be accounted for as individual anim effects. Genetics, recent physical activity and nutritional history are all precedented bases for individual variation. The magnitude of potential effects of physical activity have been shown in studies of muscle atrophy and hypertrophy (McDonald and Fitts 1995; Kim *et a* 1995). Exercise influences the structure of muscle, in terms of size (Morpugo 1897; Jokl 1964), number of capillaries and neural connections (Astrand and Rodahl 1986), endurance capacity and tensile properties (Kovanen *et al.* 1984) and at the molecular level, content and distribution of glycogen and collagen (Zimmerman *et al.* 1993; Thomas *et al.* 1992). In the context of beef cattle production variation between herds could result if one herd routinely walked a distance to get food and water, while another had limited mobility in feedlot.

Variation in nutrition is a natural and frequent phenomenon experienced by young cattle growing in areas with marked seasonal variation in pasture quality, quantity and parasite load. These cattle are, weight-for-age, older than their continuously fed counterparts and therefore have older more mature, intramuscular collagen. This impacts on tenderness (Bouton *et al.* 1978; Shorthose and Harris 1990). Variation in meat tenderness may also result from variation in growth path *per se*. One effect of growth path on tenderness is considered to be through its influence on intramuscular collagen turnover. It is thought that a high plane of nutrition and a more rapid growth rate results in greater rates of collagen synthesis (McCormick 1989). Changes in the collagen cross-link profile are also known to occur (McCormick 1994). Etherington (1987) hypothesised that recently synthesised collagen dilutes the older, heat-stable collagen making it on average more heat-labile. This results in muscle with increased collagen lability and hence more tender meat. McCormick (1994) cautions however, that even though valid under some circumstances, there is a complex relationship between collagen synthesis and changes in collagen characteristics (cross-linking) which cannot be explained satisfactorily by the dilution effect alone. Further, Liu *et al.* (1996) showed recently that collagen solubility and shear force in chicken muscle were not closely related.

In the context of this discussion, we present some recent data on the effects of growth path on the connective tissue component of muscle.

EFFECT OF GROWTH PATH ON CONNECTIVE TISSUE

Three groups of nine month old, Brahman-cross steers were grown through three different paths over 257 days. Two groups were fed a low-quality grass hay diet for 100 days to induce a reduction in liveweight (up to 15%). These groups were then regrown on 2 different levels of nutrition; 1) on a high energy feedlot diet (HE) and 2) on improved tropical pasture (P). A third group (C) were grazed continuously on improved tropical pasture for the entire period. Hip heights were measured as an estimate of long bone growth. Five g of the *semitendinosus* (ST) muscle was removed by biopsy on three occasions: on day 1; towards the end of the weight loss period (day 92); and at 8 weeks into the regrowth period (day 150). Animals were slaughtered at a commercial abattoir (day 257), and processed using 'best practice' procedures. The ST was removed 48 h post-slaughter for measurement of connective tissue toughness by two macroscopic, biophysical techniques (adhesion and compression). Techniques were as described by Allingham *et al.* (submitted). Biopsy and slaughter samples were assayed for connective tissue content, collagen content and hydroxypyridinoline cross-link (HP) to collagen molar ratio.

The growth paths of the three groups of steers are shown in Figure 1. During the restricted diet phase, HE and P steers lost on average 40kg and their long bone growth slowed (0.07 mm/d). Over the same period C steers grew at 0.53 kg/d and long bones grew steadily (0.54 mm/d). When realimented, both HE and P groups gained weight significantly faster (1.2 kg/d and 0.76 kg/d respectively; $P < 0.05$) than the C group (0.55 kg/d). Bone growth during the regrowth phase was more rapid (0.86mm/d) in the HE group compared to P and C groups (0.52 and 0.46 mm/d respectively). Heavy rain resulted in lower feed intakes and hence reduced growth rates in both the P and C groups in the last 20 days of the experiment. At slaughter, the HE group had heavier carcases, higher dressing percentages and more fat coverage ($P < 0.05$) than either of the groups on pasture. The degree of finish determined over the last three weeks of the experiment, may have had some influence on these attributes. The purpose of this experiment however, was to determine the effect of different growth paths on attributes that are not associated with level of finish: that is, connective tissue toughness. This attribute is considered to reflect animal age primarily, and animals in this experiment have all advanced in age an equal number of days.

Biophysical measurements (compression and adhesion) of connective tissue toughness, are presented in Table 1. The force used in these methods is directed in a plane perpendicular to the muscle fibre axis and reflects the connective tissue contribution to toughness (Harris and Shorthose 1988). A comparison of compression values indicated a significant reduction ($P < 0.05$) in the contribution of the connective tissue to toughness of ST muscles from the HE group compared to the C group. The STs from the P group were intermediate. Adhesion measurements indicated similar trends to the compression measurements. It should be noted that overall tenderness as measured by Warner-Bratzler shear force was insignificantly different between the groups (not shown, Allingham *et al.* submitted), suggesting that the myofibrillar component of toughness was not affected by treatment.

Biochemical characteristics of connective tissue from ST biopsies are also presented in Table 1. Overall the connective tissue content of muscle samples (as a % of tissue wet weight, CTC) increased as the animals developed over time, irrespective of treatment. There was a significant difference ($P < 0.05$) between both nutritionally restricted groups and the C group by the end of the weight loss period (93 days). By the end of experiment, the CTC of samples from the HE group was less than that of the P group, but neither re-grown groups were different from the C group samples. There was no significant treatment effect on the proportion of CTC that was collagen. This proportion remained constant throughout the experiment. There was no treatment effect on the HP / collagen ratio. There was however, an age-related increase in HP, independent of growth path.

When collagen content was expressed as a percentage of muscle dry weight, there was a significant difference ($P < 0.05$) between samples from the C group and samples from HE and P groups, after the weight-loss phase (93 days). Eight weeks into the regrowth phase (150 days), differences between the groups persisted. Samples taken after slaughter showed no significant treatment effects. While the data require confirmation, they suggest dynamic changes in tissue collagen content consistent with tissue turnover. This experimental design was inappropriate for definition of the turnover mechanisms operating.

Only the macroscopic measures of connective tissue content and collagen content, show any correlation with the biophysical measures of toughness. One can conclude that whilst advancing age is certainly a contributor to the final state of the connective tissue in ST muscle, features of the growth path are also influencial. The data suggest that a period of severe nutritional restriction in an animal's growth path, can influence the connective tissue toughness of its meat for at least 150 days after the event.

EFFECTS OF NORMAL DEVELOPMENT ON CONNECTIVE TISSUE

The metabolic basis of age variation in connective tissues has attracted a great deal of experimental attention in a number of mammalian systems (Dudhia *et al.* 1996; Thomas *et al.* 1992). Development of connective tissue toughness with age shares some temporal characteristics with, for example, changes in proteoglycan synthesis in cartilage (Sampaio et al. 1988). Age influences the thermal stability of intramuscular collagen (Horgan *et al.* 1991) . Heat-stable cross-links such as HP and perhaps higher order cross-links accumulate within collagen (King 1987; Horgan *et al.* 1990; 1991; Kuypers *et al.* 1992 McCormick 1994). The number of heat-stable

cross-links that are present in muscle at any moment in time depends on the degree of lysine hydroxylation in collagen Types I and III being synthesised, the rate of turnover of pre-existing collagen, the activity of lysyl oxidase in the extracellular space and other factors (McCormick 1994). The thermal properties of collagen provide a convenient means of estimating the total cross-links per unit muscle connective tissue (Flandin *et al.* 1984; King 1987).

In the next series of experiments, the authors sought an assay for development of connective tissue in muscle with advancing a using a method that reflects the supra-molecular properties of collagen in the tissue. Differential scanning calorimetry (DSC) was the technique chosen since it records the summation of all physical interactions between connective tissue macromolecules independent of their complexity and can be applied to the small samples of connective tissue obtainable from sequential biopsies.

The effects of advancing age on *M. longissimus dorsi* (LD) connective tissue thermostability were determined using two group cattle of different genotype: high grade Brahman steers (9 months to 24 months); male and female zebu-derived calves (1/8 Africa 3/4 Hereford x Shorthorn, 1/8 Brahman) from 3 weeks to 12 months of age. Intramuscular connective tissue, free of tendon epimysium, was isolated (Kuypers *et al.* 1992). The endothermal transition profile of the connective tissue was measured in a SETAR DSC III against an equivalent weight of PBS as reference. The thermodynamic parameter, thermal maximum (T_m), was derived using SETARAM integration software, and data were analysed using ANOVA.

Breed had no significant effect on the thermal properties of connective tissue and so all the data have been analysed together, w age as the main effect (Table 2). The average T_m of collagen within the connective tissue samples was remarkably stable in this muscl over the time period studied. This is consistent with conclusions from Shorthose *et al.* (1990) and Berg and Butterfield (1976) that LD muscle has a low postnatal growth impetus. Differences in growth rate within the groups had no significant effect on the thermal stabi of collagen (Hunter, Magner, Allingham and Harper unpublished). When the individual animal data is presented however, it is clear th there is quite marked variation within animals as a function of time (Figure 2). This variation may be due to tissue turnover between biopsy times. The drop in T_m observed at 3 months (90 days) was consistent for both sexes, and it is interesting to speculate about its relation to the nutritional stress of early weaning.

One can conclude that DSC measurements of thermostability are sufficiently sensitive to differentiate temporal effects within t muscle. Even though this variation is consistent with the concept that muscle connective tissue constantly turns-over, the magnitude o the variation is surprising. One wonders about the value of a group average in analysis of the two previous experiments. We are curr working to incorporate as many individual animal attributes as possible, in the modelling of growth path and connective tissue turnover was unfortunate, that DSC could not be applied to the samples from the growth path experiment (previous section), since those data co have reflected another aspect of tissue turnover in response to nutritional stress.

STRUCTURAL VARIATION WITHIN MUSCLES

Another possible explanation for the variation observed in Figure 2, is that the data are confounded by variation between biopsy sites. Johnson and Beattie (1973) have shown that the muscle fibre characteristics of ST muscle vary with depth. The use of sequential biop within one muscle (as was done in the previous two experiments), makes the assumption of little variation between sites separated by l than 10 cm. In a recent experiment, ST muscles were taken from 20 bulls of various ages. These muscles were systematically sliced in 60 roughly cubic sections, such that the three dimensional position of each section was known. The connective tissue characteristics o collagen content, HP / collagen and CTC were measured for each section. The X and Y axes related to the transverse section of the muscle, while the Z axis related to the axial section.

The results showed that collagen content and HP / collagen vary within quite narrow ranges (Harper *et al.* unpublished) and onl show significant differences with position when Z approaches the tendinous insertions of the muscle. This is consistent with the proportion of tendon in the meat increasing toward the ends of the muscle. CTC does vary through a greater range within the populati samples. Despite this variation, it is unlikely that differences of less than 10 cm in the position of the biopsy would lead to the differen described above. Hence, the data presented in Figure 2 is unlikely to be confounded. Over longer distances in Z(eg 20 cm in an adult muscle), variation was found to be significant ($P < 0.05$). While this could result from site specific effects such as healed wound sites, more likely to reflect functional and anatomical features of the muscle.

Structural analysis has received less attention in the literature than other aspects of tenderness. Rowe et al. (1974) have presente fine electron microscopic images showing the orientation of collagen fibrils relative to the myofibrillar long axis and how *postmortem* events can modify this structure. The group headed by Dr. H. Swatland has over an extended period, developed technological approach to characterisation of connective tissue structure in the context of meat texture (Swatland 1996 and others). The optical-probes recent described provide information about the frequency and width of perimysial seams in a one-dimensional slice. Dumont *et al.* (Schmitt *et al.*1979 and others) have described methods with which to quantify structural variation of connective tissue in more than one dimension. Given the known structural flexibility of mammalian muscle in response to external and endocrinological stimulii, it is likely that meat structure may be influenced subtly by on-farm management, and that these changes persist from farm through abattoir. The availability modern image processing tools, provides an opportunity to record structural information in three dimensions and hence investigate the influence of on-farm management on meat structure (ie backgrounding or finishing growth paths). Albrecht *et al.* (1996) have given a good example of the power of these new tools in characterising the distribution of marbling lipid.

CONCLUDING REMARKS

Modern biology allows the meat researcher to choose from a broad range of technologies for studies of muscle and meat. One can cho the appropriate structural level (molecular level through to the whole animal level), the appropriate time scale (mSec through to whole life), or the appropriate experimental system (descriptive or manipulative). Analysis of the meat toughness problem in these terms lea several conclusions. Firstly, given the physics of mastication, the molecular level may be inappropriate for studies of meat, but on the other hand the whole animal level may be unnecessarily complex. We propose that structural levels between the single muscle fibre a the whole muscle are appropriate given what is known about mammalian muscle growth and development. Secondly, there will be a hierarchy of biochemical markers for meat toughness that relates to the time-scale of variation of these markers within the animal. Fo

example, if one is interested in defining markers that are indicative of meat tenderness from on–farm production through to in–restaurant consumption , then markers that have long biological life–times would be appropriate choices. The short half–time of variation in plasma cortisol supports its use as a marker for acute stress (Nwe et al., 1996), but perhaps not growth history. Collagen in intramuscular connective tissue has a half–time of 45 days (Rucklidge *et al*. 1992), and so might be a reasonable marker for longer term effects of growth. Thirdly, experiments need to be manipulative at some level, in order to establish the principles that govern the normal state. This, as we have shown in this paper, can be accomplished within the range of practices that are considered normal by the grazing industry. Our research therefore focusses on treatments that influence growth and muscle composition, and have significance to the eating attributes of the meat when presented to the consumer.

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TABLE 1: Connective tissue attributes during and after variation of growth path. Arithmetic means (± s.e) describing attributes of and necropsy samples of ST muscles.

		Biophysical characteristics		Biochemical characteristics			
Treatment Group	Day of muscle sample[1]	Compression (kg)	Adhesion (kg)	Connective tissue content[2] (% wet wt)	Collagen content (% dry wt)[3]	Connective tissue collagen (% CTC[2])	HP / collagen ratio (10 X mole/mole)
Uninterrupted Growth Control C	1			2.0 ± 0.14[4]	9.8 ± 1.0	20.5 ± 1	0.9 ± 0.1
	93			1.7 ± 0.18[a]	9.0 ± 0.4[a]	20.0 ± 0.5	0.9 ± 0.1
	150			3.1 ± 0.34	12.1 ± 1.2[c]	18.6 ± 0.7	1.5 ± 0.2
	257	2.6 ± 0.1[a]	0.66 ± 0.04[a]	3.9 ± 0.14[d]	8.0 ± 0.4[a]	18.2 ± 0.8	3.0 ± 0.1
Weight Loss / High Energy Re-growth HE	1			2.0 ± 0.21	9.2 ± 0.9	19.2 ± 0.7	0.9 ± 0.05
	93			2.9 ± 0.39[b]	13.7 ± 0.6[b]	19.9 ± 0.9	1.1 ± 0.1
	150			2.9 ± 0.41	16.0 ± 1.9[de]	19.4 ± 0.9	1.5 ± 0.1
	257	2.1 ± 0.07[b]	0.50 ± 0.03[b]	3.3 ± 0.24[bd]	7.2 ± 0.31[a]	19.5 ± 0.7	3.2 ± 0.2
Weight Loss / Pasture Re-growth P	1			1.9 ± 0.19	9.7 ± 0.8	18.9 ± 0.6	1.0 ± 0.05
	93			3.3 ± 0.31[c]	14.8 ± 1.1[bc]	20.0 ± 0.8	1.1 ± 0.1
	150			3.1 ± 0.34	18.9 ± 3.3[d]	17.8 ± 0.8	1.7 ± 0.2
	257	2.4 ± 0.08[ab]	0.58 ± 0.04[ab]	4.5 ± 0.32[d]	7.9 ± 0.2[a]	18.5 ± 0.7	2.9 ± 0.2

[a] Within columns, values with the same superscript are not significantly different at the $P = 0.05$ level.
[1] Time measured relative to the start of the weight-loss phase.
[2] Physically extractable intramuscular connective tissue.
[3] After extraction of total lipid.
[4] Arithmetic mean ± standard error, n = 12.

TABLE 2. Thermal maximum values (T_m,, in °C) for intramuscular connective tissue collagen from the LD muscles of male and fe cattle.

	Animal Age						
Sex	7 d	3 mo	9 mo	12 mo	13 mo	18mo	20mo
Male	63.2 ± 0.1[1]	62.9 ± 0.1	63.7 ± 0.1	63.9 ± 0.1	63.4 ± 0.1	63.5 ± 0.1	63.7 ± 0.1
Female	63.4 ± 0.1	63.1 ± 0.03	nd	64.0 0.1	nd	nd	nd

nd, no data available
[1] Arithmetic means with standard error of the mean of 6–9 animals.

FIGURE 1. Mean live weight and hip height over time for 3 groups of Brahman –cross steers on different growth paths. The filled black box denotes the period of dietary restriction. The symbols are: (●), control, C group; (O),high energy feedlot diet, HE; (■), improved tropical pasture diet, P.

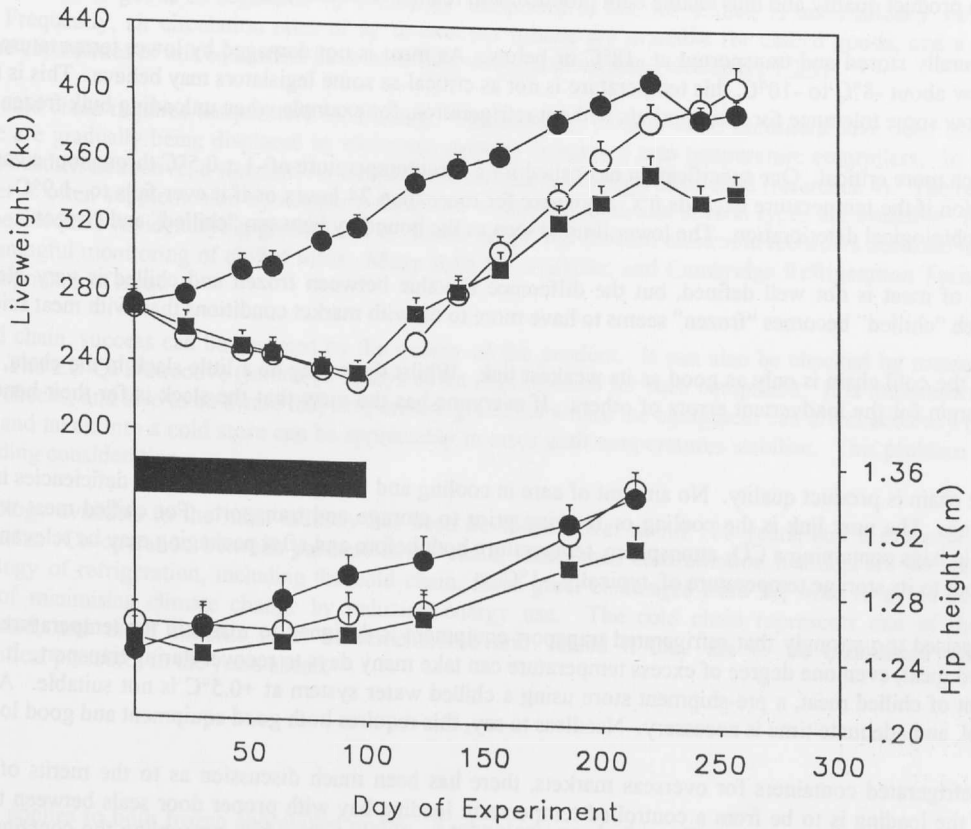

FIGURE 2. Variation in T_m in muscle sequential biopsy samples from individual animals. The dotted and solid lines indicate animals within two distinct growth experiments. The dashed lines represent the extreme trend lines encompassing the data.