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Review

The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness

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Abstract

Application of electrical stimulation in the sheep and beef processing industry has been erratic around the world and this may reflect an incomplete knowledge of how to optimise the technology. Although it is well established that stimulation increases the rate of post-mortem glycolysis, other biochemical and biophysical effects have been implicated with the use of this technology. This review seeks to examine the current theories about the effect of stimulation on post-mortem muscle. The classical view that stimulation prevents muscle from shortening excessively during rigor development has been expanded to include the possibility that it also results in physical disruption of muscle structure. The interaction of these effects with the acceleration of the rate of proteolysis through activation of the calpain protease system has not been comprehensively reviewed in the past. Thus there are two mechanisms which could explain the effect of stimulation on tenderisation, reduced 'cold-induced' shortening and alteration of protein structure. A secondary effect is the enhancement of the rate of proteolysis stimulated by release of Ca^{2+} at a higher temperature. As a result of this review we highlight several areas that may prove fruitful for further research. The challenge for further development of electrical stimulation systems is optimisation of the activation of the enzyme systems in parallel with manipulation of chilling regimes so as to ensure rigor mortis is achieved at temperatures which minimise shortening. These optimal temperatures largely established at a fixed incubation temperature for detached muscle may be different when measured in intact carcasses. The potential of regional stimulation of sections of the carcass to achieve this outcome is worthy of study given the different fibre composition of muscles and temperature gradients. In addition, to ensure that appropriate amounts of energy are applied to individual carcasses, development of self-response stimulation units, which are able to determine carcass resistance and apply appropriate durations or strengths of stimulation is worthy of future research. This would lead to more effective electrical stimulation practices.

Keywords: Lamb; Beef; Stimulation; Proteolysis; Shortening; Ultrastructure

Contents

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1. Introduction

The historical reason for the development of electrical stimulation was the acceleration of post-mortem glycolysis so that when muscle entered rigor it was prevented from shortening excessively (Swatland, 1981). Other potential effects of stimulation on post-mortem muscle were reviewed by Cross (1979) and subsequent research has attempted to clarify the importance of these effects. These effects are described as either physical disruption of the myofibrillar matrix (Ho, Stromer, Rouse, & Robson, 1997) or the acceleration of proteolysis (Uytterhaegen, Claeys, & Demeyer, 1992). It has not been agreed upon as to which of these effects is important in terms of reducing the toughness of meat.

A divergence of views may in part reflect the variety of different experimental techniques that have been used to study the effects of stimulation, but it probably also reflects sources of unknown variation. This point is illustrated by considering some recent results (Devine, Wells, Cook, & Payne, 2001; Hopkins, Littlefield, & Thompson, 2000a). In the latter experiment conducted under very controlled conditions where the same type of lambs were used and these were handled identically pre-slaughter and post-slaughter, there was a significant difference between slaughter days in the rate of decline of pH after low-voltage stimulation. There was no effect of stimulation or day of slaughter on measures of toughness. In general under commercial situations, stimulated carcasses enter rigor mortis at a higher temperature, so differences in tenderness could be attributed to the effects of faster rates of tenderisation. In one study, Devine et al. (2001) found a wide variability in the tenderisation rate for samples held at 10°C after electrical stimulation, where differences in sarcomere length were not significant and there was no detectable difference in rate of tenderisation and final tenderisation compared to non-stimulated samples. In this study, the large variability and small number of animals meant it was not possible to determine whether stimulation really did affect tenderisation rate when temperature differentials were accounted for. The issue was revisited using 350 lambs in which loin muscle entered rigor mortis at the same temperature irrespective of whether or not the carcass had been stimulated. In a preliminary paper on these results, it has been reported that electrical stimulation did result in a greater rate of tenderisation (Devine et al., 2002). It now appears that stimulation may alter tenderisation rates by another mechanism and this is an obvious area for further study. In this review we describe the results of studies that have dealt with the three classical areas by which electrical stimulation is proposed to elicit changes in post-mortem muscle. These are (1) prevention of cold-induced shortening by ensuring rigor mortis occurs under optimal conditions, (2) physical disruption of the muscle fiber and (3) acceleration of proteolysis. Acceleration of proteolysis could be classified as a secondary effect mediated through the time–temperature–pH interaction, affecting factors such as enzyme stability and activity. Research in this latter area has been active in recent years as a by-product of study on muscle enzyme systems and it is timely that this area come under review. The review will attempt to identify areas for further research and to place current knowledge in context where the ultimate aim is to reduce the toughness of sheep and beef meat.

2. Electrical stimulation and the rigor process

2.1. Why electrically stimulate?

Tenderness is a consumer evaluation where high numbers indicate greatest consumer appreciation; toughness is the inverse of this. Shear force is an objective instrumental measure where high shear values indicate increasingly unacceptable meat. Electrical stimulation of carcasses after slaughter is a process that can have a

significant effect on meat toughness, but electrical stimulation is not universally used and the reasons are not clear. While it has been used for pigs, deer, goats, sheep, cattle, and various poultry species, in this review we are only concerned with sheep and cattle.

The use of electrical stimulation for limiting meat toughness is not new (Harsham & Deatherage, 1951; Renschler, 1951), although incorporation in a practical system was first used in New Zealand and then Australia to avoid toughness resulting from cold induced shortening. The major requirement in the New Zealand situation was to accelerate the onset of rigor mortis so that it occurred at temperatures above those likely to result in cold induced shortening before the meat was frozen whether from sheep (Chrystall & Hagyard, 1976) or cattle (Davey, Gilbert, & Carse, 1976). For small, easy-to-chill lambs the improvement was dramatic ensuring that the majority of rapidly frozen meat was acceptable, especially after further ageing. For larger cattle, where conditions, which induce, cold shortening may not always be encountered, then the question of whether or not it is necessary to use electrical stimulation is not clear if the assumption is made that reduction of cold induced shortening is the main aim.

2.2. What happens with electrical stimulation?

Electrical stimulation involves passing an electric current through the body or carcass of freshly slaughtered animals. This electric current causes the muscles to contract increasing the rate of glycolysis resulting in an immediate fall in pH (ΔpH that ranges from 0.6 pH units at 35 °C to 0.018 units at 15 °C). The energy of activation of ΔpH in stimulated beef *m. sternomandibularis* is calculated to be 97 kJ/mol (Chrystall & Devine, 1980), or very similar to that for calcium-activated actomyosin ATPase (Bendall, 1969). Following the ΔpH there is a temperature dependent acceleration of glycolysis (dpH/dt) and subsequent early rigor mortis development. In non-stimulated beef *m. sternomandibularis* the energy of activation of the glycolytic process is 40–45 kJ/mol, whereas that for stimulated muscle approaches 70 kJ/mol (Chrystall & Devine, 1980). This high energy of activation means in both cases that any cooling of the muscles will markedly increase the time for attainment of rigor mortis with a larger effect in stimulated muscle.

Studies by Jeacocke (1977) characterised this temperature effect and showed that for non-stimulated muscle the rate of glycolysis was faster at higher temperatures and fell to a minimum at 12 °C and then increased again. This latter increase arises because of the extra ATP required for a cold contracture. When muscle is maintained at a constant temperature, the increased rate of pH fall after stimulation seems to occur with all stimulation parameters and even occurs as a consequence of electrical stunning (Devine, Ellery, Wade, & Chrystall, 1984). Although the pH falls are lower with low-voltage stimulation, it achieves the same rate of pH fall as high-voltage systems (Chrystall, Devine, Ellery, & Wade, 1984). The various types of parameters are covered elsewhere.

While the avoidance of the deleterious effects of cold induced shortening through the use of electrical stimulation has been established, the question arises as to whether this is the main effect or are there other effects independent of cold shortening? In order to establish this, it is essential to have a common baseline and to understand how the various shortenings occur and how they might affect tenderisation.

One factor that obscures the direct effect of stimulation, is the accelerated development of rigor mortis so that ageing commences at higher temperatures and, therefore is more rapid (Davey & Gilbert, 1976). Comparisons need to be made at a constant temperature and tenderness changes indexed to a common point such as rigor mortis (Rigor is a term applied to individual muscle fibres becoming depleted of ATP, whereas rigor mortis is a term that refers to the muscle stiffness that occurs after all muscle fibres enter rigor) at which point tenderisation is considered to commence (Devine & Graafhuis, 1995). Therefore, if the temperature conditions at rigor mortis were constant would there be any difference between stimulated and non-stimulated muscle? In commercial processing situations, this situation is difficult to achieve but can be investigated experimentally. In one case 24 lambs were either electrically stimulated or non-stimulated and chilled at 10 °C and there was no significant difference in initial shear force, rate of change of shear force or final shear force attained (Devine et al., 2001). However there was significant variability between carcasses in the ageing rate. With a larger group of 350 lambs under identical constant temperatures, where commencement of ageing was also indexed to rigor mortis, the shear force achieved was reported in a preliminary paper to be lower in stimulated muscle (Devine et al., 2002). This raises the question of the involvement of other mechanisms.

When temperature conditions are not identical there are clearly other factors that can affect the way stimulated and non-stimulated muscles tenderise. The reasons for this can be extrapolated from characteristics of the rigor process; the phenomenon of rigor shortening and the temperature related factors affecting tenderisation.

2.3. Rigor mortis and rigor contracture

Rigor mortis occurs in muscles when all supplies of energy are exhausted (depleted of ATP) (Bendall, 1969). This does not occur across all muscles simultaneously with a concomitant fall in pH and Jeacocke (1984) showed for single fibres, that there was a contracture as

the final ATP disappeared (i.e. rigor) and each fibre had its own time course depending on initial glycogen. A small temperature-dependent degree of contracture occurs for each muscle fibre as it enters rigor. The onset of the sequential progression into rigor for each muscle fibre, can be tracked by measuring isometric tension and muscle shortening (Devine, Wahlgren, & Tornberg, 1999; Hertzman, Olsson, & Tornberg, 1993; Olsson, Hertzman, & Tornberg, 1994) where the development of isometric tension increases at higher temperatures (Hertzman et al., 1993). Above 10–15 °C, the traces reveal a steady increase in tension pre-rigor and continuous shortening. Such traces can be interpreted as arising from a succession of individual muscle fibres, each exhausting their energy reserves (i.e. rigor) and each separately shortening (Jeacocke, 1984); these sum to create tension. Stiffness is a consequence of each single fibre going into full rigor, with irreversible cross bridge formation of the contractile components, actin and myosin. With increasing numbers of fibres entering rigor, the stiffness increases and is significant when the muscle reaches a pH of approximately 6.0. At some stage the bulk of stiff muscle prevents significant shortening of fibres not in rigor, from a cold contracture (if exposed to low temperatures).

The classical studies of Locker and Hagyard (1963) with muscle entering rigor at different temperatures showed minimal shortening at close to 15 °C (for excised muscle) and this correlated with minimal meat toughness (e.g. Tornberg, 1996). This correlation was fortuitous as elevated temperatures not only increase toughening due to heat shortening, but also reduce tenderisation (Devine et al., 1999). Above 12–15 °C, a contracture occurs at rigor and below this temperature a contracture occurs before rigor. Thus the shortening effects above 15 °C are a consequence of rigor shortening only and occur when muscles become depleted of glycogen. As the temperature falls below 12 °C a pre-rigor contracture takes place until rigor is completed. This arises from increased cellular calcium from the sarcoplasmic reticulum with falling temperature that in turn activates actomyosin ATPase. The rise of calcium in the cytoplasm under these conditions is due to the failure of the sarcoplasmic reticulum to sequest cytoplasmic calcium (Jaime, Beltrán, Ceña, López-Lorenzo, & Roncalés, 1992) and is different to the transitory rise in 'free' calcium that occurs after electrical stimulation. The balance of calcium under the latter conditions is potentially an important factor in the activation of enzymes such as the calpains.

2.4. Start of tenderisation

A critical aspect in unraveling the intricacies of electrical stimulation is the definitions used for tenderness, toughness, ageing and proteolysis. Tenderisation is the generalised term for the process that leads to improvement in tenderness and in reality can only be measured post-rigor. A measure of tenderness is the subjective consumer appreciation of the meat and a high score is desirable and connective tissue and the amount of intramuscular fat can influence it. An objective measure of tenderness is the force required to shear a standardised piece of meat with low shear values being desirable. Proteolysis affects all muscle proteins, including connective tissue, but its outcome is different if it affects the contractile proteins actin and myosin rather than the structural or cytoskeletal proteins. The term proteolysis is used in this review in reference to the degradation of structural proteins and this can precede tenderisation (i.e. before rigor).

Tenderisation occurs as the structural proteins are degraded (i.e. proteolysis). Proteolysis can continue even in shortened muscle without the meat-becoming tender (Locker & Wild, 1984; McDonagh, Fernandez, & Oddy, 1999). If the muscle has been stretched, then it is more tender (Hopkins, Littlefield, & Thompson, 2000b), but proteolysis may not have occurred to the same extent as non-stretched meat for the same shear force changes. Ageing is the process where meat becomes tenderer over time and involves specific degradation of the structural proteins.

The processes affecting meat tenderness start at slaughter, but changes may not be significant at that time and also measurement of tenderness at this stage is also meaningless. The endogenous enzymes responsible for tenderisation will be active throughout the rigor process. While proteolysis is taking place, significant tenderness changes are not evident until most of the muscle fibres are in rigor (Devine & Graafhuis, 1995). Because an intact muscle is a collection of individual muscle fibres entering rigor in succession, intact muscle would *appear* to start to age (tenderise) before rigor is complete in all fibres. The development of rigor and the shortening of fibres would be expected to counter early proteolysis so that the expected peak in shear force is eventually negated by the cumulative post-rigor proteolysis. Once this reverses the rise in toughness resulting from rigor contractures the process of tenderisation occurs. Under cooling conditions, the falling rigor temperatures means that those fibres at elevated temperatures will enter rigor early and will experience, initially faster tenderisation (Graafhuis, Lovatt, & Devine, 1992). Thus tenderness measured at the completion of *rigor mortis* (the earliest possible time) will be substantially different for electrically stimulated muscles than for non-stimulated muscles.

2.5. Effects of rigor mortis temperature on tenderisation

It had been initially thought that the minimal shortening which occurs when rigor mortis is attained at

15 °C was the explanation for why such meat had the lowest shear force. However, it has been shown in beef that when shortening was prevented by tight wrapping and rigor mortis occurred at a range of temperatures from 15–35 °C, that shear force was greater at the high rigor mortis temperatures. This difference was maintained with ageing at 4 °C (Devine et al., 1999). In a preliminarily paper Simmons, Singh, Dobbie, and Devine (1996) measured calpain activity throughout the rigor process and they showed that calpain activity remained constant at all temperatures until a pH of approximately 6.2, then the activity decreased. These conditions of low pH and high temperature are known to denature the contractile proteins which are more stable at rigor mortis (Offer, 1991). Such conditions, in conjunction with greater autolysis of calpains at high temperatures (Dransfield, Etherington, & Taylor, 1992) would explain how ageing enzymes are reduced in effectiveness so that both shear force increases and the ageing potential is reduced. Of some interest is the finding by Hwang and Thompson (2001b) that the most tender beef meat after 14 days of ageing was achieved when the temperature at pH 6.0 was 29–30 °C under in situ conditions. This highlights that caution is needed when extrapolating from the in vitro to in situ states. A long duration at elevated post-mortem temperatures and low pH therefore may be critical in terms of calpain inhibition and toughening.

2.6. Optimum tenderisation

The tuning of the amount of stimulation with chilling rate to reach rigor mortis at 15 °C resulted in optimum tenderisation in several studies. If there is a rapid pH fall resulting from stimulation within 2 min of slaughter, the meat is not as tender after 3 days of ageing at 4 °C as when stimulation takes place 30 min after slaughter (rigor mortis approximately 15 °C). However, both are tenderer than non-stimulated meat (Wahlgren, Devine, & Tornberg, 1997). In time, after 14 days ageing, the meat all becomes tender, but the meat stimulated at 30 min is still the tenderest. Temperature records showed that the meat stimulated at 30 min attained rigor mortis close to 15 °C. This preliminary report suggests that meat attaining rigor mortis close to 15 °C will be more tender than meat entering rigor mortis at other temperatures, but given the results of Hwang and Thompson (2001b) further studies are needed to determine the precise processing situations required to achieve optimum tenderisation.

Although there is an early increase in tenderness because of stimulation, this tenderisation from electrical stimulation alone cannot be distinguished from any ultrastructural effects; the tenderness status is merely a consequence of the interplay of muscle fibres that have or have not entered rigor early under different temperature conditions. Further to this you can get an increase in rate of proteolysis without a difference in shear force between stimulated and non-stimulated muscle (Hopkins & Thompson, 2001a) and this can even occur where both control and stimulated meat has high shear force values (Hopkins & Ferrier, 2000). Clearly dissecting out the relative contribution of each factor is a significant challenge.

3. Ultrastructural alteration and its relation to meat tenderness

3.1. Physical changes in myofibrillar structure

Electrical stimulation improves meat tenderness either through its effects on physical alteration (i.e. prevention of cold shortening or causing physical disruption) and/or acceleration of energy turnover during and after the treatment. There is potentially a powerful association between physical disruption of the myofibrillar complex and increases in tenderness (Dutson, Smith, Savell, & Carpenter, 1980; Ho, Stromer, & Robson, 1996). It is unclear whether it is the physical disruption per se or whether the physical disruption facilitates ageing in other ways. Contracture bands are not a direct consequence of electrical current passing through the muscle, but rather due to the supercontracture caused through localised excessive calcium ion release from the sarcoplasmic reticulum. It could be this extra calcium which also causes the tenderisation to proceed. This section will attempt to shed insight about the relative importance of ultrastructural alteration to the effects seen as a result of electrical stimulation.

Dutson, Yates, Smith, Carpenter, and Hostetler (1977) first reported that electrical stimulation resulted in ultrastructural changes in beef *m. longissimus*. Histological images showed the appearance of contracture bands containing predominantly stretched, ill-defined and disrupted sarcomeres similar to those illustrated in Fig. 1. This led the authors to hypothesise that physical disruption per se lowers the resistance to either chewing or mechanical shearing force. The increase in tenderness due to disruption was a plausible finding as the historical reason for the use of electrical stimulation was to prevent cold shortening, yet the applied stimulation treatment improved tenderness under circumstances where the carcasses did not suffer from cold shortening (Savell, Dutson, Smith, & Carpenter, 1978). An earlier study prior to the introduction of electrical stimulation showed that extreme fibre disruption resulted in an increase in tenderness (Marsh, Leet, & Dickson, 1974). Similarly, other studies have advocated the link between physical disruption and improved tenderness for high (300–500 volts) (Takahashi, Wang, Lochner, & Marsh, 1987; Will, Ownby, & Henrickson, 1980) and for

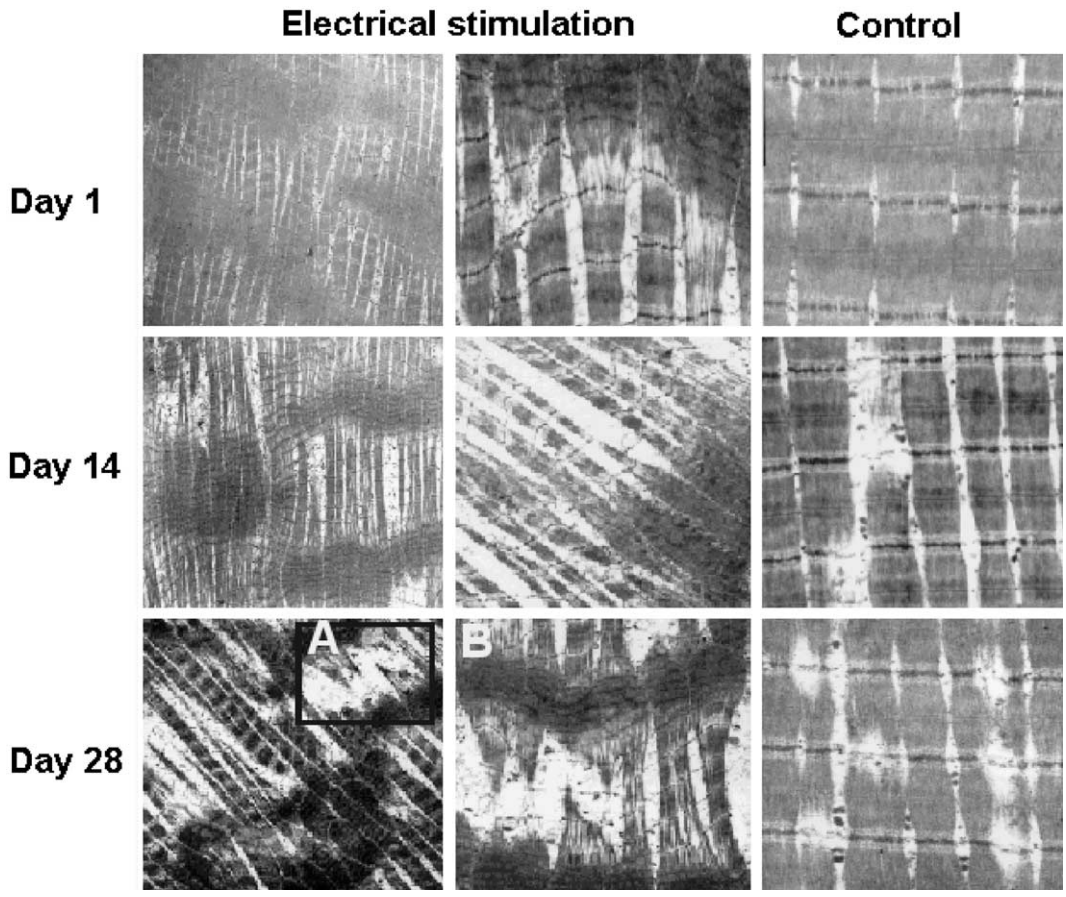

Fig. 1. Characteristics of contracture bands in electrically stimulated beef *m. longissimus* compared to controls, and changes during ageing. Photograph 'B' of Day 28 is a higher magnification of square 'A' of Day 28. Cited from Hwang and Thompson (2002).

intermediate voltage (145–250 volts) (Ho et al., 1996; Sorinmade, Cross, Ono, & Wergin, 1982) systems.

A study of the effect of electrical stimulation on both ultrastructure and pH fall for several muscle fibre types by Devine, Ellery, and Averill (1984), showed that red muscles like the masseter, did not exhibit an increase in rate of pH fall, but did show evidence of supercontracture when stimulated. This muscle was also more susceptible to cold shortening. White muscles like the cutaneous were not so susceptible to cold shortening, and had almost no supercontracture and the rate of pH fall was increased by stimulation.

3.2. Interaction of stimulation types and supercontracture

Unlike other studies (e.g. Dutson et al., 1977; Ho et al., 1996) which relied on a simple comparison between stimulated and non-stimulated muscles, Takahashi, Lochner, and Marsh, (1984) and Takahashi et al. (1987) investigated types of stimulation, formation of contracture bands and their relation to improvements in tenderness. These studies showed that stimulation at 50–60 Hz with 500 volts 40 min post-mortem resulted in severe structural alteration (examined three carcasses out of a total of seven carcasses for each treatment) and improved tenderness, whereas 2 Hz failed to improve meat tenderness with no structural alteration. Unfortunately the stimulation parameters were not adequately described and it is not clear in terms of rate and extent of pH fall how effective they were.

A number of studies, on the other hand, have questioned that alteration in the sarcomere structure results from stimulation, because contracture bands appeared to be related to denaturation of sarcoplasmic protein (George, Bendall, & Jones, 1980) or due to preparation artefacts (Fabiansson & Libelius, 1985). In the study of George et al. (1980) where beef carcasses were kept at 16 °C for 8 h after stimulation (700 volts, 25 pps, for 2 min, at 60 min post-mortem), the appearance of contracture bands had a similar appearance with these in PSE pig muscles. This raised the possibility that the formation of contracture bands in electrically stimulated muscle was related to localised protein denaturation and the authors dismissed the link between physical alteration and improved tenderness. This conclusion was based on the fact that contracture bands were not observed soon after stimulation, but there was no control to verify the conclusion. Supercontracture is not specific to stimulated muscle as it has been observed in

PSE (Bendall & Wismer-Pendersen, 1962), thaw shortened (Stromer, Goll, & Roth, 1967), cold shortened (Marsh et al., 1974) and high temperature treated muscles (Fabiansson & Libelius, 1985). Because supercontracture is considered related to the loss of calcium regulation by the sarcoplasmic reticulum or/and the mitochondria (Cornforth, Pearson, & Merkel, 1980; Davey & Gilbert, 1974; Whiting, 1980), we consider that the contracture bands result from supercontracture of the fibres rather than being due to protein denaturation. This suggests that they are a consequence of abnormal, perhaps localised calcium release from the sarcoplasmic reticulum through a tetanic contracture. Electrical stimulation may be only one of the initiators as discussed below. Such changes are fibre type specific and depend on the duration and effectiveness of the applied stimulation.

Formation of contracture bands is dependent on current frequency (Marsh, 1985; Takahashi et al., 1987) or the interaction between current frequency and voltage (Hwang & Thompson, 2002). If the time interval between successive stimuli is more than approximately 0.25 s, the muscle tetanic shortening is reversible. On the other hand, when a higher frequency of current is applied, muscle may not have enough time for relaxation between succeeding twitches, and forms irreversible contracture bands. Even though practical stimulation systems have focused on acceleration of glycolysis, the pulse frequency and voltage of electrical stimulation systems used in most studies appeared to be high enough to avoid this confounding effect. For example, in the study of Hwang and Thompson (2002), a 14.3 pps system (800 volts, for 55 s, at approximately 45 min post-mortem) and a 36 pps system (45 volts, for 45 s, soon after stunning) resulted in 89 and 55% of the specimen blocks containing contracture bands, respectively. With some exceptions in a few studies (e.g. George et al., 1980), it appears that ultrastructural alteration takes place in electrically stimulated muscle and that is related to improved meat tenderness to some extent. However, its relative magnitude compared to other factors such as enzymatic tenderisation is a subject of debate. In order to elucidate the cause of contracture bands in electrically stimulated muscle, use of a chilling regime which avoided heat or cold shortening would be required as part of the experimental design.

3.3. Interaction between physical changes in sarcomere structure and proteolysis

Electrically stimulated muscle exhibited faster degradation as assessed by examination of microscopic images (Fabiansson & Libelius, 1985) and emergence of protein degradation products (Ho et al., 1996). In the latter study, low-voltage electrical stimulation (200 volts, 20 Hz) caused contracture in beef *m. longissimus* with a concomitant increase in the degradation of titin, nebulin, desmin and troponin T. However the studies did not examine a quantitative link between physical disruption and enhanced endogenous proteolytic activity and the results of Ho et al. (1996) were different to those reported in a subsequent experiment (Ho et al., 1997).

There is no doubt that a few breaks in myofibrils at the micro-level can significantly affect meat tenderness (Dransfield, 1994) and this is seen in muscle that has been superstretched (Hopkins et al., 2000a). This concept was partly demonstrated in model systems by Purslow (1985) who found that a nick in a tensile sample significantly reduced breaking stress of cooked meat and the reduction was dependent on the length of initial nick. There are practical limitations with electron microscopic examination of muscle tissue, given that images only represent micro areas, but Fig. 1 illustrates that the extent of muscle disruption in a physically damaged area at day 1 post-mortem was already greater than that in control muscle at 28 days post-mortem. A quantitative study of beef *m. longissimus* also showed that sarcomeres adjacent to the contracture nodes had a higher frequency of I-band fracture (Ho et al., 1996). It is possible that physical stretching/tearing leads to an acceleration of proteolysis as a result of greater exposure of proteolytic substrates within muscle fibres, in addition to the direct effect of physical tearing. However, this remains to be established because there is no direct evidence available to support this notion. On the other extreme, Locker (1984) observed that when muscle was cold shortened gap-filaments were retracted into the A-band and hypothesised that this might be related to the failure of cold shortened meat to tenderise. The theory needs further examination because as outlined earlier McDonagh et al. (1999) showed that proteolysis continued in cold shortened lamb as did Locker and Wild (1984).

Unfortunately, most electron microscopic studies have presented representative photographs of the *m. longissimus,* and we have little knowledge about the frequency of structural alteration across a muscle or carcass. In a quantitative manner, Will et al. (1980) reported that electrical stimulation (300 volts DC, 400 pps with a 0.5 ms duration, for 5 min, at 30 min post-mortem) caused structural alteration in the myofibril component of beef *m. longissimus*, but not in *m. psoas*, *m. semitendinosus* or *m. supraspinatus*. Unfortunately the study did not report on tenderness or proteolysis. Sorinmade et al. (1982) commented that stimulation at 60 Hz (145–250 volts, for 2 min, at 60-min post-mortem) elicited approximately 30% physical damage in beef *m. longissimus* and that this coincided with tender meat.

A number of studies have indicated indirectly that physical disruption had less effect on tenderisation than did proteolysis (Pommier, Postes, & Butler, 1987; Unruh, Kastner, Kropf, Dikeman, & Hunt, 1986). These studies showed that stimulation treatment with a

slow chilling condition resulted in tougher meat than non-stimulated sides, consistent with the effects of elevated temperatures on toughening. In the latter study, Holstein veal calves (45 kg) were electrically stimulated (45 volts, 2 s on, 2 s off, for 80 s, during bleeding) and placed in a 12 °C cooler until the following day. In the light of more recent studies, it is clear these conditions favour autolysis of μ-calpain rather than proteolytic activity, subsequently reducing ageing potential (Dransfield et al., 1992; Hwang & Thompson, 2001b; Simmons et al., 1996). The evidence suggests that both physical disruption as well as effects on the calpain system arise as a consequence of stimulation. For example, even if there was a certain amount of physical benefit from stimulation, this might be eroded by a greater detrimental effect on the calpain system. One factor that needs to be explained is the observation that physical alteration did not improve meat tenderness when meat was relatively tender (Hwang & Thompson, 2002). In this study, shear force of beef *m. longissimus* for non-stimulated control, high-voltage and low-voltage stimulated samples were 4.8, 4.1 and 4.0 kg, respectively, at 1 day post-mortem. These observations still leave open the question of the relative importance of physical alteration in electrically stimulated carcasses to improvements in meat tenderness.

There is no direct solid evidence available at the present time to discount the importance of physical alteration in electrically stimulated carcasses, whilst some studies have shown that ultrastructural alteration coincided with improved tenderness. For more direct evidence, quantitative studies examining ultrastructural alteration, shortening and proteolysis are necessary under the same experimental conditions. Given that most electron microscopic studies have tended to focus on *m. longissimus*, it is necessary to extend the scope across muscles.

3.4. Impact of physical disruption on ions

The effects of physical disruption on the integrity of muscle structures such as cell membranes and calcium reserve organelles (sarcoplasmic reticulum, mitochondria and DNA) is also of interest with regard to calcium-dependent enzyme activity; especially myosin-ATPase and calpains. Calcium concentration in the intracellular space increases during stimulation (Westerbland & Allen, 1991), but the released calcium ions are taken back to the resting state into the sarcoplasmic reticulum if energy reserves are not completely depleted during stimulation treatment (Chrystall & Devine, 1985). If the damage is great enough to cause early release of calcium ions into the cytosol, this will have a direct effect on activation of the calpain system and muscle shortening.

Boehm, Kendall, Thompson, and Goll (1998) hypothesised that the 'free' calcium concentration can be raised to over 100 μM by an influx of extracellular calcium ions into the myofibrillar space and that the degradation of costameres and a loss of sarcolemmal integrity occurred. If physical disruption significantly increases the "leaking" of calcium ions, stimulation treatment will result in an increase in 'free' calcium ions in the cytosol. This extraordinary amount of 'free' calcium ions in the presence of a high-energy source could cause rigor shortening and a reduction in extractable μ-calpain activity in vitro. However, a number of studies have shown that μ-calpain levels were either constant or increased after stimulation treatment (Ducasting, Valin, Schollmeyer, & Cross, 1985; Morton, Bickerstaffe, Kent, Dransfield, & Keeley, 1999; Uytterhaegen et al. 1992). Horgan and Kuypers (1985) reported that electrical stimulation reduced the yield of sarcoplasmic reticulum in beef muscle, suggesting an increased release of calcium ions from electrically stimulated muscle. It has yet, however, to be determined whether the result was related to physical disruption or fast glycolysis as Hopkins and Thompson (2001b) showed that 'free' calcium concentration was a function of pH decline. This suggests that the tearing of muscle structure itself has little effect on the increase in 'free' calcium ions in the cytosol. Further even if there was a severe effect on cell membrane and calcium containing organelles, then it suggests that the binding between calcium ions and proteins was not altered and consequently the extractable levels of 'free' calcium were not affected by stimulation per se.

It has been shown in several cases that commercially used stimulation systems did not elicit activation of m-calpain (Hwang & Thompson, 2001a; Morton et al., 1999; Uytterhaegen et al., 1992). However, any physical effect on the release of calcium ions is of a particular interest because of the question about whether m-calpain activity can be influenced by electrical stimulation. There are more aspects to be understood about effect of electrical stimulation on 'free' calcium concentration, which includes its effects on the interaction between calpains and their inhibitor, and on other calcium requiring enzymes and kinases.

4. Stimulation effects on degradation of myofibrillar proteins

4.1. Effect of electrical stimulation on proteolysis

The activity of cysteine proteases enzymes, most notably the calpains has been shown to be sensitive to the prevailing pH and temperature of the meat (Dransfield, 1994; Simmons et al., 1996). Since electrical stimulation alters the post-mortem pH/temperature

relationship it is reasonable to expect that proteolysis would also be effected. The degradation of myofibrillar proteins during the post-mortem period has been studied using a number of techniques, including fragmentation of myofibrils (Olson, Parrish & Stromer, 1976), determination of 'free' amino acids (Field & Chang, 1969), measurement of protein solubility (Claeys, Uytterhaegen, Demeyer, & DeSmet, 1994), measurement of non protein nitrogen (Davey & Gilbert, 1966) and gel electrophoresis (Olson, Parrish, Dayton, & Goll, 1977; Penny & Ferguson-Pryce, 1979).

From the studies which have been summarised in Table 1 it appears that there are conflicting results with respect to whether or not stimulation increases the rate of proteolysis. However, of the techniques used to follow changes in the myofibrillar proteins, electrophoresis is the most common and this approach has some potential limitations which is exemplified by comparing the results of Ho et al. (1996, 1997). Very similar electrical inputs were used in both experiments, with different cattle breeds, but otherwise the designs were similar, yet Ho et al. (1996) in one set of results found that stimulation caused a faster degradation of proteins such as titin and troponin-T in some muscle. In another experiment, Ho et al. (1997) reported no such effect. In both cases the interpretation of the gels was based on visual assessment, whereas in the work reported by Uytterhaegen et al. (1992) and Hopkins and Thompson (2001b) techniques to quantify the change in protein bands were used. Even this approach however can produce contrary results as seen by comparing the reports of Pommier et al. (1987) and Pommier (1992).

Another deficiency in many studies is the use of single measurements of proteolysis during the post-mortem period. This could explain why stimulation was reported in several studies to have no effect on protein solubility (Bruce, Jones, & Ball, 1990; den Hertog-Meischke, Smulders, van Logtestijin, & van Knapen, 1997; Whiting, Strange, Miller, Benedict, Mozersky, & Swift, 1981). In these cases the rate of change may have been different between death and subsequent measurements which was evident in the work of Hopkins and Thompson (2001b) and also for MFIs in the work of Geesink, Smulders, van Laack, van der Volk, Wensing, and Breukink (1993) at least in the *m. longissimus*. By contrast Sonaiya, Stouffer, and Beerman (1982) found no significant effect of stimulation on the rate of change in MFIs in three muscles as reported by Geesink et al. (1993) for the *m. semimembranosus*. However the decrease after rigor of MFI values in the work of Sonaiya et al. (1982) points to a technique problem given that the effect of proteolysis becomes more marked as meat ages which should drive MFI values up (e.g. Geesink et al., 1993). Additionally the use of pre-rigor samples for MFI determination is fraught with dangers. This is because pre-rigor muscle will shorten when excised (Parrish, Young, Miner, & Andrews, 1973) confounding interpretations.

An interaction between stimulation and chilling temperature on proteolysis (Pommier, 1992; Rhee, Ryu, Imm, & Kim, 2000; Salm, Forrest, Aberle, Mills, Snyder, & Judge. 1983) signifies the complexity of post-mortem events. However in weighing up the evidence, keeping in mind the constraints of comparing different stimulation systems it appears that stimulation does increase the rate of post-mortem proteolysis. In drawing this conclusion it is worth noting that few studies have included different indicators of proteolysis in the experimental design. Where that was the case our conclusion is supported (Geesink, van Laack, Barnier, & Smulders, 1994; Hopkins & Thompson 2001b; Pommier, 1992), although results presented by Sonaiya et al. (1982) and Geesink et al. (1993) are somewhat contradictory. In this latter case Geesink, Mareko, Morton, and Bickerstaffe (2001) in a more recent study suggested that the different response was a consequence of a fast chilling regime and questions over experimental techniques raise doubts about the results of Sonaiya et al. (1982).

Unfortunately making an inference about whether this accelerated proteolysis translates into an effect on measures of tenderness is difficult. Some reports have found no effect of stimulation on shear force (Geesink et al., 1994; Ho et al., 1997; Hopkins & Thompson, 2001b), yet contrary responses in terms of proteolysis, whereas other have reported that temperature of chilling interacts to mediate the response (Pommier, 1992; Salm et al., 1983). In the case of Pommier et al. (1987) stimulation of veal carcasses actually increased toughness when judged by a panel for product aged for 8 days, which interestingly mirrored the fact that stimulation *decreased* the rate of degradation of troponin-T. The explanation for this effect may rest with the very rapid decline in muscle pH in the stimulated carcasses and the autolysis of μ-calpain. It is suggested this explains why Pommier (1992) found an exactly opposite outcome such that stimulation did decrease toughness as measured by shear force, under conditions of more moderate pH decline. Some support comes from the report of Soniya et al. (1982) who found no significant reduction in shear force in stimulated meat in which case pH decline was rapid i.e. <5.9 at 3 h post-stimulation.

Another interacting factor is the impact of the chilling regime on sarcomere length. This may explain why Geesink et al. (2001) reported that high-voltage stimulation reduced shear force, in spite of no effect on proteolysis, but in which case control muscle had significantly shorter sarcomeres. By contrast when there was no difference between treatments for sarcomere length and stimulated muscle had lower shear force values, this was mirrored by an increased change in MFI for *longissimus*, but not *semimembranosus* muscle (Geesink et al., 1993).

Table 1
Summary of reports which have measured the effect of electrical stimulation on different indicators of proteolysis

Indicator of proteolysis	Type of stimulation/conditions	Outcomes[a]	Source/species
Myofibrillar protein solubility	280 V, 60 Hz for 12 5 s pulses, 5 s apart and then 24 times at 420 V, within 45 min of death.	No effect of stimulation on protein solubility (LL) when measured at 44 h, irrespective of the chilling temperature.	Gilbert et al. (1984)—sheep
Degradation troponin-T and myofibrillar fragmentation index (MFI) changes	300 V, 60 Hz for 80, 1.5 s pulses, 0.5 s apart giving a total of 120 s, within 30 min of death. Breed of cattle unspecified.	MFI was increased by stimulation (LL, SM, TB), but not significantly and there was no effect on the degradation rate of troponin-T.	Sonaiya et al. (1982)—beef
Degradation of α-actinin and troponin-T	500 V, 60 Hz for 20 2s pulses, 1 s apart giving a total of 40 s, within 45 m of death. Breed of cattle unspecified.	Less α-actinin and troponin-T was found in stimulated muscle (LL), but there was an interaction with chilling temperature.	Salm et al. (1983)—beef
Myofibrillar protein solubility and degradation products of troponin-T	550 v, 60 Hz for 80, 1 s pulses, 0.5 s apart for a total of 2 min, within 30 min of death. Breed of cattle unspecified.	Increased protein solubility (LL) when measured at 3 days post-mortem. Reported that 30 kDa and 32 kDa subunits appeared earlier in stimulated muscle (4-h post-mortem).	Ducastaing et al. (1985)—beef
Degradation troponin-T	45 V, for 40 2 s pulses, 2 s apart giving a total of 40 s, straight after death. Used Holstein veal calves.	Stimulation *decreased* the rate of degradation of the protein (LL)	Pommier et al. (1987)—beef
Myofibrillar protein solubility	115 V, 60 Hz for 5 12 s pulses, 3 s apart giving a total of 60 s, within stimulation 1 h post-mortem. Used Charolais crossbred cattle.	No effect of stimulation on protein solubility (LL) when measured at 7 days.	Bruce et al. (1990)—beef
Degradation troponin-T and myofibrillar fragmentation index (MFI) changes	45 V, for 40 2 s pulses, 2 s apart giving a total of 40 s, straight after death. Used Holstein steers.	No effect of stimulation on the rate of degradation of troponin-T, but a product of degradation did appear earlier in stimulated muscle (LL). MFI was higher for slow chilled, stimulated muscle than for control muscle slow chilled at 24 h.	Pommier (1992)—beef
Degradation of titin, nebulin, filamin and troponin-T	600 V, 50 Hz for 120 s, within 1 h of death. Used Belgian white-red cattle.	After 1 day post-mortem less troponin-T was present in stimulated muscle (LL) and by 6 days there was less titin	Uytterhaegen et al. (1992)—beef
Degradation of troponin-T and myofibrillar fragmentation index (MFI) changes	85 V, 14 Hz for 60 s within 30 min of death. Used Friesian/Holstein veal calves.	Stimulation had no effect on the appearance rate of the 30-kDa fragment in 3 different muscles (LL; SM; PS). MFI increased faster in stimulated muscle (LL), but had no effect on MFI in the SM.	Geesink et al. (1993)—beef
Degradation of troponin-T and myofibrillar fragmentation index (MFI) changes	85 V, 14 Hz for either 8 or 64 s within 5 min of death. Breed of cattle unspecified.	Stimulation for 64 s increased the appearance rate of the 30-kDa fragment in (LL), but by 21 days there was no difference in the amount of the fragment. Day 1 MFIs were higher in stimulated muscle (64 s), but there was no difference to stimulated muscle (8 s) or control at 7, 14 or 21 days.	Geesink et al. (1994)—beef
Degradation of titin, nebulin, desmin and troponin-T	200 V, 20 Hz for 15 s 3 times with 30 s breaks between within 1 h of death. Used Angus × Jersey cattle.	Titin, nebulin and troponin-T were degraded faster in *some* stimulated muscle (LL).	Ho et al. (1996)—beef
Degradation of titin, nebulin, desmin and troponin-T	200 V, 20 Hz for 15 or 20 s, 3 times with 30 s breaks between within 1 h of death. Used Brahman × Simmental cattle.	Stimulation did not increase the degradation rate of the proteins (LL).	Ho et al. (1997)—beef
Myofibrillar protein solubility	85 V, 14 Hz for 15 s straight after death. Used Friesian/Holstein cattle.	There was no effect of simulation on protein solubility in either the LL or SM muscle.	den Hertog-Meischke et al. (1997)—beef
Degradation of titin and nebulin	50 V, 60 Hz for 20s within 3 min of death. Used Hanwoo bulls.	Degradation rate increased with stimulation and conditioning at higher temperatures (LL).	Rhee et al. (2000)—beef
Degradation product of troponin-T and of a protein designated M1, changes in MFI and myofibrillar protein solubility	45 V, with 36 pulses per s, a pulse width of 25 ms for 40 s. Used crossbred lambs.	Stimulation increased the degradation rate of protein M1, but had no effect on the appearance rate of the 30-kDa subunit. MFI and protein solubility increased faster in stimulated muscle (LL).	Hopkins and Thompson (2001a, 2001b)—sheep
Degradation products of titin, desmin and troponin-T	1130 V, 14.3 Hz for 90 s, within 30 min of death. Note also that spinal discharge was applied to the carcasses. Used Coopworth lambs.	Stimulation did not increase the degradation rate of the proteins (LL).	Geesink et al. (2001)—sheep

[a] Indicates the muscle used where LL = *longissimus*, ST = *semitendinosus*, PS = *psoas major*, SM = *semimembranosus*, TB = *triceps brachii*.

4.2. Effect of stimulation on enzyme activity

Although there is evidence that the release of lysosomal enzymes (the cathepsins) into the cytosol is accelerated by stimulation though not necessarily directly (O'Halloran, Ferguson, Egan, & Hwang, 1999; Pommier, 1992; Wu, Dutson, Valin, Cross, & Smith, 1985) a connection between these enzymes and myofibrillar proteolysis and tenderisation has not been convincingly made (Hopkins & Thompson, 2002a; Koohmaraie, 1996). Additionally although other enzyme systems may have a role in post-mortem proteolysis such as the serine peptidase's and proteasomes their contribution remains to be clarified and their activity in stimulated muscle does not appear to have been studied in any detail. On this basis the discussion here will focus on the cysteine protease enzymes from the calpain family.

There are several possible explanations why stimulation would increase the activity of specific enzymes like the calpains. It may be due to some intrinsic effect associated with the rapid pH decline that results in a low pH at elevated temperatures to affect calpain/calpastatin ratios, or it could be due to a flow on effect associated with a significant increase in 'free' calcium, which leads to activation of the calpains, particularly μ-calpain. In this regard, Dransfield (1994) *predicted*, based on his modelling of post-mortem calpain activity that calpain activity in rapidly glycolysing muscle would be increased by a factor of six compared to muscle with more 'normal' rates of glycolysis. Under these conditions however, μ-calpain is likely to undergo autolysis so the interplay with temperature and the levels of 'free' calcium will be important. Indeed Ducastaing et al. (1985) demonstrated that when beef carcasses were subjected to high-voltage stimulation (550 V, 60 Hz) for 2 min, the activity of μ-calpain at 4-h post-mortem was reduced by 80%. These authors commented that directly after stimulation the activity of μ-calpain increased by 10–15% compared to unstimulated muscle. In contrast, Hwang and Thompson (2001a) reported that μ-calpain and calpastatin activity directly post-stimulation was lower than pre-stimulation irrespective of the type of stimulation, but unfortunately there were no control measures for comparison. At 1-h post-mortem, Uytterhaegen et al. (1992) found no difference in activity due to high-voltage stimulation (see Table 1), but by 24 h post-mortem there was a significant reduction in μ-calpain and calpastatin activity, which could confer accelerated ageing. Geesink et al. (1994) showed that stimulation (LV) for only 8 s had no affect on μ-calpain and calpastatin activity at 1.5-h post-mortem, whereas stimulation for 64 s caused a significant decrease in the activity of both compounds. This reduction in calpastatin activity does not appear to be as extensive as that of μ-calpain (Ducastaing et al., 1985; Hwang & Thompson, 2001a; Uytterhaegen et al., 1992) and thus the response is similar to that reported for unstimulated muscle (Zamora, Debiton, Lepetit, Lebert, Dransfield, & Ouali, 1996).

The apparent contrary results in the literature about whether stimulation results in a rise in the activity of μ-calpain is evidenced by comparing the results of Ferguson, Jiang, Hearnshaw, Rymill, and Thompson (2000) and Rhee and Kim (2001) which highlights the importance of the interplay between sampling time and the prevailing temperature and pH. Data from Rhee and Kim (2001) sheds some light on this interplay. Samples held at 30 °C for 3-h post-mortem exhibited a larger reduction in μ-calpain activity (at 3 and 9-h post-mortem) than samples held at lower temperatures, but it was found that stimulation had a larger effect on μ-calpain activity than the elevated temperature. Unfortunately the degree of interaction between pH decline and temperature was not examined in this study. This could have verified whether the conclusion of Dransfield et al. (1992) that temperature did not affect calpain activity until the pH reached 6.2 was correct. In this regard, Hwang and Thompson (2001b) using an experimental design aimed at unravelling the impact of pH and temperature on calpain activity reported an interaction between temperature and pH at 1.5-h post-mortem. As a result when the chilling was slow the activity of the μ-calpain and calpastatin decreased when pH decline was rapid, whereas when chilling was rapid the activity of the μ-calpain was largely unaffected by pH decline. This response is consistent with the interaction between chilling regime and change in MFI's (Table 1) reported by Pommier (1992).

Electrical stimulation may confer protection to those muscle fibres that enter rigor soon after stimulation and thus avoid prolonged pre-rigor exposure to high temperatures/low pH, thus maintaining optimal calpain levels. This proposal is supported by a study that showed that stimulated muscles were more tender and aged to a greater extent (Devine et al., 2002). Further in a study of meat quality from *Bos indicus* and *Bos taurus* cattle without electrical stimulation, as the *Bos indicus* content increased, the mean shear force was higher (Hearnshaw et al., 1998). However, with electrical stimulation, the differences were reduced and all meat was more tender (Hearnshaw et al., 1998). Does this indicate that some enzymes were protected from degradation, through an as yet unknown mechanism?

4.3. Effect of stimulation on calcium levels

The evidence indicates that low-voltage stimulation per se does not lead to an increased release of 'free' calcium ions into the cytosol *after* stimulation (Hopkins & Thompson, 2001b). However stimulation accelerates pH decline which is mirrored by an increase in 'free' Ca^{2+}. This suggests that at the same temperature, stimulated

muscle will be exposed to higher levels of 'free' Ca^{2+} and this could be reflected by increased proteolysis. Given that larger extra-cellular volumes and greater cell membrane disruption was noted using NMR in meat with a faster drop in pH (due to low-voltage stimulation) (Tornberg, Wahlgren, Brondum, & Engelsen, 2000) this suggests an acceleration of the leakage of Ca^{2+} from the sarcoplasmic reticulum. It has recently been reported that the 'free' Ca^{2+} concentration in post-mortem muscle is correlated with different indicators of proteolysis (Geesink et al., 2001; Hopkins & Thompson, 2002b). It is also feasible that low and high-voltage stimulation may have different effects on the flow of Ca^{2+} ions into the cytosol from the sarcoplasmic reticulum and this could explain some the differences reported in Table 1. Related to this the response of different muscles to stimulation varies and this has been attributed to fibre type variation (Devine, Ellery, Wade, & Chrystall, 1984). Recent work has suggested that at least for L-type calcium channels in skeletal muscle there is a difference in their density between fibre types (Manttari, Pyornila, Harjula, & Jarvilehto, 2001) with the greatest density being for fast oxidative glycolytic fibres. Given the association of these calcium channels with ryanodine receptor channels in the membrane of the sarcoplasmic reticulum this may help to explain between muscle variation in response to stimulation.

Another variable in these biochemical interactions is the production of phosphate as a consequence of the conversion of ATP to ADP. It has been shown that Ca^{2+} ions precipitate with phosphate and that available Ca^{2+} therefore decreases as muscle becomes fatigued (Kabbara & Allen, 1999). The speed of this precipitation may impact on the pool of 'free' Ca^{2+} ions and affect the activation of the calpains. Related to this is has been demonstrated that as the frequency of stimulation increases the concentration of phosphate in muscle increases (Giannesini, Izquierdo, Confort-Couny, Cozzone, & Bendahan, 2001) which would impact on the extent of Ca^{2+} precipitation. Does variation in voltage also impact on the rate phosphate production as it has been reported that high-voltage stimulation reduces the level of extractable calpain at 24 h post-mortem compared to low-voltage (Hwang & Thompson, 2001a). This implies a greater level of activation and presumably a higher level of available calcium.

5. Conclusions

It is apparent that under or over stimulation can result in no or a detrimental effect on meat tenderness. Provided pre-slaughter animal status and chilling regime are taken into account when the total energy input from electrical stimulation is decided, meat tenderness will be improved. However electrical stimulation does not improve inherently tender meat beyond baseline toughness. It is apparent that electrical stimulation of carcasses hastens the onset of rigor mortis and this confers protection to muscles when exposed to chilling conditions which might otherwise cause cold-induced shortening. Commensurate with this elevation of glycolysis rate is early activation of the calpain system, which can hasten myofibrillar protein degradation and generally positively contribute to tenderness. There is a balance between early activation of the calpain system and self destruction of these enzymes exacerbated by a rapid drop in pH at high temperatures.

The challenge for further development of electrical stimulation systems is optimisation of the activation of the enzyme systems, possibly by chilling regimes to ensure rigor mortis close to say 15 °C, within the constraints of food safety concerns and bearing in mind the different fibre composition of muscles. In this sense a more targeted approach such as the use of regional stimulation of certain muscle groups, susceptible to rapid chilling effects may be more beneficial for improving tenderness. In addition, to ensure that an appropriate energy input is applied to individual carcasses, development of self-response stimulation units, which first determine carcass resistance and decide on the length of stimulation time or strength of stimulation would be another approach for best practice in electrical stimulation. It should be stressed that our knowledge of the optimum temperature at which to 'hit' rigor under in situ conditions is not complete and this represents an area worthy of more study. Related to this the observation that stimulation per se can impact on tenderisation when rates are standardised against rigor raises some interesting questions. It is possible that electrical stimulation protects a proportion of enzymes in fibres that enter rigor early?

The contribution of physical disruption to improvements in tenderness seems in the first instance to be a logical extrapolation, but remains to be verified. Linked to this, what is the activity of the calpains in disrupted fibres? In reaching these conclusions it is noted that comparison of the published studies is constrained because of the myriad of methods used by scientists and the consequent constraints in interpretation. Thus to clearly establish the contribution of mechanical disruption to improvements in tenderness quantitative studies examining ultrastructure alteration and proteolysis simultaneously are required.

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