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Review

Regulation of post-mortem glycolysis in ruminant muscle

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Abstract. As a tissue, muscle has the unique ability to switch its metabolic source of ATP, the energy currency underpinning muscle function. During oxygen debt, such as that occurring immediately following the death of animals, anaerobic metabolism is initiated in an attempt to restore homeostasis within the muscle. The cascade of biochemical events that are initiated is paramount in the context of meat quality. This review revisits this reasonably well-known subject but takes a new perspective by drawing on the understanding outside the traditional discipline of meat science. Our understanding of the intrinsic regulators of glycolytic flux has improved but knowledge gaps remain. Further efforts to understand how the glycolytic enzyme kinetics are influenced by both pre- and post-slaughter factors will be beneficial in the ongoing quest to maximise fresh meat quality.

Additional keywords: muscle, post-mortem glycolysis, ruminant.

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Introduction

Transformation of muscle to meat involves several physiological and biochemical processes evoked by the animal and its tissues in a futile attempt to reinstate homeostatic control. The magnitude, extent, and timing of these responses before, during or post-slaughter can dramatically affect meat quality development. In order to ensure consistent production of the highest quality meat possible, those involved in the meat industry must understand these biological processes and implement management practices that optimise them.

Any effort to understand post-mortem muscle biochemistry should begin with an appreciation for how energy is managed in living muscle, especially under conditions where this highly specialised tissue functions. Muscle cells are uniquely organised and designed to convert chemical energy into movement. Muscle shortens in response to neuronal stimulations that ultimately cause calcium release in the sarcoplasm. Once calcium concentration eclipses a regulatory threshold, myosin and actin interact to create movement through consumption of ATP (via myofibrillar ATPase). Because of the immediate and synchronised nature of contraction and its sensitivity to calcium, ATP-mediated calcium pumps are strategically located throughout the muscle cell on membranous vesicles that rapidly sequester calcium after such a twitch or contraction. These ATPases, as well as those responsible for maintaining membrane potentials and those participating in a myriad of other cellular processes, must function continually; yet under normal resting conditions, energy consumption (ATP turnover) is rather modest, particularly in locomotive muscles, because the major motor proteins remain idle. By contrast, working muscle cells are capable of increasing ATP turnover 100-fold (Hochachka and McClelland 1997).

This highly efficient and robust means of providing ATP rapidly in working skeletal muscle cells is retained, at least in part, during the transformation of muscle to meat. Indeed, it is exactly these unique capabilities that spring into action at slaughter in muscle; however, due to circulatory failure, muscle tissues simply lack sufficient oxygen to maintain a high level of ATP generation. When the concentration of ADP increases in living muscle, glycolytic flux increases (Cheetham *et al.* 1986; Crowther *et al.* 2002), as it does in post-mortem muscle (Kastenschmidt *et al.* 1968). Conversely, in the presence of sufficient oxygen or at a steady-state level of exercise, pyruvate is directed to the mitochondria and is further metabolised (oxidation) in the matrix to support the hydrogen ion gradient between the inner and outer membranes, which in turn synthesises ATP (Conley *et al.* 2001). This process changes rapidly under non-steady-state exercise, where consumption of ATP exceeds the capacity of the cell to re-synthesise ATP. This is quite similar to that which occurs in post-mortem muscle (Scheffler *et al.* 2011). At this point, pyruvate no longer enters the mitochondria because the electron transport chain stops functioning, and lactate and hydrogen ions accumulate. In humans that stop exercising, lactate and hydrogen ions are removed from the cell by the monocarboxylate transporter and homeostasis is once again established (Sahlin 1978; Juel *et al.* 2004). This is not the case in post-mortem muscle. Rather, lactate and protons accumulate and muscle pH decreases ultimately to a level generally found in fresh meat (Briskey *et al.* 1966).

Clearly, much is known about post-mortem energy metabolism and the conversion of muscle to meat (e.g. see reviews by Bendall 1973; Pöso and Puolanne 2005; England *et al.* 2013). However, gaps still exist in our understanding of

this critical process. Significant further insight can be garnered by taking advantage of the literature (e.g. exercise physiology) outside the discipline of meat science. Given the central importance of pH decline to meat quality development, the aim of this review was to re-examine this central dogma with a slightly different perspective, particularly with regard to the ruminant. We review the current knowledge of muscle energy metabolism, post-mortem biophysical changes and the relevance to meat quality. Further, we examine how animal-related factors and pre- and post-slaughter practices influence post-mortem glycolysis and subsequent meat quality.

Post-mortem energy metabolism and meat quality

There is substantial evidence to show that consumers rate tenderness as the most important of all palatability traits, especially for fresh beef (e.g. Huffman *et al*. 1996; Watson *et al*. 2008). Tenderness is also essential to lamb consumers but trails flavour/odour as the highest rated consumer attribute (Pethick *et al*. 2006). Post-mortem energy metabolism or glycolysis in muscle is highly relevant to ultimate meat quality, particularly tenderness. Typically, estimates of glycolytic rate in post-mortem muscle are obtained from measurements of pH over time. The rate of glycolysis post-mortem can profoundly influence two central mechanisms, which ultimately govern myofibrillar tenderness, notably, the degree of myofibrillar contraction and the rate and extent of proteolysis during ageing (Ferguson *et al.* 2001; Koohmaraie and Geesink 2006). Moreover, the combination of rapid glycolytic rates post-mortem at high muscle temperatures can compromise muscle protein integrity, which subsequently leads to losses in visual appeal and meat functionality (e.g. Simmons *et al*. 1996).

Although the rate of post-mortem glycolysis in both bovine (Butchers *et al*. 1998) and ovine (McGeehin *et al*. 2001) muscle can vary between animals, the regulatory mechanisms and extrinsic factors governing this variation are not well defined. Intrinsically, the kinetic properties of the individual ATPases govern post-mortem glycolytic rate (Bendall 1978). In muscle, several ATPases are central to muscle contraction and cell maintenance. However, the relative activities of the individual ATPases, aside from those directly responsible for calcium homeostasis, have not been investigated in detail with regard to rigor development.

After slaughter, the rate of glycolysis can be dramatically accelerated through the application of electrical stimulation, a technology specifically designed for rapid chilling of beef carcasses without the risk of cold shortening occurring. The interesting feature of electrical stimulation is that, in addition to the large initial rate change during stimulation, it also results in a faster rate of glycolysis subsequent to stimulation (Horgan and Kuypers 1985; Daly 1997).

Rigor biochemistry and associated biophysical changes in muscle

With the cessation of blood supply to the musculature, a complex cascade of biochemical events ensues, resulting in significant structural changes. The structural changes are biphasic, characterised by general stiffening or loss in inextensibility as the muscle enters rigor, followed by a phase of partial rigor attenuation. The extent and rate of these biochemical and subsequent structural changes are critical in terms of the visual appearance and/or palatability, in particular, the tenderness/toughness of meat.

The following summarises the primary biochemical and biophysical changes that occur during the conversion of muscle to meat. These are reviewed in more detail by Bendall (1973) and Tornberg (1996).

Rigor biochemistry

With respect to the biochemistry of rigor, much of our current knowledge stems from the pioneering work of Bendall and his colleagues (e.g. Bate-Smith and Bendall 1947; Bendall 1951; Bendall 1973), as well as the understanding of energy metabolism that occurs during exercise in living muscle (reviewed by Robergs *et al.* 2004). As noted above, after slaughter, anaerobic metabolism is initiated, at some point, in order to supply ATP for the continuation of cellular function. The high-energy-carrying ATP molecule forms the basis for the phosphagen system (Sahlin 1985; Kent-Braun *et al.* 1993). As for most biological reactions, hydrolysis of ATP ($ATP + H_2O \leftrightarrow ADP + P_i + H^+$) essentially provides the necessary energy (ΔG^o = 31kJ/mol) to regulate and drive muscle contraction. In living muscle, this energy value is nearly twice (60kJ/mol) as high because the steady-state metabolic state maintains ADP and Pi concentrations much lower than that at equilibrium (Kushmerick and Conley 2002). As mentioned above, bouts of exercise and events occurring in post-mortem muscle require vast amounts of energy in the form of ATP. The most immediate means of maintaining or restoring ATP levels in muscle involves phosphocreatine (PCr) as a critical 'first response' of the phosphagen system. Phosphocreatine contains a phosphate group that readily transfers to ADP via an enzyme known as creatine kinase (creatine phosphate + ADP + $H^+ \leftrightarrow$ creatine + ATP). It is important to understand that this reaction consumes a hydrogen ion and is at equilibrium because the change in free energy with this reaction is very close to zero *in vivo*. Therefore, any change on either side of the reaction will result in a compensation (balancing) by the opposite side of the reaction. This is particularly important during periods of recovery and subsequent re-establishment of the PCr pool. In exercising muscle, and certainly in the case of post-mortem muscle tissue, PCr reserves are exhausted quickly and ADP concentrations rise. There is evidence that the disappearance of PCr in muscle tissue early post-mortem may be especially relevant in development of aberrant meat quality, at least in the pig muscle (Copenhafer *et al.* 2006; Scheffler *et al.* 2011).

In non-steady-state conditions (Connett and Sahlin 1996), where consumption of ATP is greater than production, adenylate kinase (AK), or myokinase generates additional ATP through the reaction: ADP + ADP $\leftrightarrow$ ATP + AMP. Generation of AMP is particularly important as will be discussed in some detail later, because AMP is a major regulator of energy metabolism in muscle cells (Winder and Thomson 2007). The final reaction of the phosphagen system is mediated by AMP deaminase ($AMP + H^+ \leftrightarrow IMP + NH_4^+$) and is actually coupled to the previous reaction. Although the exact role of AMP deaminase is not

known, it is important to understand that the latter two reactions occur mainly when mitochondrial respiration is incapable of supplying the necessary energy (ATP) demanded by the cells (Lowenstein 1990; Tullson and Terjung 1991; Tullson *et al.* 1996). In this case, the energy status of the muscle is compromised. Moreover, some suggest that AMP deaminase prevents AMP accumulation in the cell and, thus, helps facilitate ATP generation (Hellsten *et al.* 1999), thereby retarding fatigue in exercising muscle. Korzeniewski (2006) further modelled the role of these two reactions, and showed that the effective removal of ADP reduces proton production by anaerobic glycolysis and may help 'delay' a fatigue-induced termination of glycolysis during a heavy bout of exercise. Curiously, their models predicted that changes in AMP deaminase under hypoxic (low oxygen levels) conditions were capable of altering the ultimate pH of fatigued muscle by nearly 0.3 pH units—a truly remarkable change in muscle pH, especially considering the lack of variation surrounding the ultimate pH of meat. Regardless, these data outline the critical changes that occur in muscle in response to a functioning phosphagen system during a bout of exercise and show how these reactions may alter post-mortem metabolism.

As a consequence of the aforementioned reactions, reductions in muscle PCr and, in due course, ATP occur with a concomitant increase and decrease in lactate and muscle pH, respectively (Bendall 1973). The decline in muscle pH post-mortem or in exercising muscle has commonly been attributed to the increase in lactic acid, but this is a popular misconception, as the major source of H^+ is clearly from ATP hydrolysis (see review by Robergs *et al.* 2004; discussed later). The resting pH of mammalian muscle ranges from 7.1 to 7.3 and will typically decline non-linearly to 5.5–5.6 in post-mortem muscle (Pearson and Young 1989). The attainment of ultimate pH coincides with the termination of glycolysis. This has been attributed to either a lack of available substrate (i.e. glycogen) or inactivation of one or more of the glycolytic enzymes due to the acidic conditions (Lawrie 1998), although the exact mechanism is yet unknown.

In his review, Bendall (1973) asserts that post-mortem pH decline is biphasic, characterised by an initial period of minimal change in both pH and ATP followed a phase of rapid decline in both. The initial period is termed the 'delay phase' and the conservation of ATP is attributed to the re-synthesis of ATP from PCr and ADP. As discussed previously, this reaction consumes a proton, and hence may buffer muscle pH decline. The resting concentrations of PCr vary depending on species, but a range of 18–23 μmol/g was reported by Bendall (1973). More recent estimates suggest that the concentration at slaughter may be much lower in beef (1–2 μmol/g; Hertzman *et al*. 1993) and sheep (3 μmol/g; Ferguson 2003) muscle. Given this and the lack of evidence to support a delay phase, mainly in beef *m. longissimus* (e.g. O'Halloran *et al*. 1997*a*; Butchers *et al*. 1998), the applicability and relevance of the delay phase has been questioned in conventionally slaughtered livestock (Ferguson 2003).

In many of the studies conducted by Bendall and his colleagues, the muscle relaxant myanesin was administered to the animal well before slaughter (Bendall 1973). Consequently, as Bendall (1973) observed, there was very little involuntary muscle contraction immediately following death. In commercial abattoirs, most animals endure some level of emotional stress and physical activity immediately before slaughter. It is well known that initiation of the adrenergic stress response causes elevations in cAMP, which allosterically activates glycogen phosphorylase and therefore glycolysis (Meyer and Foley 1996). Although β-adrenergic stimulation does not directly influence PCr hydrolysis (Ren and Hultman 1989), it is highly unlikely that PCr is spared during pre-slaughter handling, given its high catalytic capacity (Krause and Wegener 1996). Second, vigorous muscle contractions, particularly during the clonic phase after stunning, are evident in most, but not all animals. The absence of the delay phase in pH decline in conventionally slaughtered cattle and sheep is probably due to the combined actions of pre-slaughter stress and immediate post-stun muscle contractions, which deplete the PCr supply and rapidly activate glycolysis.

During the rapid phase, the ATP concentration declines markedly during rigor. Another key biochemical feature of the rapid phase is a rise in intracellular calcium concentration (Jeacocke 1993; Hopkins and Thompson 2001). Although there is consensus about this, the reported increases in concentration have varied considerably (Dransfield 1999), and this may in part be associated with accuracy of methods used to quantify cytosolic calcium concentration (Mickelson and Louis 1993). The increase in free calcium within the cytosol during and after rigor has been attributed to altered sarcoplasmic reticulum function specifically, leakage of calcium via ryanodine receptors, reduced activity of the calcium pumps due to depletion of ATP, and finally through disruption of membrane structures (Mickelson and Louis 1993).

The duration and rate of the rapid glycolytic phase, as is the case for most biochemical reactions, is temperature-dependent (Marsh 1954; Cassens and Newbold 1967*a*, 1967*b*; Newbold and Scopes 1967; Bendall 1973; Hertzman *et al*. 1993; Daly 1997; Ferguson 2003). However, it also important to recognise that variations in glycolytic rate can be observed, even at constant temperatures (Bendall 1978; Daly 1997; O'Halloran *et al*. 1997*a*). In the context of meat tenderness and other meat quality traits (e.g. colour, water-holding capacity), the interaction between post-mortem glycolysis and temperature in muscle is paramount.

Rigor biophysical changes

Myofibrillar shortening

Once the ATP concentration falls to ~50% of its resting level (5–6 μmol/g), ~50% of muscle elasticity has already been lost (Bendall 1951). In the absence of ATP, irreversible bonds between actin and myosin form, leading to muscle shortening and an increase in the isometric tension (Pearson and Young 1989). The degree of shortening that occurs during rigor is critical with respect to tenderness (Harris and Shorthose 1988). The seminal work of Locker and Hagyard (1963) clearly demonstrated that the degree of muscle shortening (relative to pre-rigor length) was highly dependent on the temperature at rigor. In their study, minimal shortening (~10%) was observed at 15−20°C in beef *m. sternomandibularis*. In later studies from Sweden by Hertzman *et al*. (1993), Olsson *et al*. (1994), and Devine *et al*. (1999), the evidence suggests that the optimal rigor

temperature range might be lower, at 10−15°C, in higher quality muscles such as the *m. longissimus* and *m. semimembranosus*.

While the functional relationship between the degree of shortening and either objective (e.g. shear force) and/or sensory panel assessments of tenderness/toughness is widely acknowledged (see review by Tornberg 1996), there are exceptions to this dogma (Smulders *et al*. 1990; Shackelford *et al*. 1994). Smulders *et al*. (1990) demonstrated variation in tenderness/toughness that was independent of sarcomere length. In their study, the relationship between sarcomere length and panel tenderness scores of unaged, *m. longissimus* steaks was almost non-existent for those samples where faster rates of post-mortem glycolysis (i.e. pH <6.3 at 3 h post-mortem) had occurred. The explanation to this hitherto incongruent outcome can be attributed, in part, to the contribution of proteolysis.

Attenuation of rigor

The attenuation of rigor is also known as the tenderisation phase, where the rigor-induced increase in isometric tension is reduced to some degree post-mortem through the actions of enzymatic processes. These cause a weakening of the myofibrillar matrix through the degradation of key structural proteins, which in turn, manifests as an improvement in tenderness. These degradative changes are exploited for commercial benefit via the practice known as ageing, which involves storing meat at low temperatures for periods of several days to weeks.

In his review of the proteolytic systems involved in post-mortem muscle tenderisation, Ouali (1992) identified three main endogenous protease systems: (*i*) calcium-dependent calpain system (μ-calpain, m-calpain and their inhibitor calpastatin); (*ii*) lysosomal cathepsins (cathepsin B, D, H and L); and (*iii*) ubiquitin-proteasome complex (also known as the multicatalytic proteinase). Debate has ensued regarding the relative contributions of these proteinases, particularly the most extensively studied calpains and cathepsins (Koohmaraie 1992, 1996; Roncales *et al*. 1995; Ouali *et al*. 2006). However, based on the *in vitro* data, μ-calpain appears to be responsible for the majority of the post-mortem tenderisation (Roncales *et al*. 1995; Koohmaraie 1996; Dransfield 1999; Koohmaraie and Geesink 2006).

There has been debate over when proteolysis commences post-mortem. Evidence shows that it begins quite early after stunning (Troy *et al*. 1986; Koohmaraie *et al*. 1987). Whether the initial proteolytic activity results in significant structural weakening is a matter of conjecture (Dransfield *et al*. 1992). Dransfield *et al*. (1992) argued that, under normal rigor conditions, calpain-mediated tenderisation commences approximately midway through the development of rigor (pH 6.1–6.3).

The prevailing conditions of pH and temperature post-mortem also govern the rate of calpain activation and inactivation (Dransfield 1994; Simmons *et al*. 1996). In rapidly glycolysing muscle, the rate of proteolytic activity as measured directly (Simmons *et al*. 1996) and indirectly via electrophoresis (O'Halloran *et al*. 1997*a*, 1997*b*) was accelerated. Dransfield (1994) predicted that calpain activities would be six times greater following rapid glycolysis (i.e. pH 5.5 at 2 h post-mortem) than at more standard rates of glycolysis (i.e. pH 5.5 at 20 h) under standard cooling conditions. Part of this can be explained by the decrease in inhibitory activity of calpastatin during the early post-mortem period when the pH falls below 6.4 (Dransfield 1995; O'Halloran *et al*. 1997*b*). Consequently, during rapid glycolysis, the majority of the tenderisation can occur within the first 24 h post-mortem, with very little response to further ageing (Dransfield 1995).

An important caveat here with respect to proteolytic activation during rapid glycolysis is that this only applies when the temperature decline is commensurate with rate of pH decline (Dransfield 1994). This unfortunately is not easily achieved under standard carcass-chilling regimes in commercial abattoirs. Consequently, the corollary of rapid glycolysis is, quite often, higher rigor temperatures. This is undesirable on two fronts. First, it can increase the risk of heat shortening, and second, and perhaps more importantly, it accelerates the inactivation of calpains through autolysis (Dransfield 1994, 1995). In some cases, the rate of inactivation can exceed the rate of calpain activation, thus leading to minimal tenderisation (Dransfield 1995). Consequently, the combination of high temperature and rapid glycolysis can facilitate increases in toughness with very limited ageing potential.

The study by Simmons *et al*. (1996) nicely illustrates the autolytic effect of temperature on proteolytic activity and the impact on ageing response. They showed that the different pre-rigor temperatures (15, 25 and 35°C) resulted in a 16%, 49% and 74% reduction in calpain activity, respectively, at rigor (i.e. pH 5.5). Higher rigor temperatures also resulted in increased shortening as measured by sarcomere length. Despite this, meat held at 35°C was more tender at rigor than meat held at either 15 or 25°C, indicating that rapid proteolysis helped to negate some of the loss in tenderness through increased shortening. However, any advantage was quickly lost with further ageing, as the reduction in shear force was substantially greater at lower rigor temperatures (45–50%) than at 35°C (20%). This reinforces the assertion of Dransfield (1994, 1995) that high rigor temperatures limit the ageing capacity of meat. Further evidence of this is provided in the more recent studies of Devine *et al*. (1999) and Devine *et al*. (2002).

Based on the above evidence, the fast declines in both glycolysis and temperature appear to be optimal with respect to minimising the degree of shortening and maximising the rate and extent of proteolytic tenderisation.

Glycolysis

Glycolysis in muscle is largely responsible for rephosphorylation of ADP during a bout of exercise when oxygen becomes limiting and cannot support mitochondrial respiration, or during post-mortem metabolism. Glycolysis or 'sweet (sugar) dissolution (loosening)' is a series of 10 biochemical reactions responsible for metabolising a six-carbon molecule into two three-carbon molecules and, in the process, generates ATP. Often, glycolysis is separated into two phases. The first phase, or preparatory phase, involves five reactions that metabolise the six carbon hexoses into one common product, glyceraldehyde 3-phosphate. The second set of five reactions yield ATP, yet retaining the three-carbon molecules, albeit in the form of pyruvate.

The first rate-limiting enzyme of glycolysis is phosphofructokinase (PFK), which irreversibly catalyses the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate. PFK is often cited as the most profound control point of glycolysis in living tissue, and it may facilitate termination of muscle metabolism post-mortem. To that end, PFK responds positively to AMP, fructose 1,6-phosphate, ADP, phosphate and K^+. Conversely, the action of PFK is dramatically attenuated by the presence of ATP and citrate. The latter is a product of fatty acid oxidation. Using his classic reconstituted glycolytic system, Scopes (1974) showed that even at a pH as low as 5.35, PFK remained strongly active, arguing that PFK, although possibly modulating the rate of pH decline, clearly was not responsible for dictating the cessation of glycolysis. Schwägele and Honikel (1988) further supported this notion by showing that PFK activity changed little in pig muscle, ranging in pH from 5.3 to 6.8 at 45 min post-mortem. Even so, there is doubt about whether these enzyme assays were conducted at a neutral pH or at a pH reflecting the environment from which they were extracted. Regardless, PFK remains as one of the most likely candidates in modulating post-mortem metabolism.

Pyruvate kinase (PK), one of the more distal enzymes in the glycolytic pathway, irreversibly catalyses the conversion of phosphoenolpyruvate to pyruvate. It exists in at least three different isoforms and is negatively controlled by ATP, and by acetyl-CoA and fatty acids, both substrates of the TCA cycle. Schwägele *et al*. (1996) reported that the activity of PK from pale, soft and exudative (PSE) meat was greater at lower pH values. Using isoelectric focusing they proposed that an additional isoform might exist in pigs, making the muscle more prone to a quality aberration. However, such an isoform has not been identified and may simply be an artefact of fast-glycolysing muscle.

Creation of lactate is often considered part of glycolysis. However, lactate is the ultimate product of anaerobic metabolism and may be one of the most misunderstood biochemical events in meat science and among the meat animal community (Scheffler and Gerrard 2007; Scheffler *et al.* 2011), as well as in exercise physiology (reviewed by Robergs *et al*. 2004). As outlined previously, the general perception of post-mortem metabolism is that glucose is converted to 'lactic acid' and the latter causes muscle pH to decline. However, it is important to understand that lactate formation during anaerobic metabolism serves two purposes. First, this reaction regenerates NAD+, a co-enzyme required by a more proximal glycolytic reaction using the enzyme glyceraldehyde phosphate dehydrogenase (Lehninger *et al.* 1993). If NADH is not oxidised, glycolysis stops prematurely, or proceeds at an extremely reduced rate. Second, during the reduction of pyruvate to lactate, one hydrogen from NADH and one hydrogen from solution are removed from the cytoplasm. If glycolysis were to proceed without the formation of lactate, the concentration of H^+ would increase more rapidly, overwhelming the buffering capacity of the muscle (i.e. P_i, amino acids and various proteins) and decreasing cytosolic pH quicker, thereby compromising the power surrounding contraction, which ultimately results in fatigue (Fitts 1994; Westerblad *et al.* 2002). Lactate formation essentially prolongs glycolysis by retarding the increase in free hydrogen accumulation in the cytosol (Robergs 2001). In support, the pK_a, or the negative log of the dissociation constant of lactate, is ~3.86; therefore, lactic acid is always dissociated and exists as a salt rather than an acid in the muscle tissue. So at any given time during post-mortem metabolism, most of lactate exists in an unprotonated state. Furthermore, recall that the equilibrium constant favours the development of lactate, meaning that any time pyruvate entry into the mitochondria is slightly slowed, lactate is preferentially and immediately formed. These data show the utility of lactate formation for extending anaerobic muscle metabolism and suggest that lactate accumulation is a good indicator of the extent and rate of glycolysis; however, they identify a major pitfall of using lactate directly to predict muscle pH decline.

Some of the most informative studies conducted in the area were reported by Robert K. Scopes while at the Meat Research Institute in Bristol. In particular, he showed that glycolysis was regulated by ATPase activity. Either directly or indirectly, the activities of the aforementioned enzymes are virtually controlled by cellular levels of ADP. When ATP is plentiful, minimal glycolysis is necessary. As such, glycolysis occurring post-mortem is greatly impacted by the disappearance of ATP (Scopes 1974).

Of particular significance to muscle cells are: myofibrillar ATPase (mATPase), sarcoplasmic reticulum Ca^{2+}-ATPase (SR-ATPase), plasmalemma Na^+,K^+-ATPase, plasmalemma Ca^{2+}-ATPase, and mitochondrial ATPase. Activites of mATPase, SR-ATPase and mitochondrial ATPase are similar immediately post-mortem (Greaser *et al.* 1969). However, given the abundance of mATPase in skeletal muscle, ATP consumption by the myofibrillar component likely drives post-mortem metabolism. One of the best examples of a rapid glycolysis in muscle post-mortem is that associated with halothane-positive pigs, which often results in PSE pork. In this regard and given the above discussion, the mATPase activity of muscle in PSE-generating pork muscle should be quite high. Contrary to this thesis, however, Greaser *et al*. (1969) reported mATPase activity is actually greater in myofibrils from normal than halothane-positive pigs. Honikel and Kim (1986) corroborated this finding by showing that sarcomeres of isolated myofibrils from PSE pork were unable to shorten, whereas those of normal pork remained largely functional. These data argue strongly against mATPase driving glycolysis in muscle from halothane-positives pigs. Alternatively, it is well known that the functional difference between halothane-positive and normal pigs resides largely in the ability of the muscles to maintain sarcoplasmic calcium levels. Specifically, halothane-positive pigs possess a mutation in the ryanodine receptor protein, which in muscle functions as a calcium release channel embedded in the sarcoplasmic reticulum (Mickelson *et al.* 1989). Although difficult to measure, calcium levels in muscle of mutated animals are indeed elevated over those in muscle possessing a normal calcium channel (Lopez *et al.* 1986; Iaizzo *et al.* 1988). As a result, ATP consumption by the SR-ATPase directly would remain quite high in the muscle of mutated animals. In response to this aggravated calcium homeostasis, mATPase would be elevated due to rising levels of cellular calcium, and this loss of ATP, or increase in ADP, would increase flux through glycolysis. In fact, Strasburg and Chiang (2009) and others (Allison *et al.* 2003) have argued that the bulk of variation observed in turkey and pork quality,

respectively, is manifested in polymorphisms within the ryanodine receptor gene, which is intuitive given the size and complexity of the tetrameric protein and associated genes (Rossi and Sorrentino 2002). Regardless of the exact ATPase responsible, there is little question that glycolysis responds to declining ATP levels and, by design, must have access to ADP to rephosphorylate.

Continuing the line of thought that energy status (ATP) modulates glycolytic flux, existence of cellular AMP reflects a compromised energy status and, therefore, is a logical activator of glycolysis (Lehninger *et al.* 1993). Although AMP can directly alter enzyme activity through allosteric means, the primary sensor of cellular AMP in muscle cells is AMP kinase (AMPK), especially in living tissue. Under conditions where the energy expenditure is pronounced, AMP binds to AMPK and becomes activated through various phosphorylation events involving upstream kinases. This, in turn, increases energy-producing biochemical pathways and down-regulates those pathways consuming energy (Winder and Thomson 2007), such as glucose uptake, glycolysis and oxidative phosphorylation, and fatty acid and glycogen syntheses pathways, respectively. Given the profound ability that AMPK activation has on energy metabolism in living muscle, some authors have logically argued that AMPK activation may drive post-mortem glycolysis. Shen and Du (2005) first showed that muscle of mice lacking a functional AMPK failed to experience a normal pH decline post-mortem compared with wild-type mice. They further showed that muscle of mice treated with 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR), a chemical that activates AMPK, had a greater reduction in pH than normal mice. Subsequently, they reported an association between muscle with increased AMPK activation and muscle ultimately developing PSE pork (Shen *et al.* 2006*a*). Making the connection to animal handling, Shen *et al*. (2006*b*) then reported that pigs transported greater distances had greater AMPK activity and poorer meat-pork quality characteristics. Although changes in AMPK activation before death were not ruled out as a driver of post-mortem metabolism, Shen *et al*. (2008) culminated their efforts to show AMPK was a driver of meat quality development by showing that administration of compound C, an AMPK inhibitor, to animals immediately before exsanguination was capable of severely retarding muscle pH decline. These data strongly argue that activated AMPK, possibly through modulation of phosphofructokinase 1 and 2 activities—the latter catalyses the production of fructose 2,6-bisphosphate and increases PFK1 activity—may impact post-mortem metabolism and subsequent meat quality development. Others supported this notion by showing that global phosphorylation patterns, possibly through AMPK-mediated events or otherwise, occur post-mortem and may alter the transformation of muscle to meat (Huang *et al*. 2011; Lametsch *et al*. 2011).

Alternatively and as pointed out above, it is possible that energy charge of the muscle before slaughter remains the culprit of adverse meat quality development. Although hardly a valid argument, this is more in line with theories initially proposed to underlie handling-related quality issues (Warriss 1990). In the case of the AMPK-mediated aberrations in meat-quality development, a gain-in-function mutation in the gamma 3 subunit has been identified in Rendement Napole pigs (RN) (Milan *et al*. 2000). These pigs possess muscle chronically attempting to generate extra energy (ATP) because the AMP binding portion of the enzyme is altered in such a manner that it 'thinks' it is bound to AMP. Even though, we (Copenhafer *et al.* 2006) and others (Monin and Sellier 1985; Fernandez *et al.* 1992; Milan *et al.* 2000) have shown that RN pigs possess higher glycogen levels than their normal counterparts, these pigs have greater PCr immediately post-stunning (Copenhafer *et al.* 2006). As discussed, altering the phosphagen system has clear implications on glycolysis, and this, with other as-yet unknown energy substrates, may alter the beginning energy status of muscle at the point of death and thereby alter post-mortem metabolism rather than the fact they have greater activated AMPK at slaughter.

Given that RN pigs produce 'acid meat' and have a lower ultimate pH (Monin and Sellier 1985), presumably from augmented, carbohydrate-based metabolism (Przybylski *et al*. 1994), it is odd that muscle cross-sections stain more intensely for oxidative enzymes (Lebret *et al.* 1999). This raises the question of how mitochondria contribute to post-mortem metabolism, an issue that has often been ignored in meat science. Energy is clearly stored in the electrochemical gradient that exists across the inner mitochondria membrane, and is captured through the rephosphorylation of ADP by F_1F_0-ATP synthase in the presence of oxygen. Curiously, however, this synthase reverses itself and functions as an ATPase when oxygen is limiting, in an ill-fated attempt to retain the proton motive forces within the mitochondrion (Scott and Nicholls 1980). The additional consumption of ATP in this manner has been proposed as 'mitochondrial treason' and may change the way post-mortem is classically understood, especially in muscle considered more oxidative in nature or the inherent differences between muscles varying in fibre-type composition (Hudson 2012).

Pöso and Puolanne (2005) suggested that transient oxidative metabolism may only account for a small (1–5%) fraction of ATP produced post-mortem by oxygen bound to residual haemoglobin in muscle. This follows on the evidence from Hochachka (2003), who showed that myoglobin is capable of buffering oxygen levels in the muscle during non-steady-state levels of exercise. Indeed, some level of oxidative metabolism can occur during periods of hypoxia or reduced oxygen saturation levels (Marcinek *et al*. 2003). Therefore, unless oxygen saturation of myoglobin abruptly goes to zero at exsanguination, mitochondrial function may occur during the earliest periods of post-mortem metabolism, perhaps buffering energy depletion and modifying subsequently the transformation of muscle to meat. Alternatively, mitochondria may exacerbate the process by augmenting ATP consumption. Regardless, additional work in this area is needed to understand the full impact of the mitochondria and the cessation of oxidative metabolism on post-mortem metabolism and meat quality development.

Glycogenolysis

Glycogen structure

The structure and metabolism of glycogen were recently reviewed by Roach *et al*. (2012). Glycogen exists as a

branched-chain polymer of glucose. The molecule is believed to comprise ~10 000 glycosyl units linked by α-1,4 (93%) and α-1,6 (7%) glycosidic linkages (Connett and Sahlin 1996). The basic structure of glycogen has been studied extensively, and the Whelan model (Gunja-Smith *et al*. 1970) is generally accepted (Goldsmith *et al*. 1982; Melendez-Hevia *et al*. 1993). The biogenesis of glycogen commences with a self-glycosylating protein primer glycogenin (hexosyltransferase) (see reviews by Alonso *et al*. 1995; Roach *et al*. 2012). Based on the Whelan model (Gunja-Smith *et al*. 1970), the different glycosidic linkages give rise to two different types of branched chains: B-chains, which are branched, and the non-branched A-chains. The degree of branching occurs by a uniform factor of two. Therefore, every B-chain has two branches on it. The molecule is arranged in concentric tiers where the number of chains in any single tier is double that of the previous tier. The mathematical modelling studies of Melendez-Hevia *et al*. (1993) indicate that the structure of glycogen is functionally optimal as it maximises the storage of glucose within a small volume and the number glycosyl units that are readily phosphorylated.

Histochemical studies (Fridén *et al*. 1989) reveal that glycogen particles are distributed over five subcellular locations: the subsarcolemmal, intermyofibrillar, para-Z-disk, N_2 line (located in the I band), and H zone spaces. Those authors proposed a functional association with these storage depots and were able to demonstrate that the stores near the Z disks and N_2 lines were preferentially depleted during exercise. Fridén *et al*. (1989) also observed the existence of different-sized populations of glycogen particles. Further investigation has revealed that glycogen is present in two discrete forms, which are identifiable based on their solubility in perchloric acid (PCA) and their size (Jansson 1981; Alonso *et al*. 1995). The smaller (up to 400 kDa) form, known as proglycogen (PG), is not soluble in PCA because of its higher protein/carbohydrate ratio. It is estimated that 10% of proglycogen is protein in the form of glycogenin. By contrast, the more 'mature' macroglycogen (MG), by virtue of its size (~10^3 kDa) and therefore considerably smaller protein/carbohydrate ratio, is soluble in PCA.

The relative proportions of PG and MG vary depending on the total glycogen content (Adamo and Graham 1998; Derave *et al*. 2000; Bröjer *et al*. 2002). However, the methodology used to determine the concentrations of PG and MG has been challenged, as it may lead to overestimation of the PG fraction (James *et al*. 2008). Notwithstanding this, the proportion of total glycogen as MG is recorded as 40% in horses (Bröjer *et al*. 2002), 24–25% (estimated) in pigs (Young *et al*. 2009), and 46–57% in sheep (Ferguson *et al*. 2008).

The physiological roles of these two forms and indeed their metabolic regulation are not fully understood (Alonso *et al*. 1995; Graham *et al*. 2001). Contention remains about whether they are discrete entities or whether PG merely represents a continuum of smaller glycogen particles (Alonso *et al*. 1995; Roach *et al*. 2012). There is evidence that catabolism of PG and MG may be differentially regulated (Huang *et al*. 1997; Asp *et al*. 1999; Derave *et al*. 2000; Shearer *et al*. 2001; Graham *et al*. 2001). In human studies, the results strongly indicate that PG is preferentially depleted over MG during the initial stages of physical activity in muscles with normal to high glycogen concentrations (Graham *et al*. 2001; Shearer *et al*. 2001). At lower glycogen concentrations, the two pools contribute equally to glycogenolysis (Shearer *et al*. 2001). In pigs, the results have been equivocal (Rosenvold *et al*. 2003, 2010).

Glycogen concentration in ruminant muscle

The muscle glycogen concentration at rest in normal healthy sheep and cattle ranges from 75 to 120 μmol/g (Monin 1981; Lambert *et al*. 1998; Pethick *et al*. 1999; Immonen *et al*. 2000; Gardner 2001; Ferguson *et al*. 2007). Glycogen levels will vary between muscles, a reflection of their metabolic profile, specifically, fibre type. For example, Monin (1981) reported that ovine muscles with a high proportion of type IIa fibres had higher glycogen concentrations (90–105 μmol/g) than muscles predominantly comprising either type I or IIb fibres (75–80 μmol/g). However, the differences were not as well defined between bovine muscles. Nevertheless, Monin (1981) did observe that glycogen concentration increased in muscles with a higher percentage of type II fibres, which was corroborated by Lacourt and Tarrant (1985). Furthermore, studies in cattle (Pethick *et al*. 1999; Gardner 2001) suggest, based on the histochemical classification of bovine muscles (Totland and Kryvi 1991), that muscles high in IIa fibres (fast oxidative/glycolytic) have higher glycogen contents than predominantly IIb muscles (fast glycolytic).

Glycogen concentration is, of course, central to the extent of post-mortem pH decline. In healthy animals not unduly stressed before slaughter, this should not be limiting factor, as the concentration is generally higher that that required to attain normal ultimate pH (5.5–5.6). From studies investigating the relationship between pre-slaughter glycogen levels or glycolytic potential – defined by Monin and Sellier (1985) as [lactate] + 2 ([glycogen] + [glucose-6-phosphate] + [glucose]) – and ultimate pH, it is evident that ultimate pH is only affected once the pre-slaughter glycogen level falls below the critical threshold of 45–55 μmol/g (Howard 1963; Monin 1981; Warriss 1990; Wulf *et al*. 2002).

Regulation of glycogenolysis

Although the initial substrate 'powering' glycolysis is often thought to be glucose, glycogenolysis, or the breakdown of glycogen is actually the sequential liberation of glucose 1-phosphate residues from glycogen by the enzyme glycogen phosphorylase (GP) (Lehninger *et al*. 1993). This reaction is one of the rate-limiting steps of anaerobic metabolism post-mortem as oxygen is depleted from the tissues. In living tissues, glucose originates in the blood and is immediately phosphorylated to glucose 6-phosphate (G6P) by hexokinase. Once phosphorylated, it is shuttled to storage as part of a larger glycogen molecule, or it enters glycolysis. Free glucose arising from glycogenolysis is not likely to be phosphorylated post-mortem, because G6P is a potent inhibitor of hexokinase. The bottom line is that entry of a glucose molecule into glycolysis requires energy (1 ATP), whereas glycogen residues are already in a form (G6P) to begin metabolism. Although seemingly trivial in nature, it is critical to understanding the origin and significance of various protons emanating from glycolysis and ATP hydrolysis post-mortem. This fact alone causes some

disparity in how energy metabolism in exercising muscle differs from that occurring in post-mortem muscle. After all, generation of free hydrogens is responsible for changes in muscle pH post-mortem, and whether glycolysis begins with glucose or G6P can dramatically affect calculations defining the source net hydrogen ion production (Hamm 1977; Robergs 2001). Regardless, once a glucose residue is liberated from glycogen, it is rapidly converted to G6P by an enzyme that repositions the phosphate on the sugar.

Two enzymes operate in parallel during glycogenolysis. As discussed, GP is responsible for the transfer of a glycosyl unit to inorganic phosphate to form glucose-1-phosphate (Connett and Sahlin 1996). It targets the α-1,4 glycosidic linkages on the A-chain branches only, and continues until it reaches a point where four residues remain before the α-1,6 branch point. The bi-functional enzyme amylo-1,6-glucosidase, or debranching enzyme as it is commonly known, is then required to catalyse two successive reactions that result in the transfer of a block of three residues to a nearby non-reducing end (i.e. α-1,4 chain) and the release of the final residue as free glucose (Lehninger *et al*. 1993). GP is primarily responsible during the initial phase of glycogenolysis, since 40–50% of the glycosyl units exist as α-1,4 linkages in the outer branches (Connett and Sahlin 1996). Consequently, debranching enzyme is not required until the limit dextrin has been reached on each A-chain.

Glycogen phosphorylase exists as two inter-convertible forms and it is regulated by both substrate and allosteric control mechanisms. Consequently, it is therefore recognised as a key rate-limiting enzyme for glycogenolysis (Stanley and Connett 1991; Connett and Sahlin 1996). A cyclic process of covalent modification regulates the inter-conversion between the active form of GP (GPa) and the less active form (GPb) (Meinke and Edstrom 1991). While GPb is less active, it is still allosterically capable of activation by the presence of AMP in times of great energy demand. Conversion to GPa is governed by phosphorylase kinase, which in turn is regulated by calcium (Ca^{2+}, pH, and by β-adrenergic stimulation via cyclic AMP (cAMP); Hargreaves and Richter 1988; Meinke and Edstrom 1991; Connett and Sahlin 1996). The biochemical cascade of events initiated by β-adrenergic stimulation commences with the epinephrine-mediated increase in cAMP, which results in activation of cAMP-dependent protein kinase. This enzyme phosphorylates phosphorylase kinase, which catalyses the conversion of GPb to GPa. The activation of phosphorylase kinase by cAMP and Ca^{2+} therefore couples activation of glycogenolysis to the adrenergic stress response (i.e. fight or flight response) and muscle contraction, respectively. GP is also allosterically regulated where AMP and IMP are potent activators, while ADP, ATP and glucose-6-phosphate all inhibit GP activity (Connett and Sahlin 1996).

The phosphorylation of GP (i.e. conversion to active form) does not automatically signify higher rates of glycogenolysis. Chasiotis (1988) showed that following epinephrine infusion, the percentage of GP in its active form increased from 22% to 80%. However, despite this, there was minimal effect on glycogenolytic rate. Chasiotis (1988) attributed the low glycogenolytic rate to substrate inhibition of GP by the normally low inorganic phosphate levels in resting muscle. This is overcome at the commencement of muscle contraction where the phosphate concentration rises rapidly through ATP hydrolysis and rephosphorylation of PCr.

In addition to phosphate, evidence from human and rodent studies suggests that glycogen concentration may also regulate GP activity and therefore glycogenolysis (Richter and Galbo 1986; Hespel and Richter 1990, 1992; Hargreaves *et al*. 1995, 1997; Vandenberghe *et al*. 1999; Shearer *et al*. 2001). Paradoxically, the reported K_m of GP for glycogen is reported as 1–2 mM (Newsholme and Leech 1983). Therefore, the enzyme should be fully saturated with its substrate given that the normal resting concentrations of glycogen in mammalian muscle ranges from 80 to 100 mM (Connett and Sahlin 1996). However, the relevance of the *in vitro* K_m estimates to *in vivo* conditions has been questioned (Hargreaves and Richter 1988; Connett and Sahlin 1996).

Further contention about whether glycogen concentration regulates glycogenolysis has been fuelled by equivocal results. Whereas Richter and Galbo (1986), Hespel and Richter (1990, 1992), Hargreaves *et al*. (1995), Hargreaves *et al*. (1997), Vandenberghe *et al*. (1999) and Shearer *et al*. (2001) all found a positive effect, others (Ren *et al*. 1990; Spriet *et al*. 1990; Bangsbo *et al*. 1992) could not establish an effect. Some of the disparity appears linked to methodological issues, in particular, the intensity of the exercise and the variation in glycogen concentration. According to Vandenberghe *et al*. (1999), a positive association was more apparent during prolonged exercise than short-term intense activity, although this has not always been the case (e.g. Shearer *et al*. 2001).

Of significance, however, in the studies by Vandenberghe *et al*. (1999) and Shearer *et al*. (2001) was the finding that the active form of GP was elevated in the presence of high glycogen concentrations. This agrees with earlier reports for rodent muscle by Richter and Galbo (1986) and Hespel and Richter (1992). If the reported K_m for GP and glycogen is erroneous, the coupling between GP transformation and glycogen concentration could therefore account for the higher glycogenolytic rates. However, this begs the question: what is the mechanistic basis for the coupling in the first instance? Shearer *et al*. (2001) hypothesised that the substrate-mediated increase in the transformation of GP might be linked to the association and dissociation of GP and other relevant enzymes with the glycogen particle. It is generally accepted that the glycolytic enzymes exist as bound complexes either within the cytosol or with cellular structures (see reviews by Brooks and Storey 1991; Roach *et al*. 2012). Furthermore, the state of these complexes is not fixed, and this is believed to be implicit in the cellular regulation of glycolytic flux. Using this as a basis, Shearer *et al*. (2001) put forward the view that the increased glycogenolytic rate may be because more GP is bound with glycogen when glycogen levels are high.

The question of whether the association between glycogen concentration and glycogenolysis was relevant to post-mortem biochemistry in ovine muscle was examined by Daly *et al*. (2006) and Ferguson *et al*. (2008). Only Daly *et al*. (2006) found glycogen concentration to influence the magnitude of the pH response to post-slaughter electrical stimulation. However, there was agreement between the studies regarding the positive association between glycogen concentration and rate of pH decline adjusted to a constant temperature of 38°C

(refer Bendall 1978). A significant aspect of both studies was that the muscle glycogen concentration in both studies was relatively low (40–55 μmol/g). Therefore, this does not align with the view by Shearer *et al*. (2001) that accelerated glycogenolysis is evident primarily at higher glycogen concentrations. This again raises an issue that is paramount to the study of post-mortem metabolism—'what is the energy status of the tissue prior to slaughter?'—as altered metabolism post-mortem is profoundly a function of those physiological changes that occur in the tissues preceding slaughter.

Clearly, further examination of the association between pre-slaughter muscle glycogen concentration and post-mortem glycolysis is warranted. However, the data and hypotheses of (Copenhafer *et al.* 2006; Scheffler and Gerrard 2007; Park *et al.* 2009; Scheffler *et al.* 2011), including earlier observations of Sellier and Monin (1994), strongly suggest that factors other than glycogen concentration underpin the variation in the rate and extent of post-mortem pH decline.

Factors influencing post-mortem glycolytic rate in ruminant muscle

As stated earlier, the time-dependent changes in pH are typically used to estimate glycolytic rate in post-mortem muscle. In general, depending on the rate of chilling, normal ultimate pH (i.e. 5.5–5.6) in ruminant muscle is attained within 24–48 h (Tarrant and Mothersill 1977; Lister *et al*. 1981). Glycolytic rate can vary substantially between contemporary animals (O'Halloran *et al*. 1997*a*; Butchers *et al*. 1998; McGeehin *et al*. 2001) and between muscles within the carcass (Tarrant and Mothersill 1977). The intrinsic and extrinsic factors inherent to this variance are discussed below. It is worth highlighting that, in general, this area has not received critical attention, particularly in ruminants. The opposite is true with respect to the knowledge pertaining to pig muscle, largely because of their inherently faster glycolytic rates and because this can be further accelerated in certain genotypes (e.g. differences in halothane gene expression).

Animal and ante-mortem factors

Muscle fibre type

Muscles vary with respect to their fibre-type composition. These variations in fibre type are reflected by differences between muscles in the physical appearance, mechanical properties and metabolic rates. Not surprisingly, divergent patterns in the post-mortem rates of glycolysis are also evident. Muscles with a high proportion of oxidative type I fibres display faster rates of pH decline than predominantly type IIb fibre muscles (Devine *et al*. 1984; Aalhus and Price 1991). This seems incongruous, as muscles with a higher proportion of type IIb fibres, and therefore higher activities of glycolytic enzymes (Monin 1981; Talmant *et al*. 1986), might be expected to have faster rates of pH decline. Aalhus and Price (1991) postulated that the increased buffering capacity in type IIb muscles might explain the apparent anomaly. The data of Talmant *et al*. (1986) certainly support the fact that slow-twitch, type I muscles have a lower buffering capacity than type II muscles. In their study based on 18 bovine muscles, the buffering capacity ranged from 40 to 55 μmol lactate/g.pH unit.

Importantly, glycogen concentration varies between the fibre types, and this, in turn, can influence the extent of post-mortem glycolysis and therefore ultimate pH. Muscles with predominantly type I fibres have lower glycogen concentrations than those with type II or fast twitch muscles (Monin 1981; Talmant *et al*. 1986).

Breed/genotype differences

The effects of genotype and environmental factors on the variance in meat quality traits were recently reviewed by Warner *et al*. (2010). Genetic differences are certainly evident for important traits such as tenderness in cattle (Burrow *et al*. 2001; Johnston *et al*. 2003) and sheep (Mortimer *et al*. 2009; Warner *et al*. 2010). However, it was not clear whether this could be accounted for by genetic differences in glycolytic rate, because the trait was rarely measured.

Studies contrasting *Bos taurus* and *Bos indicus* beef quality (Wheeler *et al*. 1990*a*, 1990*b*; Shackelford *et al*. 1991) suggest that the rate of pH decline may be slightly slower in *Bos indicus* muscle. However, it is not clear whether there were differences in the rates of temperature decline, which may have confounded the intrinsic rates of pH decline. This is an important point, as breed differences in maturity pattern will give rise to variations in carcass weight and fatness and, therefore, to variability in carcass cooling rates. Consequently, this may influence the rate of glycolysis, so care needs to be exercised when interpreting data on pH decline made on cooling carcasses. However, it is possible to correct mathematically for the temperature-sensitive variation in pH decline (Bendall 1978), and this will facilitate more meaningful comparisons of pH decline when measurements are made on cooling carcasses.

In a lamb study contrasting five genotypes based on Poll Dorset, Border Leicester and Merino breeds, Hopkins *et al*. (2007) did not observe differences in post-mortem glycolytic rate (*m. longissimus*).

With respect to genotype, two genes associated with muscle hyperplasia in cattle (myostatin gene) and muscle hypertrophy in sheep (callipyge gene) may also indirectly influence post-mortem glycolytic rate. Concomitant with the increase in muscle mass, the proportions of type IIb fibres and type IIa fibres increased and decreased, respectively, in double-muscled cattle and callipyge lambs (Holmes and Ashmore 1972, Carpenter *et al*. 1996; Greenwood and Dunshea 2009). Based on the observations of Devine *et al*. (1984) and Aalhus and Price (1991), slower rates of pH decline might be expected in these muscles. However, in practice, this may be offset by the increased muscle mass, which will retard muscle temperature decline.

Sex

Due to a lack of comparative studies, little can be concluded about differences in glycolytic rate between the sexes. In one study in lambs, McGeehin *et al*. (2001) observed faster rates in females than males. Differences in maturity and therefore fat cover may have been a contributing factor.

Nutrition and production system

Nutritionally mediated differences in carcass weight and fatness will influence cooling rate and, potentially, glycolytic

rate (Jacob and Hopkins 2014). Grain-fed cattle show elevated core body temperature relative to grass-fed cattle (Jacob *et al*. 2014), and this can contribute to accelerated glycolytic rate post-slaughter. Furthermore, cattle on grain-based diets show a lower pH early post-slaughter and a higher rigor temperature than grass-fed cattle, even after adjustment for carcass weight and fatness (Warner *et al*. 2014). In another example, pronounced differences in glycogen concentration can be achieved through varying either the level of feeding or the energy density of the diet (Pethick *et al*. 1999). As discussed above, glycogen concentration has been shown to influence glycogenolytic rate.

The daily level of physical activity, which can vary between extensive and intensive production systems, may also indirectly affect post-mortem glycolysis, once again through changes in the fibre-type profile. Vestergaard *et al*. (2000) investigated the effect of finishing system on muscle fibre characteristics in young bulls. In contrast to extensively reared bulls (loose housing plus pasture), the bulls that were intensively housed (tethered in stalls) had lower proportions of type I and IIa fibres and a higher proportion of type IIb fibres. A similar trend, but only for type I fibres, was observed by Moody *et al*. (1980) in their study comparing pasture and feedlot lambs. Vestergaard *et al*. (2000) attributed the transition in muscle fibre profile to differences in activity level. This assertion is supported by the general conclusion from human and animal studies that, in response to endurance exercise, there is an increase in muscle oxidative capacity, which is manifest by an increase in type I and IIa fibres (Aalhus and Price 1991; Henriksson 1992; Essen-Gustavsson 1996). The exercise study in lambs by Aalhus and Price (1991) is relevant in the context of this review, as the post-mortem pH declines were measured. Despite the increase in the percentage of type I fibres with exercise, the rate of post-mortem pH decline was comparable to that measured in non-exercised controls. Thus, small changes in fibre-type distribution might not always correlate with altered rates of post-mortem glycolysis.

Pre-slaughter stress

The impact of pre-slaughter stress on muscle glycogen loss and the deleterious consequences for meat quality have been extensively studied in meat animals (see reviews by Tarrant 1989; Lister 1989; Ferguson and Warner 2008). Stress-mediated reductions in glycogen concentration below the critical threshold of 45–55 μmol/g (Howard 1963; Monin 1981; Warriss 1990) will give rise to elevated ultimate pH (pH_u). Meat with pH_u >5.9 is typically referred to as DFD. It is characterised by a darker colour, increased water-holding capacity and, depending on the pH_u, increased toughness (especially at pH_u 5.9–6.2; see Purchas and Aungsupakorn 1993).

Glycogen loss during pre-slaughter handling of animals is mediated by the exposure to several stressors such as: fasting, dehydration, novel/unfamiliar environments, transport, increased human contact, changes to social structure (i.e. through separation and mixing), and sudden climatic changes. The magnitude of glycogen loss will depend on the intensity and duration of the various stressors and the susceptibility of the animal to stress (Ferguson *et al*. 2001). Tarrant (1989), in his review, reported glycogenolytic rates in cattle to vary between 0.05 μmol/g.h (fasting heifers) and 11 μmol/g.h (mixed penning of bulls). The intensity of physical activity is critical, as physical activity *per se* may not always result in glycogen depletion. For example, Lambert *et al*. (1998) demonstrated that fast-walking cattle at a speed of 8 km/h over 5 km did not affect glycogen concentration in *m. longissimus*.

Glycogenolysis will also vary between muscles and fibre types (Tarrant 1989). Muscles along the back and in the hind limbs appear most prone to glycogen depletion in cattle (Tarrant and Sherington 1980). The association between exercise intensity, fibre-type and glycogen mobilisation was shown in an elegant study by Richter *et al*. (1982). During high-frequency stimulation of perfused rat muscle, the effect of epinephrine on glycogenolysis was most pronounced in slow-twitch fibres, whereas there was virtually no effect in the fast-twitch fibres. By contrast, the opposite was observed when the muscle was exposed to low-frequency stimulation. Lacourt and Tarrant (1985) also showed that type I fibres were more responsive to adrenaline injection in cattle. However, in response to the combination of physical activity and sympatho-adrenal activation associated with mixed penning of bulls, glycogen loss was higher in type II fibres.

Although the association between pre-slaughter stress and muscle glycogen depletion has been extensively studied in ruminants, the same cannot be said for the association between stress pre-slaughter and post-mortem glycolytic rate. This in contrast to the large body of evidence published for pigs (e.g. Klont and Lambooy 1995; Warriss *et al*. 1995; Channon *et al*. 2000; Støier *et al*. 2001). The general finding across these studies was that stress just before stunning resulted in lower initial muscle pH, higher initial muscle temperature, and a faster rate of pH decline in the first hour after death but similar rates beyond that.

The results from several ovine studies where exercise stress was applied just before slaughter are equivocal. Simmons *et al*. (1997) exercised sheep at 7 km/h for 30 min over a 90-min period and reported that the rate of pH decline at a constant temperature (15°C) was slower in the exercised group then the non-exercised group (0.036 *v.* 0.073 pH units/h). By contrast, Ferguson (2003) observed no difference in the rate of pH decline between the exercised (running at ~8 km/h for 15 min immediately before slaughter) and non-exercised treatment groups. Pre-slaughter exercise stress also resulted in significant glycogen depletion and elevated muscle temperatures at slaughter. In stark contrast, Bond and Warner (2007) clearly showed that lambs that were exercised for 10 min pre-slaughter had much faster pH declines, particularly in the early post-mortem period.

The application of moderate exercise just before slaughter could be challenged for its relevance to commercial practice. Clearly, best practice pre-slaughter management aims to minimise the intensity and duration of stressors that typically occur during the critical pre-slaughter period. Unfortunately, compliance with best practice does not always occur, and anecdotally, animals are subjected to unnecessary bouts of physical activity before slaughter in some abattoirs. As evidenced in the above studies, increased activity, depending on the intensity and duration, leads to changes in muscle metabolite concentration (e.g. PCr, glycogen), temperature and pH at slaughter. Furthermore, exercise stress has also been shown to affect sarcoplasmic reticulum function, specifically

Ca^{2+} transport, in several animal (Byrd *et al*. 1989*a*, 1989*b*; Luckin *et al*. 1991; Favero *et al*. 1993; Ortenblad *et al*. 2000) and human (Gollnick *et al*. 1991; Booth *et al*. 1997) studies. In particular, exercise attenuates Ca^{2+} uptake and SR Ca^{2+}-ATPase activity (Byrd *et al*. 1989*a*, 1989*b*; Luckin *et al*. 1991; Gollnick *et al*. 1991; Booth *et al*. 1997) or SR Ca^{2+} release (Favero *et al*. 1993; Westerblad *et al*. 1998, Ortenblad *et al*. 2000).

Kuchenmeister *et al*. (2001) reported that pre-slaughter stress in pigs also influenced Ca^{2+} uptake. In sheep, pre-slaughter exercise elicited an 18% reduction in Ca^{2+} uptake, but this was not found to be significantly different to the uptake rates in the non-exercised controls. It was concluded that pre-slaughter exercise had minimal effect on SR functionality.

Overall, these results suggest that sarcoplasmic reticulum function might be altered by pre-slaughter stress, and consequently, may contribute to variations in post-mortem glycolytic rate. Furthermore, the stress-induced changes in metabolite concentrations, pH and sarcoplasmic reticulum function may also be implicit in the reduced glycolytic response to electrical stimulation (Chrystall *et al*. 1982; Warner *et al*. 2000).

Post-mortem factors

Method of stunning

The process of stunning an animal initiates significant changes in energy metabolism in muscle. This can be attributed to neuromuscular activation, and because the plasma concentrations of adrenaline and noradrenaline concentrations rise rapidly during stunning (van der Wal *et al*. 1999). The choice of stunning method varies between livestock species. In pigs, carbon dioxide and electric stunning are predominantly used, whereas captive bolt and electric stunning are both used to stun cattle and sheep.

In studies comparing different stunning methods, significant differences in the metabolic response have been reported. In porcine muscle, electric stunning resulted in a lower initial pH (Gregory 1995; Bertram *et al*. 2002) and faster rate of pH decline (Channon *et al*. 2002) compared with carbon dioxide stunning. Petersen and Blackmore (1982) compared captive bolt and electric stunning in lambs and found that while the rate of pH decline was not affected, electric stunning resulted in a lower initial pH, and this was maintained during rigor.

Another issue relevant to stunning is the degree of involuntary muscle contraction that occurs in animals following stunning (i.e. clonic phase). Further reductions in initial pH values at slaughter can occur depending on the level of muscle activity (Bendall 1973).

Temperature decline

The temperature dependence of post-mortem muscle glycolysis has been studied by several workers (Marsh 1954; Cassens and Newbold 1967*a*, 1967*b*; Newbold and Scopes 1967; Bendall 1973; Jeacocke 1977; Tarrant and Mothersill 1977). In the studies where the muscles were excised soon after death and incubated at different temperatures (Marsh 1954; Cassens and Newbold 1967*a*, 1967*b*), typically over the range of 1−37°C, the 10°C temperature coefficients (Q_{10}) increased from 1.25 (5−17°C) to 1.7–1.9 (15−37°C). At temperatures ≥37°C, the temperature coefficients rise dramatically. Marsh (1954) reported that the Q_{10} for the temperature range 37−43°C was nearly double (6.8) that calculated over the range 33.5−37°C (3.7).

It is important to recognise that temperature decline within any given muscle will vary depending on its anatomical location (i.e. deep *v.* superficial muscles), the weight and fatness of the carcass, and the temperature and air-speed conditions during chilling. Consequently, glycolytic rate varies enormously not only between muscles, but also within a muscle (Tarrant and Mothersill 1977; Sammel *et al*. 2002). To highlight the within-muscle variation Tarrant and Mothersill (1977) demonstrated that the rate of glycolysis in four beef muscles (*m. semimembranosus*, *m. adducter*, *m. semitendinosus and m. biceps femoris*) was, on average, 64% faster when the measurement was taken at a depth of 8 cm within the muscle compared with 5 cm. The significance of this result should be kept in mind when interpreting pH decline rates based on *in situ* pH measurements in cooling muscle.

Muscle temperature at slaughter and the subsequent rate of cooling clearly has a profound effect on post-mortem glycolysis.

Electrical stimulation

Application of electrical stimulation to carcasses was designed to accelerate post-mortem glycolysis and therefore minimise the risk of cold shortening during rapid chilling. Excellent reviews on development, scientific basis and methods of electrical stimulation are provided by Bendall (1980) and Chrystall and Devine (1983).

In summary, electrical stimulation results in a biphasic acceleration in muscle pH decline as illustrated in Fig. 1. Initially, during stimulation there is a sharp decrease in pH (ΔpH ~0.4–0.5 pH units). During this phase, the rate of glycolysis is ~100–150 times greater than the rate of normal rigor development (Chrystall and Devine 1983). In the second phase, the rate of pH decline subsequent to stimulation is generally faster (1.5–2 times) than that observed in non-stimulated muscle (Chrystall and Devine 1978; Horgan and Kuypers 1985). Although there has been debate as to whether

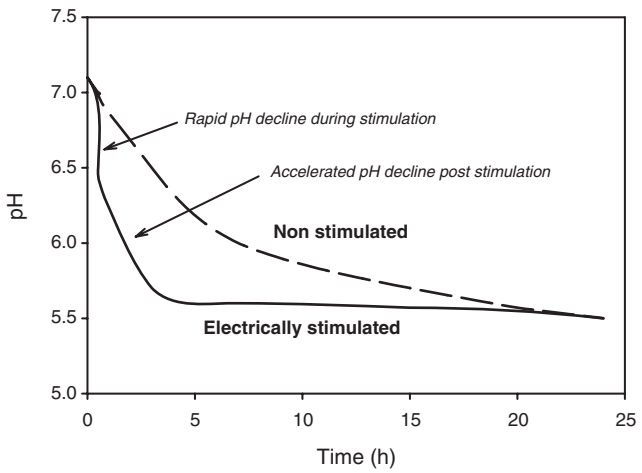

Fig. 1. Indicative post-mortem pH/time profiles for muscles from electrically stimulated (—) and non-stimulated (– –) carcasses (adapted from Ferguson *et al*. 2001).

the latter effect was merely an artefact of higher muscle temperatures (Bendall 1980), the results of Daly (1997) and Chrystall and Devine (1978) strongly suggest that temperature is unlikely to be the sole contributor. Daly (1997) reported a 50–75% increase in post-stimulation pH decline compared with non-stimulated muscle when both were held at constant temperature (35°C).

The magnitude of change in the ΔpH and/or the post-stimulation rate of pH decline is contingent on several factors including voltage (Chrystall and Devine 1978; Bendall 1980; Horgan and Kuypers 1985; Aalhus *et al*. 1994), frequency (Chrystall and Devine 1978; Bouton *et al*. 1980), current and wave form (Chrystall and Devine 1978), and duration of stimulation (Chrystall and Devine 1978; Butchers *et al*. 1998; Hwang and Thompson 2001). Moreover, intrinsic muscle properties such as the pre-stimulation pH (Chrystall and Devine 1978; Daly *et al*. 2006; Ferguson *et al*. 2007) and muscle fibre type (Devine *et al*. 1984) also influence the glycolytic response to electrical stimulation. In these studies, a larger ΔpH was evident in those muscles with a higher pre-stimulation pH and/or high proportion of glycolytic type IIb fibres. Surprisingly, however, the post-stimulation rate of decline is typically slower in muscles high in type IIb fibres than in those with predominantly type I fibres (Devine *et al*. 1984).

The mechanisms associated with the post-stimulation increase in pH decline have not been elucidated. Bendall (1980) hypothesised that electrical stimulation affected the sarcoplasmic reticulum capacity to retain Ca^{2+}, which led to an increase in sarcoplasmic reticulum pump activity. However, equivocal results have been found with respect to the immediate changes in SR Ca^{2+}-ATPase activity following electrical stimulation (Tume 1979; Horgan and Kuypers 1985; Ferguson 2003). Tume (1979) and Ferguson (2003) observed a reduction in SR Ca^{2+}-ATPase activity following stimulation in sheep muscle, which contrasts the outcomes of Horgan and Kuypers (1985) using purified sarcoplasmic reticulum from rabbit muscle. Tume (1979) concluded that the reduction in SR Ca^{2+}-ATPase activity following stimulation was permanent and attributed it to a conformational change in the ATPase, resulting in reduced affinity for ATP and inorganic phosphate.

Horgan and Kuypers (1985) speculated that another ATPase, probably actomyosin Ca^{2+}-ATPase, was implicated. However, the prerequisite for increased activity of actomyosin Ca^{2+}-ATPase is, of course, Ca^{2+}. An increase in cytosolic Ca^{2+} and, perhaps, elevated Ca^{2+} sensitivity of the regulatory proteins such as troponin would be required to activate this ATPase. Whether stimulation accelerates the normal increase in cytosolic Ca^{2+} typically observed in post-mortem muscle is not clear yet. However, Daly (1997) proposed that the stimulation-mediated increase in ADP might trigger increased leakage of Ca^{2+} from the SR.

Conclusions

Post-mortem energy metabolism plays a crucial role in the transformation of living tissue, muscle, into a high-quality food source, meat. The rate and extent of post-mortem glycolysis has a profound effect on the ultimate quality of meat. Those biochemical reactions involved in this energy metabolism are largely well known and characterised in living muscle. Despite this, there is still a widespread misconception about the role of lactate in the development of acidosis following glycolysis. Although predicated on anaerobic metabolism, there are gaps in our understanding of how muscle transitions to this type of metabolism, including the role of mitochondrial function on ATP utilisation early post-mortem. Moreover, the effects of different pre- and post-slaughter factors on the kinetics of those enzymes (e.g. AMPK) involved in carbohydrate metabolism in post-mortem muscle require more investigation. The knowledge gained from further study is likely to yield new directions and potential strategies for optimising the pre- and post-slaughter management of animals and their carcasses, respectively, so that meat quality is maximised.

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