Meat Science 95 (2013) 837–843

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Exploring the unknowns involved in the transformation of muscle to meat

E.M. England, T.L. Scheffler, S.C. Kasten, S.K. Matarneh, D.E. Gerrard *

Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, United States

ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 9 April 2013
Accepted 10 April 2013

Keywords:
Glycolysis
Mitochondria
Pork
Ultimate pH

ABSTRACT

Meat quality development, or the transformation of muscle to meat, involves a myriad of biochemical pathways that are largely well-studied in living muscle tissue. However, these pathways are less predictable when homeostatic ranges are violated. In addition, there is far less known about how various management or environmental stimuli impact these pathways, either by substrate load or altered cellular environment. Likewise, it is largely accepted that oxygen plays little to no role in the conversion of muscle to meat, as anaerobic metabolism predominates in the muscle tissue. Even so, the oxygen tension within the tissues does not fall precipitously at exsanguination. Therefore, transition to an anaerobic environment may impact energy metabolism postmortem. Antemortem handling, on the other hand, clearly impacts meat quality development, yet the exact mechanisms remain a mystery. In this paper, we will attempt to review those factors known to affect postmortem energy metabolism in muscle and explore those areas where additional work may be fruitful.

1. Introduction

Postmortem metabolism is a heavily researched topic. Many scientific and technological advances have led to improved animal welfare, feeding strategies, slaughter processes, and resulted in improved meat quality development for the consumer. Despite a myriad of advances, questions remain regarding those mechanisms controlling or impacting postmortem metabolism and how physiological and tissue-based homeostatic set points are maintained or breeched by various management practices that ultimately lead to altered meat quality development. Though the rate of postmortem metabolism is quite important in driving meat quality development, it is fairly well established. Alternatively, the biochemical mechanism(s) responsible for the cessation of postmortem metabolism, or protracted carbohydrate metabolism are particularly puzzling. The role of mitochondria and curtailed oxidative metabolism play in modulating postmortem metabolism will also be explored. Finally, we will briefly review antemortem animal handling practices in an effort to understand how these management practices alter the aforementioned.

2. Cessation of postmortem metabolism

In order to understand those mechanisms that may control an abbreviated or protracted postmortem metabolism in muscle, one must first reason why it stops. To date, this has not been unequivocally established. Though some would argue it is simply a function of glycogen abundance at harvest, this is not the case, especially when extreme deviates are removed from the population (Copenhafer, Richert, Schinckel, Grant, & Gerrard, 2006; Scheffler & Gerrard, 2007; Scheffler, Park, & Gerrard, 2011). Over sixty years have elapsed since meat scientists across the globe have known that some muscle, for whatever reason, is capable of breaching a final pH, which is otherwise relatively constant across myriad of animals managed and processed under a variety of conditions, yet little progress or even interest exists in this area. Essentially, there are two viable hypotheses, either there is a pH-mediated inactivation of glycolytic enzymes, which stops hydrogen accumulation at a constant endpoint, or there is loss of adenosine nucleotides preventing a glycolytic substrate to rephosphorylate (Dalrymple & Hamm, 1975; Greaser, 2001). In an attempt to stimulate or re-kindle an interest in this fascinating biochemical process, we will begin by reviewing the collective works of one of the great pioneers of postmortem metabolism, Robert K. Scopes.

Scopes and Lawrie (1963) first entered the area of postmortem metabolism by noting that an accelerated rate of postmortem metabolism resulted in denatured sarcoplasmic proteins and adulterated meat quality development. They predicted at that time that the antemortem 'state' of the animal likely dictated the pH decline in these muscle tissues (Scopes & Lawrie, 1963) and quickly extended these initial observations to show that differences in muscle temperature and pH combinations indeed altered the extractability, or solubility of sarcoplasmic, and myofibrillar muscle proteins (Scopes, 1964). These early data likely formed the foundation for a number of subsequent studies over the next 50 years based on the premise that enzymes, and other proteins, denature with time postmortem (Joo, Kauffman,

* Corresponding author. Tel.: +1 540 231 9157; fax: +1 540 231 3010.
E-mail address: dgerrard@vt.edu (D.E. Gerrard).

0309-1740
http://dx.doi.org/10.1016/j.meatsci.2013.04.031

Kim, & Park, 1999; Warner, Kauffman, & Greaser, 1997), and represent events now commonly known as the conversion of muscle to meat. Most likely because of animal to animal variation, Scopes then modified his approach and used minced muscle to study postmortem metabolism. Using this approach, Newbold and Scopes (1971) showed that varying the concentrations of inorganic phosphate (P_i) up to 50 mM reduced the ultimate pH (pH_u) of the mince, yet concentrations greater than 50 mM did not further facilitate greater declines in the metabolizing tissue preparation. Based on these observations, they proposed that P_i may induce greater glycogen phosphorylase (GP) activity, thereby explaining the lower pH_u observed in some muscles. This hypothesis is particularly intriguing given the intimate relationship between the phosphagen system and energy metabolism in exercising muscle (Robergs, Ghiasvand, & Parker, 2004). Recall, GP is present in muscle in two forms, *a* and *b*, the former being more active than the latter (Berg, Tymoczko, & Stryer, 2007). GP is activated by AMP and inhibited by ATP and glucose 6-phosphate. Both calcium and epinephrine are capable of shifting the inactive form *b* to the active *a* form by stimulating phosphorylase kinase. Of particular significance is that though active, GP cannot generate glucose 1-phosphate without inorganic phosphate (Morgan & Parmeggianiani, 1964). Given that added inorganic phosphate to a muscle mince caused a lower pH_u in glycolysing muscle preparations (Newbold & Scopes, 1971), it is possible to argue that inorganic phosphate may be rate-limiting during particular times postmortem metabolism. Addition of inorganic phosphate, on the other hand, would undoubtedly raise the pH of the mince early and ultimately could result in a higher pH of the reaction. After all, liberation of free phosphate via ATP hydrolysis, especially in heavily exercising muscle, is known to buffer muscle cells against massive hydrogen accumulation (Robergs, 2001). Regardless, defining the role of phosphate, especially free phosphate in postmortem muscle, may be quite enlightening.

Scopes further refined his in vitro system to include a glycolysing mixture (glycogen, ATP, NAD, P_i, etc.), organic buffers (TRIS, acetate), and purified glycolytic enzymes (Scopes, 1973). The composition of the buffer is particularly germane to the issue at hand, as buffer capacity of muscle can dramatically impact the pH_u and quality of meat (Kylä-Puhju, Ruusunen, Kivikari, & Puolanne, 2004; van Laack, Kauffman, & Greaser, 2001). Using this system, Scopes (1973) documented the extent resting muscle could rephosphorylate creatine dependent upon available inorganic phosphate, GP *a* concentration, and ATPase activity. He then showed that the rate of glycolysis is directly proportional to the amount of ATP consumed (Scopes, 1974a). Specifically, when ATPase activity was stimulated, lactate formation was increased proportionally. In addition, he noted that glycolysis stopped once adenonucleotides were metabolized. In contrast, when ATPase, or ATP consumption, was reduced or minimized, the entire system was capable of maintaining ATP concentrations in a steady-state condition, where minimal AMP is detected. As a result, a slower metabolism ensued. These results are particularly interesting as they argue that energy levels such as: phosphocreatine, ATP or the ability of ATP to be rephosphorylated (see discussion below) in the muscle tissue at harvest may shift the time at which glycolysis may begin, or even reach maximal levels. Changes in the time at which these events occur postmortem could have dramatic effects on ultimate meat quality development, as protein denaturation again is a pH-temperature phenomenon (Offer, 1991; Wismer-Pedersen, 1959). Furthermore, these data show theoretically, that removal of adenonucleotides from glycolysing muscle will arrest metabolism raising another point of control that will be discussed briefly below.

His final and arguably the most important data using this in vitro system, were those directly targeted at understanding the enzymes responsible for pacing both early postmortem metabolism and that responsible for extending carbohydrate metabolism in skeletal muscle (Scopes, 1974b). These data showed that regardless of enzymes present, ATPase concentration, or ATP consumption, drives the rate of metabolism. These findings formed the basis by which many understand the role ATPase plays in controlling the rate of postmortem metabolism (Bowker, Grant, Swartz, & Gerrard, 2004; Hamm, 1977), especially where inherent differences in ATP consumption found between muscles of different fiber types change postmortem metabolism (Fernandez & Tornberg, 1991; Klont, Brocks, & Eikelenboom, 1998). Moreover, early postmortem consumption of ATP in muscle is hallmark of halothane-positive pigs containing a mutated calcium channel protein that allows cellular calcium concentrations to rise to a point where corresponding downstream ATPases force an aggressive metabolism and aberrant meat quality development (Cheah, Cheah, Crosland, Casey, & Webb, 1984; Greaser, Cassens, Briskey, & Hoekstra, 1969; Monin, Sellier, Ollivier, Goutepongea, & Girard, 1981). Results of these studies also raised the idea that GP *a*, and to a lesser extent AMP deaminase concentrations may dictate the pH at which metabolism stops (Scopes, 1974b).

Understanding how Scopes settled on GP *a* as a driver of pH_u in porcine skeletal muscle is logical though difficult to explain in living, or dying muscle. In his studies (Scopes, 1974b), GP *a* concentrations were included at sub- and supra-physiological levels. While the increased levels of GP *a* resulted in a reduced pH_u in vitro, it also accelerated the rate of pH decline, begging the question of whether phosphorylase differs between pigs with altered muscle metabolism. GP *a* activity in resting halothane positive pig muscle is lower compared to wild-type pigs (Fernandez, Neyraud, Astruc, & Sante, 2002), yet increases above that of wild-type animals during postmortem metabolism (Monin, Talmant, Laborde, Zabari, & Sellier, 1986). This increase in phosphorylase *a* content is likely due to the calcium-mediated activation of phosphorylase kinase, the enzyme responsible for the conversion of GP *b* to *a* form (Meyer, Fischer, & Krebs, 1964). Alternatively, it could be due to increased catecholamine release and action at harvest (Althen, Ono, & Topel, 1977). However, classically halothane pigs do not differ in pH_u (Copenhafer et al., 2006; De Smet et al., 1996; Fernandez et al., 2002; Klont, Lambooy, & van Logtestijn, 1993; Kocwin-Podsiadla, Przybylski, Kuryl, Talmant, & Monin, 1995) which argues against GP *a* content driving the pH_u of meat. Even so, a number of investigators have shown that meat of halothane positive pigs results in a lower pH_u (Fisher, Mellett, & Hoffman, 2000; Hamilton, Ellis, Miller, McKeith, & Parrett, 2000; Klont & Lambooy, 1995; Klont, Lambooy, & van Logtestijn, 1994; Monin et al., 1981). The latter issue makes it difficult to study cessation of postmortem metabolism in halothane-sensitive pigs. In comparison, no differences have been noted in GP *a* activity between wild-type and Rendement Napole (RN) pigs (Estrade, Ayoub, Talmant, & Monin, 1994). Recall, RN pigs possess a gene mutation that somehow allows for a breech in the normal postmortem set points and results in lower pH_u and a type of 'acid meat'. Regardless, the aforementioned data strongly support the notion that GP may be involved in controlling the extent of postmortem metabolism and should be closer scrutinized.

Though not specifically addressed by Scopes' work, the enzyme most frequently implicated as responsible for the cessation of postmortem metabolism is phosphofructokinase (PFK) (Bendall, 1973; Hamm, 1977), due in part, to its rate-limiting status in glycolysis and its complex control in living tissues (Berg et al., 2007). Moreover, glucose 6-phosphate increases late postmortem (Copenhafer et al., 2006; Kastenschmidt, Hoekstra, & Briskey, 1968) suggesting a loss in PFK activity sometime earlier. Data to directly support this hypothesis are scant. However, a recent study comparing normal and RN pig muscle showed a number of sarcoplasmic and myofibrillar proteins experience phosphorylation events postmortem (Lametsch et al., 2011). To that end, the increased phosphorylation of PFK in RN pig muscle may increase its stability and subsequent pH inhibition during the postmortem period (Sola-Penna, Da Silva, Coelho, Marinho-Carvalho, & Zancan, 2010). This may help explain the lower pH_u of fresh pork derived from this genotype.

Schwägele and Honikel (1988) quantified a host of glycolytic enzyme activities over a wide range of pH values (5.3 to 6.8) found in postmortem tissue. Results showed the activities of PFK, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, pyruvate kinase,

and adenylate kinase varied with pH conditions studied; however no enzyme lost activity completely. Enzymes were extracted in a phosphate buffer (pH 7.0) suggesting assays were conducted at a pH close to the physiological norm, rather than those conditions found postmortem. If this was indeed the case, then the activity of these enzymes at normal postmortem conditions remains unknown, though Scopes argues that PFK remains active at pH 5.35 (Scopes, 1974b). Regardless, these findings provide some evidence that glycolytic-based enzyme inactivity may be subtle. These data do not, however, rule-out enzyme inactivation as a means of arresting postmortem metabolism.

The idea that postmortem metabolism stops in response to adenonucleotides loss is intriguing and rational. After all, the enzymes thought to be rate-limiting in glycogenolysis and glycolysis (PFK, GP, pyruvate kinase) are all allosterically activated/inactivated by ATP or its metabolites (Berg et al., 2007). Under normal circumstances, ATP is hydrolyzed to ADP, which then can be rephosphorylated to ATP by either phosphoglycerate kinase, pyruvate kinase or adenylate kinase. Adenylate kinase converts 2 ADP molecules into one ATP and one AMP molecule (ADP + ADP → ATP + AMP) (Pearson, 1971). Once formed, AMP is quickly converted by AMP deaminase (AMP → IMP) and unavailable for use by glycolysis (Pearson & Young, 1989), thus reducing the concentration of the adenonucleotide pool and eliminating a substrate for glycolysis to phosphorylate. The adenylate kinase and AMP deaminase reactions are coupled so an increase in activity of one results in an increase of the other. Though subtle differences exist between species (Fishbein, Davis, & Foellmer, 1993), AMP deaminase or adenylate kinase activity do not differ greatly, mitigating excitement for either in controlling differences in the extent of postmortem metabolism postmortem. Even so, these enzymes likely deserve some additional consideration regarding our quest to understand fully postmortem metabolism.

One means of testing whether adenonucleotides dictate the cessation of glycolysis would be to eliminate them experimentally from the tissue. A complete loss of ATP would necessarily stimulate adenylate kinase and AMP deaminase and speed up the arrest of glycolysis. The only approach readily available is electrical stimulation, which stimulates glycolysis (Hwang, Devine, & Hopkins, 2003). Theoretically, it may be possible to hydrolyze enough ATP that sufficient energy would not be available to break rigor bonds, and thereby arrest glycolysis. Then, is it possible to stimulate carcasses with electricity enough to stop postmortem metabolism? If so, would sustained electrical stimulation create a dark, firm, and dry (DFD)-like meat condition if the loss of adenosine nucleotides is solely responsible for the cessation of glycolysis and establishment of the pH_u of meat? Across numerous electrical stimulation studies reviewed, we have yet to read of any form of electrical stimulation that ever resulted in higher pH_u values or DFD-like meat, at least in pigs (Hallund & Bendall, 1965; Hammelman et al., 2003; Maribo, Ertbjerg, Andersson, Barton-Gade, & Møller, 1999; Taylor, Nute, & Warkup, 1995). In fact, electrical stimulation is a reliable tool to produce pale, soft, and exudative (PSE) meat in pigs (Bowker, Grant, Forrest, & Gerrard, 2000). Taken together, these data indicate that despite a rapid loss of ATP caused by electric stimulation, sometimes repeated, enough nucleotides remain in the muscle to facilitate glycolysis to a normal pH_u. Even so, until we find a way to eliminate ATP experimentally, we cannot rule out this hypothesis.

3. Mitochondria and meat quality

At harvest, muscle must adapt to new physiological circumstances in order to maintain ATP and homeostasis. Delivery of oxygen to muscle is eliminated, which impedes mitochondria from contributing to ATP production. Thus, the conversion of muscle to meat is traditionally viewed as an anaerobic process that is largely governed by degradation of glycogen to lactate and H^+. While fundamentally true, this has propagated a dubious conclusion that because mitochondria can no longer generate ATP, they are irrelevant to pH decline and postmortem metabolism. In fact, mitochondria are functionally complex and operate quite differently as the cellular milieu changes.

Although typically thought of as individual organelles, mitochondria are dynamic and plastic. Fusion and fission events allow mixing of mitochondrial contents and also contribute to a range of mitochondrial morphology, from small, round-oval organelles to a branched tubular network (reviewed by Chan, 2006). Structure of mitochondria is well-suited to their function. Mitochondria consist of an outer membrane and a highly folded inner membrane, resulting in two aqueous compartments: the intermembrane space and the matrix. The outer membrane is permeable to most small molecules and ions, while the inner membrane is completely impermeable to most small molecules. The inner membrane also contains the proteins of the electron transport chain, and the transfer of electrons through these protein complexes leads to protons being pumped out of the mitochondrial matrix into the intermembrane space. This results in an uneven distribution of charge or mitochondrial membrane potential (Ψ_{mt}), with the intermembrane space being more positive. Under normal physiological circumstances, the flow of protons through F_1F_0 ATP synthase (complex V) back to the mitochondrial matrix powers ATP synthesis. The rate of oxidative phosphorylation is primarily determined by the need for ATP. Thus, high ADP concentrations stimulate maximal oxygen consumption or respiration, referred to as state 3 respiration (Chance & Williams, 1955). Additionally, greater workloads contribute to increases in intracellular calcium; in turn, Ca^{2+} may be taken up by mitochondria, primarily via the Ca^{2+} uniporter in the inner membrane. Subsequently, increases in matrix Ca^{2+} enhance activity of enzymes such as pyruvate dehydrogenase, and this helps generate Ψ_{mt} (reviewed by McCormack, Halestrap, & Denton, 1990). Not all protons flow back through ATP synthase; proton leak across the inner membrane represents inefficiency because oxygen consumption is not coupled to ATP synthesis. Experimentally, an ATP synthase inhibitor can be used to eliminate the contribution of proton flow through the ATP synthase to determine basal or state 4 respiration. The ratio of oxygen consumption rate in state 3 relative to state 4 (respiratory control ratio, RCR) indicates the tightness of coupling between respiration and phosphorylation, and also serves as an index of mitochondrial integrity (reviewed by Brand & Nicholls, 2011).

While limited oxygen dictates that mitochondria have limited ability to generate ATP postmortem, they appear to remain functionally competent for some time. In fact, intact mitochondria could be isolated from ox neck muscle at 144 h postmortem (Cheah & Cheah, 1971). Furthermore, mitochondria isolated from dark-cutting beef are reasonably well-coupled at 5 d postmortem and perform better than mitochondria obtained from control animals at 24 h postmortem (Ashmore, Parker, & Doerr, 1972). Moreover, there are clear improvements in functional parameters of mitochondria when the pH of isolation and assay medium is increased to physiological pH (Ashmore et al., 1972; Tang et al., 2005). Thus, the main factor affecting function of mitochondria isolated in the postmortem period is development of low muscle pH. Similarly, mitochondria isolated from "normal" muscle immediately postmortem resemble those found in intact muscle, whereas some mitochondria from PSE muscle are already swollen and show decreased matrix density (Greaser et al., 1969). In permeabilized fibers from halothane positive muscle, mitochondrial state 3 respiration declines to a greater extent from 0 to 12 h postmortem compared to control (Werner, Natter, Schellander, & Wicke, 2010). Together, this suggests that acidic cellular environment and rapid pH decline are major factors influencing mitochondrial morphology and function postmortem.

Although mitochondria are still capable of oxidative phosphorylation postmortem, the oxygen deficient environment limits respiration. So, how do mitochondria contribute to postmortem metabolism and pH decline? It is important to consider that, at harvest, muscle is living and all metabolic systems are functionally competent; the subsequent struggle to "survive" or maintain homeostasis may parallel that of living

muscle exposed to anoxic or ischemic conditions. When oxygen is lacking, electron transport in mitochondria ceases, and responsibility for ATP production shifts to anaerobic glycolysis. The mismatch between ATP production and utilization creates several challenges for cellular homeostasis. First, cellular pH decreases, and this "deenergizes" or gradually decreases the Ψ_{mt}. Concurrently, decreased efficiency of sarcoplasmic reticulum Ca^{2+}-ATPase contributes to intracellular Ca^{2+} overload. In turn, mitochondria accumulate substantial amounts of Ca^{2+}, and sustained increases in matrix Ca^{2+} contribute to permeability transition whereby the inner membrane becomes permeable to solutes (Bernardi et al., 2006). This, in turn, can trigger mitochondrial matrix swelling and collapse of Ψ_{mt}. Under these extreme circumstances, the mitochondrial ATP synthase may actually run in reverse, hydrolyzing glycolytically-produced ATP in order to maintain a membrane potential (Scott & Nicholls, 1980). Net consumption of ATP by mitochondria further compromises cellular energy balance and viability.

As Hudson (2012) has recently suggested, this "cellular treason" by mitochondria may be applicable to postmortem muscle and meat quality development. ATP hydrolysis by the F_1F_0 ATPase would directly influence cellular ATP consumption, and could further promote glycolysis and pH decline postmortem. In turn, rate of ATP consumption would be expected to be positively correlated with mitochondrial content. Therefore, as Hudson (2012) proposed, F_1F_0 ATPase driven ATP hydrolysis could partly explain the paradoxical observation that red, oxidative muscles sometimes exhibit faster pH decline rates than whiter, more glycolytic muscles.

While pH decline and ATP consumption may be related directly to mitochondrial content, the relationship is likely much more convoluted. Specifically, the properties of mitochondria may vary not only by fiber type, but by their location within the muscle cell. Conventionally, energetic differences between red and white muscles are attributed primarily to greater mitochondrial density of slow fibers (Schwerzmann, Hoppeler, Kayar, & Weibel, 1989). Yet, others recently suggested that mitochondria exhibit a certain phenotype based on fiber type (Picard, Hepple, & Burelle, 2012). Mitochondrial phenotype encompasses not only ATP producing capacity, but also reactive oxygen species production, and sensitivity to Ca^{2+} and apoptosis. The ability of mitochondria to buffer Ca^{2+} influences the amplitude and frequency of Ca^{2+} oscillation within the cytosol. The nature of cytosolic Ca^{2+} oscillations varies by fiber type. Thus, Ca^{2+} handling properties of mitochondria and their tolerance for aberrant Ca^{2+} levels may differ according to fiber type. With net ATP consumption postmortem and reduced sarcoplasmic reticulum Ca^{2+}-ATPase function, mitochondria may play a significant role in accumulating Ca^{2+} and restraining glycolysis. Subcellular localization of mitochondria in muscle cells may also impact function and response to cellular stress. Muscle mitochondria are distributed primarily either beneath the cell membrane (subsarcolemmal) or between the myofibrils (intermyofibrillar) (Müller, 1976). Although slow and fast fibers have a similar volume of intermyofibrillar mitochondria, slow fibers have a much higher volume of subsarcolemmal mitochondria (Philippi & Sillau, 1994). Subsarcolemmal and intermyofibrillar mitochondrial likely form different networks that are not connected, which contributes to distinct functional and biochemical properties.

Another point of consideration, however, is that there are inhibitory mechanisms that prevent the ATPase from wasting ATP during cellular stress. For example, certain species, such as frogs, are exposed to extended periods of anoxia which would be expected to threaten their livelihood. However, in this situation, ATP hydrolysis is greatly reduced via inhibition of the F_1F_0 ATPase (St-Pierre, Brand, & Boutilier, 2000). In turn, this restricts cellular ATP depletion during ischemia, and delays cell damage and death. Moreover, different species possess different capacities for ATPase inhibition. This is based on content and affinity of F_1-ATPase inhibitory subunit (IF_1). The IF_1 subunit binds to the ATPase under nonenergizing conditions, when the pH of the mitochondrial matrix is low (Rouslin, Frank, & Broge, 1995). During anoxia, species possessing greater content of high affinity IF_1 demonstrate marked inhibition of ATPase, whereas species with low amounts of high affinity IF_1 exhibit little inhibition of ATPase. Additionally, IF_1 content varies by tissue, and importance of IF_1 may depend on conditions (i.e. presence of uncoupler) (Rouslin & Broge, 1996; Rouslin et al., 1995).

Despite their complex role in cellular function and metabolism, the role of mitochondria in postmortem muscle has remained largely unexplored. While the loss of oxygen impedes "normal" mitochondrial function, it does not preclude mitochondria from contributing to meat quality development. Mitochondrial content, as well as functional differences between distinct mitochondrial populations may influence the course of postmortem metabolism. Furthermore, mitochondrial properties may be intrinsically beneficial or detrimental to the course of postmortem metabolism and pH decline. For example, pro-survival or anti-apoptotic proteins may associate with mitochondrial membrane proteins and modify response to energetic depression. Understanding these aspects of mitochondria in postmortem muscle may improve our ability to predict pH decline and meat quality.

4. Antemortem handling

Feed withdrawal or fasting prior to slaughter is commonly used to improve meat quality by elevating pH_u through a reduction in liver (Warriss, 1982) and muscle glycogen (Fernandez & Tornberg, 1991; Sterten, Oksbjerg, Frøystein, Ekker, & Kjos, 2010; Wittmann, Ecolan, Levasseur, & Fernandez, 1994). A Bayesian meta-analysis using 16 studies showed fasting duration increases the pH_u of the *longissimus* muscle of pigs (Salmi et al., 2012) yet, the correlation is weak likely due to the unique relationship between glycogen and the pH_u of meat. In fact, this relationship is curvilinear where the muscle glycogen content is primarily responsible for dictating pH_u between a pH_u of 7.2 and 5.7 (Bendall, 1973; Henckel, Karlsson, Jensen, Oksbjerg, & Petersen, 2002), but below 5.7, other as yet unknown mechanisms must be responsible for the variation in this range. Some of the possible mechanisms have already been discussed in earlier sections, yet others likely remain.

During normal postmortem metabolism, the *longissimus* muscle (white, glycolytic muscle) utilizes 35–40 μmol glycogen/g tissue to achieve a pH_u near 5.5 (Copenhafer et al., 2006). Oftentimes, this muscle contains greater than necessary amounts of glycogen (60 μmol/g) resulting in 2–30% of the total glycogen remaining at 24 h (Pearson & Young, 1989). This residual glycogen content may help explain the weak relationship between fasting and pH_u. Fasting reduces glycogen content of muscle by as much as 20% in the *longissimus* muscle of pigs over a 24 h period (Wittmann et al., 1994), yet this reduction is insufficient to reduce glycogen below the aforementioned threshold that affects pH_u. Henckel et al. (2002) suggests that increases in pH_u will not develop unless muscle glycogen is reduced below 53 μmol/g tissue supporting this argument. Furthermore, red, oxidative muscles contain less glycogen and produce meat with higher pH_u (Monin & Talmant, 1987). Curiously, a 24 h fast reduces the glycogen content of the more oxidative *semispinalis* muscle by 50% and results in a higher pH_u (Wittmann et al., 1994). Thus, the percentage decrease in glycogen between the two muscles in response to a 24 h fast is quite different, but the absolute decrease of each is quite similar (~15 μmol/g) (Wittmann et al., 1994). Therefore, fasting may reduce the stored carbohydrate content of muscle below this 'critical' threshold and thus, fasting may be more effective on red muscles due to their lower resting glycogen content. This is especially apparent with RN pigs. Again, RN pig muscle contains extremely high levels of glycogen and produce meat with low pH_u. When fasted for 36 h, the pH_u of meat from RN mutant pigs still remained lower than controls (Bidner, Ellis, Witte, Carr, & McKeith, 2004), arguing that a 36 h fast is not sufficient to lower glycogen levels below the critical threshold where it can impact pH_u and recover a normal phenotype. Regardless of whether fasting impacts postmortem metabolism and meat quality characteristics, the practice of fasting should not be eliminated from the standard slaughter

procedure because of the microbiological ramifications mitigated by reducing gastrointestinal tract content (Miller, Carr, Bawcom, Ramsey, & Thompson, 1997).

Of particular significance to the issue at hand is the idea that different sources of dietary energy impact antemortem muscle glycogen concentrations (reviewed by Andersen, Oksbjerg, Young, & Therkildsen, 2005). Most intriguing is the idea that easily digestible carbohydrates may alter postmortem metabolism to prevent DFD meat. Muscle of pigs fed a diet of sucrose resulted in an accelerated pH decline, lower pH_u (Briskey, Bray, Hoekstra, Phillips, & Grummer, 1959) and elevated glycogen (Briskey, Bray, Hoekstra, Phillips, & Grummer, 1960). To explain the accelerated metabolism, a follow-up study compared the GP activity between control fed and sucrose pigs, but found no difference between the two treatments were detected (Sayre, Briskey, & Hoekstra, 1963). High-sugar diets encourage the incorporation of carbohydrate into the muscle as glycogen and alters glucose metabolism (reviewed by Fernandez & Tornberg, 1991) which may alter the enzymatic activity within the muscle. Though GP activity was not altered in sucrose-fed pigs (Sayre et al., 1963), other enzymes may have been altered to facilitate an accelerated metabolism. Alternatively, a lower pH_u in meat may have been directly a result of increased glycogen deposition. However, very high inclusion rates may be necessary to achieve this endpoint and this alone may have detrimental effects on tissue energy metabolism as up to 15% sucrose incorporation to grower and finishing pig diets has no effect on glycogen content or postmortem metabolism (Camp, Southern, & Bidner, 2003).

Regardless of the mechanism, diet can be used to mitigate low pH values by reducing carbohydrates from the diet to less than 5% and increasing fat to approximately 18% on a weight basis (Rosenvold et al., 2001). When fed three weeks prior to slaughter, these diets reduce the glycogen content of the *longissimus* dorsi by 11–26% (Rosenvold et al., 2001) and increase pH_{45} and pH_u (Rosenvold et al., 2002; Tikk, Tikk, Karlsson, & Andersen, 2006) and accelerate temperature decline (Tikk, Lindahl, Karlsson, & Andersen, 2008; Tikk et al., 2006). Presumably, this feeding strategy does not alter key glycolytic enzymes like PFK, GP *a*, or GP *b* activity, but may lower μ-calpain activity (Rosenvold et al., 2002), the enzyme responsible for postmortem proteolysis of myofibrillar proteins (Huff-Lonergan & Lonergan, 2007).

Strategic feeding protocols and fasting are intriguing because both appear to reduce glycogen content, which are thought to improve meat quality, yet there is no indication of an interaction between the two. To our knowledge, only one study has been conducted to address this issue (Partanen, Honkavaara, & Ruusunen, 2007). In this study, the longer fasting time and a fibrous diet increased pH_u and lowered muscle glycogen, and indicated that the combination of the two may produce an additive effect to elevate pH_u.

Pre-slaughter stress is complicated, but clearly reduces meat quality through altered metabolism. If an animal is stressed long-term, glycogen stores may be depleted resulting in abbreviated metabolism and a high pH_u (Briskey, 1964). Adrenaline injections prior to slaughter can produce a similar result if administered appropriately (Bendall & Lawrie, 1962; Henckel, Karlsson, Oksbjerg, & Søholm Petersen, 2000; Henckel et al., 2002; Rosenvold et al., 2001). Long-term stress requires a recovery period known as lairage. From a tissue standpoint, this is interpreted as sufficient time to re-establish the glycogen that was in the muscle, or eliminate entirely the stress hormones, mostly catecholamines, that may have been released in the body in response to the stress (Faucitano, 2010). For the most part, increased lairage times elevate pH_{45}, pH_u and thereby improve meat quality measurements, especially L^* (lightness) values (Salmi et al., 2012; Zhen et al., 2013). Alternatively, short-term stress immediately prior to slaughter is known to accelerate metabolism and result in higher muscle temperatures and a lower pH_u (van der Wal, Engel, & Reimert, 1999), a condition similar to the HAL mutation. Short-term stress is difficult to remedy due to its occurrence immediately prior to slaughter during handling or movement of the animal to the abattoir. Rosenvold and Andersen (2003) provide a much more comprehensive review about the differences between long-term and short-term stress upon postmortem metabolism.

To complicate matters further, the effects can be combined. Shen et al. (2006) induced pre-slaughter transport stress upon pigs both with and without rest. Transport resulted in decreased (glycogen + glucose + G6P) early postmortem as well as reduced muscle pH. Muscle from transported pigs, without rest, produced meat with a lower pH_u compared to control animals, exhibited an accelerated (AMP + IMP)/ATP ratio, maintained higher GP *a* activity through 4 h postmortem, and resulted in meat with a higher drip loss and a PSE condition. While muscle glycogen was indeed reduced in these animals, it was insufficient to produce DFD meat and only resulted in accelerated metabolism. Recall, muscle glycogen must be reduced below 53 μmol/g tissue to produce DFD meat. A general lack of consensus reflects the complexity of studying the effect of transportation on ultimate energy metabolism in the muscle, especially in pigs. Even so, it argues that pre-slaughter stress is capable of producing both DFD and PSE meat, yet the exact biochemical mechanisms remain quite unclear.

5. Summary

When thinking about the conversion of muscle to meat, unanswered questions still remain. The mechanism responsible for the cessation of anaerobic glycolysis postmortem has eluded researchers for the past 60 years, but a pH-mediated inactivation of glycolytic enzymes or a loss of adenosine nucleotides is likely. It is also quite difficult to accurately depict postmortem metabolism without recognizing the contribution of mitochondria, despite the loss of oxygen that inhibits "normal" function. Finally, prior to slaughter, the energy charge created in the animal's muscles dictates the rate and extent of metabolism. Fasting, diet, transport and lairage all affect postmortem metabolism and must not be overlooked when attempting to improve meat quality.

References

Althen, T. G., Ono, K., & Topel, D. G. (1977). Effect of stress susceptibility or stunning method on catecholamine levels in swine. *Journal of Animal Science*, *44*(6), 985–989.

Andersen, H. J., Oksbjerg, N., Young, J. F., & Therkildsen, M. (2005). Feeding and meat quality — A future approach. *Meat Science*, *70*(3), 543–554.

Ashmore, C. R., Parker, W., & Doerr, L. (1972). Respiration of mitochondria isolated from dark-cutting beef: Postmortem changes. *Journal of Animal Science*, *34*(1), 46–48.

Bendall, J. R. (1973). Structure and function of muscle. In G. H. Bourne (Ed.), (2nd ed.). New York: Academic Press.

Bendall, J. R., & Lawrie, R. A. (1962). The effect of pre-treatment with various drugs on post-mortem glycolysis and the onset of rigor mortis in rabbit skeletal muscle. *The Journal of Comparative Pathology and Therapeutics*, *72*, 118–130.

Berg, J. H., Tymoczko, J. L., & Stryer, L. (2007). *Biochemistry* (6th ed.). New York: W. H. Freeman and Company.

Bernardi, P., Krauskopf, A., Basso, E., Petronilli, V., Blalchy-Dyson, E., Di Lisa, F., & Forte, M. A. (2006). The mitochondrial permeability transition from in vitro artifact to disease target. *FEBS Journal*, *273*(10), 2077–2099.

Bidner, B. S., Ellis, M., Witte, D. P., Carr, S. N., & McKeith, F. K. (2004). Influence of dietary lysine level, pre-slaughter fasting, and Rendement Napole genotype on fresh pork quality. *Meat Science*, *68*(1), 53–60.

Bowker, B. C., Grant, A. L., Forrest, J. C., & Gerrard, D. E. (2000). Muscle metabolism and PSE pork. *Journal of Animal Science*, *79*(E-Suppl. 1), 1–8.

Bowker, B. C., Grant, A. L., Swartz, D. R., & Gerrard, D. E. (2004). Myosin heavy chain isoforms influence myofibrillar ATPase activity under simulated postmortem pH, calcium, and temperature conditions. *Meat Science*, *67*(1), 139–147.

Brand, M. D., & Nicholls, D. G. (2011). Assessing mitochondrial dysfunction in cells. *Biochemical Journal*, *435*, 297–312.

Briskey, E. J. (1964). Etiological status and associated studies of pale, soft, exudative porcine musculature. In E. M. M. C. O. Chichester, & G. F. Stewart (Eds.), *Advances in Food Research. vol. 13*. (pp. 89–178) : Academic Press.

Briskey, E. J., Bray, R. W., Hoekstra, W. G., Phillips, P. H., & Grummer, R. H. (1959). The effect of exhaustive exercise and high sucrose regimen on certain chemical and physical pork ham muscle characteristics. *Journal of Animal Science*, *18*(1), 173–177.

Briskey, E. J., Bray, R. W., Hoekstra, W. G., Phillips, P. H., & Grummer, R. H. (1960). Effect of high protein, high fat and high sucrose rations on the water-binding and associated properties of pork muscle. *Journal of Animal Science*, *19*(2), 404–411.

Camp, L. K., Southern, L. L., & Bidner, T. D. (2003). Effect of carbohydrate source on growth performance, carcass traits, and meat quality of growing-finishing pigs. *Journal of Animal Science*, *81*(10), 2488–2495.

Chan, D. C. (2006). Mitochondria: dynamic organelles in disease, aging, and development. *Cell*, *125*, 1241–1252.
Chance, B., & Williams, G. R. (1955). Respiratory enzymes in oxidative phosphorylation. *Journal of Biological Chemistry*, *217*, 383–394.
Cheah, K. S., & Cheah, A. M. (1971). Post-mortem changes in structure and function of ox muscle mitochondria. 1. Electron microscopic and polarographic investigations. *Journal of Bioenergetics and Biomembranes*, *2*(2), 85–92.
Cheah, K. S., Cheah, A. M., Crosland, A. R., Casey, J. C., & Webb, A. J. (1984). Relationship between Ca2+ release, sarcoplasmic Ca2+, glycolysis and meat quality in halothane-sensitive and halothane-insensitive pigs. *Meat Science*, *10*(2), 117–130.
Copenhafer, T. L., Richert, B. T., Schinckel, A. P., Grant, A. L., & Gerrard, D. E. (2006). Augmented postmortem glycolysis does not occur early postmortem in AMPKγ3-mutated porcine muscle of halothane positive pigs. *Meat Science*, *73*(4), 590–599.
Dalrymple, R. H., & Hamm, R. (1975). Postmortem glycolysis in prerigor ground bovine and rabbit muscle. *Journal of Food Science*, *40*(4), 850–853.
De Smet, S. M., Pauwels, H., De Bie, S., Demeyer, D. I., Callewier, J., & Eeckhout, W. (1996). Effect of halothane genotype, breed, feed withdrawal, and lairage on pork quality of Belgian slaughter pigs. *Journal of Animal Science*, *74*(8), 1854–1863.
Estrade, M., Ayoub, S., Talmant, A., & Monin, G. (1994). Enzyme activities of glycogen metabolism and mitochondrial characteristics in muscles of RN-carrier pigs (*Sus scrofa domesticus*). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *108*(3), 295–301.
Faucitano, L. (2010). Invited review: Effects of lairage and slaughter conditions on animal welfare and pork quality. *Canadian Journal of Animal Science*, *90*(4), 461–469.
Fernandez, X., Neyraud, E., Astruc, T., & Sante, V. (2002). Effects of halothane genotype and pre-slaughter treatment on pig meat quality. Part 1. Post mortem metabolism, meat quality indicators and sensory traits of *M. longissimus lumborum*. *Meat Science*, *62*(4), 429–437.
Fernandez, X., & Tornberg, E. (1991). A review of the causes of variation in muscle glycogen content and ultimate pH in pigs. *Journal of Muscle Foods*, *2*(3), 209–235.
Fishbein, W. N., Davis, J. I., & Foellmer, J. W. (1993). Comparative enzymology of AMP deaminase, adenylate kinase, and creatine kinase in vertebrate heart and skeletal muscle: the characteristic AMP deaminase levels of skeletal versus cardiac muscle are reversed in the North American toad. *Journal of Comparative Physiology. B*, *163*(3), 175–181.
Fisher, P., Mellett, F. D., & Hoffman, L. C. (2000). Halothane genotype and pork quality. 1. Carcass and meat quality characteristics of three halothane genotypes. *Meat Science*, *54*(2), 97–105.
Greaser, M. L. (2001). Meat Science and Applications. In Y. H. Hui, W. -K. Nip, R. W. Rogers, & O. A. Young (Eds.), New York: Marcel Dekker, Inc.
Greaser, M. L., Cassens, R. G., Briskey, E. J., & Hoekstra, W. G. (1969). Post-mortem changes in subcelllular fractions from normal and pale, soft, exudative porcine muscle. 2. Electron Microscopy. *Journal of Food Science*, *34*(2), 125–132.
Hallund, O., & Bendall, J. R. (1965). The long-term effect of electrical stimulation on the post-mortem fall of pH in the muscles of landrace pigs. *Journal of Food Science*, *30*(2), 296–299.
Hamilton, D. N., Ellis, M., Miller, K. D., McKeith, F. K., & Parrett, D. F. (2000). The effect of the halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. *Journal of Animal Science*, *78*(11), 2862–2867.
Hamm, R. (1977). Postmortem breakdown of ATP and glycogen in ground muscle: a review. *Meat Science*, *1*(1), 15–39.
Hammelman, J. E., Bowker, B. C., Grant, A. L., Forrest, J. C., Schinckel, A. P., & Gerrard, D. E. (2003). Early postmortem electrical stimulation simulates PSE pork development. *Meat Science*, *63*(1), 69–77.
Henckel, P., Karlsson, A., Jensen, M. T., Oksbjerg, N., & Petersen, J. S. (2002). Metabolic conditions in Porcine longissimus muscle immediately pre-slaughter and its influence on peri- and post mortem energy metabolism. *Meat Science*, *62*(2), 145–155.
Henckel, P., Karlsson, A., Oksbjerg, N., & Søholm Petersen, J. (2000). Control of post mortem pH decrease in pig muscles: Experimental design and testing of animal models. *Meat Science*, *55*(1), 131–138.
Hudson, N. J. (2012). Mitochondrial treason: A driver of pH decline rate in post-mortem muscle? *Animal Production Science*, *52*(12), 1107–1110.
Huff-Lonergan, E., & Lonergan, S. M. (2007). New frontiers in understanding drip loss in pork: Recent insights on the role of postmortem muscle biochemistry. *Journal of Animal Breeding and Genetics*, *124*, 19–26.
Hwang, I. H., Devine, C. E., & Hopkins, D. L. (2003). The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Science*, *65*(2), 677–691.
Joo, S. T., Kauffman, R. G., Kim, B. C., & Park, G. B. (1999). The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. *Meat Science*, *52*(3), 291–297.
Kastenschmidt, L. L., Hoekstra, W. G., & Briskey, E. J. (1968). Glycolytic intermediates and co-factors in "fast-" and "slow-glycolyzing" muscles of the pig. *Journal of Food Science*, *33*(2), 151–158.
Klont, R. E., Brocks, L., & Eikelenboom, G. (1998). Muscle fibre type and meat quality. *Meat Science*, *49*(Supplement 1(0)), S219–S229.
Klont, R. E., & Lambooy, E. (1995). Effects of preslaughter muscle exercise on muscle metabolism and meat quality studied in anesthetized pigs of different halothane genotypes. *Journal of Animal Science*, *73*(1), 108–117.
Klont, R. E., Lambooy, E., & van Logtestijn, J. G. (1993). Effect of preslaughter anesthesia on muscle metabolism and meat quality of pigs of different halothane genotypes. *Journal of Animal Science*, *71*(6), 1477–1485.
Klont, R. E., Lambooy, E., & van Logtestijn, J. G. (1994). Effect of dantrolene treatment on muscle metabolism and meat quality of anesthetized pigs of different halothane genotypes. *Journal of Animal Science*, *72*(8), 2008–2016.
Kocwin-Podsiadla, M., Przybylski, W., Kuryl, J., Talmant, A., & Monin, G. (1995). Muscle glycogen level and meat quality in pigs of different halothane genotypes. *Meat Science*, *40*(1), 121–125.
Kylä-Puhju, M., Ruusunen, M., Kivikari, R., & Puolanne, E. (2004). The buffering capacity of porcine muscles. *Meat Science*, *67*(4), 587–593.
Lametsch, R., Larsen, M. R., Essén-Gustavsson, B., Jensen-Waern, M., Lundström, K., & Lindahl, G. (2011). Postmortem changes in pork muscle protein phosphorylation in relation to the RN genotype. *Journal of Agricultural and Food Chemistry*, *59*(21), 11608–11615.
Maribo, H., Ertbjerg, P., Andersson, M., Barton-Gade, P., & Møller, A. J. (1999). Electrical stimulation of pigs—Effect on pH fall, meat quality and cathepsin B + L activity. *Meat Science*, *52*(2), 179–187.
McCormack, J. G., Halestrap, A. P., & Denton, R. M. (1990). Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiological Reviews*, *70*, 391–425.
Meyer, W. L., Fischer, E. H., & Krebs, E. G. (1964). Activation of skeletal muscle phosphorylase b kinase by Ca2+. *Biochemistry*, *3*(8), 1033–1039.
Miller, M. F., Carr, M. A., Bawcom, D. B., Ramsey, C. B., & Thompson, L. D. (1997). Microbiology of pork carcasses from pigs with differing origins and feed withdrawal times. *Journal of Food Protection*, *60*(3), 242–245.
Monin, G., Sellier, P., Ollivier, L., Goutepongea, R., & Girard, J. P. (1981). Carcass characteristics and meat quality of halothane negative and halothane positive pietrain pigs. *Meat Science*, *5*(6), 413–423.
Monin, G., & Talmant, A. (1987). A possible relation between muscle residual glycogen and yield of meat processing by curing and cooking. *Proceedings of the 33th international congress of meat science and technology* (pp. 312–315).
Monin, G., Talmant, A., Laborde, D., Zabari, M., & Sellier, P. (1986). Compositional and enzymatic characteristics of the Longissimus Dorsi muscle from large white, halothane-positive and halothane-negative pietrain, and hampshire pigs. *Meat Science*, *16*(4), 307–316.
Morgan, H. E., & Parmeggianiani, A. (1964). Regulation of glycogenolysis in muscle: III. Control of muscle GP activity. *Journal of Biological Chemistry*, *239*, 244–2445.
Müller, W. (1976). Subsarcolemmal mitochondria and capillarization of soleus muscle fibers in young rats subjected to an endurance training. *Cell and Tissue Research*, *174*(3), 367–389.
Newbold, R. P., & Scopes, R. K. (1971). Post-mortem glycolysis in ox skeletal muscle: effects of mincing and of dilution with or without addition of orthophosphate. *Journal of Food Science*, *36*(2), 209–214.
Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. *Meat Science*, *30*(2), 157–184.
Partanen, K., Honkavaara, M., & Ruusunen, M. (2007). Effects of finishing diet and pre-slaughter fasting time on meat quality in crossbred pigs. *Agricultural and Food Science*, *16*, 245–258.
Pearson, A. M. (1971). The science of meat and meat products. In J. F. Price, & B. S. Schweigert (Eds.), (2nd ed.). San Francisco, CA: W. H. Freeman and Company.
Pearson, A. M., & Young, R. B. (1989). *Muscle and meat biochemistry*. San Diego, CA: Academic Press, Inc.
Philippi, M., & Sillau, A. H. (1994). Oxidative capacity distribution in skeletal muscle fibers of the rat. *Journal of Experimental Biology*, *189*(1), 1–11.
Picard, M., Hepple, R. T., & Burelle, Y. (2012). Mitochondrial functional specialization in glycolytic and oxidative muscle fibers: Tailoring the organelle for optimal function. *American Journal of Physiology. Cell Physiology*, *302*(4), C629–C641.
Robergs, R. A. (2001). Exercise-induced metabolic acidosis: Where do the protons come from? *Sportscience*, *5*(2).
Robergs, R. A., Ghiasvand, F., & Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *287*(3), R502–R516.
Rosenvold, K., & Andersen, H. J. (2003). Factors of significance for pork quality—A review. *Meat Science*, *64*(3), 219–237.
Rosenvold, K., Laerke, H. N., Jensen, S. K., Karlsson, A. H., Lundstrom, K., & Andersen, H. J. (2002). Manipulation of critical quality indicators and attributes in pork through vitamin E supplementation, muscle glycogen reducing finishing feeding and pre-slaughter stress. *Meat Science*, *62*(4), 485–496.
Rosenvold, K., Petersen, J. S., Lwerke, H. N., Jensen, S. K., Therkildsen, M., Karlsson, A. H., Møller, H. S., & Andersen, H. J. (2001). Muscle glycogen stores and meat quality as affected by strategic finishing feeding of slaughter pigs. *Journal of Animal Science*, *79*(2), 382–391.
Rouslin, W., & Broge, C. W. (1996). IF1 Function in situ in uncoupler-challenged ischemic rabbit, rat, and pigeon hearts. *Journal of Biological Chemistry*, *271*(39), 23638–23641.
Rouslin, W., Frank, G., & Broge, C. (1995). Content and binding characteristics of the mitochondrial ATPase inhibitor, IF1 in the tissues of several slow and fast heart-rate homeothermic species and in two poikilotherms. *Journal of Bioenergetics and Biomembranes*, *27*(1), 117–125.
Salmi, B., Trefan, L., Bünger, L., Doeschl-Wilson, A., Bidanel, J. P., Terlouw, C., & Larzul, C. (2012). Bayesian meta-analysis of the effect of fasting, transport and lairage times on four attributes of pork meat quality. *Meat Science*, *90*(3), 584–598.
Sayre, R. N., Briskey, E. J., & Hoekstra, W. G. (1963). Effect of excitement, fasting, and sucrose feeding on porcine muscle phosphorylase and post-mortem glycolysisa, b. *Journal of Food Science*, *28*(4), 472–477.
Scheffler, T. L., & Gerrard, D. E. (2007). Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. *Meat Science*, *77*(1), 7–16.
Scheffler, T. L., Park, S., & Gerrard, D. E. (2011). Lessons to learn about postmortem metabolism using the AMPKγ3R200Q mutation in the pig. *Meat Science*, *89*(3), 244–250.
Schwägele, F., & Honikel, K. O. (1988). Studies in postmortem metabolism of PSE-prone pork muscles. *Proc. 34th Int. Cong. Meat Sci. and Technol.* (pp. 26–28).

Schwerzmann, K., Hoppeler, H., Kayar, S. R., & Weibel, E. R. (1989). Oxidative capacity of muscle and mitochondria: Correlation of physiological, biochemical, and morphometric characteristics. *Proceedings of the National Academy of Sciences*, *86*(5), 1583–1587.

Scopes, R. K. (1964). The influence of post-mortem conditions on the solubilities of muscle proteins. *Biochemical Journal*, *91*(1), 201–207.

Scopes, R. K. (1973). Studies with a reconstituted muscle glycolytic system. The rate and extent of creatine phosphorylation by anaerobic glycolysis. *Biochemical Journal*, *134*(1), 197–208.

Scopes, R. K. (1974a). Studies with a reconstituted muscle glycolytic system. The anaerobic glycolytic response to simulated tetanic contraction. *Biochemical Journal*, *138*(1), 119–123.

Scopes, R. K. (1974b). Studies with a reconstituted muscle glycolytic system. The rate and extent of glycolysis in simulated post-mortem conditions. *Biochemical Journal*, *142*(1), 79–86.

Scopes, R. K., & Lawrie, R. A. (1963). Post-mortem lability of skeletal muscle proteins. *Nature*, *197*(4873), 1202–1203.

Scott, I. D., & Nicholls, D. G. (1980). Energy transduction in intact synaptosomes. Influence of plasma-membrane depolarization on the respiration and membrane potential of internal mitochondria determined in situ. *Biochemical Journal*, *186*, 21–33.

Shen, Q. W., Means, W. J., Thompson, S. A., Underwood, K. R., Zhu, M. J., McCormick, R. J., Ford, S. P., & Du, M. (2006). Pre-slaughter transport, AMP-activated protein kinase, glycolysis, and quality of pork loin. *Meat Science*, *74*(2), 388–395.

Sola-Penna, M., Da Silva, D., Coelho, W. S., Marinho-Carvalho, M. M., & Zancan, P. (2010). Regulation of mammalian muscle type 6-phosphofructo-1-kinase and its implication for the control of the metabolism. *IUBMB Life*, *62*(11), 791–796.

Sterten, H., Oksbjerg, N., Frøystein, T., Ekker, A. S., & Kjos, N. P. (2010). Effects of fasting prior to slaughter on pH development and energy metabolism post-mortem in M. longissimus dorsi of pigs. *Meat Science*, *84*(1), 93–100.

St-Pierre, J., Brand, M. D., & Boutilier, R. G. (2000). Mitochondria as ATP consumers: Cellular treason in anoxia. *Proceedings of the National Academy of Sciences*, *97*(15), 8670–8674.

Tang, J., Faustman, C., Hoagland, T. A., Mancini, R. A., Seyfert, M., & Hunt, M. C. (2005). Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. *Journal of Agricultural and Food Chemistry*, *53*(4), 1223–1230.

Taylor, A. A., Nute, G. R., & Warkup, C. C. (1995). The effect of chilling, electrical stimulation and conditioning on pork eating quality. *Meat Science*, *39*(3), 339–347.

Tikk, K., Lindahl, G., Karlsson, A. H., & Andersen, H. J. (2008). The significance of diet, slaughter weight and aging time on pork colour and colour stability. *Meat Science*, *79*(4), 806–816.

Tikk, K., Tikk, M., Karlsson, A. H., & Andersen, H. J. (2006). The effect of a muscle-glycogen-reducing finishing diet on porcine meat and fat colour. *Meat Science*, *73*(2), 378–385.

van der Wal, P. G., Engel, B., & Reimert, H. G. M. (1999). The effect of stress, applied immediately before stunning, on pork quality. *Meat Science*, *53*(2), 101–106.

van Laack, R. L. J. M., Kauffman, R. G., & Greaser, M. L. (2001). Determinants of Ultimate pH of Meat. *Proceedings of the 47th international congress of meat science and technology* (pp. 22–26).

Warner, R. D., Kauffman, R. G., & Greaser, M. L. (1997). Muscle protein changes post mortem in relation to pork quality traits. *Meat Science*, *45*(3), 339–352.

Warriss, P. D. (1982). Loss of carcass weight, liver weight and liver glycogen, and the effects on muscle glycogen and ultimate pH in pigs fasted pre-slaughter. *Journal of the Science of Food and Agriculture*, *33*(9), 840–846.

Werner, C., Natter, R., Schellander, K., & Wicke, M. (2010). Mitochondrial respiratory activity in porcine longissimus muscle fibers of different pig genetics in relation to their meat quality. *Meat Science*, *85*(1), 127–133.

Wismer-Pedersen, J. (1959). Quality of pork in relation to rate of pH change post mortem. *Journal of Food Science*, *24*(6), 711–727.

Wittmann, W., Ecolan, P., Levasseur, P., & Fernandez, X. (1994). Fasting-induced glycogen depletion in different fibre types of red and white pig muscles—Relationship with ultimate pH. *Journal of the Science of Food and Agriculture*, *66*(2), 257–266.

Zhen, S., Liu, Y., Li, X., Ge, K., Chen, H., Li, C., & Ren, F. (2013). Effects of lairage time on welfare indicators, energy metabolism and meat quality of pigs in Beijing. *Meat Science*, *93*(2), 287–291.