Biological Basis for Pale, Soft and Exudative Pork

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Introduction

Pale, soft and exudative (PSE) pork was recognized a half century ago by Ludvigsen (1953). The undesirable appearance and texture, limited functionality, and inferior processing yield of PSE pork continue to make it a critical quality and economic concern (Cannon *et al.*, 1996; Cassens, 2000). Rapid postmortem muscle acidification combined with high muscle temperature, as well as low ultimate meat pH, have long been implicated as factors that induce PSE pork characteristics (Briskey, *et al.*, 1966; Sellier and Monin, 1994). Numerous reports on the development of PSE pork have focused on major gene effects, including the halothane (stress) gene (Fujii *et al.*, 1991; reviewed by Louis *et al.*, 1993) and the Napole gene (RN-; reviewed by Sellier and Monin, 1994; Milan *et al.*, 2000). Despite an abundance of research describing PSE pork characteristics, and a reduction in the frequency of major genes with known deleterious effects on pork quality, Cassens (2000) concluded that little progress has been made in reducing the incidence of PSE pork. The current paper will provide an overview of several biological processes associated with development of PSE pork, and highlight recent improvements in our understanding of these processes that may be useful for devising approaches to reduce the incidence of PSE pork.

Stress Response Associated with Development of PSE Pork

Animal stressors, such as physical exercise, handling, transportation, mixing of pigs, noise, and weather extremes accelerate antemortem muscle metabolism and have adverse effects on meat quality (Tarrant, 1989). The biology of the stress response in animals has been the subject of several recent reviews (Schaefer *et al.*, 2001; von Borell, 2001; Miller and O'Callaghan, 2002; Wurtman, 2002). Miller and O'Callaghan (2002) defined stress as any disruption of homeostasis. Following disruption of homeostasis, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) are activated in an attempt to preserve homeostasis (Miller and O'Callaghan, 2002). Initiation of the stress response via the HPA axis involves synthesis and release of corticotropin releasing factor (CRF) from the paraventricular nucleus of the hypothalamus. CRF travels down the axons of these neurons to the external layer of the median eminence. Release of CRF into the portal blood controls processing of adrenocorticotropic hormone (ACTH) in anterior pituitary corticotrophs, as well as secretion of several other pituitary hormones. Figure 1 illustrates the multi-hormonal control of ACTH release. ACTH released into the circulation stimulates the adrenal cortex to produce glucocorticoids, mineralocorticoids and adrenal androgens. One consequence of glucocorticoid release (primarily cortisol in pigs) is an elevation in blood glucose, which provides the body with fuel necessary to meet the higher metabolic demands associated with a stressful situation. Local delivery of cortisol to the adrenal medulla induces the enzyme

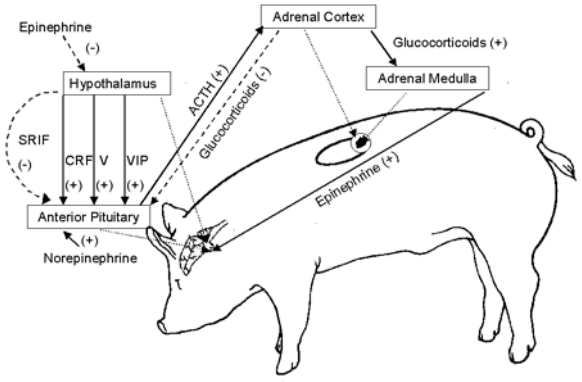

Figure 1. Multihormonal Control of the Stress Response. Hypothalamic stimulation results in the release of corticotropin-releasing factor (CRF), vasopressin (V) and vasoactive intestinal peptide. Each of these factors induces the anterior pituitary to release adrenocorticotropin (ACTH). ACTH stimulates the synthesis of glucocorticoids in the adrenal cortex. Glucocorticoids can have a stimulatory effect on the adrenal medulla and result in the synthesis of epinephrine. Glucocorticoids can also act directly on the anterior pituitary to inhibit ACTH mRNA formation or inhibit ACTH release. The secretion of epinephrine can have inhibitory effects on the hypothalamus. Additionally, epinephrine and norepinephrine can stimulate the anterior pituitary to release ACTH. Somatostatin (SRIF) has been shown to block the ACTH releasing ability of the anterior pituitary. (adapted from Axelrod and Reisine, 1984)

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The authors gratefully acknowledge support from the National Pork Board and the Michigan Agricultural Experiment Station.

***Proceedings of the 56th American Meat Science Association Reciprocal Meat Conference** (pp. 9-15)*
June 15-18, 2003, Columbia, Missouri
www.meatscience.org

phenylethanolamine-N-methyltransferase, which is rate-limiting in epinephrine synthesis from norepinephrine (reviewed by Wurtman, 2002). In turn, epinephrine stimulates glycogenolysis and is more potent than norepinephrine in raising body temperature and increasing heart rate and cardiac output. Chronic increases in epinephrine may be expected to reduce adrenomedullary norepinephrine secretion. However, this may be compensated for by the stress-induced increase in the quantity of norepinephrine released from sympathetic nerve terminals, which may actually increase plasma norepinephrine and subsequently increase peripheral resistance and raise blood pressure (Wurtman, 2002). Collectively, the release of these hormones serves to adapt the body to stressors ranging from mildly psychological to intensely physical by affecting cardiovascular, energy producing, and immune systems (Axelrod and Reisine, 1984). Antemortem activation of the HPA axis and the plethora of related catabolic biochemical events also increase heat production and the likelihood of producing PSE pork (Schaefer *et al.*, 2001).

Stress Reduction through Feed Manipulation

Schaefer *et al.* (2001) and Rosenvold and Andersen (2003) recently reviewed the role of nutrition in reducing antemortem stress and meat quality aberrations. Nutritional manipulation has included attempts to manipulate stress hormone levels (Schaefer *et al.*, 2001), as well as direct attempts to buffer the well-known decrease in pH associated with antemortem stress (Boles *et al.* 1994). With regard to the latter, feeding bicarbonate resulted in moderate improvements in texture and pH, whereas feeding an acidotic solution increased the incidence of PSE pork (Boles *et al.*, 1994).

Attempts to modify the synthesis of hormones involved in the stress response in pigs by nutritional manipulation have produced inconsistent results. Magnesium may affect an animal's response and resistance to stress by antagonizing the effects of calcium on calcium-release channel function (Zucchi and Ronca-Testoni, 1997) or by altering the release of stress hormones (Classen *et al.*, 1987). Feeding magnesium aspartate to pigs has been shown to improve pork quality and reduce the incidence of PSE carcasses in pigs subjected to inferior handling (D'Souza *et al.*, 1998; Schaefer *et al.*, 1993). Conversely, Caine *et al.* (2000) reported that supplementary magnesium exacerbated the PSE condition in pigs heterozygous for the halothane gene, leading these authors to conclude that the efficacy of magnesium aspartate hydrochloride was dependent on diet and genotype. Hamilton *et al.* (2002) observed no consistent effects of short-term feeding of magnesium sulfate on pork color and drip loss.

Exposure to stress increases catecholamine synthesis and turnover, thereby increasing the demand for tyrosine, which is the amino acid precursor of dopamine, norepinephrine and epinephrine. If catecholamine synthesis is compromised, animals become less resistant to stress and may lose the ability to respond appropriately to stimuli. Likewise, insufficient serotonin is associated with violent behavior, but supplementation with tryptophan, the substrate for serotonin synthesis, may produce sedation (Schaefer *et al.*, 2001). Adeola *et al.* (1993) reported that stress-susceptible pigs had lower brain levels of serotonin, dopamine, norepinephrine and epinephrine than stress-tolerant pigs. Similarly, Weaver *et al.* (2000) observed that boars heterozygous for the stress gene had lower basal plasma ACTH and cortisol concentrations compared to wild-type boars. However, the neuroendocrine response to stress did not differ between boars of these genotypes, despite a higher incidence of PSE meat in heterozygous boars. Feeding excess dietary tryptophan and tyrosine to pigs appears to have little effect on the incidence of PSE pork (Adeola and Ball, 1992; Schaefer *et al.*, 2001).

Genetic Basis for PSE Pork

Porcine stress syndrome (PSS), also referred to as malignant hyperthermia, is a genetic abnormality that compromises a pig's ability to cope with stressors. Malignant hyperthermia is inherited in an autosomal recessive fashion with incomplete penetrance (Mickelson and Louis, 1996). Pigs with this condition produce PSE pork more frequently than stress-resistant genotypes due to a combination of low pH and high early postmortem temperature, which results in extensive protein denaturation (Briskey *et al.*, 1966; Louis *et al.*, 1993). Initial studies to identify pigs affected by PSS utilized the anesthetic, halothane gas (Eikelenboom and Minkema, 1974; Webb and Jordan, 1978). For over a decade, halothane screening of pigs was used to identify animals that were susceptible to PSS. This test was effective at identifying homozygous positive pigs, but did not distinguish between heterozygous and homozygous normal pigs (Webb and Jordan, 1978). Cheah and Cheah (1976) showed that halothane enhanced the rate of calcium release by two-fold in pigs that were sensitive to halothane compared to those that were not. Advances in the understanding of calcium release from the sarcoplasmic reticulum (SR) led to the discovery of a substitution of T for C at nucleotide 1843 (HAL-1843) of the SR calcium-release channel cDNA (Fujii *et al.*, 1991). This substitution is responsible for an alteration in amino acid sequence from an arginine to cysteine at residue 615 in the calcium-release channel protein, also called the ryanodine receptor (RYR1; Fujii *et al.*, 1991). The polymorphism results in hypersensitive gating of the calcium release channel, which results in elevated sarcoplasmic calcium. Numerous functions of sarcoplasmic calcium ions have been reviewed by Berchtold *et al.* (2000). A few of the many critical functions of sarcoplasmic calcium ions are depicted in Figure 2. Calcium release from the SR stimulates muscle contraction, SR calcium ATPase (calcium pump) activity, mitochondrial ATP synthesis, glycolytic ATP production, heat production, as well as calpain-mediated proteolysis. Excessive SR calcium release through defective RYR1 is often associated with rapid antemortem and postmortem ATP utilization; increase rate of anaerobic glycoly-

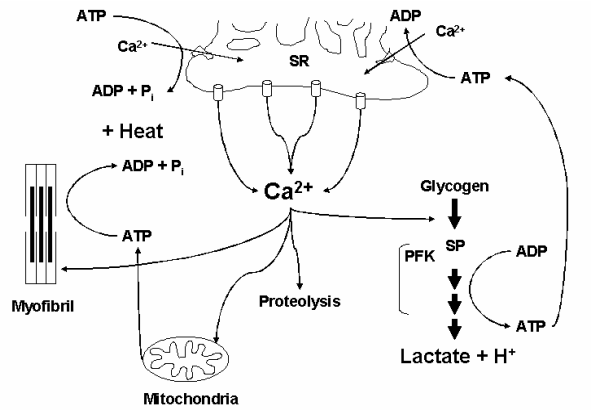

Figure 2. Calcium ions regulate skeletal muscle function. Calcium ions stimulate muscle contraction, sarcoplasmic reticulum (SR) calcium ATPase, glycogenolysis and glycolysis. Collectively, these processes result in accumulation of lactate, hydrogen ions, and heat. A rapid accumulation of hydrogen ions and heat in postmortem muscle is associated with excessive protein denaturation and development of PSE pork. Pigs with the HAL-1843 polymorphism exhibit hypersensitive calcium release channels, and therefore a compromised ability to regulate sarcoplasmic calcium ion concentrations.

sis, and the accelerated accumulation of hydrogen ions and heat associated with the development of PSE pork.

Based on the findings of Fujii *et al.* (1991), a DNA based test was established that could distinguish between homozygous positive, heterozygous carrier, and homozygous normal pigs with respect to the HAL-1843 mutation. This genetic test largely replaced halothane gas testing of swine, and efforts were made to eliminate the HAL-1843 mutation from commercial populations. Nonetheless, Murray and Johnson (1998) reported that in a population of 1006 pigs harvested in two packing plants, 90% of the PSE condition was caused by factors other than the HAL-1843 gene. Additionally, Rempel *et al.* (1993) compared the DNA-based test with the halothane challenge test and found that several pigs classified as HAL-1843 free were responsive to halothane. One explanation for these results is that other RYR1 polymorphisms may exist and result in altered halothane sensitivity and calcium regulation. Indeed, more than twenty mutations in the human RYR1 have been linked to MH (Girard *et al.*, 2001). Strasburg and Chiang (2003) provide a more complete overview of RYR function and abnormalities in these proceedings.

The Napole gene is another genetic abnormality that can lead to inferior pork quality. LeRoy *et al.* (1990) described the Napole gene as a dominant allele (RN-) and recessive allele (rn+) that is simply inherited. The dominant RN- allele results in higher than normal muscle glycogen stores and an extended postmortem pH decline that leads to pork with a lower than normal ultimate meat pH, higher reflectance (lighter meat), reduced water-holding capacity, and dramatically reduced processing yield (LeRoy *et al.*, 2000). Monin and Sellier (1985) referred to this condition as the Hampshire effect, due to its prevalence in the Hampshire breed. The causative polymorphism was identified in the *PRKAG3* gene, which encodes a muscle specific isoform of the regulatory γ subunit of adenosine monophosphate-activated protein kinase (AMPK; Milan *et al.*, 2000). Activated AMPK turns on ATP-producing pathways, inhibits ATP-consuming pathways, and can inactivate glycogen synthase (Hardie *et al.*, 1998). The RN- allele results from an R200Q substitution in AMPK. This modification has little effect on early postmortem pH values, but the inferior color and water-holding capacity of RN- pork are associated with lower 24-hour pH values. The reduced water-holding capacity of pork with low ultimate pH has been attributed to a reduced net protein charge that decreases repulsion between myofilaments (Hamm, 1994), as well as a more pronounced denaturation of myosin tails and sarcoplasmic proteins (Deng *et al.*, 2002). Conversely, Ciobanu *et al.* (2001) reported the presence of important alleles of the gene encoding AMPK that are associated with low glycogen content and improved pork quality. These findings demonstrate that additional alleles of genes involved in major mutations may be important contributors to pork quality.

Physical and Biochemical Aspects of PSE Pork

Water-holding capacity of pork is influenced by protein denaturation, myofibrillar lattice spacing, cytoskeletal links, membrane permeability, and the size of fluid channels in the extracellular space (reviewed by Purslow *et al.*, 2001; Warner *et al.*, 2001; Honikel, 2002). Recent nuclear magnetic resonance measurements on pork *longissimus* muscle indicated that drip loss is an ongoing process involving the transfer of water from myofibrils to the extracellular space, and this process is affected by structural features at several levels of organization within muscle tissue (Bertram *et al.*, 2002). The physical features of muscle that affect, or result from, fluid loss also influence pork color. Increased extracellular fluid results in a pale product due to greater light reflectance and scatter. Pale color may also be associated with low myoglobin concentration or stability (reviewed by Faustman and Cassens, 1990). Zhu and Brewer (1998) found PSE pork to have lower metmyoglobin reductase and a higher proportion of metmyoglobin at the *longissimus* muscle surface than normal pork.

Many of the structural features of meat or meat proteins that affect color and water-holding capacity are dictated by early postmortem events. Schäfer *et al.* (2002) reported that early postmortem temperature and pH were sufficient to account for 89% of the variation in drip loss from pork. Klont and Lambooy (1995) also demonstrated the effects of temperature on water-holding capacity by experimentally inducing rectal and muscle temperature differences between 36.9 and 39.6°C in anesthetized pigs. While these may be considered within the normal range of body temperature for pigs, an increase to the upper level of this normal range caused an increased incidence of PSE meat in all halothane genotypes (Klont and Lambooy, 1995). It is also interesting to note that functional tests of mitochondria from normal and HAL-1843 heterozygous or homozygous pigs revealed that homozygous HAL-1843 pigs had more than twice the exo-NADH oxidase activity compared to normal pigs, whereas heterozygous MH pigs were intermediate

(Rasmussen *et al.*, 1996). These authors speculated that exo-NADH oxidase activity might help sustain accelerated glycolysis by re-oxidizing the cytosolic NADH as an alternative to NADH shuttle activity. This process would be expected to result in production of heat, but not ATP. Whether or not differences in exo-NADH oxidase contribute to variation in heat production and pork quality in muscle of normal pigs is currently unknown.

Poulanne *et al.* (2002) indicated that heat production accounts for 69% of the energy produced during the splitting of ATP to yield ADP and P_i. Thus, the activity of the myosin and/or calcium ATPases (Figure 2) during the antemortem and early postmortem periods are likely to play an important role in determining pork quality due to the heat production and glycolytic stimulation associated with ATP utilization. Although myosin ATPase activity is associated with specific myosin heavy chain (MyHC) isoforms, the relationships between MyHC isoforms and pork loin drip loss or color are generally low (Eggert *et al.*, 2002; Huff-Lonergan *et al.*, 2002; Ritter, 2002). Our attempts to explain harvest day effects on pork loin fluid loss revealed that a lower proportion of type IIA MyHC and a higher proportion of type IIB and/or IIX MyHC appear to contribute to accelerated pH decline and increased fluid loss when less favorable antemortem or early postmortem conditions are encountered (Ritter, 2002).

Relatively low pH combined with high muscle temperature during the early postmortem period causes denaturation and reduced solubility of sarcoplasmic proteins (Sayre and Briskey, 1963; Scopes, 1964; Joo *et al.*, 1999) and myosin (Offer, 1991; Warner *et al.*, 1997). Pale color and reduced water-holding capacity associated with PSE pork have been primarily attributed to denaturation of sarcoplasmic and myofibrillar proteins, respectively (Joo *et al.*, 1999). However, Wilson and van Laack (1999) demonstrated that when myofibrils from either PSE or normal pork were combined with sarcoplasmic extract from PSE meat, the water-holding capacity of the myofibrils was lower than when combined with extract from normal pork. These authors concluded that sarcoplasmic proteins also influence water-holding capacity, through as yet undefined mechanisms. One possibility is that denatured sarcoplasmic proteins adsorb onto the surface of myofibrils, thereby shielding the charged groups available for fluid binding (Bendall and Wismer-Pedersen, 1962; Boles *et al.*, 1992). Additional research is required to elucidate the mechanisms whereby sarcoplasmic proteins may influence water-holding capacity of meat.

Since the rate and extent of hydrogen ion accumulation have profound effects on pork quality, and hydrogen ion accumulation results from anaerobic glycolysis, regulation of glycogenolysis and glycolysis are important aspects of pork quality development. Muscle glycogen stores at the time of slaughter have long been recognized to influence meat quality (Briskey *et al.*, 1966).

Proglycogen and macroglycogen can be distinguished on the basis of size and protein content, and have been described in detail by Lomako *et al.* (1993). In pigs, proglycogen is degraded preferentially during the first 45-60 minutes postmortem (Rozenvold *et al.*, 2003). Furthermore, total glycogen and the proportion of acid-insoluble proglycogen are higher in muscles that exhibit rapid postmortem pH decline and PSE pork (Briskey and Wismer-Pedersen, 1961). Although the macroglycogen pool can be reduced by dietary manipulation, a subsequent reduction in postmortem glycolysis is due to reduced metabolism of the proglycogen pool (Rosenvold *et al.*, 2003). In a recent review, Rosenvold and Andersen (2003), citing unpublished observations of B. Essen-Gustavsson, suggested that early postmortem glycolysis in RN- pigs may be similar to that of non-carriers of the RN- mutation because the increase in glycogen in RN- pigs is due to greater macroglycogen stores. Consequently, total glycogen appears to be inversely associated with ultimate meat pH, whereas the rate of pH decline appears to be positively associated with the proportion of proglycogen.

In living skeletal muscle, energy utilization and energy production are highly coordinated events. In fact, Conley *et al.* (1997) suggested that elevated sarcoplasmic calcium activates muscle contraction, glycogenolysis and glycolysis in parallel (Figure 2), since glycolytic rate is dependent on muscle stimulation frequency and independent of ADP, AMP and P_i concentrations. Once again, this highlights the importance of calcium regulation on muscle metabolism. Control over glycolytic flux, or the flow of intermediates through glycolysis, may also be controlled by covalent modification (enzyme phosphorylation and dephosphorylation), substrate control, and allosteric control mediated by changes in metabolite and co-factor concentrations (reviewed by Connett and Sahlin, 1996). The reversible binding of enzyme-enzyme and enzyme-contractile protein interactions provide additional possibilities for the regulation of glycolytic flux in skeletal muscle. The proportions of several glycolytic enzymes bound to contractile proteins increase with increased rates of glycolysis, and this may provide a mechanism for enhancing metabolite transfer rates (Parkhouse, 1992). Lee *et al.* (1989) summarized several studies demonstrating that phosphofructokinase (PFK) is phosphorylated in contracting muscle when the need for energy is high. Modification of PFK by phosphorylation favors enzyme binding to actin by increasing its apparent affinity for F-actin. It is currently not clear if enzyme modifications that occur under normal physiological conditions will also occur under postmortem conditions, or if enzyme binding has adverse consequences relevant to pork water-holding capacity and color.

In a classical study of postmortem glycolysis, Kastenschmidt *et al.* (1968) quantified levels of glycolytic intermediates and co-factors in *longissimus* muscles that exhibited fast and slow rates of postmortem glycolysis. These authors concluded that accelerated glycolytic rates resulted from coordinated stimulation of glycogen phosphorylase, PFK, and pyruvate kinase (PK). These enzymes have traditionally

been considered to catalyze rate-determining steps of glycogenolysis and glycolysis in skeletal muscle, since the reactions are far from equilibrium and reactions catalyzed by PFK and PK also proceed with a large decrease in free energy.

Phosphofructokinase has been regarded as the primary regulatory enzyme of glycolysis. It was on this premise that Sayre *et al.* (1963) investigated PFK activity in porcine muscle extracts. These authors determined that in vitro PFK and phosphorylase activities were not associated with the rate of pH decline in *longissimus* muscle of Hampshire, Poland China and Chester White pigs. Surprisingly, Allison *et al.* (2003) found that maximal in vitro PFK activity extracted from *longissimus* samples obtained at 20 minutes postmortem was inversely correlated with loin chop fluid loss. This observation may reflect early postmortem inactivation of the acid labile PFK enzyme in muscle undergoing rapid glycolysis.

Schwägele *et al.* (1996) demonstrated that muscle from halothane-sensitive pigs had four times more total PK activity than control pigs. Additionally, PK isolated from muscle of halothane-sensitive pigs lost only 30% of its activity when assayed at pH 5.5 rather than pH 7.0. In contrast, PK from control pig muscle lost >90% of its activity when assayed at pH 5.5. The higher activity and pH stability of PK from muscle of halothane-sensitive pigs were attributed to the presence of a more highly phosphorylated enzyme (Schwägele *et al.*, 1996). These enzyme properties may allow continued rapid accumulation of lactate and hydrogen ions in PSE muscle under conditions that would result in slow glycolysis in muscle producing higher quality pork. We recently reported that differences in PK capacity do not explain variation in color and water-holding capacity of pork loin muscle from HAL-1843-negative pigs (Allison *et al.*, 2003). Additionally, when we measured PK activity at pH 5.5, we observed a loss of >88% activity in all loin muscle samples at this pH compared to activity measured at pH 7.0. Thus, factors that contribute to the PSE condition of pork from halothane-sensitive pigs may not be broadly applicable to pigs that do not possess the HAL-1843 polymorphism.

Xu *et al.* (1995) suggested that ATP may be functionally compartmentalized in both skeletal and cardiac muscle cells. These authors demonstrated that the entire chain of glycolytic enzymes from aldolase onward are bound to SR membranes from cardiac and skeletal muscle. Additionally, immunogold labeling of ultrathin sections revealed that pyruvate kinase was located on SR vesicles immediately adjacent to the calcium ATPase (Xu and Becker, 1998). Aldolase and glyceraldehyde phosphate dehydrogenase were also found in close proximity to the calcium ATPase (Xu and Becker, 1998). ATP produced via SR associated glycolytic enzymes was shown to be preferentially used to fuel the calcium ATPase ion pump, suggesting that this ATP is transferred to the calcium pump in a protected microenvironment and is functionally coupled to calcium transport (Xu *et al.*, 1995). How (or if) the functional coupling of glycolytic enzymes to the major sites of energy utilization (myosin ATPase and calcium ATPase) affects pork quality is currently unknown. However, this coupling may influence glycolytic rate, ultimate enzyme location and the degree of denaturation of sarcoplasmic proteins, which, in turn, may influence the color (Joo *et al.*, 1999) and water-holding capacity of pork (Wilson and van Laack, 1999).

Postmortem muscle glycolysis is frequently monitored by measuring pH at specified times. This measurement undoubtedly reflects the pH of tissue, as opposed to that of individual muscle fibers, and may not accurately reflect the nature of postmortem glycolysis in individual muscle fibers. Oscillatory behavior of the glycolytic pathway has been well documented (reviewed by Smolen, 1995; Tornheim, 1979). In cell-free extracts of skeletal muscle, glycolytic oscillations are generated by repeated bursts of PFK activity. When the [ATP]/[ADP] ratio decreases to a trigger level, this initiates a sudden increase, or burst, in glycolytic flux that restores a high [ATP]/[ADP] ratio. In postmortem tissue, glycolytic bursts may also result in rapid and localized acidification, which could exacerbate protein denaturation. Using a protocol adapted from Tornheim *et al.* (1991), we have observed oscillatory behavior of glycolysis in sarcoplasmic protein extracts from porcine *longissimus* muscle (unpublished observations). The potential contribution of these oscillations to the development of PSE pork warrants further investigation.

Conclusions

The complexity of the PSE pork problem is highlighted by the fact that removal of genetic polymorphisms known to be associated with a higher incidence of PSE pork has not reduced the incidence of the problem. Providing biological explanations for conditions that result in production of PSE pork is essential for development of new strategies to improve the quality and consistency of pork. Calcium ions play a key role in regulating skeletal muscle metabolism and function, and much remains to be resolved regarding the regulation of calcium signaling. Additional information is also required to identify factors that contribute to rapid postmortem glycolysis. A detailed understanding of factors that regulate postmortem glycolysis in porcine skeletal muscle will improve efforts to control the rate of muscle pH decline, and reduce the incidence of PSE pork.

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