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MYOFIBRILLAR CHARACTERISTICS OF PORCINE STRESS SYNDROME

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Abstract

Porcine Stress Syndrome (PSS) is a genetic trait causing considerable economic loss to the swine industry through stress related death and the poor quality meat known as pale, soft, exudative (PSE) pork. A scanning and transmission electron microscopic examination of muscle biopsies from stress susceptible pigs revealed contracture bands, wide separation of myofibers and focal distortion and dissolution of myofibrils. The changes affecting myofibrillar characteristics and intra and intercellular accumulation of material suspected to be myoplasmic fluid in biopsies of halothane reactors suggest that the myopathic alterations presaging the carcass deterioration into pale, soft, exudative pork are integrants of this syndrome and that the PSE trait may not be a postmortem change triggered by the environmental factors just prior to or during slaughter.

Introduction

Porcine Stress Syndrome (PSS) is one of the major economic threats to modern day swine industry. The economic impact from this syndrome is two pronged with a major loss inflicted on the breeder through death in response to environmental stress, and a substantial loss to the meat industry and consumers from the inferior carcass quality of the pigs which survive until slaughter time. The undesirable carcass changes include the development of pale, soft, exudative (PSE) pork or dark, firm, dry (DFD) meat, depending upon the glycogen level in the muscles at the time of slaughter (Briskey, 1964). Previous investigations on stress susceptible pigs have shown that the trait occurs at a high frequency in some breeds and that it has a genetic etiology (Sybesma and Eikelenboom, 1969; Williams et al, 1977; Webb, 1980). The incidence is higher in breeds which exhibit rapid growth rate, high feed efficiency and heavy muscling (Nelson, 1973). The adverse and often fatal reaction is elicited by a variety of forms of stress including transport, crowding, fighting, restraint, temperature fluctuation, and exposure to drugs including halothane (Williams, 1977; Britt, 1972). The clinical symptoms of stress reaction include an increase in heart rate, muscle tone, cyanosis and body temperature (Britt, 1972; Grenert, 1980). The stress syndrome in pigs is similar to the condition recognized as malignant hyperthermia in man (Williams, 1977) and in both conditions muscle enzymes including creatine kinase (CK) leak out into the circulation because of a defect in muscle cell membrane (Britt, 1972; 1974). It has been postulated that cells other than muscle (such as erythrocytes) may have defective membranes (Harrison and Verburg, 1973) and that the syndrome in man and pigs exhibits varying degrees of myopathy (Buxton, 1980).

The objectives of our investigation were to examine the scanning and transmission microscopic images of muscle biopsies in order to determine whether or not myopathic alterations related to the "watery pork" characteristic are detectable in biopsies of boars which were previously classified as either stress susceptible or normal on the basis of their response to halothane challenge.

KEY WORDS: Porcine Stress Syndrome; Contracture Band; Myofibrillar Distortions; Pale, soft, exudative (PSE) Pork; Malignant Hyperthermia; Watery Pork; Carcass Quality.
Materials and Methods

Halothane Test

For the halothane test, the boars were restrained on their back in a V-shaped backboard and were allowed to breathe halothane (Somnothane®) in oxygen through a tight-fitting face mask for five minutes. Halothane in oxygen was administered by a Narcovet Veterinary Anesthesia Machine at a concentration of 4.0% for the first three minutes and 2.0% for the last two minutes. During the administration of halothane, the boars were carefully observed for signs of muscular rigidity, and the flexibility of the limbs was tested frequently. Boars that showed muscular relaxation during anesthesia were classified as normal or non-reactors to halothane (Eikelenboom and Minkema, 1974). Additional signs of a reactor to halothane during anesthesia included laboured, extended hindlimbs, and when the hindlimbs became stiff and extended within two minutes. During the administration of halothane, anesthetic was continued by water spray from a hose.

All halothane reactors were removed from the challenging agent and subjected to surface cooling by water spray from a hose.

Collection and Preparation of Muscle

The animals included in this study were halothane-tested boars belonging to the Yorkshire and Landrace breeds (Table I). They consisted of six normal and six halothane reactors which were revived and maintained for approximately four months prior to subjecting them to the biopsy procedure.

Skeletal muscle samples were obtained from the medial portion of the gracilis muscle. For the biopsies, the non-reactors were anesthetized with halothane in oxygen and the reactors were anesthetized by intravenous administration of thiopental sodium (Pentothal®).

Muscle samples were placed in Hanks' balanced salt solution (HBSS) immediately after collection. Pieces of muscle, approximately 10 mm in length and 4 mm in cross section were dissected out and placed in such a way that the myofibers were in longitudinal orientation when the specimen was tied at each end with a surgical silk thread prior to fixing on to a cork to prevent contraction. The specimens were immersed in fixative consisting of 5.0% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 12 hours. Some of the samples were then cut into 1 nm² pieces, washed in phosphate buffer several times, and post-fixed in phosphate buffered 1.0% osmium tetroxide for one hour. The tissue pieces were then rinsed in phosphate buffer, dehydrated in a graded series of ethanol, infiltrated in Epon via propylene oxide, flat-embedded in Epon in aluminium weigh boats and polymerized at 60°C for 48 hours.

Epon embedded specimens were cut with a jeweller's saw and affixed to blank Epon blocks with the five minute epoxy glue and 1.0 µm sections were cut with a Reichert OMU2 ultramicrotome for light microscopy. Areas of interest were selected for ultrathin sections (60-150 nm) which were mounted on uncoated 300 mesh grids and were contrasted in 2.0% ethanolic uranyl acetate and lead citrate and examined in a JEOI 100S transmission electron microscope.

Samples from glutaraldehyde fixed muscle specimens were cut obliquely with a clean razor blade and varying levels of the cut surface of the muscle cells were exposed by gently detaching the cut ends, prior to processing the samples for scanning electron microscopy according to procedures outlined previously (Basrur and Basrur, 1977)

Results

The ultrastructural features of muscle from nonreactor pigs were generally similar to those reported for normal mammalian muscle. Muscle samples from halothane reactors showed a variety of changes including hypercontraction, widened interfibrillar spaces, alterations in the Z-bands, disorientation and dissolution of myofibrils, and bizarre orientation of myofibrillar remnants at right angles to the long axis of the muscle cell (Figs. 1 to 5). Scanning electron microscopy studies showed that the surface contour of the normal muscle (from halothane nonreactors) is relatively even and that in the fractured regions the myofibrils were stacked in very close apposition to each other (Fig. 6). In halothane reactor muscle samples, individual muscle fibers were noted in hypercontracted state adjacent to relatively normal muscle fibers (Fig. 7). Aggregates of material probably including myoplasm that has segregated and leaked out of the muscle cells (Fig. 8) were noted in the intercellular space and within individual muscle fibers. The myofibrils towards the periphery of individual muscle cells were often noted to branch and stretch towards those of adjacent muscle fibers (Fig. 9) and myofibrillar bundles exhibited varying diameters and were separated from each other (Fig. 10).
Fig. 1. A muscle biopsy sample from a halothane reactor boar showing contracture bands (CB) in a few myofibrillar bundles adjacent to apparently normal bundles (MF). Note the focal nature of the contracture involving 13 to 14 bands, and the signs of detachment of the sarcomere (open arrows).

Fig. 2. A muscle biopsy sample from a reactor boar showing dissolution of contractile elements and the remnants of contractile elements (arrow) towards the plasma membrane (PM). Note the remnants of the T-system (arrowhead) and the wide space (S) between myofibrillar bundles.
Fig. 7. Surface view of muscle cells from a halothane reactor boar showing a myofiber exhibiting hypercontraction (HC), among apparently normal myofibers.

Fig. 8. An exposed view of the muscle from a halothane reactor boar. Note the bulged areas running almost parallel to the fibers, probably representing myoplasm (MP) that has segregated or leaked out of the muscle.

The ultrastructural changes observed in skeletal muscle samples of halothane reactor (stress susceptible) boars and boars which exhibited normal reaction to halothane are summarized in Table 1. Evidence of myofibrillar disorganization and the presence of vacuoles of varying size and number, were strikingly more prominent in the reactors although one of the boars classified as normal (4L; NR) also exhibited these features. Hypercontraction of individual muscle fibers, however, was noted only in the reactors (Table 1).

Fig. 9. Myofibrillar disorganization in a muscle fiber of a halothane reactor boar. Note the fibrillar branching (MF) and extension to the neighboring myofibers (arrows).

Fig. 10. Myofibrillar bundles from a halothane reactor boar showing wide space (S) between individual myofibrillar bundles. Note the contracture bands (CB) in these myofibrils.

It is worthy of note that the muscle biopsies of all reactors included in this study exhibited two or more of these alterations (Table 1). In addition to these, muscle biopsies of halothane reactors also exhibited the presence of strap-like fibers, displacement of myonuclei from their peripheral location towards the center, accumulation of glycogen granules and the presence of lamellated bodies in varying proportions of the muscle fibers.
Table 1. Comparison of Skeletal Muscle Alterations in Landrace and Yorkshire Boars Identified as Reactors and Nonreactors to Halothane Challenge.

<table>
<thead>
<tr>
<th>Boar #</th>
<th>Hyper-</th>
<th>Vacuolation</th>
<th>Myofibrillar</th>
<th>Dissolution</th>
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<td>Contraction of Myoplasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
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<tr>
<td>2Y;NR</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
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<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4L;NR</td>
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Y: Yorkshire NR: Nonreactor to Halothane
L: Landrace R: Reactor to Halothane

Discussion

Our transmission and scanning electron microscopic studies show that the biopsy specimens from stress susceptible boars exhibit features which may be causally related to the tendency of these animals to develop pale, soft, exudative (PSE) pork. The characteristic features relevant to this phenomenon are the abundance of non-myofibrillar material accumulated between individual myofibrillar bundles, hypercontraction, degeneration and dissolution of myofibrils, and the accumulation of material resembling myoplasm within and between muscle cells. The widened interfibrillar space and the disorientation of individual bundles of myofibrils are probably related to the fluid segregation within muscle cells whereas the presence of strap-like fibers and the displacement of myonuclei may be indicative of the regenerative process taking place in response to the degeneration of individual muscle fibers in stress susceptible boars.

Briskey (1964) and Briskey and Wismer-Pedersen (1961) reported that there is no change suggestive of degeneration in gross appearance or in histological features at the time of slaughter, in muscles which ultimately become PSE. These investigators believe that the PSE condition is attributable to accelerated postmortem glycolysis and that the degree of muscle hydration is related to the extent of rigor mortis. However, Williams, et al. (1977) have noted that excess heat generation occurs in PSE muscle 14 minutes before rigor mortis develops and have suggested that rigor, which also can cause heat production, is secondary to the other metabolic changes including tissue acidity, ATP depletion and the elevation of sarcoplasmic calcium levels. These investigators also demonstrated that heat production occurs through futile cycling in PSE muscle before slaughter and that the alteration in muscle cell components and the development of PSE muscle postmortem may be related to this phenomenon (Williams et al., 1977).

Histological and ultrastructural changes during postmortem autolysis of muscle samples from normal and stress reactor pigs have been examined by various investigators (Briskey, 1964; Dutson et al., 1974). The most striking post-mortem difference between PSE muscle and normal muscle was in the extent of derangement in mitochondria and sarcoplasmic reticulum and in the disruption of myofibrillar organization (Dutson et al., 1974). Myofibrillar disruption and a tendency for longitudinal splitting were generally pronounced in the white fiber type as compared to the red fibers in muscle samples subjected to postmortem autolysis (Abbott et al., 1977). A recent scanning and transmission electron microscopic study on cryofractured longissimus dorsi muscle samples collected immediately after slaughter from pigs genetically predisposed to different degrees of stress, revealed changes similar to those described in this report (Cloke et al., 1981). These investigators also noted that the connective tissue investment of individual muscle fiber is detached and that the plasma membrane is disrupted in stress susceptible pigs and postulated that the extent of plasma membrane disruption noted in stress susceptible pigs may be indicative of the relative fragility of their muscle and connective tissue components. All these studies on PSS pigs (Cloke et al., 1981; Dutson et al., 1974; Briskey, 1964; Venable, 1973) relate to the state of muscle samples at the time of slaughter or at different intervals after slaughter. Our studies were conducted on biopsies which represent the status of muscle in the live animal with the exception that they were exposed to anesthetics during biopsy collection. The reactors were anesthetized with sodium pentothal instead of halothane in order to avoid a stress reaction from halothane exposure at the time of biopsy collection. The difference noted between the muscle samples of halothane reactors and non-reactors cannot be attributed to the difference in the anesthetics since sodium pentothal or sodium thiamylal does not trigger stress reaction in pigs (Jones et al., 1972). Furthermore, the biopsy collection was accomplished within 20 minutes of induction, at which time no difference was noted in our pilot study on muscle samples collected from normal pigs anesthetized with halothane, sodium pentothal or sodium thiamylal. The similarity between the ultrastructural alterations in biopsies of stress susceptible pigs in the present study and those reported in pigs subjected to fatal stress reaction crisis (Venable, 1973) further supports our view that the myofibrillar alterations reported here represent a real difference between stress susceptible and normal pigs.

It has been hypothesized previously (Briskey, 1964; Swatland, 1980) that the PSE trait is precipitated by environmental factors that induce stress in a genetically predisposed animal. The environmental factors that
aggravate stress susceptibility include trucking, fighting and crowding trauma related to transportation to the slaughterhouse. The variable expression of PSE trait in pigs previously identified as stress susceptible has been attributed to the variability in the degree of preslaughter trauma and postmortem environmental factors (Swatland, 1980; Cassens et al., 1963; Barton-Gade, 1981). Our observations on muscle biopsies indicate that alterations relevant to the PSE trait are already present in PSS muscle in vivo, and that evidences of a membrane defect and myopathy postulated in porcine stress syndrome-malignant hyperthermia complex (Lucke et al., 1979; Britt, 1974; Williams, 1977; Gronert, 1980) are recognizable in stress susceptible pigs with the aid of scanning and transmission electron microscopy.

Acknowledgements

Our sincere thanks are extended to Dr. Dave Seeler for halothane testing the pigs used in this study. The technical assistance of Mr. C. A. Ackerley, Mr. Ed. Reyes, Mrs. Helen Randall and Mrs. Mary John are gratefully acknowledged. This investigation was supported by Natural Sciences and Engineering Research Council of Canada, Ontario Ministry of Agriculture and Food and Agriculture Canada research contract.

References


Discussion with Reviewers

M. Ashraf: Please define myoplasm as discussed in Figure 8, the myoplasm" appears to be a contracted blood vessel.

Authors: The term myoplasm is used in Figure 8 to describe the SEM image of the intracellular material similar to the TEM images free of myofibrils shown in Figures 2 and 3. Images similar to that identified as myoplasm in Figure 8 were noted scattered irregularly within and between
Authors: In our ongoing studies we have examined biopsies from a few halothane nonreactors which were subsequently challenged with succinylcholine. Biopsies from boars which were nonreactors to halothane and succinylcholine were generally free of the severe ultrastructural alterations. More notably, we detected occasional some disparity between the animal's response to halothane and the ultrastructural features of muscle sample from that animal. In one instance, the biopsy sample from a halothane nonreactor boar showed severe myopathic alterations similar to those reported here. This boar later reacted severely and fatally while being moved to another pen, exhibiting all the clinical signs of malignant hyperthermia. We are not sure whether this boar was a “heterozygote” for the halothane gene.

M. Ashraf: Are the changes discussed specific to Porcine Stress Syndrome: How are they related to other known skeletal muscle myopathies? Authors: Some of the changes noted in muscle samples of stress susceptible pigs, such as myofibrillar disintegration, hypercontraction and displacement of nuclei are also noted in a variety of human myopathies (Buxton, 1980). Williams (1977) had postulated that myopathy may be an integral part of malignant hyperthermia in man and stress susceptibility in pigs. Our biopsy material which showed myopathic changes in all stress susceptible pigs emphasizes the point that these changes may be the physical basis, or the forerunners, of the PSE trait which is generally considered to be the result of postmortem deterioration of muscle in certain genotypes.

K. Lundström: Have you checked pigs that do not react to halothane but are carriers of the halothane gene (heterozygotes)? Authors: In our ongoing studies we have examined the biopsies from a few halothane nonreactors which were subsequently challenged with succinylcholine. Biopsies from boars which were nonreactors to halothane and succinylcholine were generally free of the severe ultrastructural alterations. More notably, we detected occasional some disparity between the animal's response to halothane and the ultrastructural features of muscle sample from that animal. In

C.A. Voyle: Did the authors attempt to classify the muscle fibers, i.e. red, white or intermediate, which showed altered appearance in the halothane reactors? Authors: We did not undertake fiber typing in this investigation. The muscle sample used in this study was obtained from gracilis which is mainly of the white muscle type and consequently a majority of the muscle fibers showing ultrastructural alterations were of the white type fibers. However, disintegration of myofibrils were also noted in thinner fibers which according to the Z-line criteria used by Dutson et al (1974) may well be red fibers. We are not sure however, whether these thin fibers are red fibers or newly formed strap-like cells resulting from the regeneration of white fibers.

C.A. Voyle: What proportion of the fibers in the muscle samples were affected? Authors: The proportion of fibers detected with alterations in muscle biopsy samples varied depending upon the optical system used for examination. For example, light microscopic examination of semithin sections showed fibers exhibiting hypercontraction, displacement of nuclei and vacuolation in 5 to 10% of cells only. At the ultrastructural level, myofibrillar alterations, mitochondrial distortions and the presence of lamellated structures reminiscent of muscle degeneration were noted in 15 to 30% of the fibers. One of the striking features of these biopsies was that all samples from halothane reactors contained some fibers showing unequivocal degenerative changes although these were generally surrounded by apparent unaltered normal muscle cells.

K. Lundström: Which biopsy procedure did you use? Is it a surgical procedure with samples cut out with a scalpel or did you use some sort of biopsy needle? Authors: We used the surgical procedures using scalpels to cut out muscle samples. The procedure introduced by Lundström et al (Swedish J. Agric. Res. 3:211-213, 1973) was not used in our studies since we needed larger samples to carry out our ultrastructural studies and some of our other investigations not reported here, including in vivo caffeine contracture test.

K. Lundström: You state that the incidence of stress susceptible pigs are higher in breeds which exhibit rapid growth rate and heavy muscling. I fully agree on heavy muscling, but I cannot remember any evidence of higher growth rate in stress susceptible pigs. The contrary is, however, well known in pigs of the Pietrain breed.
Various investigators have reported that halothane reactor (stress susceptible) pigs exhibit increased muscle growth and increased feed efficiency and growth rate relative to non-stress susceptible pigs. However in a recent report, Webb (1980) states that the stress susceptible pigs are better than normal pigs on food conversion ratio and lean content criteria, while the former group performs less well in terms of daily food intake and growth rate. Even though these two statements would appear to be contradictory, it is possible that the lack of appetite of stress susceptible pigs combined with their genetic predisposition to be muscular, is responsible for the apparent superiority in food conversion ratio and carcass traits reported in stress susceptible pigs.