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Method of isolation, rate of postmortem metabolism, and myosin heavy chain isoform composition influence ATPase activity of isolated porcine myofibrils

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Abstract

The objective of this study was to determine the influence of myofibril isolation procedures and myosin heavy chain (MyHC) isoform composition on myofibrillar ATPase activity as related to postmortem muscle metabolism. Myofibrils from the *red* (RST) and *white* (WST) portions of *semitendinosus* muscles were isolated using two different methods (A and B) at 3 min and 24 h postmortem in control (NS) and electrically stimulated (ES) pork carcasses. Comparison of the relative MyHC isoform profiles between the two different myofibril isolation methods and myosin extracts from the RST and WST at 3 min showed that method B myofibrils were more similar to the myosin extract than method A. Myofibrillar ATPase activity remained constant or increased ($P<0.01$) from 3 min to 24 h postmortem in NS carcasses and decreased ($P<0.0001$) in ES carcasses. From the RST, method A myofibrils had higher ($P<0.0001$) ATPase activity compared to method B across sampling time and carcass treatment. In the WST, method A myofibrils had lower ($P<0.01$) activity at 3 min, were not different at 24 h in NS carcasses, but had higher ($P<0.05$) activity at 24 h in ES carcasses versus method B myofibrils. Compared to method B, isolation method A biased the isoform profile of myofibril samples more towards faster MyHC (2A and 2X) in the RST and towards MyHC 2X in the WST. Results suggest that the ATPase activity and MyHC isoform profile of isolated myofibril samples are influenced by method of myofibril isolation, postmortem sampling time, and the rate of postmortem metabolism. Thus, differences in MyHC isoform profile and method of myofibril isolation must be taken into account to determine accurately the relationship between myofibrillar ATPase activity and rate of postmortem metabolism.

Keywords: Myofibril; Myofibrillar ATPase; Myosin heavy chain

1. Introduction

Pale, soft, exudative (PSE) pork usually develops as the result of rapid muscle metabolism during the early postmortem period (<1 h). Accelerated metabolism gives a low muscle pH while carcass temperature remains high and results in severe protein denaturation that compromises pork quality (Briskey & Wismer-Pedersen, 1961; Charpentier, 1969; Goutefongea, 1971). Accelerated postmortem glycolysis is often triggered by an impaired Ca^{2+} homeostasis, as in Hal gene positive pigs (Eikelenboom & Nanni Costa, 1988; Mickelson et al., 1989; Monin et al., 1999). However, rapid glycolysis frequently occurs in the absence of aberrant Ca^{2+} gating. Thus, factors other than the Hal gene, such as muscle fiber type, may potentially function to determine the differing propensities of muscles to develop PSE.

One of the primary determinates of muscle fiber type is myosin heavy chain (MyHC) isoform expression which is influenced by activity, hormonal factors, and genetics (Petersen, Henkel, Oksberg, & Sørensen, 1998; Rahelic & Puac, 1981; Weiler, Appell, Kermser, Hofacker, & Claus, 1995). Muscles differing in fiber type composition demonstrate varying degrees of susceptibility to developing the PSE condition (Briskey & Wismer-Pedersen, 1961; Merkel, 1971; Warner, Kauffman, & Russell, 1993). Myosin isoform composition may be important to meat quality development from

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two perspectives: (1) myosin isoform composition may influence postmortem energy metabolism, and/or (2) myosin isoforms may differ in their susceptibility to protein denaturation. From Scopes' (1974) data, it can be inferred that myosin ATPase activity can influence overall rate of ATP breakdown postmortem, and subsequently alter the rate of glycolysis and the onset of rigor. Findings that ATPase activity varies widely across MyHC isoforms lead to the hypothesis that differences in myofibrillar ATPase resulting from different muscle fiber types, may be sufficient to influence postmortem ATP hydrolysis, energy utilization, and subsequent glycolysis.

Greaser, Cassens, Briskey, & Hoeks, (1969), however, demonstrated that myofibrillar ATPase activity does not differ between normal and PSE muscle at 0 min postmortem. Furthermore, at 24 h postmortem, myofibrillar ATPase activity of PSE muscle is significantly less than muscle undergoing a normal rate of postmortem glycolysis (Greaser et al., 1969; Sung, Ho, & Izumi, 1981; Warner Kauffman, & Greaser, 1997). These data argue against myofibrillar ATPase as the driving force behind the accelerated rate of glycolysis observed in PSE pork. However, the method used to isolate myofibrils in some of these early studies may have altered the enzymatic activity of the myofibrils, and the MyHC isoform composition of the isolated myofibrils was not characterized. In order to make definitive conclusions regarding the role of myofibrillar ATPase activity in postmortem muscle, it is paramount to have myofibril preparations that closely represent the in vivo characteristics of the myofibrils in terms of sarcomere integrity and MyHC composition of the muscle. The primary objective of this study was to determine if method of isolation influences the ATPase activity and MyHC profile of isolated myofibril samples. A secondary objective was to determine how the in vivo rate of postmortem metabolism influences the ATPase activity of isolated myofibrils and if the method of isolation influences the interpretation of such data.

To pursue these objectives, myofibrils were isolated via two different methodologies at 3 min and 24 h postmortem on porcine carcasses treated to give normal or accelerated glycolysis. The maximum, calcium activated, myofibrillar ATPase activity was measured, and the MyHC composition of the myofibrils and muscle extracts from the same muscle region were measured.

2. Materials and methods

2.1. Animals and electrical stimulation treatment

Six 110 kg gilts were slaughtered in the Purdue University Meat Science Teaching and Research Laboratory following standard slaughtering procedures. Carcasses were randomly subjected to one of two treatments: electrical stimulation (ES, $n = 3$) for 30 pulses of 450 V at 3 and 20 min postmortem (modified from Bowker et al., 1999) or non-stimulated (NS, $n = 3$) controls. Exsanguination was considered 0 min postmortem. Carcasses were placed in a chill cooler (4 °C) at 120 min postmortem.

2.2. Muscle sampling, myosin extraction, and myofibril isolation

At 3 min and 24 h postmortem, the *semitendinosus* muscles were excised from the left and right side of each carcass, respectively. The 3 min muscle samples were excised immediately prior to the ES treatment. Each muscle was separated into the *red* (RST) and *white* (WST) *semitendinosus*. From muscle samples obtained at 3 min postmortem, approximately 3 g of tissue was removed and powdered in liquid nitrogen for myosin extraction. Myosin was extracted with a high ionic strength extraction buffer (0.3 M KCl, 0.1 M KH_2PO_4, 50 mM K_2HPO_4, 10 mM EDTA, pH 6.5), diluted to 50% with glycerol, and stored at −20 °C according to the procedures of Bär and Pette (1988). Myofibrils were isolated from 3 min and 24 h muscle samples according to two different isolation methods.

- *Method A:* Myofibrils were isolated according to the procedure outlined by Greaser et al. (1969). All steps were performed at 4 °C. Samples were homogenized with six 15-s bursts in a Waring blender in 4 volumes of cold buffer (0.1 M KCl, 5 mM histidine, pH 7.2). Homogenates were centrifuged at 1000 × *g* for 20 min. Pellets were resuspended in 10–20 volumes of cold buffer, filtered through cheesecloth, and centrifuged at 1000 × *g* for 20 min. Myofibril fractions were suspended in rigor buffer (RB: 75 mM KCl, 10 mM imidazole (pH 7.2), 2 mM $MgCl_2$, 2 mM EGTA, 1 mM NaN_3), made in 50% glycerol (v/v), with 1 mM (final) dithiothreitol (DTT). Isolated myofibrils were stored at −20 °C. Thus, homogenization of method A myofibrils occurred pre-rigor in 3 min samples and post-rigor in 24 h samples.
- *Method B:* Myofibrils were isolated according to the modified procedure of Swartz, Greaser, & Marsh (1993). All steps were performed at 4 °C. Muscle bundle samples ranging from 0.2 to 0.5 cm wide and 3 to 5 cm in length were excised and placed in cold RB. Slightly stretched muscle bundles were tied to applicator sticks and bathed in RB plus 0.5% Triton X-100 (v/v) and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) at 4 °C for 24 h with stirring. The attached ends of the muscle bundles were removed and the remainder minced with scissors and homogenized in 10 volumes of RB for three 10-s bursts using a Polytron Homogenizer (speed setting 5) (Brinkman Instruments, New York, NY, USA). An additional 10 volumes of RB was added and samples were further homogenized using 50 strokes with a

Dounce homogenizer using loose-fitting pestle. The suspension was centrifuged at 1000 × *g* for 10 min, the pellet resuspended in 20 volumes of RB then homogenized for three 5-s bursts. The suspension was again centrifuged at 1000 × *g* for 10 min. The pellet was homogenized for two 4-s bursts in 20 volumes of RB and an equal volume of RB plus 0.2% Triton X-100 was added. The suspension was filtered through cheesecloth and centrifuged at 1000 × *g* for 10 min. The myofibril pellet was resuspended in 20 volumes of RB plus 0.2% Triton X-100, pelleted by centrifugation at 1000 × *g* for 10 min, resuspended in RB, and washed three times with 20 volumes of RB per wash. The myofibrils were stored as for method A. Thus, homogenization of method B myofibrils occurred post-rigor in both 3 min and 24 h samples.

Myofibrils from both methods were observed using a phase contrast, oil-immersion lens (100X, NA 1.3) on a Nikon Microphot-FXL microscope equipped with a CCD-IRIS/RGB color video camera (DXC-151-A). Digital images were acquired with and processed using IPLab Version 3.060 (Scanalytics Inc., Fairfax, VA, USA).

2.3. ATPase assay

Maximum, Ca^{2+}-activated, myofibrillar ATPase activity was measured according to procedures adapted from Carter and Karl (1982), and Swartz, Zhang, and Yancey (1999). The program of Fabiato (1988) was used to formulate base solutions of pCa 9 and 3 (10^{-9} and 10^{-3} [Ca^{2+}] respectively) and the pCa 4 buffer used for the assay. Solutions contained 20 mM PIPES (pH 7.00), 4 mM EGTA, 4 mM $MgCl_2$, 1 mM NaN_3, 1 mM DTT, 1 mg/ml bovine serum albumin (BSA), KCl to 180 mM ionic strength and either 5 (pCa 3) or 0 mM (pCa 9) $CaCl_2$.

Prior to the ATPase assay, myofibrils were washed free of glycerol by four dilution and centrifugation cycles (1000–4000 × *g* for 5 min) in pCa 9 buffer. Myofibrillar protein concentration was determined using the biuret assay with BSA standards, and the samples were adjusted to 0.5 mg/ml protein and pCa 4 using pCa 3 buffer. The maximum ATPase activity was measured at pCa 4 and a protein concentration of 0.1 mg/ml. The ATPase reaction was performed in quadruplicate 0.1 ml samples at 39 °C. The ATPase reaction was started by adding 80 μl of 2.5 mM ATP in pCa 4 buffer to 20 μl of myofibrils in pCa 4 buffer (2 mM MgATP and 0.1 mg/ml myofibrils final) and quenched after 5 min. with 20 μl of ice-cold 25% trichloroacetic acid (TCA). Samples were centrifuged at 12000 × *g* for 15 s to remove protein, and an aliquot of supernatant was removed for the measurement of inorganic phosphate (P_i) according to Carter and Karl (1982). Samples with TCA added prior to ATP were used to account for phosphate contamination and non-enzymatic hydrolysis of ATP.

To determine the time-course of ATP hydrolysis, P_i release over time was measured in WST myofibrils isolated at 3 min and 24 h postmortem from an NS carcass using isolation methods A and B. The reactions were done as above but with 5 mM MgATP and quenching at 0, 2.5, 5, 7.5, 10, 12.5 and 15 min.

2.4. MyHC isoform determination

The relative MyHC isoform composition in myofibril preparations and myosin extracts was determined by an enzyme-linked immunosorbent assay (ELISA) described in Depreux et al. (2000) with slight modifications. Biuret and bicinchoninic acid (BCA) procedures with BSA standards were used to measure the protein concentration of myofibril preparations and myosin extracts, respectively. Protein samples in 50 mM Tris (pH 8.5) were coated overnight at room temperature on 96-well ELISA plates (Corning Inc., Acton, MA, USA) using 10 μg protein/well for myofibrils and 7 μg for myosin extracts. Monoclonal antibodies against type I (A4.840) (Hughes & Blau, 1992), 2A (6B8) (Depreux et al., 2000), 2X (SC-71) (Bottinelli, Schiaffino, & Reggiani 1991), and 2B (BF-F3) (Schiaffino et al., 1989) MyHC were used in conjunction with alkaline-phosphatase linked secondary anti-mouse (IgG or IgM) antibodies (Jackson Immunoresearch Laboratories, West Grove, PA, USA). Past studies have demonstrated that these monoclonal antibodies provide an effective tool to determine MyHC in porcine muscle (Depreux, Grant, & Gerrard, 2002; Depreux et al., 2000). To control for plate-to-plate variation, ELISA absorbance values were normalized to an in-plate standard myosin extract from the RST (for type I and 2A MyHC) or WST (for type 2X and 2B MyHC) muscle. Also, for each carcass all myofibril samples (method A, B, and different time postmortem) were assayed in the same plate for each different MyHC and the same in-plate standard.

2.5. Statistical analysis

The SAS general linear model procedure was used to perform analysis of variance on ATPase activity and ELISA data (SAS, 1985). Data collected from 3 min samples (prior to ES treatment) were pooled according to isolation method (A or B) and muscle (RST or WST). Thus, $n = 6$ for 3 min samples and $n = 3$ for 24 h ES and NS samples. Significance level was set at $P < 0.05$.

3. Results

3.1. Carcass treatments

To simulate PSE development, early postmortem ES was applied to hasten postmortem pH decline. Pork

quality and pH decline data taken from the *longissimus* muscle were consistent with the results shown by Bowker et al. (1999), indicating that early postmortem ES is an effective method of stimulating a rapid postmortem metabolism. ES carcasses had lower ($P<0.05$) pH values at 45 min postmortem (5.74 ± 0.05 vs. 6.24 ± 0.11), paler ($P<0.05$) color (National Pork Producers Council Color scores: 1.33 ± 0.17 vs. 2.67 ± 0.17), and higher ($P<0.05$) drip loss (13.6 ± 0.6 vs. 4.5 ± 1.7) than control carcasses. Early postmortem ES was found to have similar effects on the pH decline and quality characteristics of the *m. semitendinosus* as the *m. longissimus* (unpublished data).

3.2. Myofibril structure

The structural features of the isolated WST myofibrils are shown in Fig. 1. Phase-contrast images of myofibrils isolated at 3 min postmortem by method A appear more aggregated and have less obvious and shorter sarcomere lengths than method B myofibrils (Fig. 1a and b). These differences are not evident when myofibrils were isolated at 24 h postmortem (Fig. 1c and d). Similar structural features were observed with the RST (image not shown). These images suggest that the method of isolation influences the structural integrity at 3 min postmortem but not at 24 h in the NS carcass.

3.3. MyHC isoform composition: myofibrils versus myosin extracts

The ELISA method of Depreux et al. (2000) was developed to analyze the relative MyHC isoform amount in crude myosin extracts from whole muscle. In the current study, this method was used to determine the relative MyHC isoform amount in isolated myofibrils. To validate the use of this method on myofibrils and to compare the different myofibril isolation methods, the MyHC isoform ratios within the myofibril samples were compared to that of myosin extracts from the same muscles. Fig. 2 shows the MyHC isoform ratios of isolated myofibrils and myosin extracts from muscles sampled at 3 min postmortem. For the RST, the ratio of type I to 2A MyHC was the same for both types of isolated myofibrils and myosin extracts (Fig. 2a). The ratios of type I to 2X and type 2A to 2X MyHC (Fig. 2b and c) were lower in method A myofibrils compared to method B myofibrils and myosin extracts, which did not differ. In the WST, the ratio of 2X to 2B was higher ($P<0.05$) in method A myofibrils

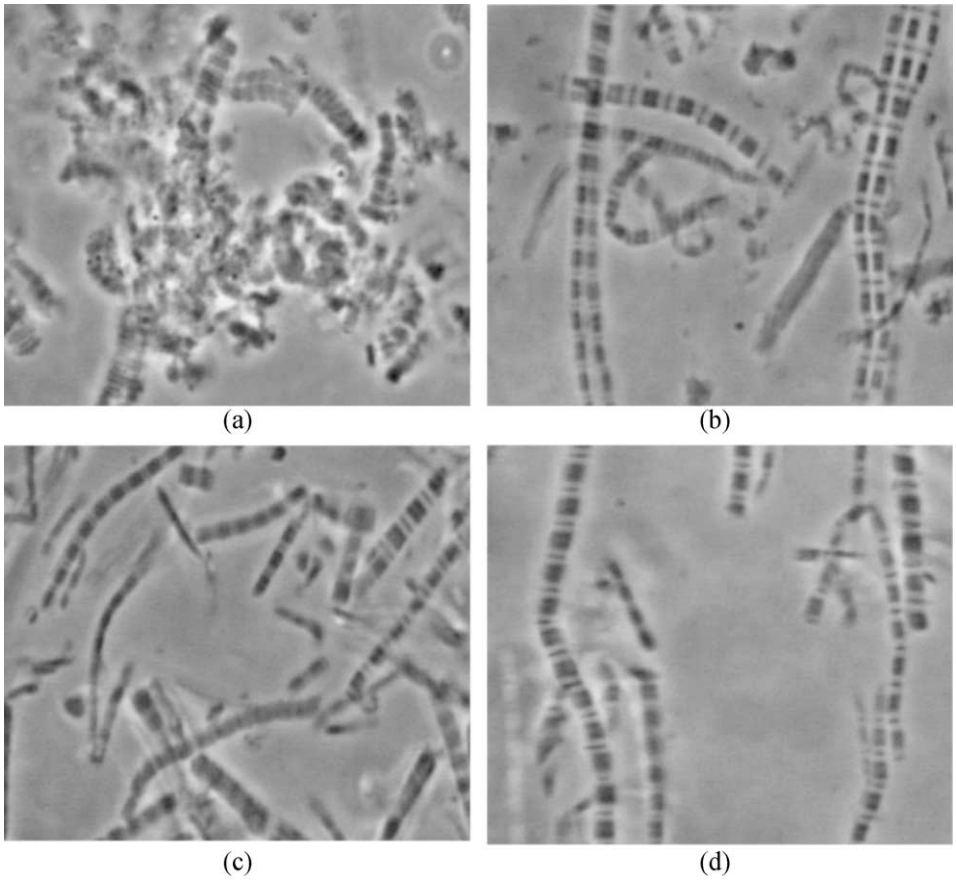

Fig. 1. WST myofibrils isolated from a non-stimulated carcass by method A at 3 min postmortem (top left, a), method B at 3 min postmortem (top right, b), method A at 24 h postmortem (bottom left, c), and method B at 24 h postmortem (bottom right, d).

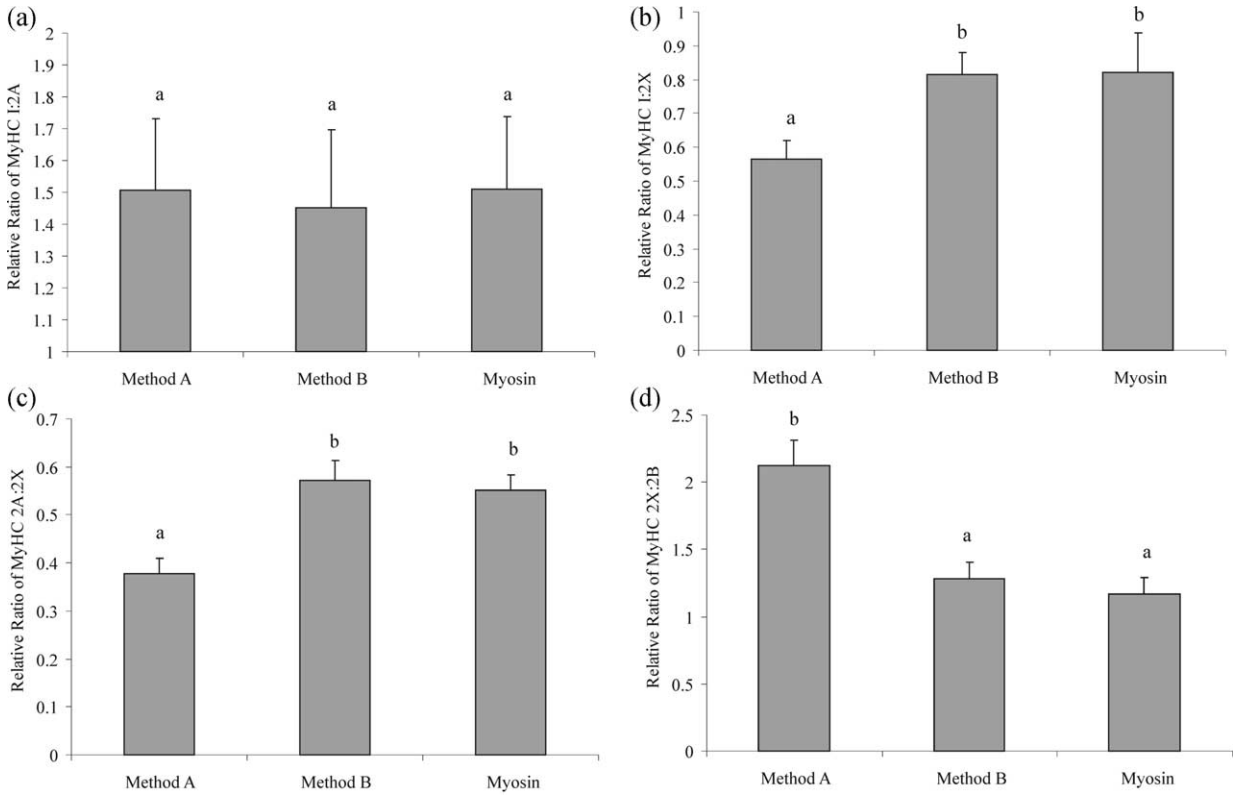

Fig. 2. Relative ratio of MyHC isoforms in myofibrils (methods A and B) and myosin extract isolated at 3 min postmortem (means±S.E.): (a) ratio of type I to type 2A MyHC in RST, (b) ratio of type I to type 2X MyHC in RST, (c) ratio of type 2A to type 2X MyHC in RST, (d) ratio of type 2X to type 2B MyHC in WST. Columns bearing different superscripts differ significantly ($P<0.05$).

than method B myofibrils or myosin extracts (Fig. 2d), which did not differ. These data show that method B myofibrils have MyHC isoform ratios that are more similar to myosin extracts than method A.

3.4. Factors influencing myofibril MyHC isoform composition

The relative amounts of the different MyHC isoforms of the RST are shown in Table 1. Only the amounts of type I, 2A, and 2X were measured because past research showed insignificant amounts of 2B MyHC in porcine RST (Lefaucheur et al., 1998). Method of isolation did not influence the amount of type I MyHC in myofibril samples regardless of sampling time or carcass treatment. For both method A and B myofibrils, the relative amount of type I MyHC was higher ($P<0.001$) in 3 min versus 24 h samples with the percentage difference being greater in ES versus NS samples. There was a significant interaction ($P<0.01$) between method, time of isolation, and carcass treatment for type 2A and 2X MyHC. Analysis of type 2A data for method of isolation shows that at 3 min, method A myofibrils had less ($P<0.01$) type 2A MyHC than method B. However, when isolated at 24 h method A myofibrils had more ($P<0.001$) 2A MyHC than method B myofibrils. For method A myofibrils, 3 min samples had less ($P<0.001$) 2A MyHC compared to NS-24 h samples and similar 2A amounts compared to ES-24 h samples. For method B myofibrils, 3 min samples had similar amounts of 2A MyHC compared to NS-24 h samples but higher ($P<0.001$) amounts compared to ES-24 h samples. Analysis of type 2X data for method of isolation shows that method A myofibrils had more ($P<0.01$) type 2X MyHC than method B myofibrils in 3 min and ES-24 h samples, but not in NS-24 h samples. Analysis of sampling time and carcass treatment shows that 3 min samples had higher ($P<0.01$) amounts of type 2X MyHC than 24 h samples regardless of isolation method. For method A myofibrils, NS-24 h and ES-24 h samples did not differ in 2X MyHC content. For method B myofibrils, NS-24 h

Table 1
MyHC isoform composition (relative A_{410}) of RST (means±S.E.)

Sample	Isolation method	Type I	Type 2A	Type 2X
3 min	A	1.305±0.126de	0.874±0.044b	2.296±0.059d
	B	1.478±0.051e	1.022±0.041c	1.802±0.031c
NS—24 h	A	1.019±0.132bc	1.325±0.025d	1.613±0.057b
	B	1.186±0.161cd	0.974±0.104bc	1.529±0.115b
ES—24 h	A	0.725±0.052a	0.944±0.096bc	1.543±0.067b
	B	0.781±0.070ab	0.472±0.022a	1.202±0.019a

1a,b Means within a column with different letters differ significantly ($P<0.05$).

samples had more ($P<0.01$) type 2X MyHC than ES-24 h samples. In general, method A biased RST myofibril samples more towards faster MyHCs (type 2A and 2X) independent of sampling time or carcass treatment.

The relative amounts of 2X and 2B MyHC isoforms in the WST myofibril samples are shown in Table 2. Only type 2X and 2B MyHC data were collected for WST samples because these two isoforms are the predominant MyHC isoforms found in WST (Lefaucheur, Edom, Ecolum, & Butter-Browne, 1995). Comparison of the isolation methods shows that there was less ($P<0.001$) 2X MyHC in method A versus method B myofibrils in NS-24 h samples but no differences in method in 3 min and ES-24 h samples. Analysis of sampling time shows that there was more ($P<0.001$) 2X MyHC in 3 min versus 24 h samples in both NS and ES carcasses regardless of isolation method. NS-24 h samples had more ($P<0.01$) type 2X MyHC than ES-24 h samples for both isolation methods. For type 2B MyHC, there was a significant ($P<0.0001$) interaction among carcass treatment and sampling time with isolation method. Compared to method B myofibrils, method A myofibrils had less ($P<0.01$) type 2B MyHC in 3 min and ES-24 h samples but similar 2B amounts in NS-24 h samples. For method A myofibrils, there was less ($P<0.001$) type 2B MyHC in 3 min versus 24 h samples. For method B myofibril samples there were no differences in type 2B content regardless of sampling time or carcass treatment.

3.5. Factors influencing myofibrillar ATPase activity

To determine the linearity of the ATPase assay and the influence of isolation method and sampling time on this linearity, P_i release as a function of time was measured in myofibrils isolated by methods A and B from WST at 3 min and 24 h in NS carcasses (Fig. 3). Independent of isolation method or sampling time, the slope (rate) appeared linear to about 5 min and was less linear at reaction times greater than 5 min. Thus, an incubation time of 5 min. was used for subsequent ATPase assays. Myofibrils isolated at 3 min using method A released less P_i than myofibrils isolated by method B over a 15 min reaction time (Fig. 3a). When isolated at 24 h, myofibrils did not differ in P_i release over time regardless of isolation method (Fig. 3b). Thus, enzymatic activity of myofibrils was influenced by method of isolation.

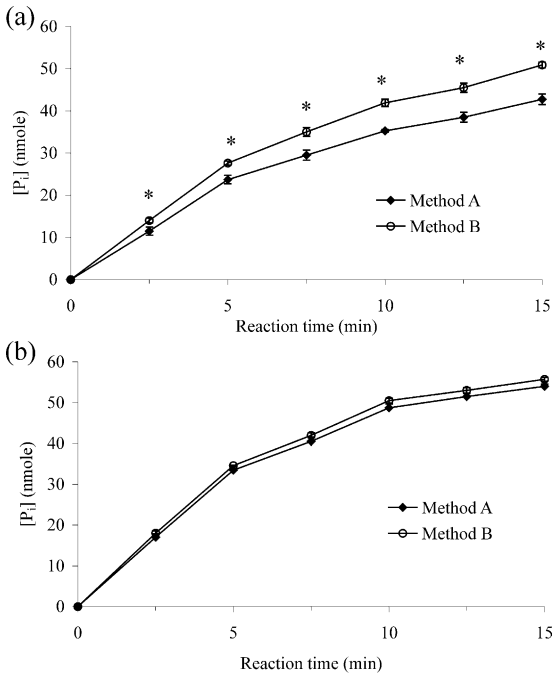

Fig. 3. Time-course of P_i release by WST myofibrils isolated from a non-stimulated carcass at (a) 3 min postmortem and (b) 24 h postmortem utilizing methods A and B (means±S.E.). * indicates that means differ ($P<0.05$) between methods A and B at a given reaction time.

Myofibrillar ATPase activity was measured to determine if method of isolation, carcass treatment, and postmortem sampling time influence myofibril enzymatic capacity. Maximum Ca^{2+}-activated myofibrillar ATPase activity of the RST is shown in Table 3. ATPase activity remained constant from 3 min to 24 h in NS muscle and decreased from 3 min to 24 h postmortem with ES, regardless of isolation method. RST myofibrils isolated by method A had greater ($P<0.0001$) ATPase activity in both 3 min and 24 h samples compared with method B myofibrils. The influence of ES on ATPase activity was less severe in method A versus method B myofibrils. ES samples were 59% ($P<0.01$) of NS samples at 24 h postmortem for method A and 37% ($P<0.01$) for method B myofibrils.

Table 2
MyHC isoform composition (relative A_{410}) of WST (means±S.E.)

Sample	Isolation method	Type 2X	Type 2B
3 min	A	1.622±0.079d	0.761±0.028a
	B	1.663±0.041d	1.300±0.081c
NS—24 h	A	1.243±0.088b	1.329±0.041c
	B	1.398±0.088c	1.376±0.046c
ES—24 h	A	0.932±0.036a	1.092±0.022b
	B	1.051±0.041a	1.264±0.029c

1a,b Means within a column with different letters differ significantly ($P<0.05$).

Table 3
ATPase activity (nmole P_i/μg protein/min) of RST (means±S.E.)

Isolation method	Time of sampling and carcass treatment		
	3 min	NS—24 h	ES—24 h
A	0.534±0.015d	0.540±0.019d	0.319±0.048b
B	0.391±0.007c	0.334±0.046bc	0.122±0.021a

Means with different letters differ significantly ($P<0.05$).

Thus, the ATPase activity of RST myofibrils was influenced by method of isolation, time postmortem, and carcass treatment.

Myofibrillar ATPase activity of the WST is shown in Table 4. ATPase activity increased from 3 min to 24 h in NS muscle and decreased from 3 min to 24 h postmortem with ES, regardless of isolation method. In 3 min samples, ATPase activity was less ($P<0.01$) in method A than in method B myofibrils. In NS-24 h samples, myofibrils from the two isolation methods did not differ in their activity. In ES-24 h samples, method A myofibrils had higher activity ($P<0.05$) than method B myofibrils. ES had a greater influence on the ATPase activity of myofibrils isolated by method B than A. ES samples were 45% ($P<0.01$) of NS samples at 24 h postmortem for method A and 29% for method B. As with RST myofibrils, the ATPase activity of WST myofibrils was influenced by method of isolation, time postmortem, and carcass treatment.

4. Discussion

The role myofibrillar ATPase activity play in modulating postmortem metabolism and PSE development is not fully understood. Interpretation of past data argues that early postmortem myofibrillar ATPase activity from PSE muscle does not differ or is lower than that from normal muscle (Greaser et al., 1969). Additionally, data demonstrating that PSE myofibrils lose their contractibility and functionality early postmortem (Honikel & Kim, 1986) argue against myosin acting as the predominant ATPase pushing rapid postmortem glycolysis. Failure of earlier studies to document the underlying MyHC isoform composition, which is a primary determinant of myofibrillar ATPase activity, raises questions about their conclusions, however. Data from the present study demonstrate that the procedure utilized to isolate myofibrils can influence MyHC isoform composition and the subsequent ATPase activity of myofibril samples. By documenting MyHC isoform profiles, this study helps further explain the fluctuations previously observed in myofibrillar ATPase activity in normal and rapidly metabolizing postmortem muscle.

Procedural differences in the two isolation methods could potentially account for the differences observed in the ATPase activity and MyHC isoform profile between myofibril samples utilized in this study. The most critical difference between the two procedures is the rigor state of the myofibrils at the time of homogenization. In method B, myofibrils are put into rigor prior to homogenization and the myofibrils are isolated in the presence of EGTA (Ca^{+2}-chelator) as well as a non-ionic surfactant (Triton X-100) and a serine protease inhibitor (PMSF). The isolation procedure (method A) used by Greaser et al. (1969) is a more crude methodology in which the muscle is immediately homogenized pre-rigor (if sampled early postmortem) and the myofibrillar fraction is isolated using differential centrifugation and suspension in a salt buffer. Such homogenization of muscle pre-rigor has previously been shown to result in structural damage to myofibrils (Locker, Daines, & Leet, 1976). This effect is clearly observed by visually assessing the differences in the integrity of the myofibrils from the two isolation procedures at 3 min and 24 h postmortem (Fig. 1). At 3 min postmortem, method B myofibrils (Fig. 1b) had a higher proportion of distinguishable sarcomeres than method A myofibrils, which appeared to be highly contracted (Fig. 1a). Such contractions could have caused physical disruption of the myofilament organization within the myofibrils and altered the subset of myofibrils isolated, as well as impairing myofibril functionality. Fewer visual differences were detected between method A and B myofibrils when isolated at 24 h compared to 3 min postmortem, as method A had less visible structural damage and more distinguishable sarcomeres when isolated at 24 h (Fig. 1c) versus 3 min (Fig. 1a). This was attributed to the fact that when homogenized at 24 h postmortem the muscle was in full rigor, which acts as a protective mechanism and prevents the contraction and structural damage observed in 3 min isolations in which the muscle is homogenized pre-rigor (method A). Furthermore, if denaturation of myofibrillar proteins interferes with myofibril isolation, the variation in MyHC isoform profiles observed in myofibril samples from a given isolation procedure across carcass treatments and time postmortem could be a function of MyHC isoforms differing in their susceptibility to pH and temperature denaturation.

Data regarding the ability of the myofibrils to hydrolyze ATP (Fig. 3) further support the argument that the integrity and enzymatic capacity of the myofibrils are influenced more by method of isolation early postmortem than when the muscle tissue is in full rigor at 24 h postmortem. When isolated at 24 h postmortem, WST myofibrils isolated by method A and B did not differ in their ability to hydrolyze ATP. When isolated at 3 min, however, method A myofibrils exhibited a reduced enzymatic capacity. Thus, postmortem time of sampling and rigor state are important factors to consider in the interpretation of ATPase data on isolated myofibrils.

Table 4
ATPase activity (nmole P_i/μg protein/min) of WST (means±S.E.)

Isolation method	Time of sampling and carcass treatment		
	3 min	NS—24 h	ES—24 h
A	0.444±0.019c	0.643±0.050e	0.292±0.008b
B	0.534±0.017d	0.655±0.044e	0.196±0.026a

Means with different letters differ significantly ($P<0.05$).

Data from the current study support earlier findings regarding the behavior of myofibrillar ATPase in postmortem muscle. Myofibrillar ATPase activity was found to increase with time postmortem in WST myofibrils from NS carcasses. This is consistent with findings in the *longissimus* muscle by Greaser et al. (1969). In muscle undergoing a rapid postmortem glycolysis, increased myofibrillar ATPase activity with postmortem time was not observed. In the current study myofibrillar ATPase activity declines between 3 min and 24 h postmortem when ES is used to hasten pH decline and simulate PSE development. Similarly, previous data have shown that *longissimus* muscle taken from PSE carcasses at 24 h or later postmortem exhibits lower myofibrillar ATPase activity than normal muscle (Greaser et al., 1969; Honikel & Kim, 1986; Sung et al., 1981; Warner et al., 1997). This decrease in enzyme activity is likely the result of protein denaturation due to adverse pH and temperature conditions present in PSE muscle. This is supported by data showing a decrease in the solubility of the myofibrillar protein fraction taken from PSE muscle as compared with normal muscle (Honikel & Kim, 1986; Joo, Kauffman, Kim, & Park, 1999; Warner et al., 1997). It is not known, however, if this denaturation manifests itself in a subset of the myofibrils present in the myofibrillar protein fractions.

The increase in myofibrillar ATPase activity at 24 h versus 3 min postmortem in normal, non-stimulated muscle can be partially explained by drastic differences in MyHC composition between the myofibril preparations. In the WST method A myofibrils, there was approximately a 2-fold increase in the relative amount of 2B MyHC present in the NS-24 h versus 3 min samples. Thus, the preferential shift towards extracting a population of myofibrils possessing more fast-contracting MyHC isoforms at 24 h may partially explain the elevated ATPase activity present in 24 h samples from normal muscle. This argument does not hold true, however, for WST method B myofibrils. For WST method B myofibrils, there was not an increased amount of 2B MyHC in NS-24 h samples, yet there was an elevated myofibrillar ATPase activity at 24 h compared to 3 min. Clearly, differences in MyHC isoform composition do not fully explain the elevated myofibrillar ATPase activity observed in normal muscle at 24 h postmortem.

The lower ATPase activity observed in ES-24 h samples is thought to be due to the dual effects of irreversible pH inactivation of ATPase activity and the differential extraction of MyHC isoforms at 3 min and 24 h postmortem. There was a lower amount of type 2X MyHC in ES-24 h versus 3 min in both RST and WST myofibril samples. This alone, however, probably does not account for the drastic reduction in ATPase activity. In fact, in ES samples there was a decrease in the amount of type I MyHC extracted from the RST and an increased amount of 2B MyHC in WST myofibrils isolated at 24 h. Intuitively, such shifts towards fast isoforms would theoretically cause an increase in the observed ATPase activity, under the assumption that the MyHC molecules present retain their ability to hydrolyze ATP despite adverse pH conditions induced by the ES treatment. This is not a valid assumption however, as others have reported that there is more myofibrillar protein denaturation in PSE meat at 24 h postmortem (Sung et al., 1981; Warner et al., 1997). This raises the question of whether or not MyHC isoforms differ in their susceptibility to adverse pH and temperature conditions. The fact that WST myofibril samples isolated at 24 h from ES carcasses had significantly more 2B MyHC than 3 min samples, yet a much lower ATPase activity would indicate that 2B MyHC is highly susceptible to pH inactivation. Studies to address this hypothesis are currently underway in our laboratory.

In both the RST and WST, there was an overall decrease in the relative amount of MyHC measured in myofibrils isolated at 24 h compared to 3 min postmortem, with the decrease being more severe in ES carcasses. These trends are consistent across muscle and MyHC isoform with the exception of type 2B MyHC from the WST and the type 2A MyHC from the RST method A myofibrils. Besides the differential extraction of various populations of myofibrils at different times postmortem, such results could also be the result of a decline in the antigenicity of the MyHC isoforms with postmortem time. The exaggerated effect observed in ES carcasses would lend further evidence to the hypothesis that low pH and high temperature has a direct effect on the MyHC molecule. Additional research is needed, however, to directly address the issue of loss in antigenicity.

Data from this study also demonstrate that different isolation methods influence MyHC isoform composition and ATPase activity of myofibril preparations. In the RST, the isolation methods appeared to vary in their ability to extract type 2A and 2X MyHC from muscle regardless of time of sampling or carcass treatment. For WST, method A tended to result in isolation of a lower proportion of type 2B MyHC than method B across all sampling times and carcass treatments. Although WST method B myofibrils isolated from ES carcasses at 24 h had similar amounts of 2X MyHC and more 2B MyHC than method A myofibrils, method B myofibrils exhibited a lower maximum ATPase activity. Hence, further support is given to the hypothesis that 2B MyHC is more susceptible to the loss of enzyme activity as the result of adverse pH conditions postmortem.

Data from this experiment illustrate that the procedure of Depreaux et al. (2000) can be adapted to quantify the relative amount of MyHC isoforms in myofibril preparations. Obviously the two methods utilized to isolate myofibrils resulted in myofibril preparations with

differing MyHC isoform profiles. In order to interpret correctly these data in regards to postmortem myofibrillar ATPase activity, the key question is which MyHC profile is most indicative of the in vivo composition of the muscle. The MyHC profiles of solubilized myosin extracts, as determined by the ELISA protocol developed by Depreux et al. (2000), were utilized as the standards to which the isoform profiles of the various myofibril preparations were compared. Fig. 2 demonstrates that method B myofibrils possessed MyHC profiles that more closely resembled those found in myosin extracts. Therefore, the MyHC composition of myofibrils isolated using the methodology of Swartz et al. (1993) (method B) is more reflective of the in vivo composition of the muscle than the MyHC profile of myofibrils isolated using the protocol of Greaser et al. (1969). Thus, myofibrils should be isolated according to method B for use in subsequent studies regarding myofibrillar ATPase activity and postmortem metabolism.

5. Conclusions

Data from this study provide evidence that the method of myofibril isolation influences the interpretation of data regarding the functionality of myofibrils postmortem. Method of isolation, sampling time, and carcass pH decline influence MyHC isoform profile and the ATPase activity of isolated myofibril samples. Thus, differences in myofibril isolation procedures must be accounted for to interpret accurately results regarding the role of myofibrillar ATPase in postmortem muscle metabolism. If myofibrillar ATPase activity drives in vivo glycolysis, these data indicate that the myofibrillar ATPase must be active early postmortem before it is inactivated by the pH and temperature conditions of the muscle. Furthermore, these data suggest that MyHC isoforms strongly influence myofibrillar ATPase activity and the apparent loss of activity with postmortem time and rate of metabolism. More specifically, these data suggest that fast MyHC isoforms may have higher myofibrillar ATPase capacity early postmortem, but they are more susceptible to denaturation and inactivation than slow isoforms.

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