

# Myosin heavy chain isoforms influence myofibrillar ATPase activity under simulated postmortem pH, calcium, and temperature conditions

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## Abstract

The pH and Ca<sup>2+</sup> sensitivity of myofibrillar ATPase activity plays an integral role in regulating postmortem muscle ATP utilization and likely paces postmortem glycolysis. The objective of this study was to determine the influence of pH and Ca<sup>2+</sup> concentration on the ATPase activity of myofibrils from red *semitendinosus* (RST) and white *semitendinosus* (WST) porcine muscles. Myofibrillar ATPase was measured at 39 °C over a pH range 5–7.5 and a [Ca<sup>2+</sup>] range pCa 4–9 (10<sup>-4</sup>–10<sup>-9</sup>M). At maximum Ca<sup>2+</sup>-dependent activation (pCa 4), RST myofibrils had lower ( $p < 0.0001$ ) ATPase activity than WST myofibrils. This maximum activity of myofibrils from both muscle regions was not influenced from pH 7.5 to 6.5, declined between pH 6.5 and 5.75 (Hill coefficient,  $n_H = 2.7$ –3.4; pH at half maximum activity,  $pH_{50} = 5.97$ ) and was near zero at pH 5.5. At pH 7, pCa-activity relationships showed that RST required less Ca<sup>2+</sup> for half-maximum activation (higher pCa<sub>50</sub>; 6.50) than WST myofibrils (pCa<sub>50</sub> = 6.35) but had no difference in  $n_H$ . At pH 7, both RST and WST myofibrils had maximum Ca<sup>2+</sup>-dependent, actin-activated ATPase activity at pCa ≤ 6 and Ca<sup>2+</sup>-independent myosin ATPase activity at pCa ≥ 6.75. pCa-activity relationships at different pH levels indicated that pCa<sub>50</sub> decreased with pH from pH 6.5 to 6.125 in both RST and WST myofibrils. At pH < 5.75, [Ca<sup>2+</sup>] did not influence ATPase activity in RST or WST myofibrils. These data show that myofibrils with predominantly fast MyHC (WST) have a higher actin-activated myosin ATPase activity than myofibrils with primarily slow MyHC isoforms (RST) at Ca<sup>2+</sup> concentrations and pH values characteristic of postmortem muscle.

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## 1. Introduction

The rate of postmortem muscle metabolism plays a key role in determining pork quality. Accelerated glycolysis early postmortem (<1 h) results in adverse pH and temperature conditions within muscle, leading to the excessive protein denaturation responsible for inferior meat quality (Briskey, 1964; Briskey & Wismer-Pedersen, 1961; Charpentier, 1969; Goutefongea, 1971). Consequently, the lack of uniformity in pork quality can

be largely attributed to the fact that rate and extent of postmortem glycolysis varies widely from carcass to carcass and among muscles. Muscles with a high proportion of fast-twitch, glycolytic fibers are typically more susceptible to an accelerated pH decline and development of poor quality meat compared to muscles with an abundance of slow-twitch, oxidative fibers (Dildey, Aberle, Forrest, & Judge, 1970; Warner, Kauffman, & Russell, 1993).

In a reconstituted in vitro muscle system, Scopes (1974) showed that rate of postmortem glycolysis is directly proportional to the rate of ATP hydrolysis. Accelerated ATP breakdown during the first 30 min postmortem in pig muscles (Bendall & Wismer-Pedersen, 1962; Briskey & Wismer-Pedersen, 1961) is related to the

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development of PSE (pale, soft, exudative) meat (Briskey, 1964). Due to the abundance of myosin in muscle, the myofibrillar ATPase, primarily actin-activated myosin ATPase or myosin ATPase, is a major source of ATP utilization postmortem. Cytoplasmic  $\text{Ca}^{2+}$  and  $\text{H}^+$  concentrations are critical factors controlling myofibrillar ATPase activity. In myofibrils, ATPase activity increases 20–100-fold with  $\text{Ca}^{2+}$  activation (Goodno, Wall, & Perry, 1978). Unfortunately, postmortem pCa within the cytoplasm is not known, but is likely low (pCa 5) in PSE pigs as they may be trembling or in tetany early postmortem.  $\text{Ca}^{2+}$  regulation of myofibrillar ATPase activity is the result of  $\text{Ca}^{2+}$  binding to troponin which shifts the position of the tropomyosin allowing for the interaction of actin and myosin (Potter & Gergely, 1975) and a further, myosin-dependent, tropomyosin movement resulting in full actin-activation of myosin (Lehman et al., 2000). Studies with skinned, single muscle fibers show that pH influences myofibrillar ATPase activity and  $\text{Ca}^{2+}$ -sensitivity (Blanchard, Pan, & Solaro, 1984; Cooke, Franks, Luciani, & Pate, 1988; Godt & Kentish, 1989; Kentish & Nayler, 1979; Potma, Van Graas, & Stienen, 1994). Subsequently, it can be postulated that  $\text{Ca}^{2+}$  and pH sensitivity of myofibrillar ATPase plays an integral role in regulating postmortem muscle ATP utilization and may pace postmortem glycolysis.

Myofibrillar ATPase activity is also a function of the muscle fiber type from which myofibrils are isolated and the expression of different myofibrillar protein isoforms, particularly troponin (Tn) and myosin heavy chain (MyHC). It can be postulated that TnC and TnI isoforms influence the pH effect on the pCa/activity relationship due to changes in the  $\text{Ca}^{2+}$ -affinity of Tn. The TnC-fast isoform, which is associated with fast MyHC expression, has two low affinity  $\text{Ca}^{2+}$ -binding sites, whereas TnC-slow/cardiac isoform only has one low affinity  $\text{Ca}^{2+}$ -binding site (as reviewed by Berchtold, Brinkmeier, & Muntener, 2000; Schiaffino & Reggiani, 1996). Four MyHC isoforms have been identified in adult porcine skeletal muscle: MyHC-I/slow, MyHC-2A, MyHC-2X, and MyHC-2B (Lefaucheur et al., 1998). Muscle fibers expressing 2B MyHC have the highest rate of ATPase activity, followed by 2X, 2A, and type I fibers (Bottinelli, Canepari, Reggiani, & Stienen, 1994). Thus, it can be hypothesized that MyHC and Tn isoform composition may alter postmortem ATP utilization and influence the rate of postmortem metabolism by regulating the pH and  $\text{Ca}^{2+}$ -sensitivity of postmortem myofibrillar ATPase activity. Data on the influence of MyHC on myofibril ATPase activity at the pH and temperature conditions found in postmortem muscle are lacking. Therefore, the objective of this study was to determine the influence of MyHC isoform composition on myofibrillar ATPase activity as a function of  $[\text{Ca}^{2+}]$  and pH at early postmortem temperature conditions.

## 2. Materials and methods

### 2.1. Myofibril isolation

Pooled myofibril samples were prepared from the *semitendinosus* muscles from three market weight pigs according to the procedure of Swartz, Greaser, and Marsh (1993) with slight modifications. Our previous studies showed that this method gives higher quality and more representative myofibrils than another method (in press). At 3 min post-exsanguination, muscle was excised from the carcass and dissected into the red (RST) and white (WST) *semitendinosus* portions. RST samples were dissected from the medial 1/3 of the *semitendinosus* muscle, and WST samples were obtained from the superficial 1/3 of the muscle. All steps of the myofibril isolation procedure were performed at 4 °C. Muscle bundles, 4–7 cm in length, were dissected out and placed in cold rigor buffer (RB: 75 mM KCl, 10 mM imidazole (pH 7.2), 2 mM  $\text{MgCl}_2$ , 2 mM EGTA, 1 mM  $\text{NaN}_3$ ). Bundles were attached to applicator sticks and bathed in RB plus 0.5% Triton X-100 (v/v) and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) with stirring. After 24 h, bundles were minced with scissors and homogenized in 10 volumes of RB for three 10-s bursts using a Polytron Homogenizer (Brinkman Instruments, New York, NY, USA). An additional 10 volumes of RB was added and samples were further homogenized using 50–100 strokes with a Dounce homogenizer and loose-fitting pestle. After centrifuging the suspension at 1000g for 10 min, the pellet was suspended in 20 volumes of RB and homogenized for three 5-s bursts. Following centrifugation at 1000g for 10 min, the pellet was homogenized for two 4-s bursts in 20 volumes of RB. To the suspension, an equal volume of RB containing 0.2% Triton X-100 was added. Suspension was filtered through cheesecloth and centrifuged at 1000g for 10 min. Myofibril pellet was then suspended in 20 volumes of RB containing 0.2% Triton X-100 and centrifuged at 1000g for 10 min. Myofibril pellet was then washed three more times with 20 volumes of RB per wash and a 10 min centrifugation at 1000g following each wash. The final pellet was suspended in RB made in 50% glycerol (v/v), and 1 mM DTT (final concentration). Glycerinated myofibrils were stored at –20 °C and used within 3 months.

### 2.2. Myofibrillar ATPase assay

Myofibrillar ATPase activity was determined using procedures adapted from Carter and Karl (1982) and Swartz, Zhang, and Yancey (1999). Base solutions of pCa 9 and 3 were formulated using the program of Fabiato (1988). For solutions of pH  $\geq$  6.5 buffers were composed as follows: 20 mM PIPES, 4 mM EGTA, 4 mM  $\text{MgCl}_2$ , 1 mM  $\text{NaN}_3$ , 1 mM DTT, 1 mg/ml BSA,

180 mM ionic strength with KCl, and either 5 (pCa 3) or 0 mM (pCa 9) CaCl<sub>2</sub>. For solutions at pH < 6.5, 20 mM MES was substituted for PIPES. Solution compositions were calculated for use at 39 °C. Since the pK<sub>a</sub> of the selected buffers changed little with temperature, the pH of the solutions was adjusted at room temperature. Prior to the ATPase assay, myofibrils were washed free of glycerol by four dilution and centrifugation cycles (1000–4000g for 5 min) in pH 7, pCa 9 buffer. The washed myofibril pellets were then resuspended to approximately 5 mg/ml in pCa 9 buffer at the desired pH, and the protein concentration was measured using the biuret assay with bovine serum albumin (BSA) standards. The myofibrils were then diluted to 0.5 mg/ml in pCa 9 buffer at the desired pH to create a working solution that was further diluted for the ATPase assay. The working solution was diluted with different proportions of pCa 9 and 3 buffers in order to yield varying Ca<sup>2+</sup> concentrations (pCa 4–9) in the final reaction mixture.

The myofibrillar ATPase activity of the RST and WST were determined at pH 5–7.5 and pCa 4–9 at 39 °C. The ATPase activity of the myofibrils was measured at a total protein concentration of 0.1 mg/ml in a total assay volume of 100 µl. The ATPase reaction was initiated by adding 80 µl of 2.5 mM ATP buffer to 20 µl of myofibrils (0.5 mg/ml). The reaction was quenched by the addition of 20 µl of cold 25% trichloroacetic acid (TCA). Reaction time varied from 5 to 30 min depending upon pCa and pH levels. After the reaction was stopped, samples were centrifuged at 12,000g for 15 s, and an aliquot of supernatant was removed for the determination of inorganic phosphate (P<sub>i</sub>) according to Carter and Karl (1982). Non-enzymatic hydrolysis of ATP during the reaction was accounted for by controls with TCA added prior to myofibrils. All samples were assayed in quadruplicate and the assays were repeated three times with freshly deglycerinated myofibril samples each time.

### 2.3. MyHC isoform determination

The relative MyHC isoform composition of the RST and WST myofibrils was determined by the enzyme-linked immunosorbent assay (ELISA) procedure for myosin extracts described by Depreux et al. (2000) with modifications. We previously showed that there is no difference in relative isoform composition between myofibrils (isolated as described above) and myosin extracts from the same muscle region (in press). Myofibrils were diluted in 0.05 M Tris buffer (pH 8.5) and coated on 96-well ELISA plates (Corning Inc., Acton, MA, USA) at 10 µg of protein per well. Plates were incubated overnight at room temperature. 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). ELISA absorbance values were normalized to the MyHC isoform content of standard myosin extracts from the RST (for type I and 2A antibodies) and WST (for type 2X and 2B antibodies) muscle in order to account for variation between plates. All samples were assayed on the same plate for each isoform.

### 2.4. Data analysis

Data were fit with a sigmoid logistic equation (SigmaPlot, 1994) of the form

$$\text{Activity} = \frac{(a - d)}{1 + (x/c)^b} + d,$$

where  $a$  = maximum activity (activity at infinite H<sup>+</sup> or Ca<sup>2+</sup> concentration),  $d$  = minimum activity (activity at 0 concentration),  $c$  = concentration of H<sup>+</sup> or Ca<sup>2+</sup> at half maximum activity,  $x$  = concentration of H<sup>+</sup> or Ca<sup>2+</sup>, and  $b$  = Hill coefficient ( $n_H$ ). This equation was used previously to fit data on myosin S1 binding to myofibrils for estimation of binding at infinitely low levels of S1 (Zhang, Yancey, & Swartz, 2000). An advantage of this form of a Hill equation is that it allows the software to estimate the maximum and minimum activity using the equation rather than normalizing the data to assumed minimum and maximum activity.

The SAS general linear model procedure was used to perform analysis of variance on the titration curve parameters and ELISA data (SAS, 1985). Significance level was set at  $p < 0.05$ .

## 3. Results and discussion

Past data suggest postmortem pH decline and metabolism are strongly influenced by myofibrillar ATPase activity, and this activity is altered by pH and pCa. Krzywicki (1971) found that myofibrillar ATPase activity of the *longissimus dorsi* muscle (LD) sampled at 35 min postmortem was higher in carcasses with a more rapid pH decline. An extremely rapid pH decline, however, may result in a decreased myosin ATPase activity prior to sampling. Penny (1967) showed that diminished ATPase activity due to myofibrillar protein denaturation is observed in pork LD muscle that declines to pH < 5.9 at 90 min postmortem while muscle temperature is still 33–35 °C. Similarly, Krzywicki (1971) found that myosin ATPase activity from LD samples was drastically reduced in muscles with pH < 5.8 and 40 °C at 35 min postmortem. In vivo, myofibrillar ATPase activity is largely a function of sarcoplasmic pCa, which is affected

by pH. As pH declines the sarcoplasmic reticulum (SR) loses its  $\text{Ca}^{2+}$ -accumulating ability more rapidly in PSE muscle compared to normal muscle (Greaser et al., 1969a, Greaser, Cassens, Hoekstra, & Briskey, 1969b, 1969c; Krzywicki, 1971). Subsequently, an increase in the sarcoplasmic pCa can enhance myosin ATPase activity, which accelerates pH decline leading to further denaturation. It has not been determined how myofibrils respond to pCa at different pH levels and if MyHC isoform differences mediate these effects.

The RST and WST vary widely in postmortem metabolism with the white portion undergoing a more rapid rate of postmortem glycolysis (Beecher, Briskey, & Hoekstra, 1965a). Differences in muscle fiber type between the two portions of the muscle are thought to play a role in this observation. Muscle fiber types differ in numerous characteristics that may contribute to this phenomenon, such as ATPase activity, metabolic enzyme profile, and  $\text{Ca}^{2+}$  metabolism. Due to the vital role ATPase activity plays in driving glycolysis (Scopes, 1974), the current study was designed to investigate the pH and pCa dependence of myofibrillar ATPase activity, which is greatly influenced by the various isoforms of myosin (Bárány et al., 1967). The relative amount of each MyHC isoform in the isolated RST and WST myofibrils used in this study is shown in Table 1. RST myofibrils had a greater ( $p < 0.0001$ ) amount of type I MyHC than WST myofibrils. Conversely, WST myofibrils had more 2B MyHC than RST myofibrils, as RST muscle does not express the 2B MyHC isoform (Lefaucheur et al., 1998). RST myofibrils also had more ( $p < 0.0001$ ) 2A and 2X MyHC compared to WST myofibrils. Such results are consistent with past studies that characterized muscle fiber type in the *semitendinosus* using metabolic enzyme profiles (Beecher, Cassens, Hoekstra, & Briskey, 1965b; Kiessling & Hansson, 1983).

Although it is well established that muscle fibers expressing fast MyHC isoforms have higher rates of ATPase activity than slow muscle fibers (Bárány, 1967), it is not known how MyHC isoforms influence myofibrillar ATPase activity over the range of pH decline (7.0–5.5) observed in postmortem porcine muscle. Much of the past data regarding pH and MyHC isoform effects on actin-activated myosin ATPase activity have been collected on skinned muscle fibers over a pH range 6–7.5 (Blanchard et al., 1984; Cooke et al., 1988; Godt & Kentish, 1989; Kentish & Nayler, 1979; Potma et al., 1994). To determine the effects of pH on myofibrillar,

$\text{Ca}^{2+}$ -dependent, actin-activated myosin ATPase activity, the ATPase activity of RST and WST myofibrils was measured at pCa 4 at pH levels from 5 to 7.5. The resulting sigmoidal-shaped curve and the fitted parameters are shown in Fig. 1 and Table 2, respectively. Maximum ATPase activity was lower ( $p < 0.0001$ ) in RST myofibrils than WST (0.385 and 0.597, respectively). Myofibrils from both muscles had a steep decline in activity between pH 6.5 and 5.5 with  $n_H$  being 3.4 and 2.7 (RST and WST) and  $\text{pH}_{50}$  being 5.97 but there was no difference between the samples. Below pH 5.75, the ATPase activity did not differ between the RST and WST myofibrils. Thus, overall pH sensitivity did not differ between RST and WST myofibrils at pCa 4.

The effect of pH on myofibrillar myosin ATPase activity was determined by measuring ATPase activity at various pH levels at pCa 9 (Fig. 2). The data for both muscle region myofibrils did not fit a sigmoid logistic response, rather there was essentially a sharp change in activity at pH 6.0 with it being higher at higher pH and lower at lower pH. The ATPase activity was negligible at  $\text{pH} < 5.5$ , similar to that observed at pCa 4. The transition point in the pH activity relationship is similar between that measured at pCa 4 and 9, suggesting that the loss in activity at and below pH 6.0 is myosin related not thin filament (troponin) related. Comparison between muscle regions at pH levels greater than 6.0 showed that RST myofibrils had lower activity ( $p < 0.01$ ) than WST fitting with the higher proportion of slow MyHC in the RST.

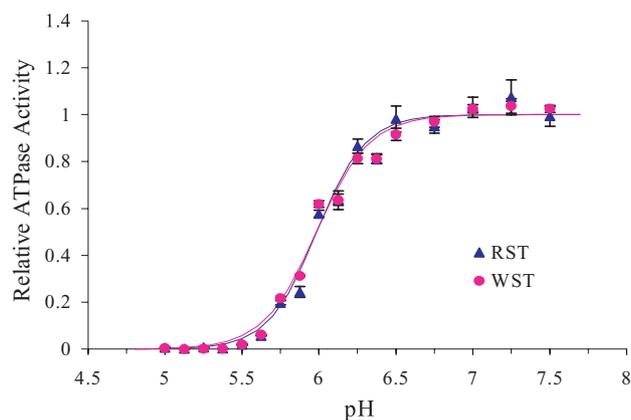


Fig. 1. Myofibrillar ATPase activity (mean  $\pm$  SE) of RST and WST at pCa 4 as a function of pH. Activity is plotted as the relative ATPase with the maximum activity set at 1.

Table 1  
Relative abundance ( $A_{410}$ ) of MyHC isoforms in RST and WST myofibrils (mean  $\pm$  SE)

	I	2A	2X	2B
RST	0.86 $\pm$ 0.02 <sup>b</sup>	0.87 $\pm$ 0.02 <sup>b</sup>	1.24 $\pm$ 0.03 <sup>b</sup>	–
WST	0.17 $\pm$ 0.01 <sup>a</sup>	0.76 $\pm$ 0.02 <sup>a</sup>	0.98 $\pm$ 0.04 <sup>a</sup>	1.89 $\pm$ 0.06 <sup>b</sup>

<sup>a, b</sup> Means within a column with different superscripts differ significantly ( $p < 0.01$ ).

Table 2  
Parameters of pH titration curves at pCa 4 of RST and WST (mean  $\pm$  SE)

	Maximum activity*	Hill coefficient ( $n_H$ )	pH <sub>50</sub>	Minimum activity*
RST	0.385 $\pm$ 0.028 <sup>a</sup>	3.4 $\pm$ 0.4 <sup>a</sup>	5.96 $\pm$ 0.02 <sup>a</sup>	7.3E-4 $\pm$ 6.8E-4 <sup>a</sup>
WST	0.597 $\pm$ 0.015 <sup>b</sup>	2.7 $\pm$ 0.6 <sup>a</sup>	5.98 $\pm$ 0.02 <sup>a</sup>	0.5E-4 $\pm$ 1.1E-5 <sup>a</sup>

\* Values expressed as nmol P<sub>i</sub>/μg protein/min.

<sup>a, b</sup> Means within a column with different superscripts differ significantly ( $p < 0.05$ ).

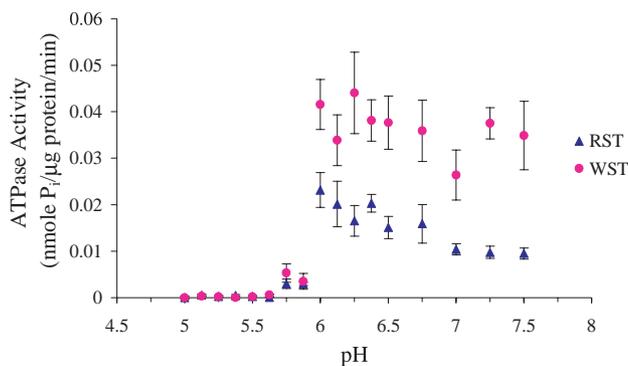


Fig. 2. Myofibrillar ATPase activity (mean  $\pm$  SE) of RST and WST at pCa 9 as a function of pH.

Low pH may decrease myofibrillar ATPase activity by at least two mechanisms: via the thick filament (myosin) and the thin filament (troponin). Low pH may directly alter the catalytic activity of the myosin molecule contained within the thick filaments. Low pH may also influence the thin filament by altering the ability of calcium to shift the position of tropomyosin via troponin and thus influence the degree of actin-activation of myosin. Data showing that ATPase activity of purified myosin is reduced by a low in vitro pH support the notion that pH effects are occurring directly on the myosin molecule (Penny, 1967). Additionally, this hypothesis is supported by data demonstrating that the maximum velocity of myosin ATPase and actin-activated heavy meromyosin (HMM) ATPase activity of normal muscle were greater than that of PSE muscle with a rapid pH decline (Sung, Ito, & Izumi, 1981). From the current study, the fact that pH influences myofibrillar ATPase at pCa 9 in the absence of Ca<sup>2+</sup>-activation (Fig. 2) suggests pH directly influences the ability of the myosin molecule to hydrolyze ATP. The effect of low pH on the thin filament and Ca<sup>2+</sup>-activation of myofibrils cannot be completely disregarded, however, as Park, Ito, and Fukazawa (1977) showed that the Ca<sup>2+</sup>-sensitivity of actomyosin isolated from PSE muscle was partially lost due to the inactivation of troponin.

The intracellular pCa is not likely at 9 or 4 post-mortem but likely changes in addition to the change in pH. To determine the influence of Ca<sup>2+</sup> on myofibrillar ATPase activity, RST and WST myofibrillar ATPase activity was measured as a function of pCa. Fig. 3 shows the sigmoidal-shaped curve characterizing the effects of

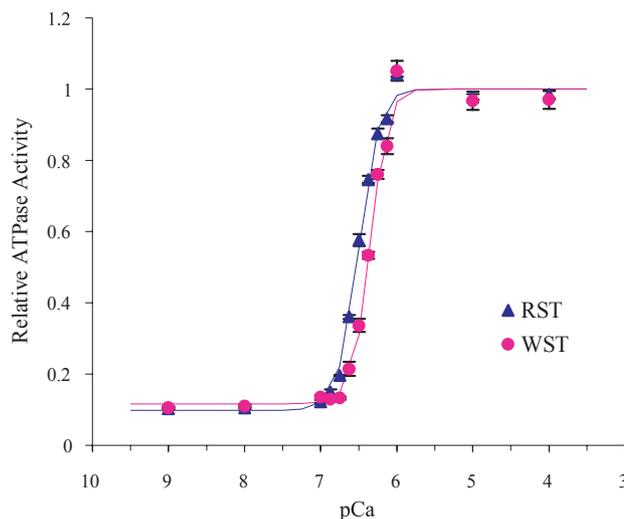


Fig. 3. Myofibrillar ATPase activity (mean  $\pm$  SE) of RST and WST at pH 7 as a function of pCa. Activity is plotted as the relative ATPase with the maximum activity set at 1.

pCa on myofibrillar ATPase activity at pH 7. Parameters describing the curve are shown in Table 3. RST myofibrils had lower ( $p < 0.05$ ) fitted values for the maximum and minimum activity than WST myofibrils. The relative activation by calcium (maximum/minimum activity) was 8.5–10-fold for both muscle region samples. Both RST and WST myofibrils displayed maximum actin-activated ATPase activity at pCa  $\leq$  6 and basal myosin ATPase activity at pCa  $\geq$  6.75.

The Ca<sup>2+</sup>-sensitivity and cooperativity of Ca<sup>2+</sup> activation of ATPase activity were estimated by the pCa<sub>50</sub> and Hill coefficient ( $n_H$ ), respectively, obtained from curve fitting. Data from skinned single fibers show that slow fibers generally have a higher Ca<sup>2+</sup>-sensitivity ( $\sim$ 0.2–0.3 pCa units) but lower Hill coefficients than fast fibers (as reviewed by Schiaffino & Reggiani, 1996). Also, in rabbit fast muscle fibers, the pCa<sub>50</sub> and Hill coefficient vary between the different types of fast fibers (Schachat, Diamond, & Brandt, 1987). For porcine muscle, myofibrils from the RST were more Ca<sup>2+</sup>-sensitive than WST myofibrils as indicated by a higher ( $p < 0.05$ ) pCa<sub>50</sub> value in agreement with data from rabbit and rat muscle. However, there was no difference in the apparent cooperativity of Ca<sup>2+</sup> activation ( $n_H$ ) between myofibrils from the RST and WST although the values fit with previous observations in other species with the RST being lower

Table 3  
Parameters of pCa titration curves at pH 7 of RST and WST (mean  $\pm$  SE)

	Maximum activity*	Hill coefficient ( $n_H$ )	pCa <sub>50</sub>	Minimum activity*
RST	0.475 $\pm$ 0.010 <sup>a</sup>	3.3 $\pm$ 0.5 <sup>a</sup>	6.50 $\pm$ 0.04 <sup>b</sup>	0.047 $\pm$ 0.004 <sup>a</sup>
WST	0.682 $\pm$ 0.049 <sup>b</sup>	3.8 $\pm$ 0.6 <sup>a</sup>	6.35 $\pm$ 0.04 <sup>a</sup>	0.080 $\pm$ 0.005 <sup>b</sup>

\* Values expressed as nmol P<sub>i</sub>/μg protein/min.

<sup>a, b</sup> Means within a column with different superscripts differ significantly ( $p < 0.05$ ).

than the WST. Differences in the Ca<sup>2+</sup>-sensitivity between the myofibrils of the RST and WST may arise from differences in troponin C (TnC) isoforms, which vary in Ca<sup>2+</sup>-affinity. The lower magnitude of the differences in Ca<sup>2+</sup>-sensitivity and cooperativity in the current study compared to other studies is likely the result of having myofibrils from a mixed population of fiber types (and thus MyHC isoforms) compared to single muscle fibers expressing predominantly one MyHC isoform. From these results it can be postulated that MyHC determines the basal (pCa 9) and maximal (pCa 4) myofibrillar ATPase activity while the Tn isoforms may influence the pCa relationship.

During the early postmortem period, however, pH does not remain at a constant pH 7. In pork carcasses with a rapid rate of postmortem glycolysis the pH at 45 min is usually below 6, while it is usually above 6 in carcasses with normal postmortem metabolism. In the current in vitro study, the effects of acidic pH (pH < 7) on the Ca<sup>2+</sup> activation of myofibrillar ATPase were determined by measuring the pCa-activity relationship at acidic pH levels (5.5–6.5). Between pH 6.125 and 6.5, RST and WST myofibrils had sigmoidal-shaped pCa-activity relationships, similar to that observed at pH 7 (Fig. 4(a) and (b), respectively). Parameters describing these curves are shown in Table 4. Maximum myofibrillar ATPase activity and the pCa required for half-maximal activation (pCa<sub>50</sub>) decreased with pH from pH 6.5–6.125 in both the RST and WST myofibrils. The pCa-activity curves at pH levels between 6.125 and 6.5 show that RST and WST myofibrils had similar pCa<sub>50</sub> values, but that WST myofibrils had higher actin-activated myosin ATPase activity than RST myofibrils at acidic pH and submaximal pCa.

At pH  $\leq$  6, the pCa-activity curves did not fit a sigmoid–logistic relationship as observed at higher pH levels. Fig. 5(a) and (b) show the effects of pCa on ATPase activity at pH 6 and 5.875. In both the RST and WST myofibrils, at pH 6 there was a peak in activity at pCa 6.5. A similar trend was observed in the RST myofibrils at pH 5.875, but not in the WST myofibrils. The cause of this peak in activity at pH 6 and pCa 6.5 is not known. This peak was observed several times using new pCa buffers, suggesting that it was not experimental error. If this is true, it has important implications to postmortem metabolisms as it suggests that the myofibrils become more sensitive to pCa when pH is between 6.0 and 5.5. At pH 5.5 (data not shown), pCa levels did

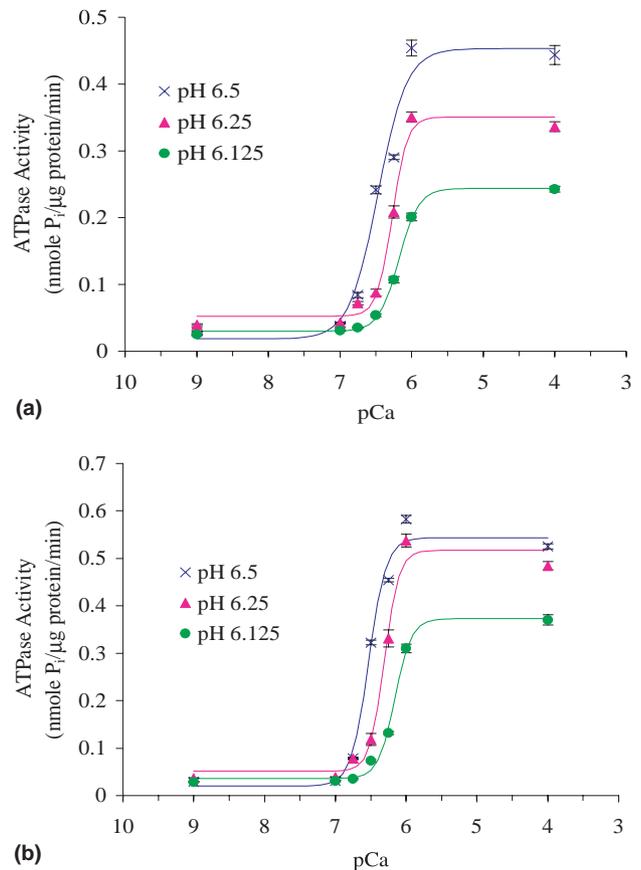


Fig. 4. Myofibrillar ATPase activity (mean  $\pm$  SE) as a function of pCa at pH 6.5, 6.25, and 6.125 for the RST (a) and WST (b).

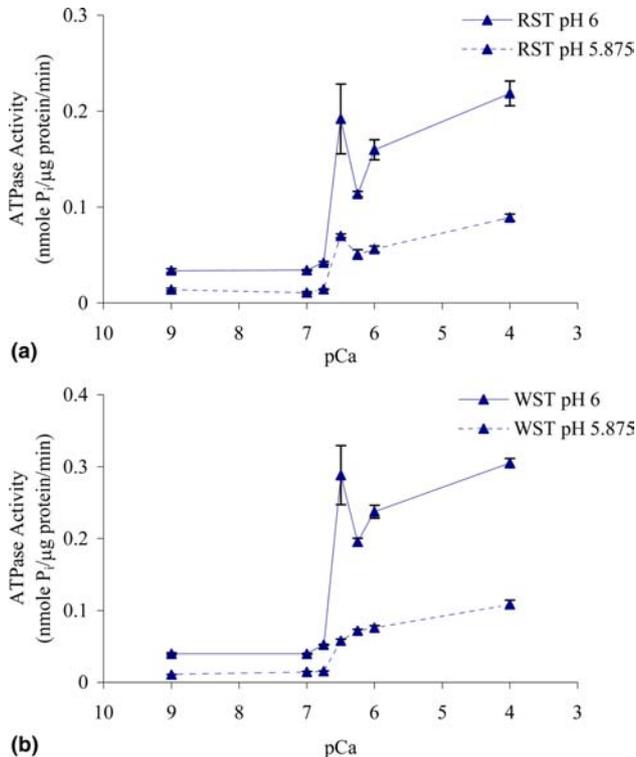
not affect ATPase activity in either the RST or WST myofibrils in agreement with the data in Figs. 1 and 2 where the myosin ATPase activity is dramatically inhibited at pH 5.5 and below.

The diminished response of the myofibrillar ATPase activity to pCa at acidic pH values is consistent with other data showing that acidosis reduces Ca<sup>2+</sup>-sensitivity of the pCa-tension relationship in skinned muscle fibers (Godt & Nosek, 1987; Metzger, 1996; Metzger & Moss, 1987). In skinned fibers, the reduction in Ca<sup>2+</sup>-sensitivity is in part due to acidosis decreasing the Ca<sup>2+</sup>-affinity of TnC in an isoform specific manner, with the effect being greater in the TnC-slow isoform than the TnC-fast isoform (Palmer & Kentish, 1994; Solaro, El-Saleh, & Kentish, 1989). Using myofibrils, several studies have shown that acidic pH decreases the Ca<sup>2+</sup>-binding of troponin, which diminishes ATPase activity (Blanchard

Table 4

Parameters for pCa titration curves of RST and WST at pH 6.5, 6.25, and 6.125 (mean  $\pm$  SE)

	pH	Maximum activity*	Hill coefficient ( $n_H$ )	pCa <sub>50</sub>	Minimum activity*
RST	6.5	0.453 $\pm$ 0.010 <sup>d</sup>	2.2 $\pm$ 0.2 <sup>a</sup>	6.47 $\pm$ 0.08 <sup>c</sup>	0.019 $\pm$ 0.010 <sup>a</sup>
	6.25	0.351 $\pm$ 0.005 <sup>b</sup>	4.2 $\pm$ 0.3 <sup>c</sup>	6.27 $\pm$ 0.04 <sup>b</sup>	0.053 $\pm$ 0.004 <sup>c</sup>
	6.125	0.244 $\pm$ 0.005 <sup>a</sup>	3.2 $\pm$ 0.2 <sup>b</sup>	6.18 $\pm$ 0.04 <sup>a</sup>	0.030 $\pm$ 0.010 <sup>ab</sup>
WST	6.5	0.544 $\pm$ 0.007 <sup>f</sup>	3.5 $\pm$ 0.3 <sup>b</sup>	6.53 $\pm$ 0.12 <sup>c</sup>	0.020 $\pm$ 0.007 <sup>a</sup>
	6.25	0.517 $\pm$ 0.009 <sup>e</sup>	4.2 $\pm$ 0.3 <sup>c</sup>	6.31 $\pm$ 0.04 <sup>b</sup>	0.051 $\pm$ 0.006 <sup>c</sup>
	6.125	0.373 $\pm$ 0.005 <sup>c</sup>	3.7 $\pm$ 0.2 <sup>b</sup>	6.16 $\pm$ 0.04 <sup>a</sup>	0.036 $\pm$ 0.005 <sup>b</sup>

\* Values expressed as nmol P<sub>i</sub>/μg protein/min.a, b Means within a column with different superscripts differ significantly ( $p < 0.05$ ).Fig. 5. Myofibrillar ATPase activity (mean  $\pm$  SE) of the RST (a) and WST (b) as a function of pCa (6.25–6.5) at pH 6 and 5.875.

et al., 1984; Blanchard & Solaro, 1984). Similarly, a reduction in the Ca<sup>2+</sup>-affinity of the TnC may account for the loss of Ca<sup>2+</sup>-activation of the myofibrils at the pH conditions used in the current study. In order to determine more precisely the role of thin filament protein isoforms on the influence of pH on the pCa-activity relationship, further studies substituting TnC or whole Tn on the myofibrils would be useful.

Because low pH conditions reduce the Ca<sup>2+</sup>-sensitivity (pCa<sub>50</sub>) of the myofibrillar ATPase activity and not just the maximum activity, these data imply that pH not only acts directly on the myosin but also influences Ca<sup>2+</sup>-activation of the myofibrils by acting on the regulatory components of the thin filament. This is further supported by the fact that myofibrils were unresponsive to elevated Ca<sup>2+</sup> concentrations below pH 5.75. In order

to understand more precisely the nature of the effects of acidic pH on myofibrillar ATPase activity, further research is needed. The degree to which thin filament inactivation contributes to the reduced ATPase activity at low pH can be tested more rigidly by stripping the myofibrils of their regulatory proteins and performing low pH ATPase assays, or by measuring the effects of pH on the enzymatic activity of isolated myosin subfragment 1 and its activation by actin.

Data from a previous study (in press) on RST and WST myofibrils sampled at 24 h postmortem from carcasses with or without rapid pH decline showed that those with rapid pH decline had lower maximum Ca<sup>2+</sup>-dependent, actin-activated ATPase activity than those without and the effect was greater in WST than RST myofibrils. Thus, it can be hypothesized that the decrease in myofibrillar ATPase activity at low pH and high temperature in vitro is more profound in myofibrils with fast MyHC isoforms (WST) versus slow MyHC isoforms (RST). From the current data, however, the individual contributions of MyHC isoforms to the overall myofibrillar ATPase activity at various pH and Ca<sup>2+</sup> conditions could not be assessed due to the fact that myofibril preparations contained mixed populations. Thus, it could not be determined if changes in ATPase activity with pH and Ca<sup>2+</sup> were due to alterations in the entire population of myofibrils or a subset of myofibrils expressing predominantly fast MyHC isoforms. In order to fully address the notion that in vivo postmortem myofibrillar ATPase activity is different between MyHC isoforms, data are required describing the effect of in vivo muscle conditions on the ATPase activity of individual fibers classified by MyHC isoform.

As with any in vitro study, precautions must be taken in extrapolating the data to in situ phenomenon. Although this study shows MyHC isoform plays a large role in determining in vitro myofibrillar ATPase activity, it must be noted that pH decline and glycolytic rate often vary more between PSE and normal muscle than does the proportion of the different MyHC isoforms. Thus, although MyHC isoform composition seems to be a key component, this factor alone is not the sole determinate of the rate of postmortem metabolism. Overall, data from this study demonstrate that WST

myofibrils have a greater myofibrillar ATPase activity than RST myofibrils at pH >5.5, but it should be emphasized that the degree of Ca<sup>2+</sup>-activation of the myofibrils is extremely important in determining the effects of MyHC isoform on postmortem ATPase activity. At conditions conducive to maximum enzyme activity, RST and WST myofibrils have a ~1.5–2-fold difference in ATPase activity, whereas a shift in Ca<sup>2+</sup> concentration of ~0.5 pCa units can change activity levels 10-fold. Due to the fact that fast-twitch muscle fibers have a more extensive SR with a higher density of SR Ca<sup>2+</sup>-ATPase pumps (Damiani & Margreth, 1994) and express different isoforms of SR Ca<sup>2+</sup>-ATPase than slow-twitch fibers (as reviewed by Wuytack et al., 1992), it can be postulated that fluctuations in cytosolic Ca<sup>2+</sup> may be different between fiber types during the early postmortem period which could lead to varying degrees of Ca<sup>2+</sup>-activation of myofibrillar ATPase. Data describing the intracellular fluxes in cytosolic Ca<sup>2+</sup> during the early postmortem period are lacking, however.

#### 4. Conclusions

Data from this in vitro study demonstrate that the Ca<sup>2+</sup>-sensitivity and ATPase activity of myofibrils decrease with pH conditions similar to those occurring in postmortem muscle. Furthermore, the data suggest that myofibrils with predominantly fast MyHC isoforms have a higher actin-activated myosin ATPase activity than myofibrils with primarily slow MyHC isoforms at Ca<sup>2+</sup> concentrations and pH values characteristic of postmortem muscle. Such differences may partially explain the variation observed in the postmortem metabolism between various muscles.

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