Excess glycogen does not resolve high ultimate pH of oxidative muscle

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26 **Abstract**

27 Skeletal muscle glycogen content can impact the extent of postmortem pH decline. Compared
28 to glycolytic muscles, oxidative muscles contain lower glycogen levels antemortem which may
29 contribute to the higher ultimate pH. In an effort to explore further the participation of
30 glycogen in postmortem metabolism, we postulated that increasing the availability of glycogen
31 would drive additional pH decline in oxidative muscles to equivalent pH values similar to the
32 ultimate pH of glycolytic muscles. Glycolysis and pH declines were compared in porcine
33 *longissimus lumborum* (glycolytic) and *masseter* (oxidative) muscles using an *in vitro* system in
34 the presence of excess glycogen. The ultimate pH of the system containing *longissimus*
35 *lumborum* reached a value similar to that observed in intact muscle. The pH decline of the
36 system containing *masseter* samples stopped prematurely resulting in a higher ultimate pH
37 which was similar to that of intact *masseter* muscle. To investigate further, we titrated
38 powdered *longissimus lumborum* and *masseter* samples in the reaction buffer. As the
39 percentage of glycolytic sample increased, the ultimate pH decreased. These data show
40 oxidative muscle produces meat with a high ultimate pH regardless of glycogen content and
41 suggest inherent muscle factors associated with glycolytic muscle control the extent of pH
42 decline in pig muscles.

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48 **Keywords**

49 Ultimate pH, Glycolytic capacity, Glycogen, Meat quality, Pork

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51 **Introduction**

52 Two classical fresh meat quality problems prevail in the meat industry: pale, soft and exudative
53 (PSE) meat and dark, firm and dry (DFD) meat. Dark, firm and dry meat displays a dark red or
54 purple color with a high ultimate pH. The ultimate pH cutoff for classifying meat as DFD is
55 traditionally thought to be above pH 6.0 (Briskey, 1964), yet some argue as low as 5.87 (Page,
56 Wulf, & Schwotzer, 2001). While this condition is more prevalent in meat from ruminants, it
57 also occurs in non-ruminants (McVeigh & Tarrant, 1982; Warriss, 1982; Warriss, Bevis, & Ekins,
58 1989; Warriss, Kestin, Brown, & Wilkins, 1984). Historically, DFD has been attributed to low
59 glycogen content at death (usually due to stress). Stress prior to slaughter often results in a
60 negative linear relationship between ultimate pH and pre-slaughter muscle glycogen, especially
61 when muscle glycogen content is between 0 and 53 mol/g glycogen in pigs and cattle (Henckel,
62 Karlsson, Jensen, Oksbjerg, & Petersen, 2002; Immonen & Puolanne, 2000). Therefore, muscle
63 glycogen content early postmortem is useful in predicting postmortem pH decline (Warriss, et
64 al., 1989).

65 Data describing this linear relationship between ultimate pH and glycogen content are
66 collected from studies using the *longissimus lumborum*, after all this muscle is the most
67 economically relevant to consumers interested in fresh meat quality. In pigs, this muscle
68 consists largely of the fast-contracting fibers that are glycolytic in nature. As a result, the

ultimate pH of meat produced by this muscle is often near pH 5.5 – 5.6. A strong relationship between glycogen and ultimate pH suggests that glycogen, or more specifically the lack thereof, is responsible for arresting postmortem glycolysis and leading to pork with an elevated ultimate pH. In pigs, especially, a number of muscles produce meat with an ultimate pH at or above pH 5.9, including the adductor, semitendinosus (red portion), gracilis, semimembranosus, gastrocnemius, and masseter (Huff-Lonergan, et al., 2002; Porcine Myology, 2005; Realini, et al., 2013). These muscles, however, contain lower antemortem glycogen and a higher proportion of slow-contracting fibers than in the *longissimus lumborum*. Therefore, if a linear relationship exists between antemortem muscle glycogen and ultimate pH in all muscles, we hypothesized that providing glycogen in excess will produce meat with an ultimate pH near 5.5 – 5.6. Testing this hypothesis *in vivo* is difficult. Increasing glycogen content of muscle through high starch diets has proven unreliable (reviewed by Fernandez & Tornberg, 1991; Rosenvold, Essén-Gustavsson, & Andersen, 2002; Rosenvold et al., 2001, 2002). Therefore, we tested the aforementioned hypothesis using our *in vitro* system designed to recapitulate postmortem glycolysis (England, Matarneh, Scheffler, Wachet, & Gerrard, 2014; England, Matarneh, Scheffler, Wachet, & Gerrard, 2015).

To conduct this study, a muscle was needed that met two criteria. First, it must be a predominantly oxidative muscle with high ultimate pH. Second, it must be readily available for easy sampling postmortem. The potential options were the *masseter*, the *diaphragm* or red portion of the *semitendinosus*. All three muscles are predominantly slow-contracting and oxidative, but the *masseter* alone exhibits exclusively type I and type IIA fibers (Johnson, White, & Lawrie, 1986; Realini, et al., 2013; Toniolo, et al., 2004; Tuxen & Kirkeby, 1990). Therefore,

the *masseter* was selected over the *diaphragm* or red *semitendinosus*. While the porcine *masseter* does not contain nearly as high a proportion of type I fibers as cattle (~100%) (Johnson, et al., 1986), it met both criteria for this study.

Materials and Methods

Sample collection

Market-weight pigs (100 – 125 kg) were harvested in the Virginia Tech Meat Center using accepted commercial processing procedures. Following exsanguination, porcine *longissimus lumborum* and *masseter* samples were excised at 5 and 1440 min (24 hr) postmortem. Samples were used for meat quality characterization or snap frozen in liquid nitrogen and stored at – 80 °C.

Meat quality characteristics and muscle metabolites

The frozen intact 24 h samples of the *longissimus lumborum* and *masseter* were analyzed for meat quality and muscle metabolites. Specifically glycogen, glucose 6-phosphate, glucose, and lactate were measured according to Bergmeyer (1984) using a method modified for microplate design (Hammelman, et al., 2003). Muscle pH was measured by powdering the tissue and homogenizing it using a solution containing 150 mM KCl and 5 mM sodium iodoacetate at a 1:8 ratio (wt/vol) (Bendall, 1973). Samples were equilibrated to 25 °C, centrifuged at 13,000 x g for 5 min at room temperature and measured immediately thereafter using a Thermo Scientific Orion Ross Ultra Semi-Micro glass electrode (Thermo Scientific, Pittsburgh, PA). Both the unfrozen *longissimus lumborum* and *masseter* were measured for objective color (L*, a*, and

b*) using a Minolta colorimeter CR-300 (Konica Minolta Inc., Osaka, Japan) with 8 mm aperture, C illuminant, and 0 observer. The mean value of three separate measurements was used for comparisons between muscles.

In vitro buffer system

The 5 min porcine *longissimus lumborum* and *masseter* muscles were powdered in liquid nitrogen and homogenized at a 100 mg / mL (1:10 ratio) into an anaerobic glycolysis buffer containing 10 mM Na₂HPO₄, 5 mM MgCl₂, 60 mM KCl, 5 mM ATP, 0.5 mM ADP, 0.5 mM NAD⁺, 30 mM glycogen, 25 mM carnosine, 30 mM creatine and 10 mM sodium acetate (pH 7.4) (England, et al., 2014; England, et al., 2015). Reaction vessels were stored at 25 °C for the duration of the trial. Aliquots were removed from the muscle and reaction buffer homogenate at 0, 60, 120, 240 and 1440 min for further analysis. In this study, one *masseter* and one *longissimus lumborum* sample was used from six separate pigs.

In a separate study, the ratio of total muscle mass to buffer volume was maintained, but the percent composition of *masseter* and *longissimus lumborum* (n=4 per treatment) were varied in order to titrate ultimate pH of the system. In this study, one *masseter* and one *longissimus lumborum* sample was used from four separate pigs.

In another study, 24 h *masseter* (n=4 per treatment) was added to the *in vitro* buffer system with changes. The pH was adjusted pH 6.1 to match the *in vivo* pH of the 24 h *masseter* and ATP was included at 3 mM or omitted (control). Samples were removed at 0 and 1440-min for pH and lactate. In this study, four separate *masseter* muscles from four pigs were used, but powdered muscle from each *masseter* was split between treatments.

In the final study, the extent of pH decline for the *masseter* (n=6 per treatment) was compared with and without a cocktail of mitochondria electron transport chain inhibitors (2 M rotenone (Complex I), 1 mM potassium cyanide (Complex IV), and 2 M oligomycin (Complex V)). In this study, six separate *masseter* muscles from six pigs were used, but powdered muscle from each *masseter* was split between treatments.

Metabolite analysis

Aliquots were removed from the homogenate for glycogen analysis and mixed with an equal volume of 2.5 M HCl, heated at 90 °C for 2 h, centrifuged at 13,000 x g for 5 min at room temperature, and the resulting supernatant was neutralized with 1.25 M KOH (Bergmeyer, 1984). Samples were removed from the homogenate for lactate, glucose 6-phosphate, ATP analysis and treated with an equal volume of ice-cold 1 M perchloric acid, centrifuged at 13,000 x g for 5 min at room temperature, and the resulting supernatant was neutralized with 2 M KOH (Bergmeyer, 1984).

Glycogen, lactate, glucose, and glucose 6-phosphate were measured from muscle homogenates using previous methods (Bergmeyer, 1984) modified for microplate volumes (Hammelman, et al., 2003). All reactions occurred in 5 mL borosilicate glass tubes and were analyzed spectrophotometrically at 340 nm in triplicate using 96-well microplates. ATP was separated with an HP Agilent 1100 (Agilent Technologies, Santa Clara, CA) using an Accucore C18 2.6 μ m 50 mm x 4.6 mm column (Thermo Scientific, Pittsburgh, PA), detected at 254 nm with gradient separation (Bernocchi, et al., 1994; Williams, Vidt, & Rinehart, 2008) and quantified using commercially available ATP (Sigma-Aldrich, St. Louis, MO) as a standard.

pH Analysis

Measurement of muscle pH was conducted according to (Bendall, 1973). The muscle homogenate pH was measured similarly with a slight modification. Four volumes of homogenate were transferred to a new tube and one volume of 25 mM sodium iodoacetate and 750 mM KCl (pH 7.0) was added. Samples were equilibrated to 25 °C, centrifuged at 13,000 x g for 5 min at room temperature and measured immediately thereafter using a Thermo Scientific Orion Ross Ultra Semi-Micro glass electrode (Thermo Scientific, Pittsburgh, PA).

Acid phosphatase activity assay

Acid phosphatase activity was measure according to a modified procedure by Bergmeyer (1974). Briefly, muscle samples were homogenized in 100 mM K₂HPO₄ (pH 7.4) and centrifuged at 13,000 x g for 5 min at room temperature. Aliquots of the supernatant were added to the reaction buffer containing 41 mM sodium citrate (pH 4.8) and 6.9 mM p-nitrophenylphosphate heated to 37 °C. Following a 10 min incubation, 4 volumes of 100 mM NaOH were added to stop the reaction. The activity was measured in triplicate using 96-well microplates spectrophotometrically at 410 nm and reported as units of activity defined as 1.0 µmole of p-nitrophenylphosphate per minute per g whole tissue at pH 4.8 and 37 °C. In this study, one *masseter* and one *longissimus lumborum* sample was used from eight separate pigs.

Gel electrophoresis and immunoblotting

Muscle tissue was powdered in liquid nitrogen and the proteins solubilized with a buffer containing 8M urea, 2M thiourea, 3% SDS (w/v), 75mM DTT, 0.05M Tris-HCl (pH 6.8), and 0.03% bromophenol blue (w/v) according to Warren, Krzesinski, & Greaser (2003). Solubilized proteins

from the *longissimus lumborum* and *masseter* (n = 6 for each muscle) were separated by SDS-PAGE (7%, 10% or 14% acrylamide), transferred to nitrocellulose membranes and immunoblotted with primary antibodies specific for phosphofructokinase, myoglobin, citrate synthase (Abcam, Cambridge, MA), lactate dehydrogenase A, glyceraldehyde 3-phosphate dehydrogenase (Novus Biologicals, Littleton, CO), myosin heavy chain I, and normalized to actin (Developmental Studies Hybridoma Bank, Iowa City, IA). Bands were visualized with IRDye fluorescent secondary antibodies (LI-COR Biosciences, Lincoln, NE) and quantified using LI-COR Biosciences Odyssey imaging system and software.

Statistical Analysis

Data are presented as means $\pm$ SE. All data were analyzed with a mixed model in JMP (SAS Institute Inc., Cary, NC). Individual animals were recognized as an experimental unit and time-course data were analyzed with a split-plot design. All differences are presented within time. The means were evaluated using a Student's t-test and considered significant at $P \leq 0.05$.

Results and discussion

3.1 Meat quality and muscle fiber-type

Meat quality profiling was compared between the intact *longissimus lumborum* and *masseter* (Table 1). At 24 hr, the *masseter* exhibited lower lactate formation, lower lightness (L*) value, higher redness (a*) and a greater ultimate pH. These data are consistent with previous characterization (Realini, et al., 2013). In addition, the abundance of specific metabolic and contractile proteins was measured (Figure 1). The *masseter* contained a higher content of myosin heavy chain type I isoform (slow-contracting), lower glycolytic enzyme abundance

(lactate dehydrogenase, phosphofructokinase-1, and glycerol 3-phosphate dehydrogenase; $P < 0.0001$), greater mitochondrial enzyme capacity (citrate synthase; $P < 0.0001$) and greater myoglobin ($P < 0.0001$). All proteins analyzed further affirms the *masseter* is a red muscle that contains a high proportion of slow-contracting muscle fibers that possess a more oxidative metabolic profile than the *longissimus lumborum*.

3.2 In vitro buffer system

Using this system, we compared the extent of glycolysis and pH decline between the *longissimus lumborum* and *masseter*. Glycogen was included at 30 mM and decreased ($P < 0.0001$) with time in both treatments. However, no treatment differences ($P = 0.8067$) were found at any time-point (Figure 2). Furthermore, glycogen content was not depleted in either treatment at 1440 min which indicated that glycogen was not limiting.

The extent of pH decline differed between the muscles. The *masseter* pH was higher at 0 min and the difference was maintained through the entire period (Figure 3A; $P \leq 0.0145$). The reaction buffer was initially adjusted to pH 7.4. When added, the *longissimus lumborum* decreased the initial pH to 6.8. However, the *masseter* only decreased the pH to 7.0 either because the tissue had a higher initial pH or a reduced amount of buffering compounds with a pK_a below 7.2 compared to the *longissimus lumborum*. Regardless, when initial pH was normalized to 0 min, net pH decline still maintained the difference with time (Figure 3B). In a previous study, we concluded that phosphofructokinase-1 activity ultimately terminates postmortem glycolysis and pH decline between pH 5.9 – 5.5 (England, et al., 2014). The ultimate pH of the *masseter* treatment was 5.92 ± 0.09 . In comparison to the 24 h intact

220 *masseter* (Table 1), there is a greater pH decline *in vitro*. Yet, glycogen was not depleted *in vivo*
221 and indicates postmortem glycolytic flux could have continued. This premature termination of
222 pH decline *in vivo* was likely due to the cold temperatures used in pork processing. Lactate
223 formation followed pH decline. Both total and net lactate was greater in the *longissimus*
224 *lumborum* than the *masseter* ($P \leq 0.05$; Figure 4). Lactate also correlated strongly with pH
225 across the entire reaction period for the *longissimus lumborum* ($R^2 = 0.71$; $P < 0.0001$) and
226 *masseter* ($R^2 = 0.79$; $P < 0.0001$). Combined, these data show that pH and glycolysis is arrested
227 in the *masseter* and stops at a higher ultimate pH even in the presence of excess glycogen.

228 One surprising finding was the discrepancy between glycogen disappearance and lactate
229 formation. Lactate formation was dramatically greater in the *longissimus lumborum*, but
230 glycogen catabolism was not different between treatments. Therefore, we investigated glucose
231 6-phosphate and glucose content (Figure 5). In the *longissimus lumborum*, glucose 6-phosphate
232 was greater ($P < 0.0001$) at 120, 240 and 1440 min. However, glucose was greater ($P < 0.001$) in
233 the *masseter* at 240 and 1440 min. To explain this phenomenon, we postulated that glucose
234 was being produced in a manner not normally present during *in vivo* postmortem metabolism.
235 Normally, glycogen debranching enzyme (EC 2.4.1.25; EC 3.2.1.33) releases glucose molecules
236 that are not phosphorylated by hexokinase postmortem (Young, Humphrey, & Wild, 1988) and
237 thus glucose accumulates with time postmortem. Alternatively, an additional enzyme may be
238 producing glucose in our system. The two likely enzymes responsible were glucose 6-
239 phosphatase (EC 3.1.3.9) or acid phosphatase (EC 3.1.3.2). Glucose 6-phosphatase is a
240 gluconeogenic enzyme that catalyzes the conversion of glucose 6-phosphate to glucose and is
241 found in liver and kidney tissue, but has little significant activity (if any) in muscle tissue (van

Schaftingen & Gerin, 2002). Alternatively, acid phosphatase is a lysosomal enzyme that functions to liberate inorganic phosphate from orthophosphoric monoesters like glucose 6-phosphate as pH declines. Indeed, the *masseter* contained greater ($P < 0.001$) acid phosphatase enzymatic activity (Figure 6). Others showed that acid phosphatase is more abundant in oxidative muscles of guinea pigs and mice (Peter, Kar, Barnard, Pearson, & Edgerton, 1972; Vihko, Salminen, & Rantamaki, 1979) and these data further support the oxidative status of the *masseter* and suggest that if lysosomes are disrupted postmortem, the conversion of glucose 6-phosphate to glucose may occur, altering the notion that glucose formation is exclusively caused by glycogen catabolism. However because other lysosomal-based enzymes like cathepsin play little role in postmortem proteolysis (Ouali, 1992), it suggests that the increased activity in our system is an *in vitro* manifestation only and acid phosphatase likely plays little role *in vivo*.

The *masseter* had lower ($P = 0.0305$) ATP at 0 min but greater ($P = 0.0419$) ATP at 240 min (Figure 7). However, no ATP treatment effect was detected ($P = 0.3941$) between the muscles. These results further suggest ATP hydrolysis is not substantially different between the muscles. Our lab has previously established that while myofibrillar ATPase activity was significantly greater in a predominantly glycolytic muscle (white *semitendinosus*) than an oxidative muscle (red *semitendinosus*) (Bowker, Grant, Swartz, & Gerrard, 2004), the magnitude of difference in ATPase activity between glycolytic and oxidative muscles is small. In an attempt to further establish the role ATP disappearance plays in terminating pH decline in oxidative muscle, we added 24 h *masseter* to the *in vitro* reaction buffer with and without 3 mM ATP. In the presence of additional ATP, the 24 h *masseter* was able to reactivate glycolysis by producing

additional lactate and pH decline (Figure 8). Thus, these data show that ATP became a limiting reagent in the *in vitro* oxidative masseter muscle and may be responsible for the termination of postmortem glycolysis.

Combined with the pH and lactate data (Figures 3 and 4), the ATP data suggests that in oxidative muscle, postmortem glycolysis is terminated because ATP becomes limiting. In glycolytic muscle, glycolysis functions postmortem until muscle pH is lowered to pH 5.9 – 5.5 where phosphofructokinase activity is inhibited (England, et al., 2014). The increased glycolytic flux results in an ultimate pH near the lower pH in the functional range of phosphofructokinase. The increased glycolytic flux (lactate) by the glycolytic *longissimus lumborum* (Figure 4) translates into a greater overall number of ATP molecules generated by glycolysis was greater. The glycolytic capacity of oxidative muscle is low and unable to produce the same glycolytic flux prior to pH inhibition of phosphofructokinase. Even in the presence of excess glycogen, pH decline of the oxidative *masseter* was terminated near the upper end of the pH range near pH 5.9 (Figure 3A). Therefore, the high ultimate pH of the oxidative *masseter* is due to glycolytic capacity or more specifically the lack thereof.

To follow-up, we tested whether glycolytic capacity linearly controlled ultimate pH. The percent composition of muscle was titrated in the *in vitro* system. We maintained a consistent muscle mass to volume ratio, but varied the percent composition between powdered pre-rigor *longissimus lumborum* and *masseter* and measured ultimate pH. The ultimate pH of the 0% *longissimus lumborum* (i.e. 100% *masseter*), was 5.95 ± 0.03 . The ultimate pH decreased ($P < 0.05$) in a curvilinear fashion until a 50:50 ratio was reached and did not decrease further from that point (Figure 9). These data show that small increases in glycolytic enzymatic capacity in a

red muscle impact ultimate pH of the muscle. These data may help further the correlation between increased oxidative or fast-oxidative fibers and elevated ultimate pH (Chang, et al., 2003; Gil, et al., 2003; Kang, et al., 2011; Maltin, et al., 1997; Ryu, et al., 2008). In these studies, animals with increased oxidative (type I) or fast-oxidative fibers (type IIA) produced meat with an elevated ultimate pH. Because the muscle studied was the *longissimus lumborum*, the elevated ultimate pH improved fresh meat quality. Therefore, better pork quality can be produced by selecting for an increase in the percentage of oxidative fibers in the *longissimus lumborum*.

These results must also be addressed in light of the AMPK $\gamma 3^{R200Q}$ (RN⁻) mutant pigs. Compared to control animals, muscle from these mutant pigs exhibits a slower contractile speed (Park, Gunawan, Scheffler, Grant, & Gerrard, 2009) and possesses lower lactate dehydrogenase activity with greater citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activity in the *longissimus lumborum* (England, et al., 2015; Lebret, et al., 1999; Scheffler, et al., 2014). In addition, these mutant pigs have 50% greater muscle mitochondria number (Scheffler, et al., 2014), epitomizing an oxidative type of muscle. Based on this study, therefore, mutant pigs should produce meat with a higher ultimate pH than their wild-type counterparts or at least not different. However, these animals consistently produce meat with an abnormally low ultimate pH of 5.2 - 5.3 that is often classified as acid meat (Milan, et al., 1996). The low ultimate pH is produced from a net increase in glycolytic flux (Matarneh, England, Scheffler, Oliver, & Gerrard, 2015). Therefore, an overriding mechanism must be able to extend pH decline, postmortem glycolysis or both. To date, the exact mechanism is unknown. While these animals deposit 70% more glycogen in the *longissimus lumborum* than wild-type pigs (Milan, et al., 2000), the muscle

also contains greater glycogen phosphorylase and lower AMP deaminase activity (England, et al., 2015; Granlund, Jensen-Waern, & Essén-Gustavsson, 2011). Both enzymes have been implicated in extending postmortem pH decline (Scopes, 1974). Alternatively, additional mitochondria may play a role in postmortem metabolism and extend postmortem pH decline. Our lab has recently reported that mitochondria can extend pH decline and postmortem glycolysis *in vitro* (Scheffler, Matarneh, England, & Gerrard, 2015). This is an exciting finding because RN⁻ muscle contains greater number of mitochondria and the mitochondria have greater maximal, ADP-stimulated oxygen consumption rate compared to wild-type animals (Scheffler, et al., 2014). To address this issue, we investigated the role the mitochondria play in the *in vitro* glycolysis of the *masseter*. We included a cocktail of mitochondrial inhibitors in our *in vitro* glycolytic system with the *masseter* muscle. As evident by the difference in citrate synthase abundance (Figure 1), the *masseter* contains substantially more mitochondria. This study was conducted with frozen *masseter* muscle with no added mitochondria. The pH declines of those treatments including mitochondria inhibitors were arrested early at an ultimate pH approximately 0.2 higher than controls (Figure 10). Furthermore, the difference in ultimate pH deviated late postmortem which is also consistent with the RN⁻ pig (Copenhafer, Richert, Schinckel, Grant, & Gerrard, 2006). While not specifically outlining the role the mitochondria play in postmortem metabolism, these data raise the possibility that mitochondria participate in energy production postmortem and play a role in dictating fresh meat quality and controlling ultimate pH.

Conclusions

These data indicate that in the presence of excess glycogen, oxidative muscle still produces high ultimate pH meat. Prior to this study, high ultimate pH meat in oxidative muscles has been suggested to be directly caused by a lack of antemortem glycogen levels. Rather, both the *in vivo* and *in vitro* studies suggest that porcine oxidative muscles can and do stop postmortem pH decline in the presence of excess glycogen. Instead, the glycolytic capacity of the muscle dictates ultimate pH and subsequently quality. To that end, we have updated the working model (England, et al., 2014; England, et al., 2015; Matarneh, et al., 2015) that outlines the factors that control both the rate and extent of postmortem pH decline (Figure 11).

Acknowledgments

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349 **Figure 1.** Representative western blot of protein abundance (A) of *longissimus lumborum* (LM)
 350 and *masseter* (M) muscles (n = 6 per muscle). MyHC-1: Myosin heavy chain type I isoform. CS:
 351 Citrate synthase. MB: Myoglobin. GAPDH: Glycerol 3-phosphate dehydrogenase. LDH: Lactate
 352 dehydrogenase. PFK: Phosphofructokinase-1. Relative immunoblot band density (B) between
 353 muscles. Data are mean $\pm$ SE. * $P < 0.0001$.

354 **Figure 2.** Mean glycogen levels (mM) of reactions consisting of buffer and *longissimus lumborum*
 355 or *masseter* muscle homogenates (n = 6 per muscle). Data are mean $\pm$ SE. * $P < 0.05$ within time.

356 **Figure 3.** Mean pH decline (A) and normalized to 0 min pH decline (B) of reactions consisting of
 357 buffer and *longissimus lumborum* or *masseter* (n = 6 per muscle). Data are means $\pm$ SE. * $P <$
 358 0.05 within time.

359 **Figure 4.** Mean lactate (mM; A) and normalized to 0 min lactate (mM; B) of reactions consisting of
 360 buffer and *longissimus lumborum* or *masseter* (n = 6 per muscle). Data are means $\pm$ SE. * $P < 0.05$
 361 within time.

362 **Figure 5.** Mean glucose 6-phosphate (A; mM) and glucose (B; mM) of reactions consisting of
 363 buffer and *longissimus lumborum* or *masseter* (n = 6 per muscle). Data are means $\pm$ SE. * $P < 0.05$
 364 within time.

365 **Figure 6.** Mean acid phosphatase activity of the *longissimus lumborum* or *masseter* (n = 8 per
 366 muscle). One unit of activity is defined as 1.0 μ mole of p-nitrophenylphosphate per minute per g
 367 whole tissue at pH 4.8 and 37°C. * $P < 0.05$

368 **Figure 7.** Mean ATP (mM) of reactions consisting of buffer and *longissimus lumborum* or
 369 *masseter* (n = 6 per muscle). Data are mean $\pm$ SE. * $P < 0.05$ within time.

370 **Figure 8.** Mean lactate (mM; A) and pH (B) of reactions consisting of buffer and 24 h *masseter*
 371 with 3 mM ATP and without ATP (control). Data are means $\pm$ SE. Mean values without a
 372 common superscript (a,b,c) are different ($P < 0.05$).

373 **Figure 9.** Mean ultimate pH of the *longissimus lumborum* and *masseter* mixtures and reaction
 374 buffer at specified ratios (n = 4 per muscle). Data are means $\pm$ SE. Mean values without a
 375 common superscript (a,b,c,d) are different ($P < 0.05$).

376 **Figure 10.** Mean of the reaction buffer and *masseter* mixture with and without the
 377 mitochondrial electron transport chain inhibitor cocktail (n = 6 per treatment; 2 M rotenone, 1
 378 mM potassium cyanide, and 2 M oligomycin). Data are means $\pm$ SE. * $P < 0.05$ within time.

379 **Figure 11.** Working model of the factors controlling the rate and extent of postmortem
 380 metabolism.

Figure 1.

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426 **Figure 11.**

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	Longissimus lumborum	Masseter
pH ₂₄	5.47 ± 0.01 ^a	6.12 ± 0.04 ^b
Glycogen ₂₄	16.41 ± 1.98	10.37 ± 3.53
G6P ₂₄	6.76 ± 1.37	5.42 ± 1.15
Glucose ₂₄	7.06 ± 0.66 ^a	2.89 ± 0.41 ^b
Lactate ₂₄	94.14 ± 1.77 ^a	46.77 ± 3.58 ^b
L*	64.14 ± 1.60 ^a	37.89 ± 0.65 ^b
a*	11.14 ± 0.73 ^a	25.48 ± 0.95 ^b
b*	7.06 ± 0.62	6.74 ± 0.24

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429 **Table 1.** Meat quality characteristics of the *longissimus lumborum* and *masseter* (n = 5 per
430 muscle) at 24 h postmortem. All measurements are from 24 h samples. Data are mean ± SE.
431 Mean values without a common superscript (a,b) within a row are different ($P < 0.05$).

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