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Presence of oxygen and mitochondria in skeletal muscle early postmortem

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ABSTRACT

Anaerobic glycolysis dominates energy metabolism postmortem. Even so, however, recent studies suggest mitochondria can modify postmortem energy metabolism and may contribute to pH decline, possibly affecting the transformation of muscle to meat and fresh meat quality development. Because oxygen is a necessary component of mitochondrial function, oxygenation of porcine and bovine *longissimus thoracis et lumborum* was determined postmortem using NIR spectroscopy. The ratio of oxy- to deoxymyoglobin decreased with time postmortem in both species. Metabolic analyses of muscle samples collected over the same timeframe also revealed fluctuations in TCA intermediates. Finally, mitochondria collected from muscle of electrically stimulated carcasses differed from those of non-stimulated muscle. Collectively, these data support the thesis that muscle mitochondria function early postmortem and may play a more active part in pH decline and possibly meat quality development.

1. Introduction

During the transformation of muscle to meat, ADP is urgently rephosphorylated using the phosphagen system and glycolysis in a futile attempt to maintain ATP in the tissues (Scheffler & Gerrard, 2007). Because neither requires oxygen and lactate accumulation over time postmortem closely reflects changes in glycogen depletion, postmortem metabolism is generally considered an anaerobic event (England, Scheffler, Kasten, Matarneh, & Gerrard, 2013). Recently, however, we showed mitochondria extended pH decline in an *in vitro* system simulating postmortem metabolism conditions and indicted these organelles as partially responsible for the altered glycolytic flux in pigs known to have an exaggerated pH decline and lowered pork quality (Matarneh et al., 2017). Even so, however, their contribution to energy metabolism postmortem remains a salient point of some contention. Hamm (1977) argues the contribution of hydrogen ion production to pH decline mainly arises from glycolysis and lactate formation, a process minimally occurring when functioning mitochondria are present. However, the totality of glycogen degradation and lactate formation fail to account for all variation in the ultimate pH of muscle postmortem (Scheffler, Scheffler, Kasten, Sosnicki, & Gerrard, 2013). Granted, there is little question that tissue oxygenation is compromised once the circulatory system fails (Pearson & Young, 1989). Yet, prior to stunning and even immediately post-exsanguination, muscle oxygenation likely remains capable of supporting mitochondrial function, albeit at reduced levels. Indeed, oxygen is critical for optimal mitochondrial function as it serves as the final electron acceptor in the electron transport chain. If functioning optimally and well-coupled, one mitochondrion can oxidize one pyruvate molecule into 15 molecules of ATP (Stenesh, 1998). Therefore, even the smallest degree of mitochondrial activity could have a profound impact on postmortem energy metabolism and perhaps fresh meat quality development. Though the aforementioned studies showed mitochondria can alter glycolysis, the availability of tissue oxygenation over time postmortem is not well-documented, nor are continued oxidative phosphorylation events well-understood in dying tissue.

Near infrared reflectance spectroscopy (NIRS) is a noninvasive technology used to predict pork (Andersen, Borggaard, Rasmussen, & Houmøller, 1999; Balage, da Luz e Silva, Gomide, de Bonin, & Figueira, 2015; Chan, Walker, & Mills, 2002; Cozzolino, Barlocco, Vadell, Ballesteros, & Gallieta, 2003; Josell, Martinsson, Borggaard, Andersen, & Tornberg, 2000; Kapper, Klont, Verdonk, & Urlings, 2012; Kapper,

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Klont, Verdonk, Williams, & Urlings, 2012; Savenije, Geesink, van der Palen, & Hemke, 2006) and beef quality (Andrés et al., 2008; Cozzolino & Murray, 2002; Hoving-Bolink et al., 2005; Leroy et al., 2004; Ripoll, Albertí, Panea, Olleta, & Sañudo, 2008). Though the exact means by which this technology is capable of predicting fresh meat quality remain obscure, NIR-based technologies generally scan wavelengths between 400 and 2500 nm, which correlate well with a number of physical and chemical parameters of meat (Prieto, Roehe, Lavín, Batten, & Andrés, 2009). This technology can also be used to assess muscle oxygenation by scanning a specific set of near infrared wavelengths between 590 and 890 nm that correspond to absorption spectra of hemoglobin and myoglobin (Schmitz, 2012). Using this approach, spectral scans can be used to quantify oxygenated hemoglobin and myoglobin when compared against the total oxygenated and deoxygenated forms in muscle (Boushel & Piantadosi, 2000). Commercially, these technologies are reliably used by athletes as a portable means of monitoring blood oxygenation levels during bouts of exercise. While it is not possible to delineate between the contribution of myoglobin and hemoglobin using this approach (Boushel et al., 2001), muscle contains only 2–3% residual blood (*i.e.* hemoglobin) from properly exsanguinated animals (Warriss, 1977). Therefore in muscle postmortem, decreases in muscle oxygenation are mainly due to the transition of oxy- to deoxymyoglobin. In this regard, changes in tissue oxygenation may prove useful in understanding more thoroughly the possibility of mitochondria functioning in muscle postmortem. Additionally, metabolomics is a powerful approach to assess temporal changes in energy metabolism that occur in tissues, in this case muscle over time postmortem. To that end, we used NIR spectroscopy and metabolomics to create additional information to support the concept that mitochondria can function postmortem. Finally, previous studies suggested that mitochondria may participate in postmortem energy metabolism during the conversion of muscle to meat (Matarneh, Beline, de Luz e Silva, Shi, & Gerrard, 2017; Matarneh, England, et al., 2017; Scheffler, Matarneh, England, & Gerrard, 2015). Because electrical stimulation is an effective technique to reliably accelerate postmortem energy metabolism and pH decline (Hammelman et al., 2003; Hwang, Devine, & Hopkins, 2003), a follow-up study was conducted to evaluate the ability of electrical stimulation to modulate postmortem muscle oxygenation and mitochondrial respiration.

2. Materials and methods

2.1. Soluble protein extraction and myoglobin oxygenation

Ground beef from the Virginia Tech Meat Center and two local retailers (n = 3) was mixed with 50 mM KH_2PO_4 (pH 7.4) (wt/vol) at room temperature using a stir plate at a 1:2 ratio (wt/vol). The mixture was filtered with two layers of cheesecloth and the resulting solution was stored on ice thereafter. An aliquot of the mixture (200 mL) was placed in a re-closable plastic bag with air removed. Muscle oxygenation (%) was measured with the muscle oxygen monitor (Moxy Muscle Oxygen Monitor, Fortiori Design LLC, Hutchinson, MN) with and without added sodium dithionite. Muscle myoglobin redox state was also measured spectrophotometrically (Tang, Faustman, & Hoagland, 2004). Muscle oxygenation (%) from the myoglobin redox state analysis was expressed as oxymyoglobin % / (oxymyoglobin % + deoxymyoglobin %).

2.2. Muscle oxygenation monitor testing

Two beef strip steaks (*longissimus lumborum*) were collected from three different animals. One steak from each animal was packaged in Styrofoam trays with PVC overwrap (n = 3) and one vacuum-sealed (n = 3). All steaks were stored at 4 °C overnight. After, muscle oxygenation was determined through the packaging material, overwraps were removed and CIELAB color was assessed immediately using a Konica Minolta CR-300 with an 8 mm aperture, an illuminant D65, and a 0° angle observer.

2.3. Postmortem muscle oxygenation

Initially, market weight cattle (n = 5; 550–700 kg live weight) and pigs (n = 6; 100–125 kg live weight) were harvested for *in vivo* postmortem muscle oxygenation, pH, and metabolomics testing at the Virginia Tech Meat Center using commercial processing procedures under state inspection. Immediately after exsanguination, skin, fat, and epimysium were removed to expose the initial measurement sites of at the posterior end of the *longissimus thoracis et lumborum* (LTL). The muscle oxygenation monitor was sealed in a clear, re-closable plastic bag (provided by the manufacturer) and pressed against the exposed muscle until the muscle oxygen output equilibrated (duration 10–15 s). After measuring muscle oxygenation, samples were collected from the underlying muscle, frozen in liquid nitrogen, and stored at − 80 °C for pH and metabolomics analysis. Additional LTL sites were exposed and muscle oxygenation and muscle samples were collected at 0, 60, 120, 240, 1440, and 2880 min postmortem in beef and 0, 60, 120, and 1440 min postmortem in pork. These time-points were chosen based on the normal time to resolve rigor mortis and achieve ultimate pH in beef (24–48 h) and pigs (12–24 h) (Bendall, 1973). Fiber orientation was consistently measured in each study as suggested by a previous investigation (Mohan et al., 2010).

In a follow-up study to further evaluate postmortem metabolomics changes in the LTL, *masseter*, and *cutaneous trunci*, additional cattle (n = 4; 550–700 kg live weight) were harvested for a separate study at the Ohio State University Meat Center using commercial processing procedures under USDA-FSIS inspection. Muscle samples were collected from the LTL, *masseter*, and *cutaneous trunci* at 0, 240, and 1440 min and frozen in liquid nitrogen for metabolomic analysis.

2.4. Muscle pH analysis

Frozen beef and pork LTL samples from the initial slaughter at Virginia Tech were powdered in liquid nitrogen, added to a buffer containing 5 mM sodium iodoacetate and 150 mM KCl (pH 7.0) at a 1:8 ratio (wt/vol), and homogenized (Bendall, 1973). Muscle homogenates were warmed to 25 °C, centrifuged, and measured using a calibrated Orion Ross Ultra pH electrode (Thermo Scientific, Pittsburgh, PA).

2.5. Metabolomics

Metabolites were extracted from samples from both slaughters designed to evaluate postmortem metabolomics changes in skeletal muscle. Briefly, 0.4 g of muscle was kept in 1.2 mL of methanol (GC–MS grade, J.T Baker, USA) in borosilicate glass vials with PTFE lined caps. Vials were then vortexed for 30 s and kept for incubation for 20 h at room temperature (22–26 °C). Following incubation, samples were vortexed again for 10 s and centrifuged for 5 min at 2000 rpm. From the supernatant, 200 μL was transferred to amber colored vials. Ribitol (2 μg) was added as the internal standard to all the samples. Samples were dried under a gentle stream of nitrogen gas.

2.6. Metabolomic profiling

Metabolomic profiling was conducted using gas chromatography – mass spectrometry (GC–MS). Samples were derivatized prior to GC–MS analysis, using a modified procedure described by (Abraham, Dillwith, Mafi, VanOverbeke, & Ramanathan, 2017; Rudell, Mattheis, & Curry, 2008). Dried samples were reconstituted with 100 μL methoxyamine (2% methoxyamine hydrochloride in pyridine; Pierce, IL, USA) and were incubated at 50 °C for 2 h. Silylation was done with 100 μL of N, O-bis (Trimethylsilyl) trifluoroacetamide with 1% trichloromethylsilane (BSTFA + 1%TMCS; ThermoScientific, PA, USA)