DOI:10.1021/jf1002842

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effects of Lactate on Bovine Heart Mitochondria-Mediated Metmyoglobin Reduction

RANJITH RAMANATHAN,[†] RICHARD A. MANCINI,*[,†] AND NAVEENA B. MAHESWARAPPA[‡]

[†]Department of Animal Science, University of Connecticut, Storrs, Connecticut 06269-4040, and [‡]National Research Centre on Meat, Chengicherla, Hyderabad, Andhra Pradesh 500039, India

Our objective was to determine the combined effects of lactate, LDH, and NAD on metmyoglobin reduction in mitochondria isolated from bovine cardiac muscle. Mitochondria were reacted with various combinations of lactate, LDH, NAD, and mitochondrial inhibitors, and oxygen consumption was measured using a Clark oxygen electrode. Mitochondria (3 mg/mL) and bovine metmyoglobin (0.15 mM) also were reacted with substrates/enzymes/inhibitors to determine mitochondria-mediated metmyoglobin reduction in vitro. Combining lactate-LDH-NAD with isolated mitochondria increased oxygen consumption as well as metmyoglobin reduction compared with those of either control mitochondria (without lactate) or mitochondria with added lactate, at pH 5.6 and 7.4 ($p < 0.05$). The addition of mitochondrial and LDH inhibitors to lactate-LDH-NAD decreased oxygen consumption and metmyoglobin reduction ($p < 0.05$). NADH formed from lactate-LDH-NAD can be used for nonenzymatic (via the electron transport chain) and enzymatic (NADH-dependent metmyoglobin reductase) metmyoglobin reduction.

KEYWORDS: Lactate; mitochondria; myoglobin; MRA; oxygen consumption

INTRODUCTION

In living tissue, myoglobin provides oxygen to mitochondria via interaction between oxymyoglobin and the outer membrane (*1*, *2*). Although myoglobin is oxidized during this process, there is no apparent accumulation of metmyoglobin in vivo because of inherent mechanisms present in mitochondria that reduce oxidized myoglobin to deoxymyoglobin (*3*−*6*). In post-mortem muscle, mitochondria influence meat color by regenerating reducing equivalents necessary for metmyoglobin reduction and competing with myoglobin for oxygen (*4*, *7*). Nevertheless, metmyoglobin reducing activity (MRA) in muscle is an important intrinsic property that prolongs the color stability of meat during storage (*8*).

Among the substrates and cofactors involved in color stability, NADH is the key component involved in both enzymatic and nonenzymatic metmyoglobin reduction (*9*, *10*). Although processes capable of producing NADH are continually depleted post-mortem, NADH can be regenerated by either lactate or succinate dehydrogenase present in post-mortem muscle (*4*, *11*, *12*). For example, Kim et al. (*13*) reported that NADH produced by lactate dehydrogenase activity can be used to nonenzymatically reduce equine metmyoglobin. To date, no research has assessed if NADH produced via LDH can be used by mitochondria for enzymatic reduction of bovine metmyoglobin.

Recent studies have shown that isolated bovine cardiac mitochondria can utilize exogenous lactate at pH 7.4, resulting in increased oxygen consumption (*14*). Furthermore, addition of lactate-LDH-NAD to isolated mitochondria significantly increased oxygen consumption and NADH production compared with those of lactate alone (*14*). No research has determined if this NADH can be utilized by mitochondria for metmyoglobin reduction.

NADH-mediated metmyoglobin reduction in mitochondria can occur: (1) via NADH entry into complex I, which results in electron movement within the electron transport chain and oxygen consumption, or (2) enzymatically via NADH-dependent cytochrome *b5* reductase (located within mitochondrial fraction) (*7*, *15*). To date, neither of these mechanisms have been assessed in meat science. Hence, the objectives of this study were to assess the effects of lactate, LDH, and NAD on metmyoglobin reduction in mitochondria isolated from bovine cardiac muscle in vitro. More specifically, experiments were designed to determine if NADH formed via lactate-LDH-NAD (1) can result in electron movement within the electron transport chain and subsequent nonenzymatic metmyoglobin reduction and (2) can be used by the mitochondria for enzymatic metmyoglobin reduction.

MATERIALS AND METHODS

Materials and Chemicals. Bovine hearts from market age cattle were obtained locally from an abattoir within 30 min of exsanguination, placed on ice, transported to the laboratory, and used for isolating mitochondria and myoglobin. Magnesium chloride ($MgCl_2$), bovine serum albumin (BSA), sucrose, tris-hydroxymethyl aminomethane hydrochloride (Tris-HCl), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), 2-(*N*-morpholino) ethanesulfonic acid (MES), ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA), *N*-[2-hydroxyethyl] piperazine-*N'*-[2-ethanesulfonic acid] (HEPES), adenosine 5'-diphosphate (ADP), nagarase protease (10.5 Units/mg), sodium lactate,

*Corresponding author. Tel: +1 860 486 1775. Fax: +1 860 486 4375. E-mail: richard.mancini@uconn.edu.

 Published on Web 04/21/2010 © 2010 American Chemical Society

Table 1. Various Treatment Combinations Added to Bovine Cardiac Mitochondria Used to Determine if NADH Produced by Lactate-LDH-NAD Can Result in Mitochondrial Respiration and Metmyoglobin Reduction at 25 °C (n = 5)[a]

		substrate/enzyme/inhibitors[b]					
	treatments	lactate (50 mM)	LDH (95 Units)	NAD (0.2 mM)	antimycin (0.01 mM)	rotenone (0.02 mM)	oxalate (50 mM)
1	control	−	−	−	−	−	−
2	lactate	+	−	−	−	−	−
3	lactate + LDH	+	+	−	−	−	−
4	lactate + NAD	+	−	+	−	−	−
5	lactate + antimycin	+	−	−	+	−	−
6	lactate + LDH + NAD (LLN)	+	+	+	−	−	−
7	LLN + antimycin + rotenone	+	+	+	+	+	−
8	LLN + oxalate	+	+	+	−	−	+

[a] LDH = lactic dehydrogenase; NAD = β-nicotinamide adenine dinucleotide; NADH = β-nicotinamide adenine dinucleotide-reduced dipotassium salt; LLN = lactate-LDH-NAD. [b] Substrate/enzyme/inhibitors present (+) or absent (−) either in the incubation chamber of the Clark electrode or for metmyoglobin reduction.

antimycin A, rotenone, lactic acid dehydrogenase (LDH; 662 Units/mg of protein), β-nicotinamide adenine dinucleotide (NAD), Sephacryl 200-HR, ammonium sulfate, potassium chloride, EDTA, and Bicinchoninic Acid Protein Assay Kit were purchased from Sigma Chemical Co. (St. Louis, MO); and maleic acid was purchased from Fisher Scientific (Fair Lawn, New Jersey). PD-10 columns were obtained from GE Healthcare (Piscataway, NJ). All chemicals used in the experiment were of reagent grade or greater purity.

Bovine Mitochondria Isolation. Mitochondria were isolated from bovine cardiac muscle according to Smith (*16*) with minor modifications. Briefly, 100 g of ground cardiac muscle was washed twice with 250 mM sucrose and suspended in 200 mL of mitochondria isolation buffer (250 mM sucrose, 10 mM HEPES, 1 mM EGTA, and 0.1% BSA, pH 7.2). The suspension was stirred slowly and hydrolyzed with nagarase protease (protease/tissue, 0.5 mg/g) for 20 min; the pH was maintained between 7.0 and 7.2. After proteolytic digestion, the suspension was diluted to 1000 mL with mitochondria isolation buffer and subjected to two homogenization processes. The first of these was accomplished in a Kontes Duall grinder (Vineland, New Jersey) with three passes, whereas the second homogenization was performed using a Wheaton Potter-Elvehjem grinder (Millville, New Jersey) with three passes. Pestles for these grinders were driven by a heavy duty drill at 1400 rpm. The homogenate was centrifuged (1200*g*) for 20 min with a Sorvall refrigerated RC-5B centrifuge (Thermo Fisher Scientific, Waltham, MA), and the resulting supernatant was again centrifuged (26000*g*) for 15 min. The pellet was washed twice and suspended in mitochondria suspension buffer (250 mM sucrose, 10 mM HEPES, pH 7.2). All steps were performed at 0−4 °C. Mitochondrial protein content was determined using a bicinchoninic acid protein assay.

Reaction of Substrates with Mitochondria. To determine if NADH produced by lactate-LDH-NAD can result in beef mitochondrial oxygen consumption and metmyoglobin reduction, mitochondria were reacted with various combinations of lactate, LDH, and NAD (**Table 1**). To assess the role of mitochondrial inhibitors, antimycin and rotenone treatments also were included.

Oxygen Consumption Measurement. Methodology similar to that of Tang et al. (*7*, *17*) was used for reacting isolated mitochondria with substrates or enzymes. All treatments (**Table 1**) were added to mitochondria through a 1 mm port in the incubation chamber of a Clark oxygen electrode, which was used to measure mitochondrial oxygen uptake (polarizing voltage of 0.6 V and an 8 mL incubation chamber). The incubation chamber was maintained at either pH 5.6 or 7.4, and the reaction temperature was kept at 25 °C by a water jacket and Lauda RE120 circulating water bath (Westbury, NY). The chamber was stirred with a 10 mm Teflon-covered bar at 600 rpm. The electrode was attached to a Rank Brothers digital model 20 oxygen controller (Cambridge, England) and connected to a personal computer and data logger. Oxygen consumption was recorded over time by suspending mitochondria either in pH 5.6 (250 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1% BSA, and 20 mM maleic acid) or 7.4 (250 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1% BSA, and 20 mM HEPES). State IV oxygen consumption rate (OCR) was calculated on the basis of the method of Estabrook (*18*). State IV oxygen consumption measures the OCR of isolated mitochondria in the presence of added substrate.

Myoglobin Isolation and Purification. Myoglobin was purified via ammonium sulfate precipitation and gel filtration chromatography according to Faustman and Phillips (*19*). Briefly, cardiac muscle devoid of fat and connective tissue was homogenized in buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0, 4 °C) and centrifuged at 5000*g* for 10 min. The supernatant was brought to 70% ammonium sulfate saturation, and the resulting solution was stirred for 1 h at 4 °C and later centrifuged at 18000*g* for 20 min. The resulting supernatant was saturated with ammonium sulfate (100%) and centrifuged at 20000*g* for 1 h. The precipitate was resuspended in homogenization buffer and dialyzed (3 volumes) against 10 mM Tris-HCl and 1 mM EDTA, at pH 8.0, 4 °C for 24 h. Myoglobin was separated from hemoglobin using a Sephacryl 200-HR gel filtration column (2.5 × 100 cm). The elution buffer contained 5 mM Tris-HCl and 1 mM EDTA at pH 8.0, and the flow rate was 60 mL/h.

Electron Transport Mediated Metmyoglobin Reduction. Methodology similar to that of Tang et al. (*7*, *20*) was used for assessing metmyoglobin reducing activity. In order to adjust the pH of the metmyoglobin solution, samples were passed through PD-10 columns precalibrated with either MES (pH 5.6) or phosphate buffer (pH 7.4) (*21*). Metmyoglobin (2.5 mg/mL) reduction was conducted in a glass open top tube at pH 7.4 (120 mM KCl, 5 mM KH_2PO_4, and 30 mM K_2HPO_4) or 5.6 (120 mM KCl, 5 mM KH_2PO_4, and 30 mM maleic acid) and 25 °C. Bovine heart mitochondria (3 mg/mL) were combined with both metmyoglobin and substrate/enzyme treatments (**Table 1**). At specific time points, samples were removed and centrifuged (12000*g*) with an Eppendorf 5415D centrifuge (Westbury, NY) for 5 min. The resulting supernatant was scanned from 650 to 500 nm with a Shimadzu UV-2101PC spectrophotometer (Kyoto, Japan). The relative proportions of deoxymyoglobin, oxymyoglobin, and metmyoglobin were calculated according to Tang et al. (*22*).

In order to assess the ability of lactate to reduce metmyoglobin without mitochondria, bovine metmyoglobin was incubated with lactate, lactate-LDH, lactate-NAD, lactate-LDH-NAD, rotenone, and antimycin at 25 °C for 3 h. At specific time points, samples were removed, and the change in spectra was assessed by scanning the metmyoglobin-substrate/enzyme solution from 650 to 500 nm with a Shimadzu UV-2101PC spectrophotometer (Kyoto, Japan). The relative proportions of deoxymyoglobin, oxymyoglobin, and metmyoglobin were calculated according to Tang et al. (*22*).

Enzymatic Metmyoglobin Reductase Activity. Methodology similar to that of Lanari and Cassens (*23*) was modified and used for estimating the metmyoglobin reductase activity of mitochondria in the presence of lactate, LDH, and NAD. Briefly, mitochondria (3 mg/mL) were preincubated with antimycin (0.01 mM) and rotenone (0.02 mM) for 10 min in incubation buffer at either pH 7.4 (120 mM KCl, 5 mM KH_2PO_4, and 30 mM K_2HPO_4) or 5.6 (120 mM KCl, 5 mM KH_2PO_4, and 30 mM maleic acid) at 25 °C in order to inhibit ETC-mediated metmyoglobin reduction. Following incubation, mitochondria were combined with bovine metmyoglobin (0.15 mM), potassium ferrocyanide (3 mM), and EDTA (5 mM) at pH 5.6 and 7.4. The reaction was initiated by the addition of either (1) NADH (0.2 mM) or (2) a combination of lactate (50 mM), LDH (95 Units), and NAD (0.2 mM). Absorbance at 580 nm (the wavelength at which the difference in absorbance for oxymyoglobin and metmyoglobin is maximal) was measured for 30 min. The metmyoglobin reduction rate

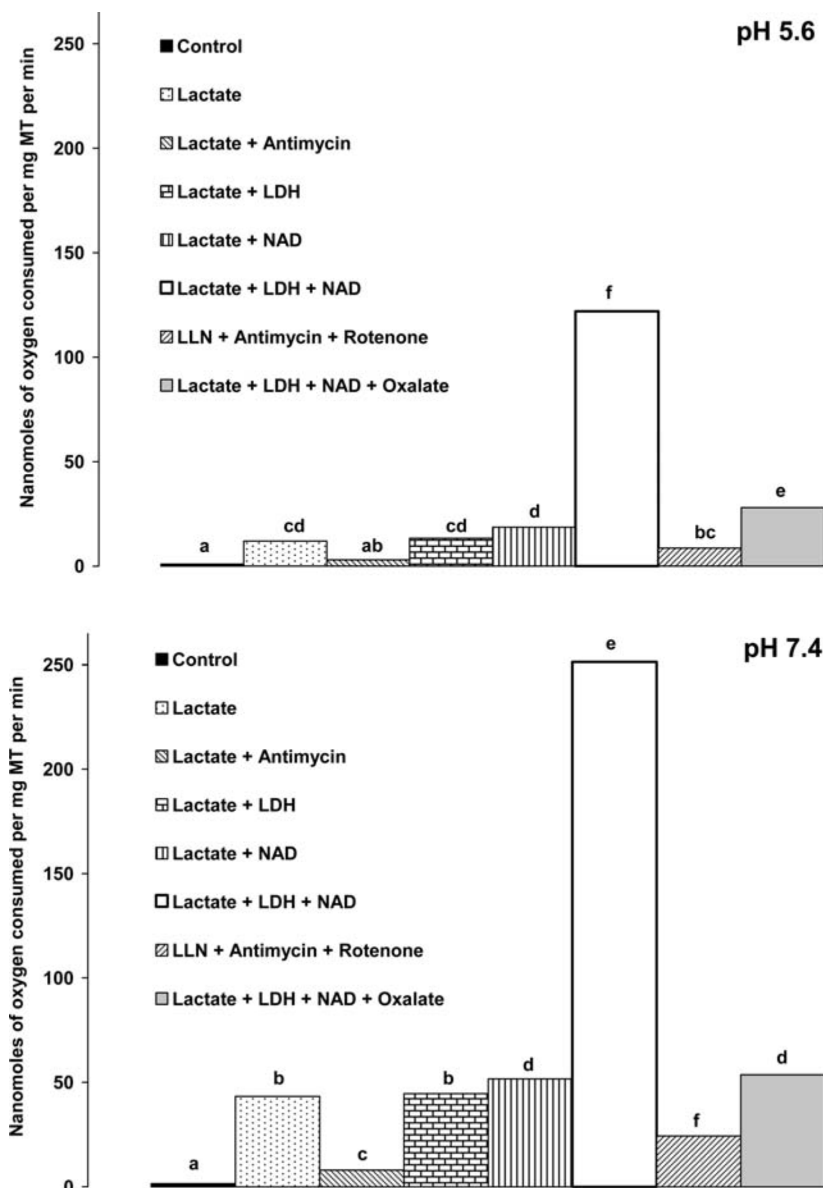

Figure 1. Effect of lactate, LDH, NAD, and NADH on oxygen consumption of beef cardiac mitochondria (MT) at pH 5.6 and 7.4, 25 °C ($n = 5$). Least square means within a pH with different letters (a−f) are significantly different ($P < 0.05$); LLN represents lactate-LDH-NAD. Standard error for treatment comparison at pH 5.6 = 3.2. Standard error for treatment comparison at pH 7.4 = 3.8.

was calculated according to Faustman et al. (*24*) and indicated by nanomol of metmyoglobin reduced per min per mg of mitochondria.

Estimating the Formation of NADH in Vitro. NADH formation resulting from the addition of lactate, LDH, and NAD, was determined as follows. Lactate (50 mM), LDH (95 Units), and NAD (0.2 mM) were reacted in incubation buffer at either pH 7.4 (120 mM KCl, 5 mM KH_2PO_4, and 30 mM K_2HPO_4) or 5.6 (120 mM KCl, 5 mM KH_2PO_4, and 30 mM maleic acid) using a quartz cuvette. The formation of NADH was measured as an increase in absorbance at 340 nm immediately after the addition of NAD to lactate, LDH, and incubation buffer (*25*), using a Shimadzu UV-2101PC spectrophotometer (Shimadzu Inc., Columbia, MD) combined with a temperature-regulated (25 °C) 6-cell position chamber (CPS 260, Shimadzu, Kyoto, Japan). Oxalate (50 mM) was used as an inhibitor for LDH. For these samples, LDH was preincubated for 3 min at either pH 7.4 or 5.6, before the addition of lactate and NAD to the reaction mixture.

Statistical Analysis. The overall experiment was replicated on five separate occasions. The experimental design was a randomized complete block design where hearts served as blocks. Treatments were assigned to isolated mitochondria within a heart (each treatment assigned once per heart per replicated experiment). Duplicate subsamples used for oxygen consumption analyses were averaged for statistical analysis.

The primary objective was to assess the effects of lactate, LDH, and NAD on metmyoglobin reduction. Because it is well established that pH influences mitochondrial activity, data within each of the 2 pH categories were analyzed separately. Fixed effects for oxygen consumption and enzymatic reductase measurements had a 1-way treatment structure. Fixed effects for electron transport mediated metmyoglobin reduction measurements had a 2-way treatment structure of substrate, time, and their interaction (measurements were taken repeatedly on samples at 0, 1, 2, and 3 h of incubation). Type-3 tests of fixed effects were performed using the MIXED procedure of SAS (version 9.1, SAS Institute Inc. Cary, NC). For all analyses, random terms included the heart (block) and unspecified residual error. Electron transport mediated metmyoglobin reduction measurements were analyzed with the addition of a Repeated Statement within Proc Mixed. Least square means for protected F-tests ($p < 0.05$) were separated using the diff option (least significant differences) and were considered significant at $p < 0.05$. The results were expressed as the least-squares mean values of five independent trials.

RESULTS AND DISCUSSION

Oxygen Consumption. *Effect of Lactate on Oxygen Consumption.* At both pH 5.6 and 7.4, the addition of lactate to isolated

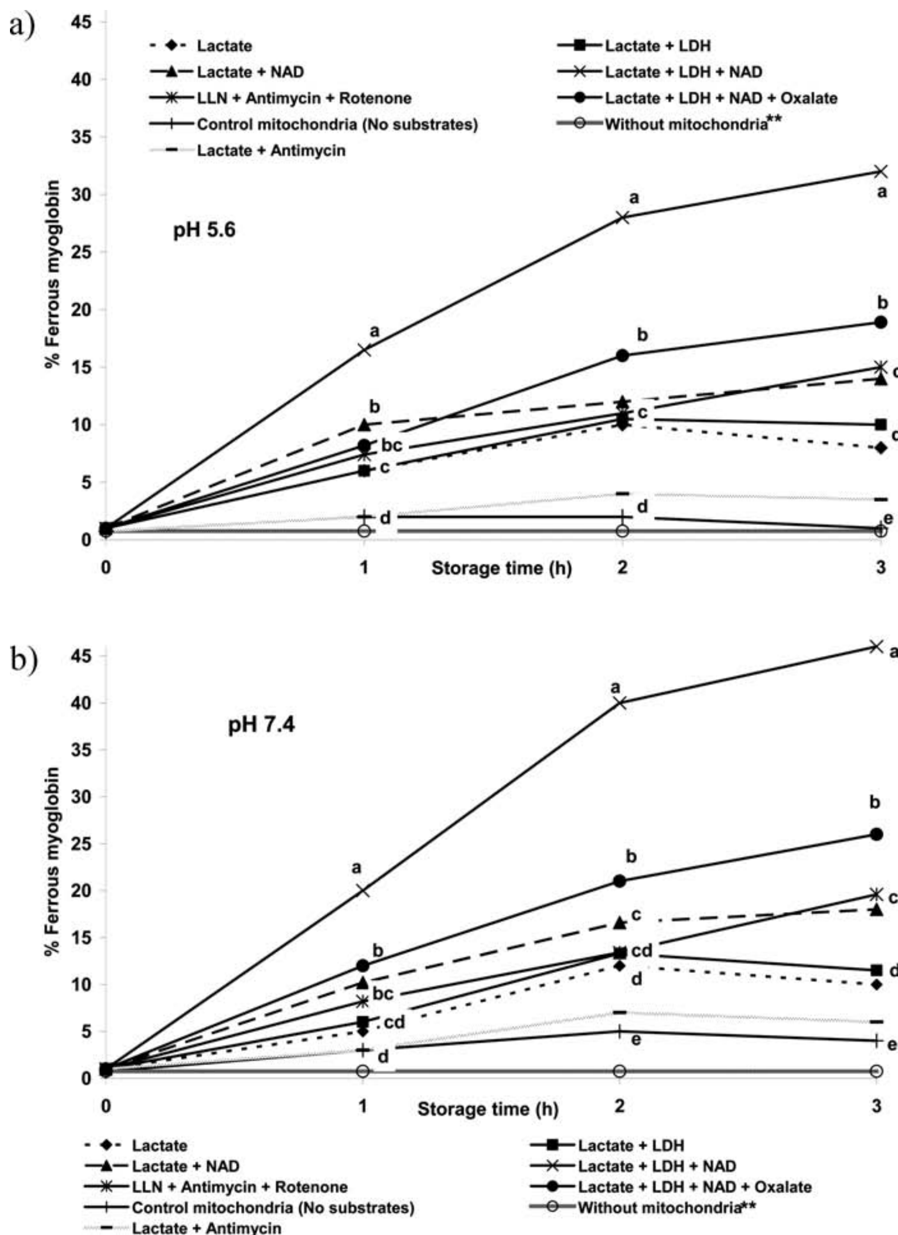

Figure 2. Effect of lactate, LDH, NAD, and NADH on bovine heart mitochondria-mediated metmyoglobin reduction at pH 5.6 and 7.4, incubated for 3 h at 25 °C (*n* = 5). Least square means within both a time point and a pH with different letters (a−e) are significantly different ($P < 0.05$). Mitochondria (MT) = 3 mg/mL; myoglobin (Mb) = 2.5 mg/mL; LLN represents lactate-LDH-NAD. ** represents the common line for treatments without mitochondria, including myoglobin alone, myoglobin with lactate, myoglobin with lactate-LDH-NAD, or myoglobin with mitochondrial inhibitors. There was no significant difference among these treatments. Hence, the common line was represented. All other treatments in the figure legend contain mitochondria. Standard error for treatment × time comparison at pH 5.6 = 1.4. Standard error for treatment × time comparison at pH 7.4 = 2.1.

mitochondria resulted in oxygen consumption when compared with ($p < 0.05$) that of control mitochondria without added lactate (**Figure 1**). This is supported by previous research at pH 7.4 using mitochondria isolated from rat skeletal muscle and beef cardiac muscle, which have the ability to use lactate as a substrate for oxygen consumption (*14*, *26*, *27*). Baldwin et al. (*28*) also reported increased oxygen consumption in rat skeletal and cardiac muscle homogenates following the addition of lactate. In the current study, the addition of antimycin (mitochondrial inhibitor that blocks complex III) reduced ($p < 0.05$) oxygen consumption resulting from lactate.

Formation of NADH in Vitro. Combining lactate, LDH, and NAD in a separate experiment without mitochondria increased absorbance at 340 nm, indicating the formation of NADH. At pH 7.4 and 5.6, change in absorbance at 340 nm per min was 0.026 ± 0.003 and 0.008 ± 0.002, respectively. Addition of oxalate to inhibit LDH reduced the absorbance to 0.005 ± 0.001 and 0.002 ± 0.001 at pH 7.4 and 5.6, respectively.

Effect of Lactate-LDH-NAD on Oxygen Consumption. The addition of lactate-LDH-NAD to isolated mitochondria resulted in significant oxygen consumption (**Figure 1**). However, addition of an LDH inhibitor to the lactate-LDH-NAD treatment decreased ($p < 0.05$) oxygen consumption compared with that of lactate-LDH-NAD. Results of the current study at pH 5.6 are supported by previous research at pH 7.4, which reported that lactate-LDH-NAD resulted in beef mitochondrial oxygen consumption (no published research at pH 5.6). Previous research also reported that the addition of either NADH or lactate to muscle homogenates resulted in increased oxygen consumption (*28*, *29*).

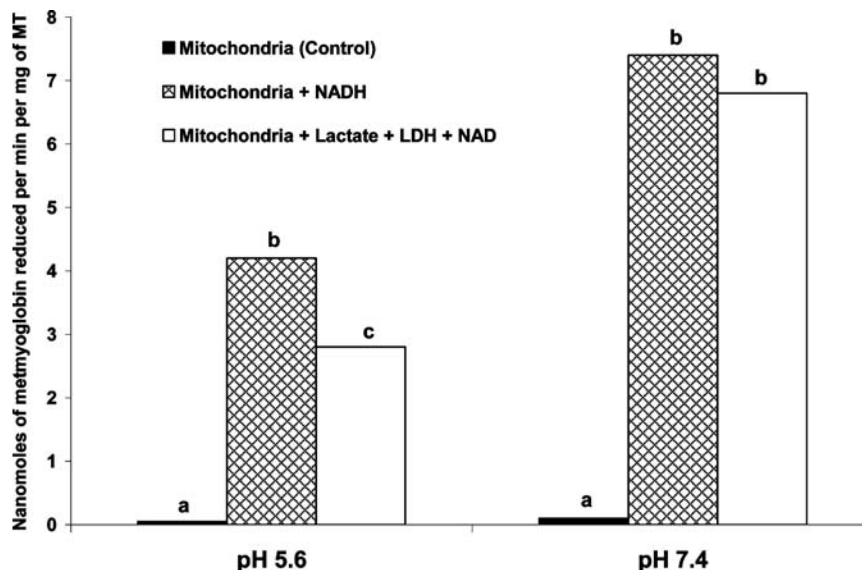

Figure 3. Comparison of metmyoglobin reductase activity of bovine cardiac mitochondria (MT) in the presence of either NADH or lactate-LDH-NAD. Least square means within a pH with different letters (a−c) are significantly different ($P < 0.05$). Standard error for treatment comparison at pH 5.6 = 0.6. Standard error for treatment comparison at pH 7.4 = 0.9.

NADH can initiate oxygen consumption by serving as a substrate for complex I of the electron transport chain. In the current study, incubation of mitochondria with antimycin and rotenone also decreased oxygen consumption resulting from the lactate-LDH-NAD combination. Both antimycin and rotenone inhibit electron transport, causing mitochondria to lose their ability to consume oxygen at the terminal step (*30*). In support, Mole et al. (*31*) reported decreased oxygen consumption in rat muscle homogenates at pH 7.4 following the addition of rotenone.

Role of Mitochondria in Metmyoglobin Reduction. There was a significant treatment × time interaction for mitochondria-mediated metmyoglobin reduction at pH 5.6 and 7.4 (**Figure 2a** and **b**). Without mitochondria, the incubation of metmyoglobin with either lactate alone or with lactate-LDH-NAD had no effect on reduction ($p > 0.05$). This suggests that the formation of NADH formed from LDH cannot reduce metmyoglobin without a reductase enzyme or an electron carrier. In support of this, Faustman et al. (*24*) reported that the addition of NADH to metmyoglobin had no effect on reduction when cytochrome *b5* and metmyoglobin reductase were not present.

Effect of Lactate on Electron Transport Linked Metmyoglobin Reduction. Addition of lactate to mitochondria resulted in metmyoglobin reduction when compared with ($p < 0.05$) that of mitochondrial controls without lactate at both pH 5.6 and 7.4 (**Figure 2a** and **b**). Antimycin decreased the metmyoglobin reduction associated with lactate (antimycin specifically blocks electron availability after complex III). Zhu et al. (*32*) reported mitochondria-mediated equine metmyoglobin reduction in the presence of lactate at pH 7.4.

Electron-transport chain mediated equine metmyoglobin reduction was first reported by Tang et al. (*7*) using succinate as a substrate. Metmyoglobin reduction following the addition of succinate to isolated mitochondria resulted from the entry of succinate into the ETC via complex II. This results in the movement of electrons, which become available to myoglobin between complex III and IV via cytochrome *c* and outer membrane cytochrome *b5*. Moreover, Tang et al. (*7*) also reported that mitochondria-mediated metmyoglobin reduction can remain active up to 45 days post-mortem after the addition of succinate. This supports the role of substrates such as lactate and succinate in ETC-mediated metmyoglobin reduction.

Effect of Lactate-LDH-NAD on Electron Transport Linked Metmyoglobin Reduction. Addition of lactate-LDH-NAD to isolated mitochondria resulted in the greatest ($p < 0.05$) metmyoglobin reduction compared with that of all other treatments (**Figure 2a** and **b**). Tang et al. (*7*) reported that electron transport chain-mediated metmyoglobin reduction occurs at a lower oxygen partial pressure, resulting from increased oxygen consumption. In the current study, combining lactate-LDH-NAD with mitochondria increased oxygen consumption, creating an oxygen partial pressure that supports the availability of electrons within the electron transport chain. These electrons are able to reduce oxidized myoglobin via an electron carrier present in mitochondria. Various researchers reported that lowered partial pressure facilitates metmyoglobin reduction (*7*, *11*).

Metmyoglobin reduction can be attributed to lactate's role in the production of NADH via LDH. This NADH can enter complex I, which produces electrons that become available to metmyoglobin between complexes III and IV. In the present study, the addition of mitochondrial and LDH inhibitors to the lactate-LDH-NAD combination decreased ($p < 0.05$) metmyoglobin reduction. However, mitochondrial inhibitors did not completely inhibit metmyoglobin reduction. This suggests that in addition to ETC-mediated nonenzymatic reduction (electrons available between complexes III and IV), NADH produced by lactate-LDH-NAD also can be used for enzymatic metmyoglobin reduction within mitochondria (via NADH-metmyoglobin reductase-cytochrome *b5*).

Effect of Lactate-LDH-NAD on Enzymatic Metmyoglobin Reduction. The role of NADH in enzymatic metmyoglobin reduction was assessed using metmyoglobin reductase activity in mitochondria following the addition of lactate, LDH, and NAD (**Figure 3**). Regardless of pH, lactate-LDH-NAD resulted in significant mitochondrial metmyoglobin reductase activity. At pH 7.4, lactate-LDH-NAD produced metmyoglobin reductase activity similar ($p > 0.05$) to the direct addition of NADH to mitochondria. Although lactate-LDH-NAD resulted in significant metmyoglobin reductase activity compared with that of mitochondrial controls (without any substrates), the direct addition of NADH had a greater activity at pH 5.6.

Metmyoglobin reductase requires NADH and an electron carrier (*24*, *33*). Moreover, Arihara et al. (*15*) reported the presence of NADH-cytochrome *b5* reductase and an electron carrier (OM cytochrome *b5*) in the mitochondrial fraction. Thus, our results suggest that mitochondria possess the components necessary for metmyoglobin reduction when NADH is regenerated post-mortem. The current study provides another mechanism by which lactate can improve color stability. More specifically, NADH regenerated by lactate-LDH interaction can be used for either ETC-mediated or enzymatic metmyoglobin reduction.

In conclusion, NADH produced via lactate-LDH-NAD will result in mitochondrial oxygen consumption and metmyoglobin reduction. More specifically, NADH formed from this process can be used in either enzymatic (NADH- dependent metmyoglobin reductase) or electron transport mediated metmyoglobin reduction. Hence, mitochondria have the ability to regenerate reducing equivalents when substrates such as succinate and lactate are available. These reducing equivalents can influence oxygen consumption, metmyoglobin reduction, and beef color stability.

ABBREVIATIONS USED

OCR, oxygen consumption rate; ETC, electron transport chain; Tris-HCl, Tris [hydroxymethyl] aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; BSA, bovine albumin; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]; ADP, adenosine 5′-diphosphate; LDH, lactic acid dehydrogenase, NAD, β-nicotinamide adenine dinucleotide, NADH, nicotinamide adenine dinucleotide-reduced; OM, outer membrane; MES, 2-(*N*-morpholino) ethanesulfonic acid.

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Received for review January 22, 2010. Revised manuscript received March 25, 2010. Accepted April 7, 2010.