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Effects of Lactate on Beef Heart Mitochondrial Oxygen Consumption and Muscle Darkening

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The mechanism of lactate-induced beef color darkening is unclear. Our objective was to evaluate the ability of mitochondria isolated from bovine cardiac muscle to utilize lactate as a fuel for respiration. Addition of lactate (4, 8, and 16 mM) to isolated bovine cardiac mitochondria resulted in state IV oxygen consumption at pH 7.2 and 25 °C measured using a Clark oxygen electrode. Combining mitochondria with lactate, LDH, and NAD increased state IV oxygen consumption compared with that of lactate alone ($p < 0.05$). Moreover, oxygen consumption resulting from the addition of lactate−LDH−NAD (0.2 mM each) was comparable to oxygen consumption resulting from the direct addition of NADH (0.2 mM) to mitochondria at pH 7.2. Rotenone reduced ($p < 0.05$) lactate-mediated darkening in bovine cardiac muscle homogenates. Lactate-induced beef color darkening may be due to increased oxygen consumption by mitochondria, which out-competes myoglobin for oxygen and results in dark colored muscle.

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

INTRODUCTION

Centralized case-ready packaging has allowed beef purveyors to enhance beef with various ingredients that increase shelf-life and color-life. Of the various ingredients used, lactate is often added to beef products as an antimicrobial (*1, 2*). However, lactate also exhibits a muscle darkening effect when used in beef injection enhancement (*3*−*6*). No research has assessed the potentional biochemical mechanism by which lactate enhancement darkens beef color.

Numerous processes compete for oxygen in postmortem muscle. In general, competition for oxygen between mitochondria and myoglobin is the primary determinant of bright cherry-red surface color development. Mitochondrial oxygen consumption can out-compete myoglobin for oxygen and produce dark colored muscle (*7*−*9*). More specifically, mitochondria can affect myoglobin redox stability in meat by either (1) decreasing pO2 via respiration or (2) electron-transport-chain-mediated metmyoglobin reduction postmortem (*10, 11*).

Mitochondria continue to metabolize oxygen in postmortem muscle (*12*). More specifically, Cheah and Cheah (*13*) were able to isolate intact mitochondria at 10 days postmortem (storage at 2 °C), and Tang et al. (*10*) reported mitochondrial oxygen consumption at 60 days postmortem (storage at 4 °C).

Mitochondria isolated from various model systems, such as potato, rat liver, sperm, and yeast, are capable of using exogenous lactate as an energy substrate, resulting in cellular respiration and increased oxygen consumption (*14*−*16*). Monocarboxylic transporters move lactate into the mitochondria, where lactate dehydrogenase (LDH) oxidizes lactate to pyruvate, producing NADH that can (1) enter the electron transport chain and (2) result in cellular respiration (*17*−*19*). If these findings also occur in postmortem bovine muscle, a lactate-mediated increase in mitochondrial respiration could subsequently lead to decreased myoglobin oxygenation and the dark color associated with lactate-enhanced beef. However, no published meat science research has assessed the effects of lactate on postmortem beef muscle oxygen consumption. Thus, our objectives were to (1) assess the ability of mitochondria isolated from bovine cardiac muscle to utilize lactate as a fuel for respiration, (2) determine if NADH produced by lactate−LDH can result in beef mitochondrial oxygen consumption, and (3) evaluate the effects of lactate and rotenone on darkening in bovine cardiac muscle homogenates.

MATERIALS AND METHODS

Materials and Chemicals. Bovine hearts from market age cattle were obtained locally from an abattoir within 0.5 h of exsanguination, placed on ice, and transported to the laboratory. Experiments were repeated on three separate occasions with each replicated trial using duplicate subsamples from $n = 5$ hearts. Magnesium chloride ($MgCl_2$), bovine serum albumin (BSA), sucrose, tris-[hydroxymethyl] aminomethane hydrochloride (Tris-HCl), potassium phosphate monobasic (KH_2PO_4), 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 U/mg), sodium lactate, sodium pyruvate, antimycin A, lactic acid dehydrogenase (LDH), β-nicotinamide adenine dinucleotide (NAD), β-nicotinamide adenine dinucleotide-reduced dipotassium salt (NADH),

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and polystyrene cuvettes were purchased from Sigma Chemical Co. (St. Louis, MO); sodium succinate was purchased from Fisher Scientific (Fair Lawn, New Jersey). All chemicals were reagent grade.

Bovine Mitochondria Isolation. Mitochondria were isolated from bovine cardiac muscle according to Smith (*20*) 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 RCB 5 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 Kit from Sigma Chemical Co. (St. Louis, MO).

Oxygen Consumption Measurement. Mitochondrial oxygen uptake was measured using a Clark oxygen electrode (polarizing voltage of 0.6 V and an 8 mL incubation chamber). Reaction components were added to the incubation chamber, which was maintained at 25 °C by a water jacket and Lauda RE120 circulating water bath (Westbury, NY). Oxygen was not directly added to the incubation chamber but was made available to mitochondria via the pre-equilibrated incubation buffer. 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 at pH 7.2 by suspending mitochondria in incubation buffer (250 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1% BSA, and 20 mM HEPES). The time period for OCR measurement varied on the basis of the amount of time required to stabilize the respiratory rate.

Calculations for states III and IV oxygen consumption rates (OCRs), and respiratory control ratio (RCR) were based on the method of Estabrook (*21*). State IV data was used in experiments 1 and 2 to measure the OCR of isolated mitochondria in the presence of added substrate (succinate, pyruvate, and lactate). State III data indicates the OCR of isolated mitochondria in the presence of both substrate and ADP, and was used to calculate the RCR for experiment 1. The respiratory control ratio determines the functional integrity of isolated mitochondria and is calculated as state III ÷ state IV. Tang et al. (*10*) reported a RCR value of 2.2−3.6 for beef heart mitochondria with functional integrity.

Experiment 1: Reaction with Substrates and Inhibitor. Methodology similar to that of Tang et al. (*10, 11*) was used for reacting isolated mitochondria with either succinate (positive control), pyruvate, or lactate at 4, 8, and 16 mM. All substrate additions were made with Hamilton syringes through a 1 mm port in the incubation chamber of the Clark oxygen electrode. In nonenhanced bovine *longissimus* muscle, Immonen et al. (*22*) reported lactate concentrations between 13 and 18 mM. Lactate concentrations in injection-enhanced beef have not been reported. In order to assess the effect of inhibitor on mitochondrial respiration, mitochondria were incubated with antimycin A (0.01 mM; ETC inhibitor). Oxygen consumption was measured as described in the previous section.

Experiment 2: Reaction with Lactate, LDH, NAD, NADH, and Inhibitor. To determine if NADH produced by lactate−LDH can result in beef mitochondrial oxygen consumption, mitochondria were reacted with lactate, LDH, NAD, NADH, and inhibitor using 8 combinations of substrates and inhibitors (summarized in **Table 1**). Substrate addition and oxygen consumption measurements were performed as previously described.

Table 1. Experiment 2: The Effect of Lactate, LDH, NAD, and NADH on the State IV Oxygen Consumption Rate of Beef Cardiac Mitochondria at pH 7.2 and 25 °C ($n = 5$)[a]

	substrate[b]					
treatments	lactate (8 mM)	LDH (20 μL)	NAD (0.2 mM)	NADH (0.2 mM)	antimycin A (0.01 mM)	state IV OCR[c]
1	+	−	−	−	−	33.4 bc[d]
2	−	+	−	−	−	5.1 a
3	−	−	+	−	−	7.4 a
4	+	+	−	−	−	36.9 c
5	+	−	+	−	−	49.5 d
6	+	+	+	−	−	240.9 e
7	−	−	−	+	−	254.5 f
8	+	+	+	−	+	27.2 b

[a] LSD for treatment comparisons = 6.4. [b] Substrate present (+) or absent (−) in the incubation chamber of the Clark electrode. LDH, lactic acid dehydrogenase; NAD, β-nicotinamide adenine dinucleotide; NADH, β-nicotinamide adenine dinucleotide-reduced dipotassium salt. [c] State IV oxygen consumption rate = oxygen consumed by isolated mitochondria in the presence of added substrate [lactate (8 mM), LDH (20 μL), NAD (0.2 mM), NADH (0.2 mM)] or inhibitor, [Antimycin A (0.01 mM)] and expressed as nmol of oxygen consumed/min per mg mitochondrial protein. [d] Least square means within a column with a different letter (a−f) are significantly different ($P < 0.05$).

NADH production resulting from the addition of lactate, LDH, and NAD was determined separately as follows. Lactate (4, 8, and 16 mM), LDH (20 μL), and NAD (0.2 mM) were reacted in incubation buffer at pH 7.2 using polystyrene disposable cuvettes. The formation of NADH was measured as an increase in absorbance at 340 nm immediately after the addition of LDH to lactate, NAD, and incubation buffer (*23*), 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).

Statistical Analysis. Each experiment was replicated three times (on three separate occasions, $n = 5$ hearts per occasion). The experimental design for experiments 1 and 2 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). Duplicate subsamples used for Clark oxygen electrode analyses were averaged for statistical analysis.

Fixed effects for experiment 1 included a two-way treatment structure of substrate, concentration, and their interaction. Fixed effects for experiment 2 included a 1-way treatment structure consisting of 8 treatments created from combinations of lactate, LDH, NADH, and inhibitor. Type-3 tests of fixed effects were performed using the MIXED procedure of SAS (Version 9.1, SAS Institute Inc. Cary, NC). For both experiments, random terms included heart (block) and unspecified residual error. Least square means for protected *F*-tests ($p < 0.05$) were separated by 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 three independent trials.

Experiment 3: Effects of Lactate and Rotenone on Cardiac Muscle Homogenates Darkening. Chemicals used in the experiment were obtained as follows: potassium lactate (PURASAL HiPure P, 60% potassium lactate/40% water; PURAC America, Inc., Lincolnshire, IL) and rotenone (Sigma Chemical Co., St. Louis, MO).

Preparation of Cardiac Muscle Homogenates. A procedure similar to that described by Cornforth and Egbert (*24*) was used to assess the inhibitory effect of rotenone on lactate-induced meat color darkening. In the present study, bovine cardiac muscle was used because its mitochondrial content is significantly greater than that of most skeletal muscles commonly used in retail beef (*25*).

Twenty-five grams of minced bovine heart ($n = 5$) visually devoid of fat and connective tissue were homogenized with one of the following treatments, each at 75 mL: (1) distilled water, (2) distilled water plus 1.5% lactate, (3) distilled water plus 3% lactate, (4) distilled water plus 0.2 g of rotenone, (5) distilled water, 1.5% lactate, and 0.2 g of rotenone, and (6) distilled water, 3% lactate, and 0.2 g of rotenone. Samples were homogenized in a Waring table-top blender (Dynamics Corp. of America, New Hartford, CT) for 30 s.

Color and pH Measurement. Homogenates were poured into glass sample cups to a level of 1.5 cm thick and covered with a black tile (Hunter Associates Laboratory, Inc., Reston, VA). CIE L* values were measured immediately after samples were poured into the sample cup and then at 10 min intervals during a 60 min incubation at room temperature (25 °C). Reflectance color measurements were performed using an inverted HunterLab MiniScan XE Plus Spectrophotometer stabilized by a stand (45/0 LAV, 2.54-cm-diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). CIE L* values measure lightness on a scale of 0 (black) to 100 (white). A lower value indicates darker color, and an increased value indicates lighter color.

Sample pH was recorded after color measurement using an Accumet combination glass electrode connected to an Accumet 50 pH meter (Fisher Scientific, Fairlawn, NJ). The pH of each treatment solution added to the minced heart also was measured.

Statistical Analysis. The experimental design was a randomized complete block with repeated measurements ($n = 5$ hearts, which served as blocks). The six treatments within the randomized complete block included a control, 1.5% lactate, 3.0% lactate, rotenone, 1.5% lactate plus rotenone, and 3.0% lactate plus rotenone. Each of the six treatments was assigned once within a heart. Seven color measurements were taken repeatedly on the same experimental unit during the incubation period. Duplicate color measurements taken on the same experimental unit were averaged for statistical analysis.

Fixed effects included treatment, incubation time, and their interaction. Type-3 tests of fixed effects for changes in L* during incubation were evaluated using the Mixed procedure of SAS. The repeated option in Proc Mixed was used for darkening analyses in order to model covariance/variance structure resulting from repeated measurements taken on the same sample during incubation. The most appropriate model was determined using Akaike's Information Criterion (AIC). Least square means were generated for significant *F*-tests ($p < 0.05$) and separated using least significant differences (diff option).

RESULTS

Experiment 1: Mitochondrial Oxygen Consumption. The addition of lactate to isolated mitochondria from bovine cardiac muscle resulted in measurable oxygen consumption (**Table 2**). Succinate (positive control) resulted in greater states III (oxygen consumption in the presence of substrate and ADP) and IV (oxygen consumption in the presence of substrate alone) oxygen consumption compared with that of pyruvate and lactate ($p < 0.05$). Increasing succinate and pyruvate concentration increased state IV oxygen consumption, whereas increasing lactate concentration tended to decrease state IV oxygen consumption. The addition of inhibitor decreased oxygen consumption for all three substrates ($p < 0.05$). The average RCR for mitochondria used in this experiment was 3.0. After the addition of inhibitor, RCR decreased to 0.6, indicating that functional integrity was not retained after the addition of antimycin A.

Experiment 2: Reaction with Lactate, LDH, NAD, NADH and Inhibitor. Oxygen consumption by isolated mitochondria in the presence of lactate, LDH, NAD, and NADH is shown in **Table 1**. Compared with lactate alone as a substrate, the addition of either LDH or NAD alone to isolated mitochondria resulted in negligible oxygen consumption. Addition of lactate in combination with LDH resulted in oxygen consumption comparable to that of lactate alone. However, combining lactate, LDH, and NAD resulted in a significant increase in oxygen consumption ($p < 0.05$). The increase in oxygen consumption resulting from a combination of lactate, LDH, and NAD was similar to that experienced when adding NADH to isolated mitochondria. Addition of antimycin A to the lactate−LDH−NAD combination significantly decreased oxygen consumption relative to that of lactate−LDH−NAD ($p < 0.05$).

Table 2. Experiment 1: States III and IV Oxygen Consumption Rates (nmol O/min mg/Mitochondrial Protein) and Respiratory Control Ratio (RCR) of Bovine Cardiac Muscle Mitochondria with Either Added Succinate, Pyruvate, or Lactate as Substrate at pH 7.2 and 25 °C[a]

substrate/ treatments	concentration of substrate (mM)	state III[b]	state IV[c]	RCR[d]
succinate	4	142.25 a,z[e]	47.40 a,x	3.00 a,x
succinate	8	181.71 b,z	56.94 b,x	3.19 a,x
succinate	16	200.42 c,z	65.10 c,x	3.07 a,x
succinate + antimycin (0.01 mM)	8	18.54 d,x	34.46 d,x	0.53 b,x
pyruvate	4	97.29 a,x	33.98 a,y	2.86 a,x
pyruvate	8	109.90 b,y	37.92 b,y	2.90 a,x
pyruvate	16	128.75 c,y	42.96 c,y	3.00 a,x
pyruvate + antimycin (0.01 mM)	8	14.04 d,x	19.56 d,y	0.72 b,y
lactate	4	114.35 a,y	38.96 a,y	2.98 a,x
lactate	8	90.47 b,x	30.60 b,z	2.96 a,x
lactate	16	75.92 c,x	24.98 c,z	3.04 a,x
lactate + antimycin (0.01 mM)	8	8.15 d,x	12.43 d,z	0.66 b,y

[a] LSD for state III substrate comparisons = 14.0, and concentration comparisons = 7.2. LSD for state IV substrate comparisons = 6.0, and concentration comparisons = 2.2. LSD for RCR substrate comparisons = 0.3, and concentration comparisons = 0.2. [b] State III: oxygen consumption rate of isolated mitochondria in the presence of substrate and ADP. [c] State IV: oxygen consumption rate of isolated mitochondria in the presence of only added substrate. [d] RCR: ratio between state III and state IV. [e] Within a substrate, values in each column with different letters (a−d) are significantly different ($P < 0.05$). Within a concentration, values in each column with different letters (x−z) are different ($P < 0.05$).

Table 3. Experiment 3: Effect of Treatment Solutions and Rotenone[a] on the pH of Bovine Cardiac Muscle Homogenates Used to Assess Lactate-Mediated Darkening Effects

treatments	pH
control[b]	6.4[c]
potassium lactate (1.5%)	6.4
potassium lactate (3%)	6.5
rotenone	6.4
potassium lactate 1.5% + rotenone	6.5
potassium lactate 3% + rotenone	6.5

[a] Treatment solutions contain potassium lactate or rotenone, added to bovine cardiac muscle during homogenization, to get final concentrations of 1.5% or 3% (wt/wt) and (0.2 wt %/wt) lactate and rotenone, respectively. LSD for treatment comparisons = 0.4. [b] Control = bovine cardiac muscle homogenate and distilled water. [c] No significant effect of treatment on homogenate pH (*P* value for *F*-test = 0.88).

Experiment 3: Effects of Lactate and Rotenone on Cardiac Muscle Homogenate Darkening. The addition of treatment solutions to the cardiac homogenates had no effect on pH ($p > 0.05$; **Table 3**). There was a significant treatment × incubation time interaction for L* values ($p = 0.003$). Lactate addition to bovine cardiac muscle darkened (decreased L* values; $p < 0.05$) homogenates within 10 min of incubation compared with control samples (**Figure 1**). There was no difference ($p > 0.05$) in L* values between 1.5% and 3% lactate. Rotenone addition resulted in lighter color (greater L* values; $p < 0.05$) compared with that of other treatments. More specifically, addition of rotenone to lactate treatments reversed the darkening effect.

DISCUSSION

Experiment 1: Mitochondrial Oxygen Consumption. Oxygen consumption associated with succinate is attributed to the substrate's ability to enter directly into complex II of the electron transport chain, by donating two electrons to coenzyme Q via

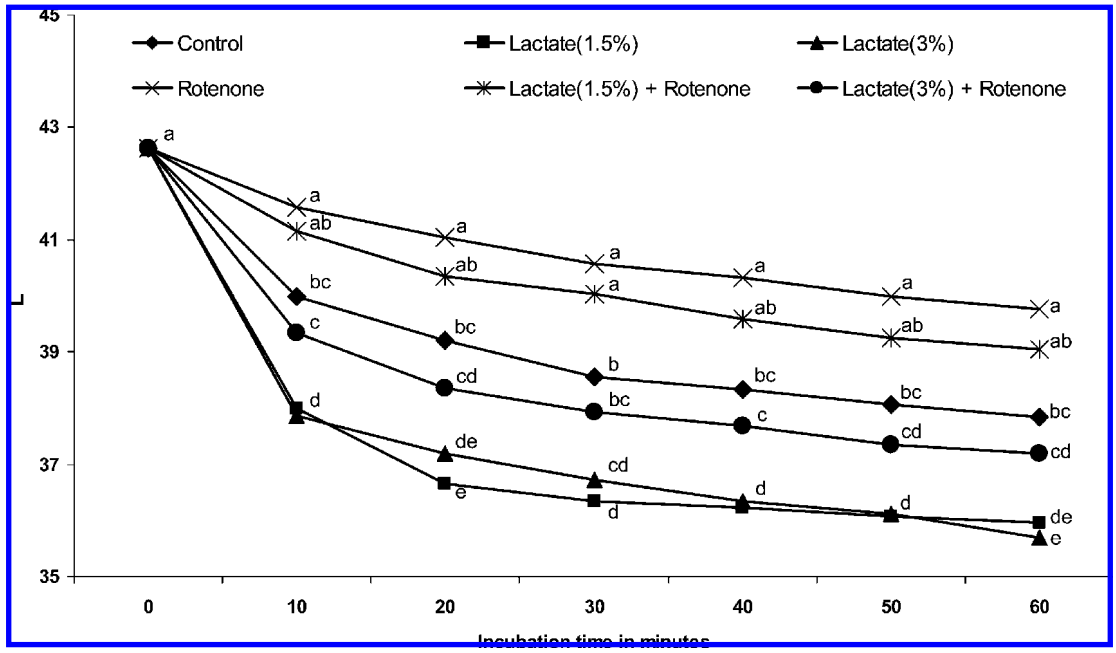

Figure 1. Experiment 3: Effects of a lactate−rotenone interaction on bovine cardiac homogenate darkening. Darkening was measured after the addition of treatment solutions containing either 33% potassium lactate, rotenone, or a combination of lactate and rotenone. Final concentrations were 1.5% or 3% (wt/wt) and 0.2 (wt %/wt), potassium lactate and rotenone, respectively. Control = bovine cardiac muscle homogenate and added distilled water. L* represents darkening. A higher value indicates a lighter color, whereas a lower value indicates a darker color. LSD for treatment comparisons = 1.8. *P* value for treatment × time interaction = 0.003.

FAD (*26*). This could be responsible for the greatest OCR associated with succinate addition compared with the other two substrates (**Table 2**).

Oxygen consumption associated with the addition of lactate and pyruvate is consistent with previous research that reports that mammalian liver and striated muscle mitochondria can oxidize exogenous lactate because of an internal LDH pool that facilitates lactate oxidation (*27*−*29*). Previous research suggests that when mitochondrial redox status decreases, lactate enters the mitochondria, where it is oxidized to pyruvate via mitochondrial LDH (*17*, *18*). Pyruvate can then be oxidized via the tricarboxylic acid cycle and electron transport chain (ETC). Monocarboxylic transporters (MCT) are involved in the intracellular lactate shuttle, which moves substrate from cellular pools into the mitochondrial matrix (*17*). In addition, a L-lactate/H^+ symporter also helps to transport lactate across the inner mitochondrial membrane (*14*). Gladden (*30*) reported that the transfer of lactate into the mitochondria also results in shuttling of NADH across the mitochondrial membrane, providing substrate for electron transport.

Gallina et al. (*31*) also reported that addition of lactate to rat spermatozoa mitochondria resulted in a lower state IV OCR compared with that of pyruvate. These authors attributed this to a difference in substrate uptake, with pyruvate uptake being 22 times greater than that of lactate. This could also be partially responsible for the observed difference in OCR between lactate and pyruvate in the current study. In addition, compared with pyruvate as a substrate, limited lactate transport within the mitochondria could be responsible for the decrease in OCR observed as lactate concentration increased.

Nevertheless, results from our present study implicate lactate in beef cardiac mitochondrial oxygen consumption and are in agreement with previous research using mitochondria isolated from (1) liver, skeletal, and cardiac muscles of rats, (2) spermatozoa of bulls, mice, rats, rabbits, and boars, (3) potato, and (4) yeast (*15*, *17*, *31*−*35*).

Experiment 2: Reaction with Lactate, LDH, NAD, NADH, and Inhibitor. The lactate−LDH system influenced beef mitochondrial oxygen consumption (**Table 1**). Lactate resulted in a significantly greater OCR than that of either LDH or NAD alone ($p < 0.05$). This likely occurred because neither LDH nor NAD can be used as a substrate for oxygen consumption. Adding lactate and LDH did not change OCR compared with that of lactate alone, whereas adding lactate and NAD increased OCR compared with that of lactate alone. This could be due to residual LDH (*14*, *17*, *36*) present in mitochondria, which can form NADH by transferring an electron to NAD.

Adding lactate, LDH, and NAD significantly increased ($p < 0.05$) OCR compared with that of combinations of either (1) lactate and NAD or (2) lactate and LDH. This increase in OCR resulting from lactate−LDH−NAD addition was similar to that observed when NADH was added directly to mitochondria, which suggests that NADH produced via lactate−LDH−NAD is involved in beef mitochondrial oxygen consumption. The formation of NADH via the lactate−LDH−NAD treatment combination at pH 7.2 was monitored in a separate experiment and measured spectrophotometrically using the change in absorbance at 340 nm. Our results indicated that the formation of NADH was concentration dependent. More specifically, absorbance (Δabs at 340 nm per min) increased with increasing lactate concentration: 0.013 ± 0.002 at 4 mM, 0.028 ± 0.004 at 8 mM, and 0.064 ± 0.004 at 16 mM.

Atkinson et al. (*37*) reported a significant increase in oxygen uptake after the addition of NADH to lamb *semimembranosus* muscle. Larsson et al. (*19*) reported greater respiratory activity in yeast after the addition of NADH and attributed this to an external NADH dehydrogenase that faces the outer side of the inner mitochondrial membrane; thus, enabling direct oxidation of cytoplasmic NADH (*38*, *39*).

Antimycin A is a mitochondrial inhibitor that blocks the flow of electrons from semiquinone to ubiquinone in the Q cycle of complex III in oxidative phosphorylation, thus preventing the

consumption of oxygen at complex IV. In the current study, addition of antimycin A resulted in decreased OCR by all three substrates.

Experiment 3: Effects of Lactate and Rotenone on Cardiac Muscle Homogenate Darkening. Addition of lactate to cardiac muscle homogenates darkened samples (**Figure 1**) without influencing pH (**Table 3**). In support of this, Papadopoulos et al. (*6*) concluded that sodium lactate darkened color in cooked, vacuum-packaged top rounds through a mechanism other than increased pH. Similar results were reported in fresh beef loins, where lactate influenced color but did not alter muscle pH (*3*−*5*). These results suggest that a mechanism other than altered pH might have resulted in the observed lactate-mediated darkening (lower L*). This could be due to the use of lactate as a fuel for beef mitochondrial respiration in muscle homogenates. Increased oxygen consumption by beef mitochondria can out-compete myoglobin for oxygen and result in dark-colored meat (*7*–*9*). Although meat color was not the focus of previous research, similar results were reported in studies by Mole et al. (*40*) and Baldwin et al. (*41*), both of which concluded that the addition of lactate to rat heart and skeletal muscle homogenates resulted in oxygen consumption via lactate metabolism.

Rotenone is an electron transport chain complex I inhibitor that blocks mitochondrial respiration by inhibiting the transfer of electrons to the flavin mononucleotides prosthetic group of NADH-Q reductase, an enzyme involved in oxidative phosphorylation (*42*). In the current study, the addition of rotenone to cardiac homogenates reversed the darkening effect of lactate ($p < 0.05$). Egbert and Cornforth (*9*) also reported greater L* values (less dark) after the addition of rotenone to prerigor *longissimus* muscle homogenates. Rotenone's ability to prevent lactate-induced darkening is likely due to the inhibition of mitochondrial oxygen consumption, which promotes myoglobin oxygenation.

The purpose of this study was to assess the ability of mitochondria isolated from bovine cardiac muscle to utilize lactate as fuel for respiration, either directly or via the lactate−LDH−NAD system. Lactate addition to isolated beef mitochondria at pH 7.2 will result in measurable oxygen consumption. In addition, oxygen consumption resulting from the addition of lactate−LDH−NAD to beef mitochondria was equivalent to oxygen consumption resulting from the direct addition of NADH at pH 7.2. This suggests that the lactate−LDH−NAD system can produce NADH, which can be used by beef mitochondria for oxygen consumption. The addition of inhibitors such as antimycin A and rotenone will minimize lactate-induced oxygen consumption and darkening, respectively. The inter-relationship between lactate and mitochondrial oxygen consumption may be associated with the darkening of lactate-enhanced beef products.

ABBREVIATIONS USED

pO2, partial oxygen pressure; OCR, oxygen consumption rate; RCR, respiratory control ratio; ETC, electron transport chain; Tris-HCl, Tris [hydroxymethyl] aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; BSA, bovine albumin; EGTA, ethylene glycol-bis(*ss*-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, β-nicotinamide adenine dinucleotide-reduced dipotassium salt.

LITERATURE CITED

(1) Mbandi, E.; Shelef, L. A. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. *Int. J. Food Microbiol.* **2002**, *76*, 191–198.

(2) Miller, R. K.; Acuff, G. R. Sodium lactate affects pathogens in cooked beef. *J. Food Sci.* **1994**, *59*, 15–19.

(3) Kim, Y. H.; Hunt, M. C.; Mancini, R. A.; Seyfert, M.; Loughin, T. M.; Kropf, D. H.; Smith, J. S. Mechanism for lactate-color stabilization in injection-enhanced beef. *J. Agric. Food Chem.* **2006**, *54*, 7856–7862.

(4) Lawrence, T. E.; Dikeman, M. E.; Hunt, M. C.; Kastner, C. L.; Johnson, D. E. Effects of calcium salts on beef *longissimus* quality. *Meat Sci.* **2003**, *64*, 299–308.

(5) Mancini, R. A.; Hunt, M. C.; Hachmeister, K. A.; Seyfert, M.; Kropf, D. H.; Johnson, D. E.; Cusick, S.; Morrow, C. The utility of lactate and rosemary in beef enhancement solutions: effects on *longissimus* color changes during display. *J. Muscle. Foods* **2005**, *16*, 27–36.

(6) Papadopoulos, L. S.; Miller, R. K.; Acuff, G. R.; Vanderzant, C.; Cross, H. R. Effect of sodium lactate on microbial and chemical composition of cooked beef during storage. *J. Food Sci.* **1991**, *56*, 341–347.

(7) Ashmore, C. R.; Doerr, L.; Foster, G.; Carroll, F. Respiration of mitochondria isolated from dark cutting beef. *J. Anim. Sci.* **1971**, *33*, 574–577.

(8) Ashmore, C. R.; Parker, W.; Doerr, L. Respiration of mitochondria isolated from dark-cutting beef: Postmortem changes. *J Anim. Sci.* **1972**, *34*, 46–48.

(9) Egbert, W. E.; Cornforth, D. P. Factors influencing color of dark cutting beef muscle. *J. Food Sci.* **1986**, *51*, 57–65.

(10) Tang, J.; Faustman, C.; Hoagland, T. A.; Mancini, R. A.; Seyfert, M.; Hunt, M. C. Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. *J. Agric. Food Chem.* **2005**, *53*, 1223–1230.

(11) Tang, J.; Faustman, C.; Mancini, R. A.; Seyfert, M.; Hunt, M. C. Mitochondrial reduction of metmyoglobin: dependence on the electron transport chain. *J. Agric. Food Chem.* **2005**, *53*, 5449–5455.

(12) Faustman, C.; Cassens, R. G. The biochemical basis for fresh meat discoloration: A review. *J. Muscle. Foods* **1990**, *1*, 217–243.

(13) Cheah, K. S.; Cheah, A. M. Post-mortem changes in structure and function of ox muscle mitochondria. 1. Electron microscopic and polarographic investigations. *J. Bioenerg. Biomembr.* **1971**, *2*, 85–92.

(14) de Bari, L.; Atlante, A.; Valenti, D.; Passarella, S. Partial reconstruction of in vitro gluconeogenesis arising from mitochondrial L-LAC uptake/metabolism and oxaloacetate export via novel L-LAC translocators. *Biochem. J.* **2004**, *380*, 231–242.

(15) Calvin, J.; Tubbs, P. K. Mitochondrial transport processes and oxidation of NADH by hypotonically-treated boar spermatozoa. *Eur. J. Biochem.* **1978**, *89*, 315–320.

(16) Pallotta, M. L.; Valenti, D.; Iacovino, M.; Passarella, S. Two separate pathways for D-lactate oxidation by *Saccharomyces cerevisiae* mitochondria which differ in energy production and carrier involvement. *Biochim. Biophys. Acta* **2004**, *1608*, 104–113.

(17) Brooks, G. A.; Dubouchaud, H.; Brown, M. A.; Sicurello, J. P.; Butz, C. E. Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1129–1134.

(18) Gladden, L. B. Lactate metabolism: A new paradigm for the third millennium. *J. Physiol.* **2004**, *558*, 5–30.

(19) Larsson, C.; Pahlman, I.; Ansell, R.; Rigoulet, M.; Adler, L.; Gustafsson, L. The Importance of the glycerol 3-phosphate shuttle during aerobic growth of *Saccharomyces cerevisiae*. *Yeast* **1998**, *14*, 347–357.

(20) Smith, A. L. Preparation, properties, and conditions for assay of mitochondria: Slaughterhouse material, small-scale. *Methods Enzymol.* **1967**, *10*, 81–86.

(21) Estabrook, R. W. Mitochondrial respiratory control and the polarographic measurement of ADP: O ratios. *Methods Enzymol.* **1967**, *10*, 41–47.
(22) Immonen, K.; Kauffman, R. G.; Schaefer, D. M.; Puolanne, E. Glycogen concentration in bovine *longissimus dorsi* muscle. *Meat Sci.* **2000**, *54*, 163–167.
(23) Bergmeyer, H. U. *Methods of Enzymatic Analysis*; Academic Press: New York, 1974; pp 545−546.
(24) Cornforth, D. P.; Egbert, W. R. Effect of rotenone and pH on the color of pre-rigor muscle. *J. Food Sci.* **1985**, *50*, 34–35.
(25) Moyes, C. D.; Hood, D. A. Origins and consequences of mitochondrial variation in vertebrate muscle. *Annu. Rev. Physiol.* **2003**, *65*, 177–201.
(26) Hederstedt, L. Respiration Without O_2. *Science* **1999**, *284*, 1941–1942.
(27) Brooks, G. A. Mammalian fuel utilization during sustained exercise. *Comp. Biochem. Physiol., B* **1998**, *120*, 89–107.
(28) Davies, K. J.; Packer, L.; Brooks, G. A. Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. *Arch. Biochem. Biophys.* **1981**, *209*, 539–554.
(29) Donovan, C. M.; Brooks, G. A. Effect of endurance training on glucose kinetics during exercise. *Am. J. Physiol.* **1983**, *244*, E83–E92.
(30) Gladden, L. B. Lactic acid: New roles in a new millennium. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 395–397.
(31) Gallina, F. G.; Burgos, N. M.; Burgos, C.; Coronel, C. E.; Blanco, A. The lactate/pyruvate shuttle in spermatozoa: operation in vitro. *Arch. Biochem. Biophys.* **1994**, *308*, 515–519.
(32) Inskeep, P. B.; Hammerstedt, R. H. Endogenous metabolism by sperm in response to altered cellular ATP requirements. *J. Cell. Physiol.* **1985**, *123*, 180–190.
(33) Burgos, G, N. M.; Burgos, C.; Montamat, E. E.; Moreno, J.; Blanco, A. A shuttle system for the transfer of reducing equivalents in mouse sperm mitochondria. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 644–649.
(34) Daff, S.; Ingledew, W. J.; Reid, G. A.; Chapman, S. K. New insights into the catalytic cycle of flavocytochrome b_2. *Biochemistry* **1996**, *35*, 6345–6350.
(35) Paventi, G.; Pizzuto, R.; Chieppa, G.; Passarella, S. L-Lactate metabolism in potato tuber mitochondria. *Eur. J. Biochem.* **2007**, *274*, 1459–1469.
(36) Valenti, D.; de Bari, L.; Atlante, A.; Passarella, S. L-LAC transport into rat heart mitochondria and reconstruction of the L-LAC/pyruvate shuttle. *Biochem. J.* **2002**, *364*, 101–104.
(37) Atkinson, J. L.; Follett, M. J.; Ratcliff, P. W. Postmortem changes in the oxygen uptake and NAD content of lamb semimembranosus. *Nature* **1969**, *223*, 1372–1373.
(38) de Vries, S.; Marres, C. A. M. The mitochondrial respiratory chain of yeast. Structure and biosynthesis and the role in cellular metabolism. *Biochim. Biophys. Acta* **1987**, *895*, 205–239.
(39) von Jagow, G.; Klingenberg, M. Pathways of hydrogen in mitochondria of *Saccharomyces carlsbergensis*. *Eur. J. Biochem.* **1970**, *12*, 583–592.
(40) Mole', P. A.; Van Handel, P. J.; Sandel, W. R. Extra O_2 consumption attributable to NADH2 during maximum lactate oxidation in the heart. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 1143–1149.
(41) Baldwin, K. M.; Hooker, A. M.; Herrick, R. E. Lactate oxidative capacity in different types of muscle. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 151–157.
(42) Ueno, H.; Miyoshi, H.; Ebisui, K.; Iwamura, H. Comparison of the inhibitory action of natural rotenone and its stereoisomers with various NADH-ubiquinone reductases. *Eur. J. Biochem.* **2005**, *1*, 411–417.

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