LACTATE-MODULATED BEEF MITOCHONDRIAL OXYGEN CONSUMPTION INFLUENCES OXYMYOGLOBIN

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Abstract – In post-mortem skeletal muscles, NADH formed from lactate addition can increase mitochondrial oxygen consumption. However, the effects of lactate-mediated mitochondrial oxygen consumption on myoglobin redox state have not been examined. Therefore, the objective of the present study was to evaluate the effects of beef mitochondrial oxygen consumption on myoglobin redox stability in the presence of lactate, LDH, and NAD. Beef longissimus mitochondria (n = 5) were incubated with oxymyoglobin (2.5 mg/ml) in the presence of lactate (40 mM), lactate dehydrogenase (LDH; 100 units), and NAD (0.02 mM) at pH 5.6 and 25°C for 24 h. Myoglobin redox state and mitochondrial oxygen consumption were monitored. The addition of lactate-LDH-NAD increased ($P < 0.05$) mitochondrial oxygen consumption and promoted formation of deoxymyoglobin compared with control samples without substrates. The results indicate that increased mitochondrial oxygen consumption can influence myoglobin redox state and that this could be partially responsible for the darkening effect observed in lactate-enhanced whole-muscle beef cuts.

Key Words – darkening, beef color, substrate.

I. INTRODUCTION

Mitochondria continue to consume oxygen in post-mortem muscles, and the competition between mitochondria and myoglobin is a key component in the development of bright-red color [1]. Mitochondrial respiration influences beef color by decreasing oxygen partial pressure, and thus, minimizing the formation of oxymyoglobin from deoxymyoglobin. In addition, mitochondria can interact with oxymyoglobin, resulting in the transfer of oxygen from myoglobin to mitochondria [2]. However, conversion of oxymyoglobin to deoxymyoglobin is not direct, but through metmyoglobin. Deoxymyoglobin is generated when oxygen consumption is accompanied by metmyoglobin reduction [3]. Therefore, the conversion of oxymyoglobin to deoxymyoglobin is dependent on the ability of mitochondria to consume oxygen and reduce metmyoglobin. However, limited research has been undertaken to assess mitochondria-mediated conversion of oxymyoglobin to deoxymyoglobin.

Previous studies [1] concluded that mitochondrial respiration resulted in the conversion of oxymyoglobin to deoxymyoglobin following the addition of succinate, a mitochondrial substrate. Furthermore, addition of lactate to isolated beef heart mitochondria resulted in oxygen consumption [4]. On the other hand, the influence of lactate-mediated oxygen consumption on myoglobin redox state has not been explored. Therefore, our objective was to examine the effect of beef skeletal muscle mitochondrial oxygen consumption on oxymyoglobin in the presence of lactate, lactate dehydrogenase (LDH), and NAD as substrates.

II. MATERIALS AND METHODS

Beef longissimus muscles (n = 5) from market age cattle were obtained from a local abattoir within 30 min of exsanguination, placed on ice, transported to the laboratory, and used to isolate mitochondria and myoglobin. Mitochondria were isolated as previously described [5], and all steps were performed at 4°C. Mitochondrial pellets were washed twice and suspended in suspension buffer (250 mM sucrose, 10 mM HEPES, pH 7.2). Mitochondrial protein content was determined using bicinchoninic acid protein assay. Myoglobin was purified via ammonium sulfate precipitation and gel-filtration chromatography [6]. Oxymyoglobin was prepared by sodium hydrosulfite-mediated reduction, and residual hydrosulfite was removed using PD-10 columns.

Mitochondrial oxygen uptake was measured using an oxygen electrode attached to a

digital oxygen controller (Rank Brothers, Cambridge, England). Reaction components (incubation buffer, mitochondria, and substrates) were added to the incubation chamber maintained at 25°C and stirred continuously. Oxygen consumption was recorded by suspending mitochondria in the oxygen electrode with lactate-LDH-NAD (LLN) at pH 5.6 (incubation buffer containing 250 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1% BSA, and 20 mM maleic acid). The concentration of lactate, NAD, and LDH were 40 mM, 0.02 mM, and 100 units, respectively. Control samples consisted of only mitochondria.

To determine the effects of mitochondria on myoglobin redox stability, mitochondria (2 mg/ml) and oxymyoglobin (2.5 mg/ml) were incubated in presence of LLN. Control samples consisted of mitochondria combined with only oxymyoglobin. The samples were incubated in screw capped vials (to prevent the potential diffusion of air from outside into the mitochondria-oxymyoglobin mixture) at 25°C and scanned (at 0, 6, 12, and 24 h) spectrophotometrically from 650 to 500 nm with an integrating sphere assembly (Shimadzu UV-2100U, Kyoto, Japan). The relative proportion of myoglobin redox forms were calculated using wavelength maxima at 503, 557, and 582 nm, representative of metmyoglobin, deoxymyoglobin, and oxymyoglobin, respectively [7].

The experimental design was a randomized complete block design with repeated measures, and beef longissimus muscle served as blocks. The experiment was replicated five times (n = 5). Treatments were assigned to isolated mitochondria within a muscle. Type-3 tests of fixed effects were performed using the PROC MIXED [8]. 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 5% level.

III. RESULTS AND DISCUSSION

Addition of LLN to mitochondria increased oxygen consumption ($P < 0.05$). In the presence of LLN, oxygen consumption was 50 nanomoles of oxygen per mg of mitochondria per minute compared to 2 nanomoles of oxygen per mg of mitochondria per minute in the absence of LLN. Previous research suggested that beef cardiac mitochondria can use lactate as a substrate for oxygen consumption primarily due to NADH formed via lactic dehydrogenase activity [4].

The addition of LLN decreased ($P < 0.05$) oxymyoglobin content (Fig. 1), and the decrease in oxymyoglobin was supported by a concomitant increase ($P < 0.05$) in deoxymyoglobin (Fig. 2). Previous investigations [1] reported that succinate facilitated the formation of deoxymyoglobin when mitochondria and oxymyoglobin were combined in a closed system. In agreement, other researchers [9] also reported a decrease in oxymyoglobin content and oxygen partial pressure when rat mitochondria and sperm whale oxymyoglobin were incubated with succinate. These authors concluded that a direct interaction between myoglobin and mitochondria is necessary for the transfer of oxygen.

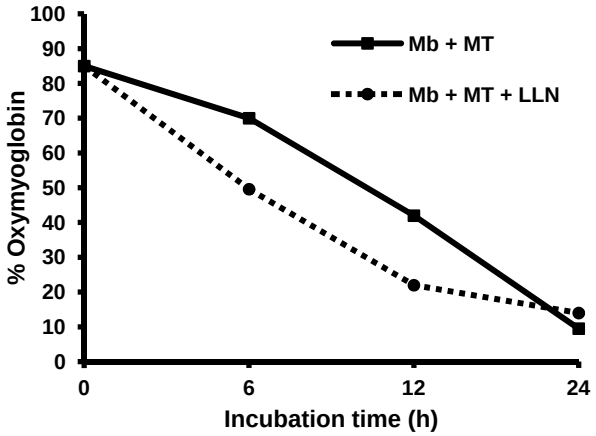

Figure 1. Effects of beef longissimus mitochondrial oxygen consumption on % oxymyoglobin in the presence of lactate, LDH, NAD at pH 5.6 and 25°C. MT = mitochondria (2 mg/ml); Mb = myoglobin (2.5 mg/ml); LLN = lactate (40 mM) + LDH (100 units) + NAD (0.02 mM)

The observed increase in mitochondrial oxygen consumption due to LLN can influence myoglobin redox state by decreasing the oxygen partial pressure, which leads to the transfer of oxygen from oxymyoglobin to mitochondria for maintaining metabolic activity. However, the conversion of oxymyoglobin to deoxymyoglobin is not direct, but through metmyoglobin. Deoxymyoglobin is generated when oxygen consumption is accompanied by subsequent metmyoglobin reduction [3]. Therefore, the

conversion of oxymyoglobin to deoxymyoglobin is dependent on the ability of mitochondria to consume oxygen and reduce metmyoglobin.

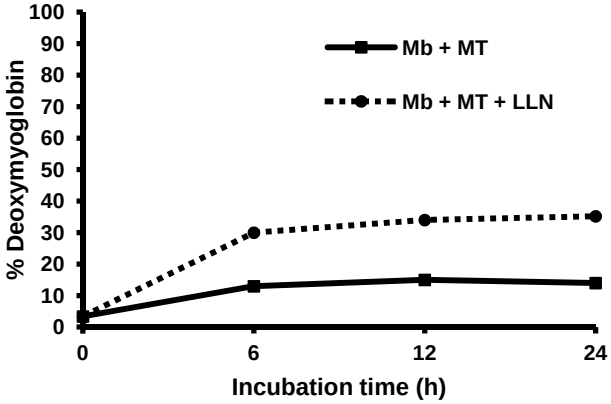

Figure 2. Effects of beef longissimus mitochondrial oxygen consumption on % deoxymyoglobin in the presence of lactate, LDH, NAD at pH 5.6 and 25°C. MT = mitochondria (2 mg/ml); Mb = myoglobin (2.5 mg/ml); LLN = lactate (40 mM) + LDH (100 units) + NAD (0.02 mM)

Addition of LLN resulted in less metmyoglobin (Fig. 3) than mitochondria controls without added substrates at 12 and 24 h ($P < 0.05$). It is likely that continued use of oxygen by mitochondria favored metmyoglobin reduction by either the electron transport chain or reductase enzymes within the outer membrane.

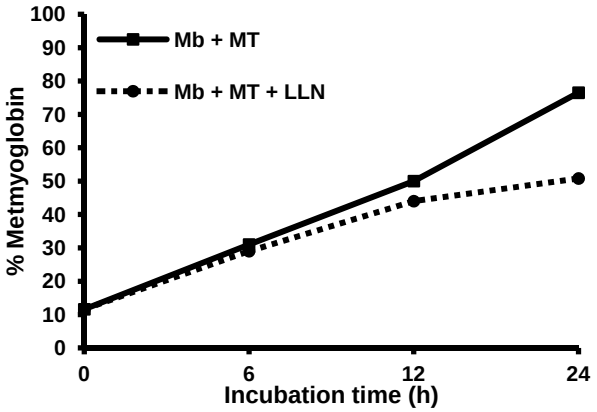

Figure 3. Effects of beef longissimus mitochondrial oxygen consumption on % metmyoglobin in the presence of lactate, LDH, NAD at pH 5.6 and 25°C. MT = mitochondria (2 mg/ml); Mb = myoglobin (2.5 mg/ml); LLN = lactate (40 mM) + LDH (100 units) + NAD (0.02 mM)

Our results suggest that increased mitochondrial oxygen consumption limits myoglobin oxygenation. This might be, in part, responsible for the darkening effect observed in lactate-enhanced beef. In support, limited myoglobin oxygenation (decreased bloom) was reported in lactate-enhanced beef loins [10]. Furthermore, previous research [11] suggested that actively respiring mitochondria can limit oxygen availability to myoglobin, and thus meat fails to bloom and does not achieve a consumer-desirable cherry-red color. These authors also concluded that the addition of rotenone (a complex I inhibitor) to pre-rigor meat (with high pH favoring mitochondrial activity) reversed darkening via the inhibition of mitochondrial respiration. Thus, mitochondrial respiration can out-compete myoglobin for oxygen resulting in low oxygen partial pressure and darker meat due to deoxygenated myoglobin.

IV. CONCLUSION

Addition of LLN increases beef mitochondrial oxygen consumption and favors the conversion of oxymyoglobin to deoxymyoglobin. The NADH generated by LLN can be used for mitochondrial oxygen consumption and enzymatic metmyoglobin reduction. Post-mortem muscles are biochemically active, and therefore addition of mitochondrial substrates can influence myoglobin redox state and thus affect beef color stability.

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