Feeding regime alters mitochondrial metabolism in beef muscle

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I. INTRODUCTION

Beef cattle feeding regimes vary greatly depending on country, feed resources and markets. Finishing systems alter whole body fatty acid composition and skeletal muscle energy metabolism impacting meat quality traits, such as color and tenderness [1-4]. Cattle finished in US feedlots are typically fed high carbohydrate diets, which result in maximal average daily gains and bright cherry red lean development. In contrast, beef cattle finished on forage systems are generally fed low energy diets with lower average daily gains and result in darker lean. Differences in diet-induced growth rate also alter skeletal muscle metabolism [2-3]. Regardless, there is a general lack of knowledge regarding the impact of nutrition on mitochondrial substrate utilization. We hypothesized that changes in bovine skeletal muscle mitochondria reflect changes in metabolism as predicated by differences in dietary energy intakes.

II. MATERIALS AND METHODS

Sixteen crossbred Angus steers were randomly assigned to either a forage- or carbohydrate-based maintenance diet for 60 d (n=8). After feeding, cattle were harvested using industry standards. Glycolytic *longissimus lumborum* (LL) and oxidative *masseter* (MS) muscle samples were collected approximately 5 min post-exsanguination. Muscle samples were processed for mitochondrial isolation as described by Scheffler et al. (2014). Mitochondria respiration was measured from freshly isolated mitochondria using Seahorse XFe96 (Agilent). Mitochondria were provided with saturating amounts of the following substrates: palmitoyl-carnitine/malate (40μM/1mM) and acetoacetate/malate (10mM/5mM). Baseline values represent basal respiration of isolated mitochondria with substrates. OXPHOS capacity was determined using ADP (5 mM) stimulated respiration. Proton leak was evaluated using 2 μM oligomycin, while maximal respiration was achieved with the uncoupler FCCP (4 μM). All data were normalized to total loaded mitochondrial protein.

Data were analyzed as a complete randomized design in a 2 x 2 factorial arrangement, considering the diet, muscle, and their interaction as a fixed effect. Harvest date was considered a random effect and animals served as experimental units. Least square means and standard error bars were obtained with SAS Proc Mixed procedure. Significance is denoted as $P<0.05$, $P<0.01$, $P<0.001$, unless otherwise stated.

III. RESULTS AND DISCUSSION

To understand the impact of nutrition on mitochondiral substrate utilization, saturating concentrations of long chain fatty acid (palmitoyl-carnitine) or short chain fatty acid (acetoacetate) were provided to isolated mitochondira to assess their oxygen consumption rate. In the presence of long chain fatty acids, higher maximal respiration were observed in mitochondira isolated from MS of forage fed cattle, followed by those from the MS of carbohydrate-fed cattle, while LL mitochondria had the lowest maximal respiration regardless of feeding regime (Table 1). These data show that feeding regime alters mitochondrial function in the presence of long chain fatty acids and suggest that mitochondria of oxidative muscles are more metabolically malleable compared to those of cattle fed a high carbohydrate diet. Whether this ability is due to the availability of carnitine transporters on the mitochondrial membrane remains to be determined.

Table 1. Mitochondria oxygen consumption rate (pmol/(sec*mg mito)) isolated from the *longissimus lumborum* (LL) and *masseter* (MS) of cattle fed a forage- or carbohydrate-based diet and incubated under saturating concentrations of palmitoyl carnitine/malate.

	Carbohydrate		Forage		p-value		
Injection	LL	MS	LL	MS	TRT	Muscle	T*M
Baseline	20.2±5.5	14.5±5.5	20.6±5.5	9.1±5.5	0.6571	0.1296	0.5991
OXPHOS Capacity	69.3±12.7	223.3±12.7	74.1±12.7	242.3±12.7	0.3601	<.0001	0.582
Proton Leak	8.2±4.9	8.6±4.9	6.7±4.9	8.9±4.9	0.9045	0.7857	0.8536
Maximal Respiration	31.1±13.1	172.8±13.1	39.3±13.1	239.8±13.1	0.0074	<.0001	0.0324

Table 2. Mitochondria oxygen consumption rate (pmol/(sec*mg mito)) of isolated from the *longissimus lumborum* (LL) and *masseter* (MS) of cattle fed a forage- or carbohydrate-based diet and incubated under saturating concentrations of acetoacetate/malate.

	Carbohydrate		Forage		p-value		
Injection	LL	MS	LL	MS	TRT	Muscle	T*M
Baseline	9.2±7.52	BD±7.52	3.8±7.52	BD±7.52	0.1917	0.0016	0.5402
OXPHOS Capacity	26.9±9.7	77.3±9.7	23.9±9.7	136.2±9.7	0.0073	<.0001	0.0034
Proton Leak	9.3±7.2	2.6±7.2	6.9±7.2	BD±7.2	0.3984	0.1586	0.5928
Maximal Respiration	9.7±9.9	80.38±9.9	9.5±9.9	150.8±9.9	0.0014	<.0001	0.0013

When isolated mitochondria were provided saturating concentrations of short chain fatty acids, a treatment by feed effect was noted in OXPHOS capacity and maximal respiration (Table 2). Both assays show that mitochondria from the MS of forage fed cattle have greater respiration than those of carbohydrate fed cattle, with LL mitochondria from either feeding treatment having the lowest respiration. Together these data show that oxidative muscle mitochondria are more responsive to feeding regime than their glycolytic muscle counterparts. Isolated mitochondria from oxidative muscle of may have the ability to utilize more long chain and short chain fatty acids, suggesting that they are more metabolically flexible in terms of substrate utilization.

IV. CONCLUSION

Together these data show that oxidative muscle mitochondria are more responsive to feeding regime than those from glycolytic muscles and that oxidative mitochondria from forage fed animals are able to respire more long chain and short chain fatty acids compared to oxidative mitochondria of carbohydrate fed animals. Future directions will involved studying the mechanisms responsible for this change in mitochondria metabolism.

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