Exogenous microRNA kinetics and the importance of animal protein intake in muscle maintenance

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I. INTRODUCTION

Dietary patterns have a profound impact on animal metabolic outcomes and physiological responses. It's well-established that reduced protein intake can disrupt muscle health by increasing muscle protein catabolism [1]. The pathways behind this are intricately linked to the function of macro, micro, and molecular nutrients that epigenetically modulate protein expression at the translation level [2]. MicroRNAs (miRs), small RNA molecules that silence gene expression, are key players in every cellular process leading to cell differentiation, development, and homeostasis [3]. Our previous research has shown that meat-derived miRs can withstand aging, cooking, and digestion and are potentially available for absorption in the duodenum. In this study, we have identified beef-derived miRs not homologous to murine and examined their absorption kinetics and organ distribution in mice. We've also investigated the effects of the absence of animal protein on lean tissue maintenance, presenting novel findings that contribute to our understanding of dietary patterns and muscle health.

II. MATERIALS AND METHODS

Animals and diets: Twelve-weeks-old, C57BL/6J background mice (male, 24.5 $\pm$ 0.4) were randomly assigned into two groups comprising four experimental units (n=4). Within each group, the mice were further randomized into two subgroups. One subgroup was fed a BEEF extract (6 µl/g), while the other received a carbohydrate diet (CHO). Mice in both dietary groups were allowed to have a regular chow diet and water ad libitum. Mice were gavaged twice a day within a 12-hour interval for a duration of seven consecutive days. Magnetic Resonance Imaging MRI (EchoMRI^{TM-} 100 System, Echo Medical Systems, Houston, TX) data were acquired from each mouse to establish baseline physiological parameters on day 1 and subsequently on day 7 to evaluate the effect of beef on lean muscle maintenance. On the fifth day of the experiment, all mice were individually housed in Promethion cages (High-Resolution Metabolic and Behavioral Phenotyping Systems for Rodents, Sable Systems International) for 35 hours to evaluate physiological activities. The same experiment was replicated to improve power, totaling 8 experiment units per treatment (n=16). **Sample collection and processing**: Post-euthanasia, organs including the liver, stomach, small and large intestine, muscle, kidney, and cecum samples were collected from each mouse and snap-frozen in liquid nitrogen to preserve tissue integrity until further analysis. **Total RNA extraction, cDNA synthesis and RT-PCR:** To understand the kinetics of beef-derived miRs, total RNA was extracted from each sample type using TRIzol method. MicroRNA expression was quantified using qRT-PCR with the selected primers and 18s as the housekeeping gene. **Statistical analysis:** MicroRNA presence in organs was analyzed as a binomial distribution using a Qui-square test. Resonance Imaging MRI and Promethion metabolic / behavior data were analyzed as a CRD, whereas behavior data was designed as a 2x2 factorial (diet x time). Data were analyzed using SAS.

III. RESULTS AND DISCUSSION

The expression of 10 beef-derived miRs, including bta-miRs 2484, 2340, 2453, and 2440, and miRs 2284w, 2284x, 3432a, 3431, 2422, and 11988 was verified in mice liver, kidney, stomach, large and

small intestines, cecum and skeletal muscle. This study did not find evidence that beef-derived miRs were absorbed in the mice GIT. Beef supplementation did not alter mice behavior and metabolic parameters such as O_2 consumption, CO_2 excretion, kcal/hr, food and water uptake, and physical activity. On the other hand, Mice that received BEEF retained lean mass during a period of 8 days. Mice that did not receive animal protein lost muscle mass (Table 1). Mice fed BEEF also retained more free water than mice fed CHO, possibly due to the maintenance of lean mass.

Table 1. Lean and free water % of mice fed short-term BEEF and CHO diets.

	Day	BEEF[1]	CHO[2]	*P*-value[3]	Std. error[4]
Lean	1	24.78	24.63[A]	0.03	0.30
	8	24.86	24.02[B]		
Free water	1	0.06[B]	0.08	0.01	0.01
	8	0.10[A]	0.06		

[1]Bovine m. semimembranosus extract in DDW.
[2]Starch = 29.81% + Maltodextrin = 9.90% + Sucrose = 11.25% + Soybean oil = 5.28% + 43.76% DDW.
[3]Significant at ≤ 0.05.
[4]Standard error of the mean.

Despite the absence of detectable ruminant-specific miR derived from beef in mice tissues, the observed lean maintenance in beef-fed mice suggests a potential role of dietary protein sources in modulating muscle tissue metabolism. At the end of the experiment, the lack of significant differences in physiological parameters between the carbohydrate and beef-fed groups illustrates the need for long-term dietary treatment for metabolic adaptation and the importance of long-term assessments.

IV CONCLUSION

The absence of the expression of beef-derived miRs in mice organs suggests that those small RNA molecules may not be absorbed in the GIT or the life span of exogenous miRs in the blood, after absorption is short. Based on previous research suggesting that milk and vegetable-derived miRs can be absorbed in in the GIT, it is possible that the short-term approach and post-mortem collection time used in this trial did not allow the detection of miRs in mice organs. On the other hand, the absence of animal protein in diets is detrimental to muscle maintenance. The presence of animal protein in diets is essential to avoid sarcopenia.

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