71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

DIETARY BEHAVIOR AFFECTS MICE'S METABOLISM AND PHYSIOLOGY: A COMPARISON STUDY USING PROTEIN-ONLY, CARBOHYDRATE-BASED, AND COMBINED FEEDING STRATEGIES

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I. INTRODUCTION

Diet plays a vital role in metabolic health, lean muscle mass maintenance, energy expenditure, and overall physiological state. Protein plays a crucial role in muscle maintenance and metabolic regulation among macronutrients. High-protein diets, especially those sourced from animal products like beef, support lean muscle mass and promote thermogenesis, contributing to favorable body composition outcomes [1]. Emerging research explores the effects of macronutrient composition on metabolic activity and energy homeostasis. For instance, high-protein, low-carbohydrate diets have been associated with improved fat oxidation and lean mass retention. In contrast, carbohydrate-rich diets may contribute to greater adiposity when not carefully controlled [2]. However, the effects of protein-only and balanced diets on activity level, physiology, and muscle integrity require more in-depth studies. In this study, we investigated the impact of a protein-only diet based on beef, a standard carbohydrate-based diet based on chow, and a diet combining beef and chow on mouse activity and physiology.

II. MATERIALS AND METHODS

Animals and diet: Twelve-week-old, C57BL/6J background mice were randomly assigned into three groups comprising nine experimental units per treatment (n=9, total n=27). Groups were fed sous vide beef pellets (BEEF), the carbohydrate-based diet (CHOW), or a combination of beef and chow diet (BEEFCHOW). Each treatment group consisted of three cages, with three mice housed per cage (3 mice × 3 cages = 9 mice per treatment). Mice in all dietary treatments had an ad libitum water supply and their respective diet. 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 30th to evaluate the effect of beef on lean muscle maintenance. On the day 23rd of the experiment, one mouse from each cage was randomly selected and individually housed in Promethion cages (High-Resolution Metabolic and Behavioral Phenotyping Systems for Rodents, Sable Systems International) for 7 days to evaluate physiological activities including energy expenditure, activity, food and water intake, and respiratory exchange ratio (RER). The same experiment was replicated to improve power, totaling 27 experiment units per treatment (n=54). **Data collection and processing**: At the end of the experiment, the data from metabolic cages were withdrawn for statistical analysis. **Statistical analysis:** Food consumption, resonance imaging MRI and Promethion metabolic / behavior data were analyzed as a CRD, whereas behavior data were designed as a 2x2 factorial (diet x time) and repeated measures were analyzed using suitable statistical matrices. Data were analyzed using SAS.

III. RESULTS AND DISCUSSION

Dietary treatments led to significant changes in metabolic data obtained from Promethion cages. Oxygen consumption (VO_2) was highest in the BEEF fed group and lowest in the CHOW group (P<0.0001), suggesting that the beef diet may stimulate metabolic rate, possibly due to higher protein content. This is consistent with the highest energy expenditure (Kcal/hr), observed in the same treatment, which may have led to a higher overall metabolic activity. Lower CO_2 production (P<0.0001) in the BEEF group suggests fat oxidation as the primary energy source compared to carbohydrate oxidation in the CHOW group, and this is further supported by a significantly lower respiratory quotient (RQ) compared to other groups. Water uptake data shows a significant difference (P=0.0007), with higher water intake in BEEF and BEEFCHOW groups, suggesting increased hydration requirements, possibly due to higher protein content, which requires more water for excretion (waste removal). Regarding physical activity, mice fed BEEFCHOW and CHOW were more active than mice fed BEEF, showing higher overall travelled distance in the cage and higher locomotion (P≤0.0001). Conversely, mice fed BEEF and BEEFCHOW were more active in the cage wheel (P<0.0001). The highest activity in mice fed BEEFCHOW and CHOW could be due to the

combination of macronutrients, which provided mice with a more complete and readily available energy source. Also, protein can stimulate diet-induced thermogenesis, potentially increasing energy expenditure and activity level. When examining the MRI results, mice fed CHOW and BEEFCHOW showed more lean tissue than those fed BEEF (P=0.02). This is possibly due to carbohydrates' long-term energy input, allowing muscle mass development. Consequently, mice showing higher lean content also showed higher total water content (P=0.04). Regarding fat tissue, although mice fed CHOW had higher numerical values than mice fed BEEF and BEEFCHOW, no statistical difference was detected (P=0.10).

IV. CONCLUSION

The findings from both metabolic cages and MRI measurements demonstrate the effects of dietary composition on metabolism, body composition, hydration, and physical activity. A beef-only diet enhanced oxygen consumption, energy expenditure, and fat oxidation, while reducing respiratory quotient, suggesting a shift toward lipid metabolism. The BEEFCHOW diet influenced physical activity and preserved lean mass and hydration better than beef alone. These results suggest that a high-protein beef-based diet may increase metabolic activity. However, it is imperative to include carbohydrate sources in long-term diets to improve muscle maintenance and activity.

ACKNOWLEDGEMENTS

This research was funded by the Alice and Fred Ottoboni Meat Science Endowment Fund.

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