Investigating Metabolic Reprogramming in Adipocytes via ^{13}C-based Techniques

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Metabolic disorders such as type II diabetes and obesity are some of the greatest health challenges facing our world today. Although several genetic, lifestyle, and environmental risk factors contribute to the pathogenesis of these disorders, a common feature shared by many is the phenomenon of metabolic reprogramming. Metabolic reprogramming is the process in which cell's normal metabolic pathway activities are altered resulting in dysregulated systems-level behavior. Understanding the function and consequences of metabolic disease rewiring on a systems-level could thus lead to new therapeutic targets for obesity, type II diabetes, and other related disorders.

Currently, the gold-standard systems-level metabolic analysis techniques are all based on stable-isotope tracing and ^{13}C-metabolic flux analysis (^{13}C-MFA). Stable-isotope tracing allows investigators to qualitatively track the carbon flow through metabolic pathways, while ^{13}C-MFA allows for a more comprehensive quantitative analysis of metabolic fluxes. Together, these ^{13}C-based techniques have the power to deconvolute environmental effects on metabolic pathway activities and identify novel intervention targets. Thus far, few studies have applied these methods to study metabolic reprogramming in adipocytes (fat cells). The main objective of this thesis is to demonstrate the potential of ^{13}C-based techniques as tools for investigating metabolic rewiring in adipocytes. Specifically, we apply stable-isotope tracing methods and ^{13}C-MFA to investigate metabolic rewiring in adipocytes exposed to three perturbations implicated in the pathogenesis of metabolic disorders: differentiation, hypoxia, and co-culture interactions. The results of these

studies demonstrate the unique metabolic insights that ^{13}C-based methods can provide. Additionally, we develop a new experimental platform that can be used to improve ^{13}C-MFA techniques for future applications in a broader range of mammalian systems.

Hyperplasia (increase in cell number) and hypertrophy (increase of cell size) are two mechanisms that allow adipocytes to store excess nutrients. However, the transition between these two states requires complex metabolic interactions that are incompletely understood. Understanding this metabolic interplay is critical as uncontrollable hyperplasia and hypertrophy have been connected to the pathogenesis of obesity. To address this knowledge gap, we applied parallel labeling experiments and ^{13}C-MFA to characterize the metabolic shifts that occur between proliferating (hyperplastic) and lipid-accumulating (hypertrophic) 3T3-L1 cells. Through the combination of these techniques, we quantified the intracellular metabolic fluxes, biomass specific rates, and lipid accumulation of these two cell phenotypes. Taken together, our results suggest a functional role for metabolic reprogramming in the transition between proliferating pre-adipocytes and lipid-accumulating adipocytes.

Next, we investigated the impact of hypoxia (low oxygen level) on adipocyte metabolism. Hypoxia has been directly attributed to the pathogenesis of adipose tissue inflammation, a hallmark of obesity and obesity-linked type II diabetes, and thus implicated in inducing adverse metabolic reprogramming events within adipocytes. Given that the metabolic pathogenesis of this dysfunction is poorly understood, we once again applied ^{13}C-based techniques to characterize the impact of hypoxia on lipid-accumulating 3T3-L1 adipocytes. Our investigations revealed that hypoxia directly suppresses branched chain amino acid catabolism and causes significant shifts in carbohydrate, amino acid, and cofactor metabolism. Overall, these results highlight the profound impact that microenvironments have on adipocyte metabolic activity and function.

Finally, we developed a co-culture platform that is compatible with co-culture ^{13}C-MFA (^{13}C-co-MFA), an advance form of ^{13}C-MFA specifically designed to track metabolite exchange. To understand disease pathogenesis resulting from failed metabolite cross-feeding interactions, we must consider how individual cellular metabolic reprogramming rewires cell-cell interactions. However, currently no co-culture platforms exist that can support ^{13}C-co-MFA techniques. Therefore, we aimed to design and validate a transwell mammalian co-culture system between adipocytes (fat cells) and hepatocytes (liver cells) emulating fasting state metabolism. Design features considered during co-culture platform development included: 1) maximizing adipocyte lipolytic and hepatocyte gluconeogenic activity; 2) investigating the unidirectional/bidirectional nature of hepatocyte glucose and adipocyte glycerol production; and 3) identifying an experimental transwell system compatible with adipocyte-hepatocyte co-culture. Through this process, we identified optimal co-culture medium, tracers, and transwell system for adipocytes and hepatocytes. Performing co-culture labeling experiments, we confirmed specific adipocyte-hepatocyte metabolite exchanges. Overall, this work highlights the complexities of mammalian co-culture studies and the importance of optimal tracer selection, and ultimately, provides a blueprint for designing future ^{13}C-co-MFA studies.