**Biomarker Discovery and Metabolic Profiling in Plasma for Non-Alcoholic Steatohepatitis**

Sunny, N.E. (UF, Medicine) and Cusi, K. (UF, Medicine)

**Introduction**

The hepatic tri-carboxylic acid (TCA) cycle is central to integrating macronutrient metabolism, and is closely coupled to cellular respiration, free radical generation and inflammation. Oxidative flux through the TCA cycle is induced during hepatic insulin resistance, in mice and humans with simple steatosis, reflecting early compensatory remodeling of mitochondrial energetics. We hypothesized that progressive severity of hepatic insulin resistance and the onset of nonalcoholic steatohepatitis (NASH) would impair oxidative flux through hepatic TCA cycle. Mice (C57/BL6) were fed a high trans-fat high fructose diet (TFD) for 8-weeks to induce simple steatosis and NASH by 24-weeks. In vivo fasting hepatic mitochondrial fluxes were determined by 13C-nuclear magnetic resonance (NMR) based isotopomer analysis. Hepatic metabolic intermediates were quantified utilizing mass spectrometry based targeted metabolomics.

**Experimental Protocol**

Male mice (C57BL/6) purchased from Jackson Laboratories (Bar Harbor, ME) at 6-8 wks were fed either a control diet: (C; 10% fat calories; Research Diets, Inc. # D09100304) or a high trans-fat high fructose diet (TFD; 40% fat calories; Research Diets, Inc.# D09100301) for 8 or 24 weeks. At 8 weeks of TFD feeding, mice had already developed simple steatosis, whereas 24 weeks on TFD resulted in development of NASH (1). We are determined in vivo hepatic mitochondrial fluxes by infusion of stable isotopes via the chronic indwelling jugular vein catheter in conscious unrestrained mice (2). On the morning of the infusion, a cocktail of [13C3]propionate and [3,4-13C2]glucose were infused into the mice for 90 min to determine TCA cycle activity and endogenous glucose production respectively (2). Upon completion of the infusion, blood was collected by exsanguination under anesthesia and processed for NMR isotopomer analysis of mono acetone glucose as described earlier (2). The samples were run on the 1.5mm probe at the AMRIS facility at UF operating at 14.1T optimized for 13C detection (3). The isotpomer analysis of the multiples arising from 13C labeling of carbon-2 of glucose will be used to determine direct functional activity of multiple nutrient pathways (2) including a) gluconeogenic flux through TCA cycle, b) mitochondrial anaplerotic flux which drives gluconeogenesis, c) pyruvate cycling.

**Results and Discussion**

As elaborated above 13C-NMR based isotopomer analysis allowed us to determine the activity of multiple pathways through the hepatic mitochondrial TCA cycle, during the transition from simple steatosis to NASH. In spite of hepatic insulin resistance, EGP (Fig. 1A), absolute TCA cycle flux (Fig. 1B), total anaplerosis (Fig. 1C), and pyruvate cycling (Fig. 1D), remained unchanged after 8 weeks of TFD. Thus during early stages of diet induced insulin resistance and simple steatosis, mitochondrial fluxes could remain apparently normal (2). We then tested the hypothesis that onset of NASH will impair mitochondrial fluxes through the TCA cycle. As expected, onset of NASH was accompanied by higher rates of EGP (Fig. 1A). Interestingly, mitochondrial TCA cycle metabolism was upregulated in mice with NASH. There was an approximate 2-fold induction in absolute TCA cycle flux (Fig. 1B), total anaplerosis (Fig. 1C), and pyruvate cycling (Fig. 1D) in mice with NASH, relative to their age matched controls. Furthermore, a robust correlation between TCA cycle flux and anaplerosis was evident, indicating the dependence of anabolic pathways of glucose production on TCA cycle flux (Fig. 1E).

**Conclusions**

Induction of the TCA cycle activity during NASH was concurrent with blunted ketogenesis, and accumulation of hepatic diacylglycerols (DAGs), ceramides (Cer) and long chain acylcarnitines suggesting inefficient oxidation and disposal of excess free fatty acids (FFA). Sustained induction of mitochondrial TCA cycle failed to prevent accretion of “lipotoxic” metabolites in the liver, and could hasten inflammation and the metabolic transition to NASH.

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**References**

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