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

Sunny, N.E. (UF, Medicine); Cusi, K. (UF, Medicine); Edison A.S. (UF, Medicine)

Introduction

Elevated levels of branched chain amino acids (BCAAs) during insulin resistance have been relevant in the prediction of type II diabetes onset, but their role in the etiology of hepatic insulin resistance remains uncertain. Our objective was to determine the link between BCAAs and dysfunctional hepatic tri-carboxylic acid (TCA) cycle, which is a central feature of hepatic insulin resistance and nonalcoholic fatty liver disease (NAFLD). Towards this objective, we determined the enrichments of isotopomers arising from ^{13}C labeling of carbon-2 of glucose from a U^{13}Cpropionate tracer. This then allowed us to determine the direct functional activity of hepatic mitochondrial pathways as described below.

Experimental Protocol

We are determining in vivo hepatic mitochondrial fluxes by infusion of stable isotopes via the chronic indwelling jugular vein catheter in conscious unrestrained mice (1, 2). On the morning of the infusion, a cocktail of [$^{13}C_3$]propionate and [3,4-$^{13}C_2$]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 (1, 2). The samples were run on the 1.5mm probe at the AMRIS facility at UF operating at 14.1T optimized for ^{13}C detection (6). The isotopomer analysis of the multiples arising from ^{13}C 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. A sample spectrum of the ^{13}C multiplets at position carbon-2 of mono acetone glucose obtained on the 1.5mm probe is provided in **Figure 1**.

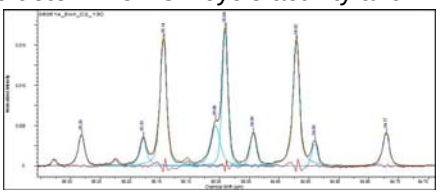

Figure 1: ^{13}C multiplet pattern in carbon-2 of glucose arising from incorporation of [$^{13}C_3$] propionate into glucose. The relative enrichments of these multiplets will allow us to calculate the functional activity of the mitochondrial TCA cycle metabolism.

Results and Discussion

Dysfunctional TCA cycle activity is intrinsic to both humans (3) and mice (2) with hepatic insulin resistance and NAFLD. Using NMR based ^{13}C isotopomer analysis, we identified that a 2-3 fold elevation in systemic BCAAs did not change endogenous glucose production in either control (**C**) or high *trans*-fat diet (**TFD**) fed mice (**Figure 2A**). However, elevated BCAAs induced hepatic TCA cycle activity (gluconeogenesis, **Figure 2B**; and total anaplerosis, **Figure 2C**) relative to citrate synthase flux in **C** mice. However, this induction was blunted in mice with NAFLD (**TFD**), clearly illustrating that BCAAs contribute to dysfunctional TCA cycle activity during NAFLD (**Figure 2**). Thus a fatty acid rich environment may be a prerequisite for manifesting defects in BCAA mediated regulation of hepatic mitochondrial metabolism. Our results open up a new avenue in understanding the interaction between BCAAs and hepatic mitochondrial fat metabolism, in turn identifying a new role of BCAAs in the etiology of NAFLD.

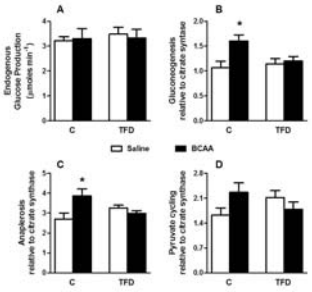

Figure 2

Conclusions

We conclude that BCAAs mediate efficient channeling of carbon substrates for oxidation through mitochondrial TCA cycle. Impairment of BCAA mediated up regulation of hepatic TCA cycle activity could be a significant contributor to mitochondrial dysfunction during NAFLD.

Acknowledgements

This work was also funded by the 2013 Research Opportunity Seed Fund Award, University of Florida.

References

[1] Sunny, N.E., *et al.*, Am J Physiol Endocrinol Metab. **298**, (6):E1226-1235 (2010).
[2] Satapati, S., *et al.*, Journal of lipid research. **53**, (6):1080-1092 (2012).
[3] Sunny, N.E., *et al.*, Cell metabolism. **14**(6):804-810 (2011)