Effects of High Carbohydrate Diet Supplementation on Hepatic Mitochondrial Metabolism

Christine Zhang, Nathan Kattapuram, Muhammed S. Muyyarikkandy, Meghan Maguire, Vaishna Muralidaran, Chaitra Surugihalli, Nishanth E. Sunny

University of Maryland, College Park

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a major public health issue affecting over 75 million patients and over 70% of patients that suffer from Type 2 Diabetes Mellitus and obesity.[1] Previous research has demonstrated that progression of NAFLD is accompanied by liver mitochondria adaptation and eventual dysfunction as they fail to respond to the influx of free fatty acids and the metabolic actions of insulin.[2-4]

The aim of this study was to elucidate the effects of high carbohydrate supplementation on mitochondrial metabolism of hepatocytes utilizing a metabolomics centered approach. Data obtained through gas chromatography mass spectrometry (GC/MS) suggest that LF and HC fed animals exhibit higher mitochondrial activity compared to their HF counterparts. Short-term increased mitochondrial activity suggests an increased robust metabolic response, however, long-term effects may be detrimental to metabolic flexibility through increased production of reactive oxygen species (ROS). While the comprehensive effects of high carbohydrate supplementation on metabolism are still under investigation, these results suggest that diets high in carbohydrates may lead to exacerbation of mitochondria, and ultimately metabolic dysfunction.

Materials and Methods

C57BL/6N male mice (n=29) were randomly placed on 1 of 3 diets: LF (10% kcal fat, 70% kcal carb.), HF (60% kcal fat, 20% kcal carb.), or HC (25% kcal fat, 34.9% kcal fructose) diet for 10 weeks. Whole livers were extracted from mice at 10 weeks to be used in GC/MS analysis. Samples were collected under fed conditions as well as overnight fasted conditions. Select organic acids and amino acids integral to the tricarboxylic acid (TCA) cycle were analyzed.

Liver Tissue Analysis

- Tissue was prepared with isotopomer standards of select organic acids and amino acids
- Metabolites were extracted and concentrations were collected via GC/MS

Mitochondrial Incubation

- Mitochondria were isolated from liver tissue and incubated in a solution of either ^{12}C pyruvate or ^{13}C pyruvate for 0 minutes, 5 minutes, and 10 minutes
- Media was collected from incubations and prepared for GC/MS analysis
- Percent enrichment was calculated for 10 min. incubations in ^{13}C pyruvate.

Results

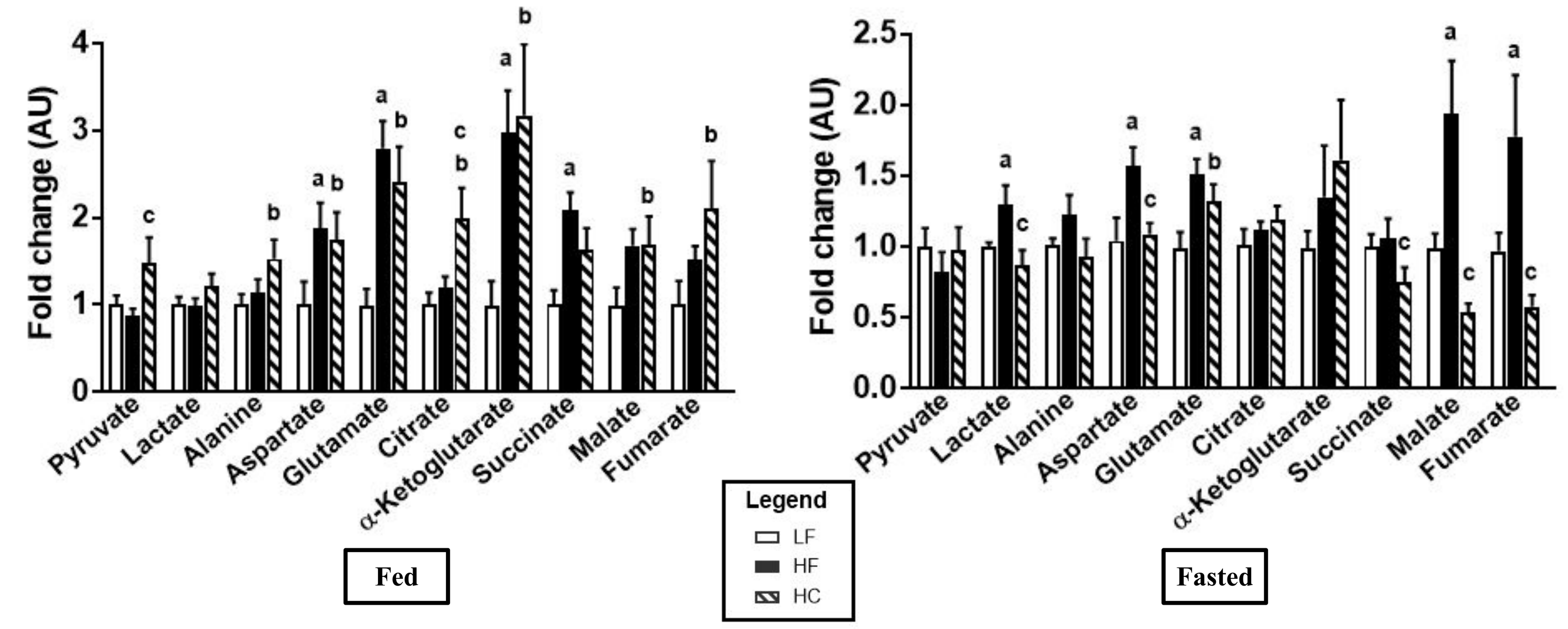


Figure 1: Concentrations of select organic acids and amino acids in fed and fasted liver tissue. Concentrations are graphed as fold change (arbitrary units) relative to LF fed mice. Superscripts indicate $p \leq 0.05$. 'a' - LF vs. HF 'b' – LF vs. HC and 'c' – HF vs. HC.

- Significant alteration in concentrations of several amino acids and organic acids in both HF and HC diet groups
- Amino acids and organic acids significantly altered *differ* between fed and fasted groups (Citrate and α-Ketoglutarate in fed, Malate and Fumarate in fasted)
- Various statistically significant changes between diet groups, however changes do not seem to follow a discernable pattern

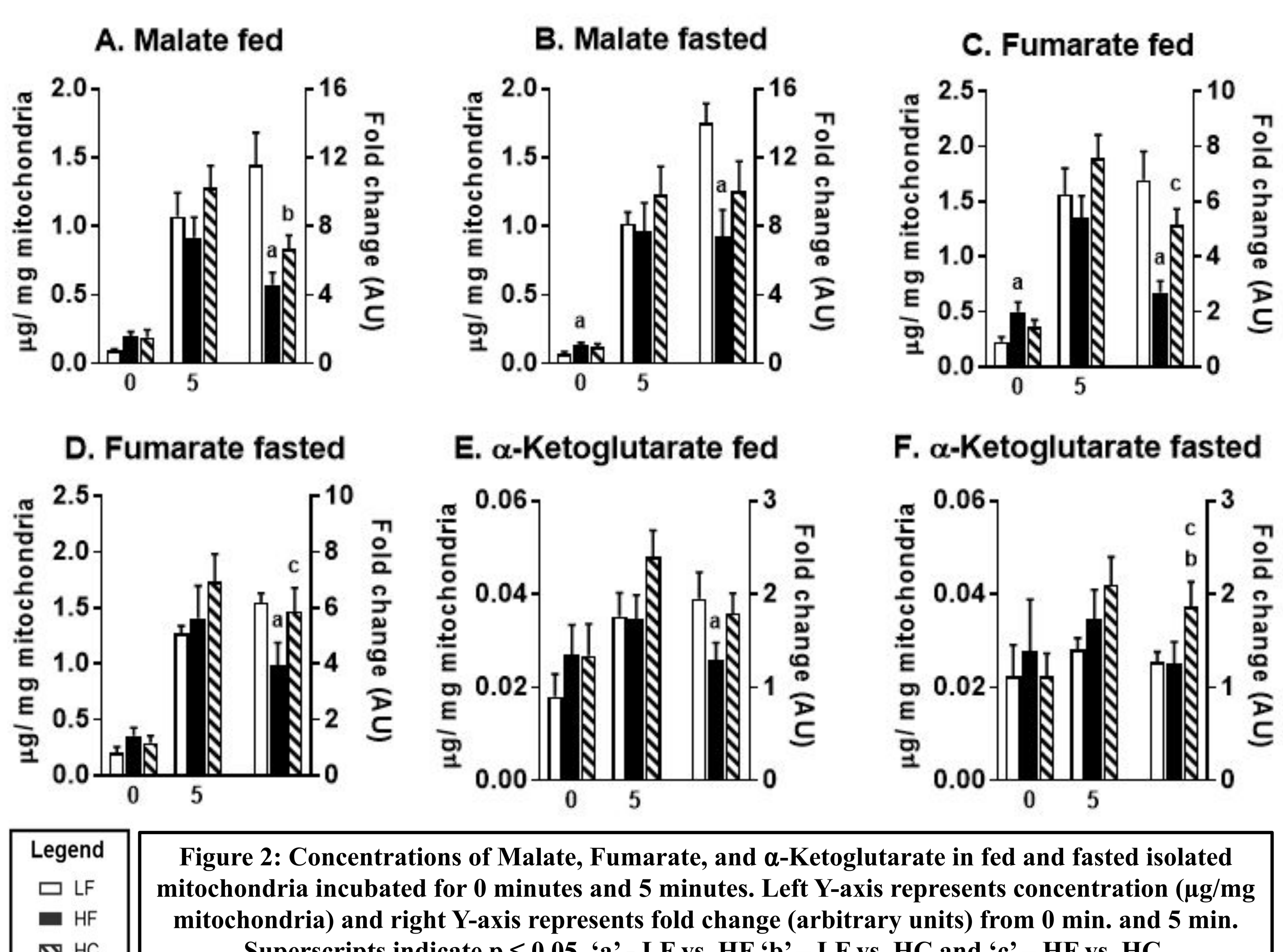


Figure 2: Concentrations of Malate, Fumarate, and α-Ketoglutarate in fed and fasted isolated mitochondria incubated for 0 minutes and 5 minutes. Left Y-axis represents concentration (μg/mg mitochondria) and right Y-axis represents fold change (arbitrary units) from 0 min. and 5 min. Superscripts indicate $p \leq 0.05$. 'a' - LF vs. HF 'b' – LF vs. HC and 'c' – HF vs. HC.

- Increase in concentrations of all organic acids between 0 min. and 5 min. of all diet groups
- HC groups showed a higher rate of increase in concentrations from 0 min. to 5 min. compared to HF groups
- Statistically significant *decrease* between the fold changes of LF and HF groups except in α-KG fasted

Results (cont.)

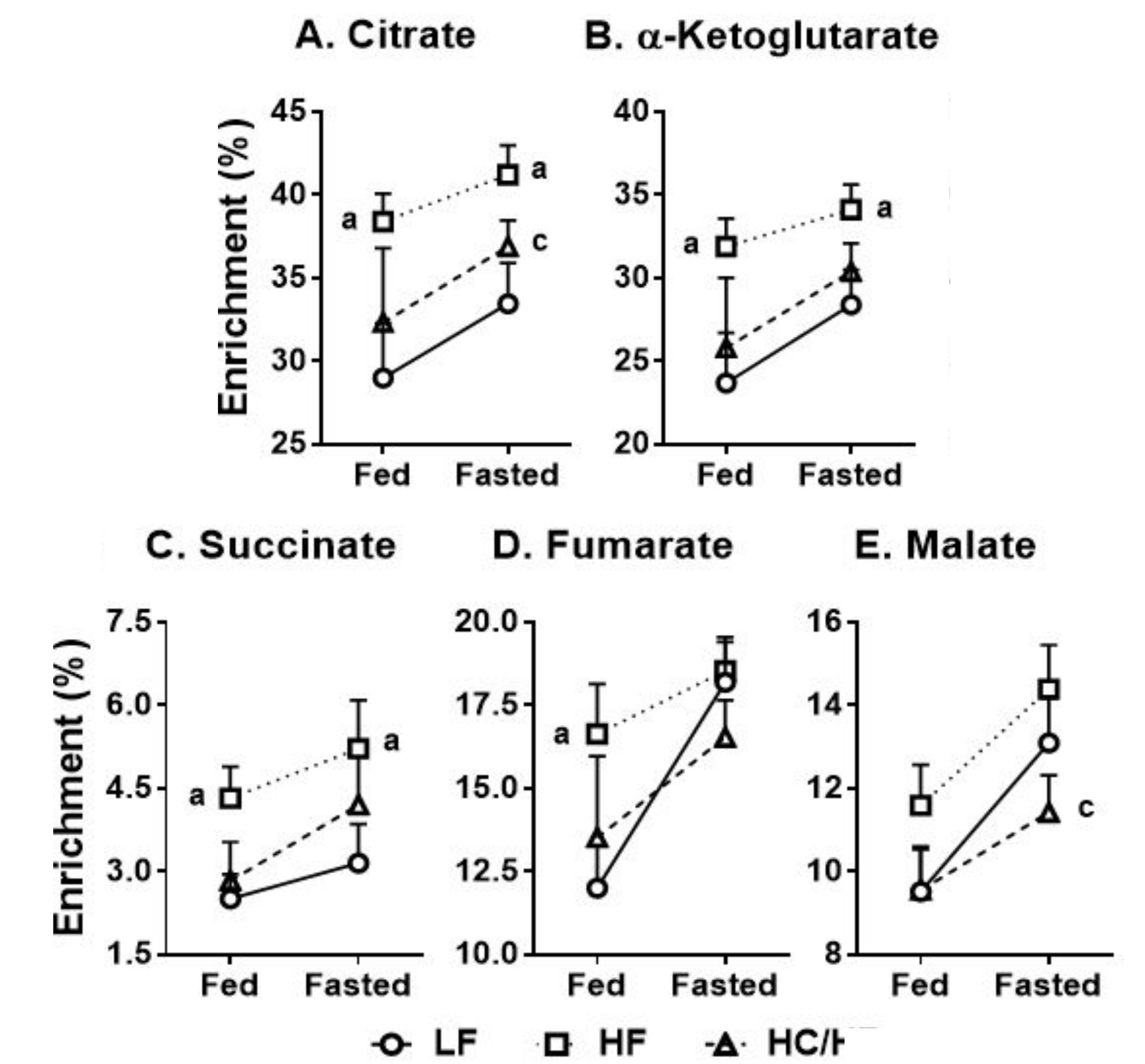
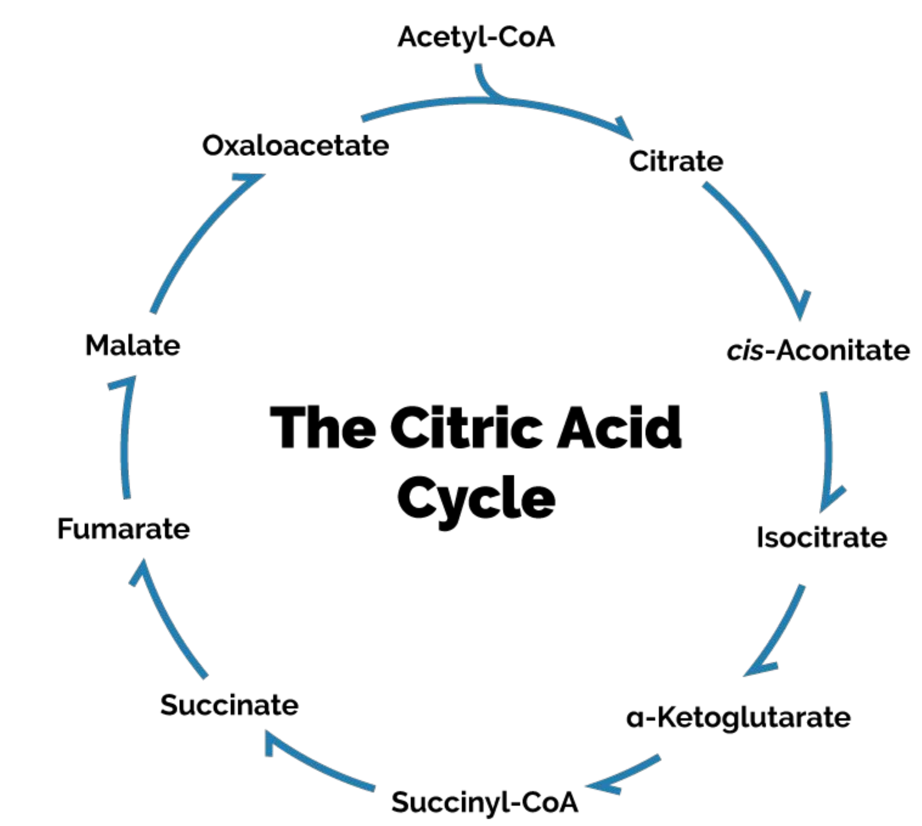


Figure 3: Organic acid enrichments of fed and fasted isolated mitochondria following 10 min. incubation in ^{13}C pyruvate. Enrichment was measured as summation of M+1, M+2, and M+3 isotopomers of each organic acid after normalization for natural abundance. Superscripts indicate $p \leq 0.05$. 'a' - LF vs. HF 'b' – LF vs. HC and 'c' – HF vs. HC.

- Overall, enrichment is higher in fed and fasted conditions of HF diet groups
  - Multiple statistically significant changes between LF and HF diet groups in various organic acids
- Change in enrichment from fed → fasted was more robust in LF and HC diet groups as shown in the steeper slope

Discussion



Simplified diagram of the citric acid (TCA) cycle denoting the major organic acids utilized.[5]

Discussion (cont.)

This preliminary investigation revealed that:

- Diet composition (LF, HF, HC) results in significant changes to organic acid concentrations and production
- LF and HC diet groups seem to exhibit robust metabolic activity compared to HF diet counterparts

As shown in the previous diagram, the organic acids selected for this study are integral to the TCA cycle, and thus, changes in concentrations or production of these molecules are likely to be indicative of TCA cycle alterations. Figure 1 suggests various changes, however, these concentrations are in whole liver tissue and many metabolic pathways may be involved. Figure 2 and 3 suggest that HC mitochondrial activity may be upregulated in comparison to HF counterparts, in both fed and fasted (metabolic stress) conditions. In fact, HC activity seems to be "rescued" to the levels of the LF control, and even beyond. While this adaptation seems to suggest a robust mitochondrial reaction, it may be maladaptive in the long-term. *High mitochondrial activity may point to decreased regulation of the TCA cycle and increased ROS production, which can lead to diseased states*. Future work includes correlation with mitochondrial respiratory function and gene expression as well as investigating the prolonged effects of high carbohydrate supplementation beyond 10 weeks.

References

1. Rinella, M. E. (2015) Nonalcoholic fatty liver disease: a systematic review. *Jama* 313, 2263-2273
2. Sunny, N. E., Parks, E. J., Browning, J. D., and Burgess, S. C. (2011) Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell metabolism 14, 804-810
3. Kim, J. A., Wei, Y., & Sowers, J. R. (2008). Role of mitochondrial dysfunction in insulin resistance. Circulation research, 102(4), 401-14.
4. Satapati, S., Kucejova, B., Duarte, J. A., Fletcher, J. A., Reynolds, L., Sunny, N. E., He, T., Nair, L. A., Livingston, K., Fu, X., Merritt, M. E., Sherry, A. D., Malloy, C. R., Shelton, J. M., Lambert, J., Parks, E. J., Corbin, I., Magnuson, M. A., Browning, J. D., and Burgess, S. C. (2015) Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. The Journal of clinical investigation 125, 4447-4462.
5. Rigby, B. (2018). The Citric Acid Cycle [JPEG]. Retrieved from https://www.climbingnutrition.com/diet/a-ketosis-biochemistry-primer/

Acknowledgments

I would like to thank Dr. Sunny for his help and guidance throughout the course of this project and for his mentorship throughout my undergraduate research career. I would also like to thank Nathan as my co-researcher throughout this project. Additionally, I would like to thank Shafeekh, Megan, Chaitra, and Vaishna for their help and support in the lab.

Lastly, I would like to thank my friends and family for their continued support in my academic and research ventures.