The Impact of Lipid Accumulation on Mitochondrial Metabolism in the Liver

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ABSTRACT

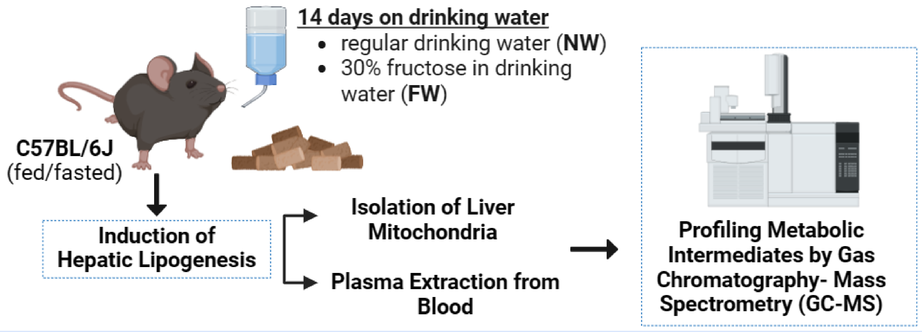
High rates of new lipid synthesis (*de novo* lipogenesis) and dysfunctional mitochondrial metabolism are central features of non-alcoholic fatty liver disease (NAFLD). However, it is not clear whether sustained induction of *de novo* lipogenesis will alter the activity of mitochondrial oxidative networks during progressive severity of NAFLD. We tested the alterations in mitochondrial metabolism in livers with high rates of *de novo* lipogenesis. Male mice (C57BL/6J) were reared on normal chow with either regular drinking water (NW) or 30% fructose in drinking water (FW) to induce lipogenesis, for 2-wks. Liver mitochondria were isolated from fed and overnight fasted (~16-hrs) mice. Mitochondria were allowed to respire at 37°C for either zero or 10-min. Changes in mitochondrial metabolites were determined via gas chromatography-mass spectrometry (GC-MS). Plasma samples were used for GC-MS based targeted metabolomics. Liver tissue was utilized to profile lipogenic gene expression. After 2-wks of treatments, lipogenic gene expression in the liver (*Acc*, *Fasn*, *Elovl6*) was significantly higher in the FW group (p≤0.05). While plasma β-hydroxybutyrate levels were induced by overnight fasting in both the groups, the fed-to-fasted fold change was significantly higher in the FW group compared to the NW group (p=0.001). Furthermore, levels of mitochondrial TCA cycle intermediates (pyruvate, citrate, fumarate) were induced with fasting in FW compared to NW (p≤0.07) after 10-mins of mitochondrial respiration. Taken together, these results suggest that the mitochondrial oxidative function is higher in livers sustaining higher rates of *de novo* lipogenesis.

INTRODUCTION

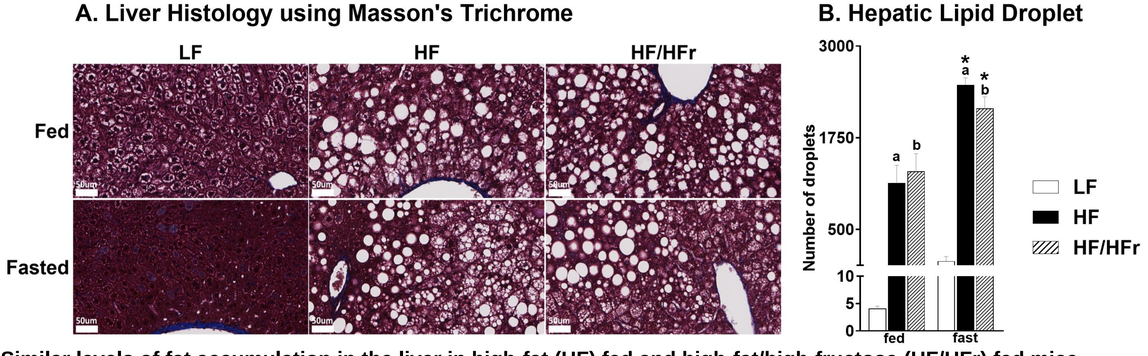
- Non-alcoholic fatty liver disease (NAFLD), characterized by excess lipid accumulation, is the most common chronic liver disease and is a common co-morbidity of obesity and type-2 diabetes mellitus [1, 2, 3].
- Dysfunctional hepatic mitochondrial metabolism has a central role in the pathophysiology of NAFLD [4,5].
- Sustained induction of hepatic *de novo* lipogenesis (DNL) is a characteristic feature of NAFLD [6].
- While both lipid-rich and carbohydrate-rich diets lead to lipid accumulation in the liver, how these dietary macroenvironments that upregulates differentially alter hepatic mitochondrial functions is not clear.

Hypothesis: Fructose-rich dietary environment will induce hepatic *de novo* lipogenesis and will alter the activity of the mitochondrial oxidative networks, contributing to the pathophysiology of NAFLD.

EXPERIMENTAL DESIGN

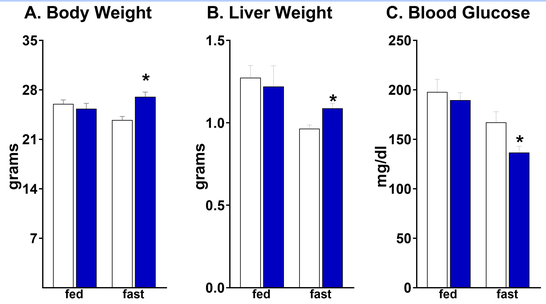


Preliminary Results: Different dietary macronutrient environment resulted in similar hepatic lipid accumulation



Similar levels of fat accumulation in the liver in high-fat (HF) fed and high-fat/high-fructose (HF/HFr) fed mice compared to the low fat-fed (LF) counterparts. (A). Liver tissue sections stained with Masson's Trichrome (40X) and **(B).** Lipid droplet count indicated elevated lipid accumulation in both high-fat (HF) fed and high-fat/high-fructose (HF/HFr) fed mice compared to low-fat (LF) fed counterparts (n=4). p≤0.05; 'a'-LF vs. HF, 'b'- LF vs. HF/HFr, 'c'- HF vs. HF/HFr; '*'- Fed vs. Fasted

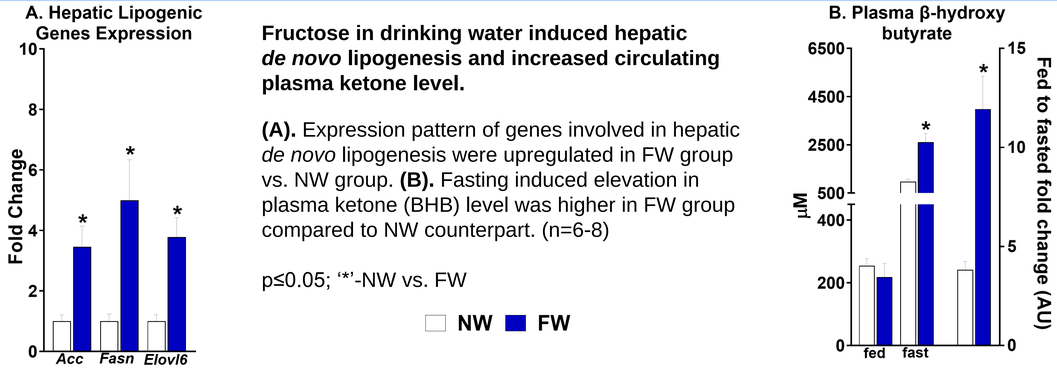
Figure 1. Metabolic characteristics of the mice following 14-days of fructose in drinking water



(A). Body weights, **(B).** Liver weights and **(C).** Blood glucose levels after 14 days of dietary treatment in fed and overnight fasted mice in NW and FW groups. (n=6-8)

p≤0.05; '*'-NW vs. FW

Figure 2. Hepatic lipogenesis and plasma ketones following 14-days of fructose in drinking water

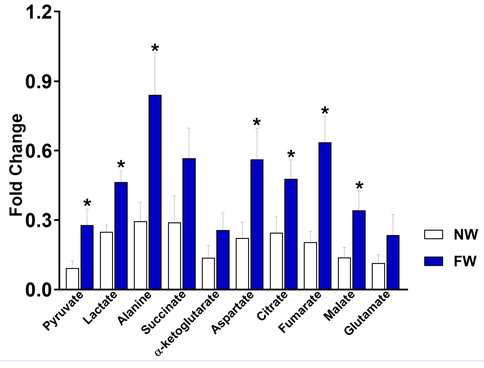


Fructose in drinking water induced hepatic *de novo* lipogenesis and increased circulating plasma ketone level.

(A). Expression pattern of genes involved in hepatic *de novo* lipogenesis were upregulated in FW group vs. NW group. **(B).** Fasting induced elevation in plasma ketone (BHB) level was higher in FW group compared to NW counterpart. (n=6-8)

p≤0.05; '*'-NW vs. FW

Figure 3. Alterations in mitochondrial intermediates following 14-days of fructose in drinking water



Induction of hepatic *de novo* lipogenesis was accompanied by elevated levels of mitochondrial metabolites in FW group.

Fed to fasted fold change of mitochondrial metabolite concentration after 10 minutes of respiration was higher in FW mice vs. NW mice. (n=6-8)

p≤0.05; '*'-NW vs. FW

CONCLUSIONS

- Both lipid-rich and fructose-rich diets contribute to lipid accumulation and NAFLD.
- Fructose-rich dietary macroenvironment upregulates hepatic *de novo* lipogenesis.
- Sustained induction of lipogenesis differentially induces mitochondrial oxidative functions, contributing to progressive severity of NAFLD.

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