The Comparative Effects of Carbohydrates and Lipids on Mitochondrial Function

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Abstract

Non-alcoholic fatty liver disease (NAFLD) and Type 2 Diabetes affect approximately 24% and 8% of the global population respectively. The Western diet, high in both carbohydrates and lipids, is believed to have a role in the development of chronic metabolic disease. Dysfunctional hepatic mitochondrial metabolism is also known to be a central feature of disordered metabolism. The objective of this study was to compare the roles of dietary carbohydrates vs. lipids in inducing dysfunctional hepatic mitochondrial metabolism. Mice, C57BL/6NJ were kept on one of three diets: low-fat control (LF; 10% kcal fat), high-fat (HF; 60% kcal fat), and high-carbohydrate (HC; 25% kcal fat and 34.9% kcal fructose) for 10 weeks. Half of the mice were randomly selected to undergo a 16 hour fast prior to sacrifice, while the other half were not fasted. Within the fasted subset, the HC diet induced significantly elevated basal mitochondrial respiration (pmol Oxygen consumed/min) when compared with HF (HF, 70.1±5.8; HC,108.6±17.4; p<.05). Among fasted mice, the HC diet also appeared to induce significantly higher maximal respiration and ATP production when compared with HF. Expression of hepatic mitochondrial complex proteins were also higher in fasted HC mice than fasted HF mice. Overall, these results suggest that the high-carbohydrate diet enhanced mitochondrial activity when compared with the high-fat diet.

Materials and Methods

C57BL/6NJ male mice were fed one of the following three diets: low-fat control (**LF**; 10% kcal fat), high-fat (**HF**; 60% kcal fat), and high-carbohydrate (**HC**; 25% kcal fat and 34.9% kcal fructose) for 10 weeks. Half of the mice were randomly selected to undergo a 16 hour overnight fast prior to sacrifice while the other half retained access to their diet. Liver samples were collected from each specimen and hepatic mitochondria were isolated. The Seahorse XF Cell Mito Stress Test was used to measure the oxygen consumption rate of isolated mitochondria. Oligomycin, FCCP, Rotenone, and Antimycin were added to mitochondrial samples during the assay. Changes in oxygen consumption rate were used to calculate basal respiration, maximal respiration, ATP production, proton leak, and coupling efficiency. A fraction of isolated mitochondria was designated for determination of mitochondrial protein content. Total mitochondrial protein was estimated using a BCA protein estimation kit. Western blotting was used to determine levels of mitochondrial protein expression.

Results

Hepatic Mitochondrial Respiration

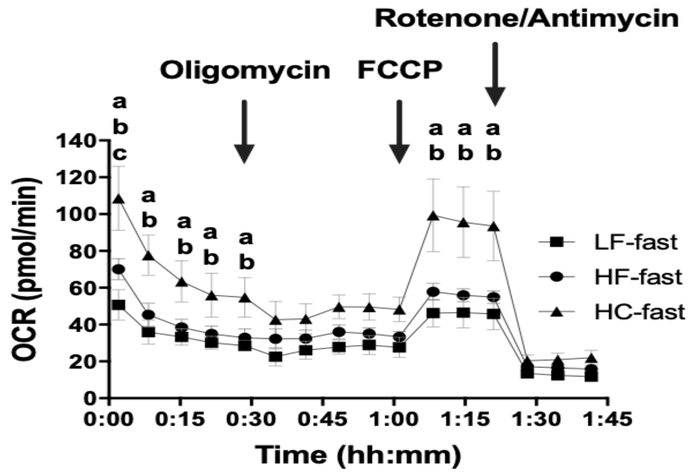

Figure 1: Dynamic mitochondrial oxygen consumption rates (n=6/group), before and after the addition of modulators, plotted with their standard error of means (SEM).

Indicators of Fasted Mitochondrial Function

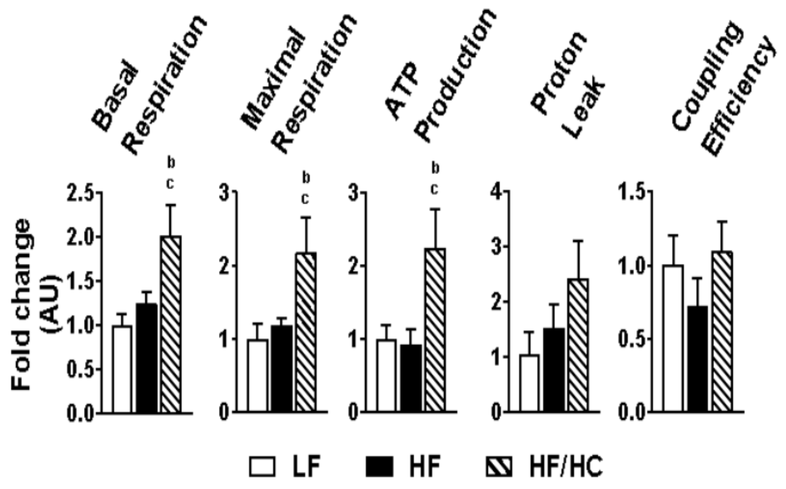

Figure 2: Average Basal respiration, Maximal respiration, ATP production, Proton Leak, and Coupling Efficiency (n=6/group) expressed as a factor of the respective LF value and plotted with their standard error means (SEM)

Fasted Hepatic COX-IV Expression

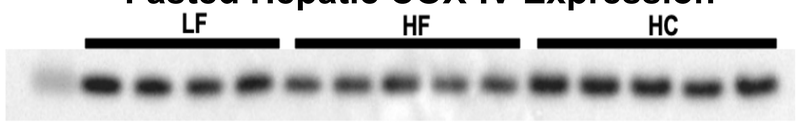

Figure 3: Western blotting for the mitochondrial protein , COX-IV, in liver samples indicated differences in fasted mitochondrial content between groups (n=4-5/group).

OXPHOS Complex Protein Expression

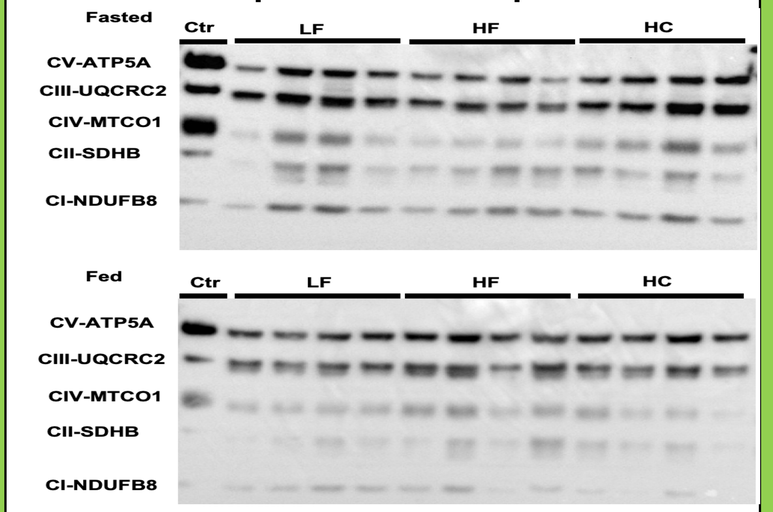

Figure 4: : Western blotting for the mitochondrial electron transport chain (ETC) complexes (n=4/group).

Summary Points

- Isolated liver mitochondria from fasted HC mice exhibited significantly higher basal respiration compared with HF and LF counterparts
- Isolated liver mitochondria from fasted HC mice have significantly elevated ATP production rates when compared with HF and LF mice
- Although fed ETC complex expression was similar between HF and HC mice, HC mice appeared to have higher ATP synthase, Complex III, and Complex IV protein expression than HF mice in response to fasting
- Comparison of hepatic Cox-IV protein expression suggests that fasted HC mice had significantly elevated mitochondrial content when compared with LF and HF mice
- **Conclusion**: HC mice appeared to have an enhanced mitochondrial response to fasting when compared with HF mice. Whether chronic induction of mitochondrial activity is beneficial for the overall metabolic health of the liver is unclear

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