A Diet High in Fat and Fructose Induces Early Hepatic Mitochondrial Aging.

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Background

Non-alcoholic fatty liver disease (NAFLD) and its more serious manifestation, non-alcoholic steatohepatitis (NASH), are recognized as one of the leading causes of liver transplantation in the US. While the molecular mechanism behind NASH is not fully understood, studies suggest that mitochondrial dysfunction and endoplasmic reticulum (ER) stress may play an important role in the progression of the disease. We studied the impact of a high fat-high carbohydrate (HFHC) diet, that induces a NASH phenotype in mice, on changes in hepatic mitochondrial morphology, function, and content.

Hypothesis

We hypothesized that mice exposed to the HFHC diet will have higher levels of ER stress markers, fibrosis, and progressive mitochondrial dysfunction.

Experimental Design

6-8 weeks old male C57Bl6/J mice were randomized to chow or high-fat-high-fructose (HF2) diet for 32 weeks. Body weights were monitored bi-weekly until 32 weeks. Plasma alanine aminotransferase (ALT) and fasting blood glucose were measured at 8, 18 and 32 weeks on diet. Liver was harvested at 8, 16 or 32 weeks to measure hepatic triglyceride, mitochondrial DNA content, gene expression and mitochondrial morphometrics using electron microscopy.

Significance

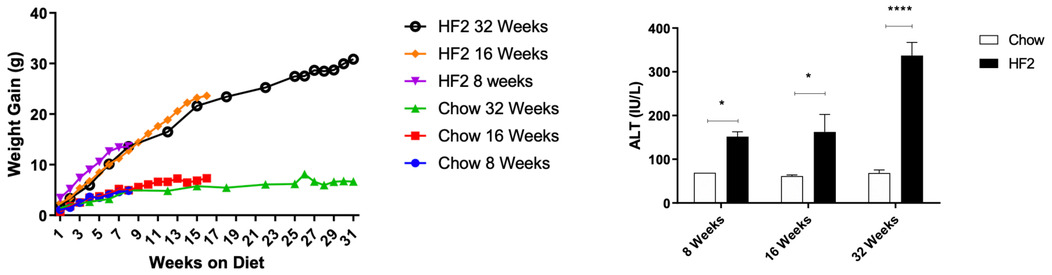
What is known?

- High fat-high fructose diet-fed mice gain body weight to develop obesity.
- High fat high fructose diet induces nonalcoholic steatohepatitis in mice.

What is new?

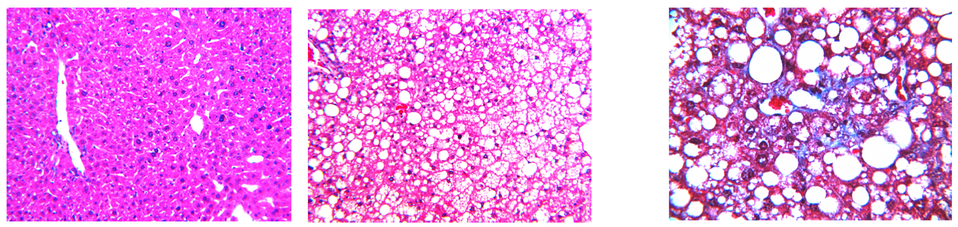
- High fat-high fructose diet promotes faster hepatic mitochondrial aging.
- High fat-high fructose diet causes depletion and dysfunction of hepatic mitochondria.

Obesity Assessment



HF2 mice gained more weight and had higher levels of ALT at 8, 16, and 32 weeks than C mice.

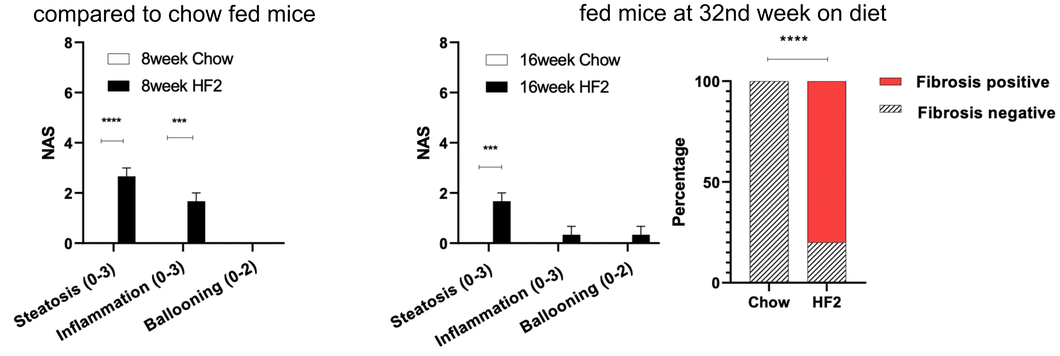
Liver Histology



Chow HF2 HF2

Representative histological image of liver section showing increased hepatic steatosis HF2 fed mice compared to chow fed mice

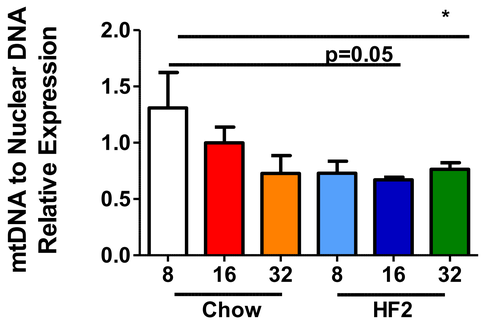
Representative Masson's Trichrome stained liver section showing hepatic fibrosis in HF2 fed mice at 32nd week on diet



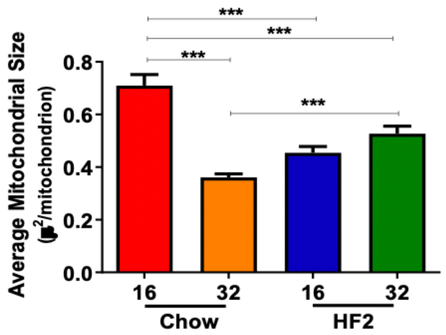
HF2 fed mice have shown progressive increase in hepatic steatosis, inflammation and ballooning score at 8th,16th and 32nd week compared to chow fed mice

80% of mice fed on HF2 diet for 32 weeks have developed hepatic fibrosis..

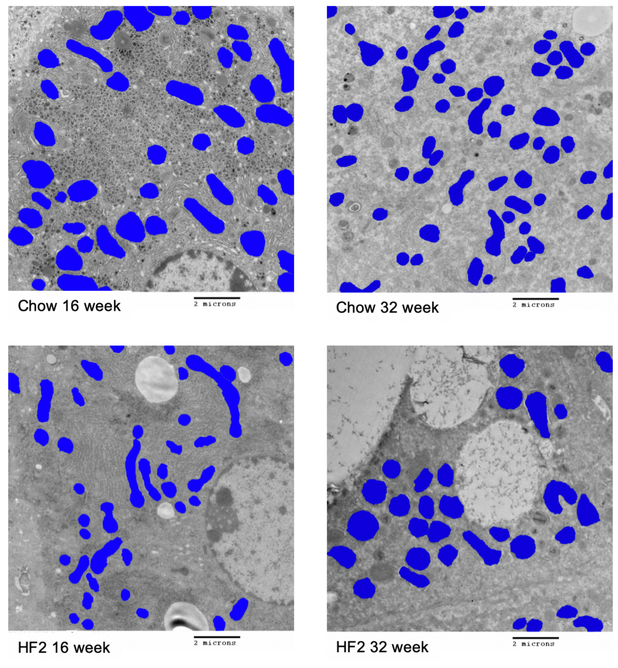
Mitochondrial DNA Content and Size



HF2 mice had lower mitochondrial DNA to nuclear DNA ratio compared to chow fed mice. Mean ±SEM. *** P< 0.001; * P<0.05.

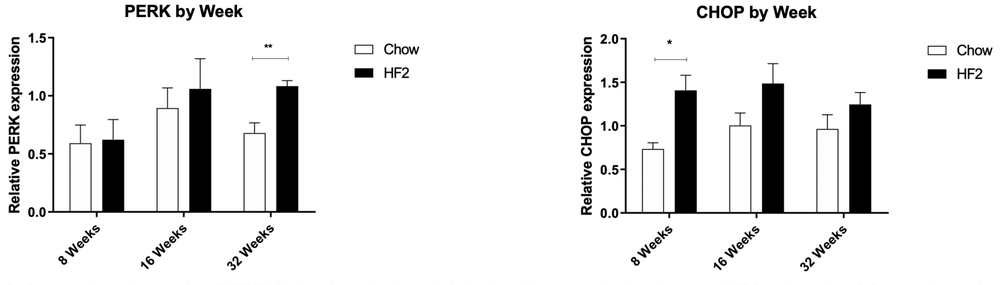


HF2 fed mice have lower average mitochondrial surface area in the liver compared to chow fed mice at 16th week of diet.



Representative Digital electron microscopy image of liver section from chow fed and HF2 fed mice at 16th week and 32nd week on diet. HFC mice had decreased mitochondrial surface area at 16 weeks in comparison to chow mice. However, HFC mice at 32 weeks show an increase in mitochondrial surface area in comparison to control mice.

Endoplasmic Reticulum Stress



6-8 weeks old male C57Bl6/J mice fed on high fat high carbohydrate (HF2) diet for 32 weeks. (A) HF2 fed mice have shown higher expression of hepatic ER stress marker PERK compared to chow fed mice at 32nd week on diet. (B) HF2 fed mice have shown higher expression of Chop, a downstream target of PERK mediated ER stress pathway at 8th, 16th and 32nd week compared to chow fed mice. Mean±SEM.** P<0.05.

Conclusions

Our HF2 diet fed mice demonstrate quick, persistent decreases in mitochondrial DNA while developing histologic findings consistent with NASH. In our model we observed an early decrease in mitochondria size. We simultaneously observed increased expression of ER stress markers in liver of HF2 fed mice. Our data demonstrate that mitochondrial dysfunction plays a key role in promoting aging of hepatic mitochondria. Deciphering the molecular mechanism triggered by HF2 diet that causes decreases in hepatic mitochondria content and mitochondrial dysfunction would provide a therapeutic option to treat NASH.

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