Dietary docosahexaenoic acid (DHA) attenuates nonalcoholic steatohepatitis (NASH) progression, but does not promote NASH remission in Ldlr−/− mice.

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**Abstract**

Nonalcoholic fatty liver disease (NAFLD) is a major public health concern. Nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD, is characterized by hepatosteatosis, inflammation, oxidative stress and fibrosis. NASH is a risk factor for cirrhosis, hepatocellular carcinoma and liver failure. We previously established that DHA (22:6,ω3) attenuated the onset of western diet (WD)-induced NASH in Ldlr−/− mice. Herein, we evaluate the efficacy of DHA to slow progression and promote remission of disease in mice with pre-existing NASH. Mice maintained on the WD for 22 wks developed mild NASH. These mice were randomized to 3 groups: (Group 1) WD-Base [equivalent to 22 wks]; (Group 2) WD + Olive oil; and (Group 3) WD + DHA. Olive oil was added to ensure isocaloric diets and DHA was at a therapeutic dose, i.e., 2% of total calories. A Control Group was maintained on laboratory chow for the duration of the study. Mice in Groups 2 and 3 were euthanized after 8 wks on the new diets. DHA had no effect on WD-induced changes in body weight, food intake, plasma glucose, TLR4 agonists or leptin. DHA, however, decreased blood lipids (triglycerides, cholesterol) and Toll-like receptor 2 agonists. Analysis of hepatic histology, lipids and gene expression established that the NASH phenotype worsened in Group 2, but not Group 3. Hepatic gene expression markers of inflammation, oxidative stress and fibrosis in the WD-Base and WD + DHA groups were comparable. While DHA supplementation increased hepatic omega-3 fatty acids, it failed to ameliorate all NASH markers. Taken together, these studies establish that DHA supplementation, at physiologically relevant levels, attenuates disease progression in mice with pre-established NASH, but does not promote NASH remission.

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**Background**

- The prevalence of diet induced NAFLD is a major U.S. public health concern. It is estimated that ~30-40% of patients with simple steatosis will progress to NASH.
- There is no optimal standard of care for treating NASH in humans. General clinical advice is weight loss and treating underlying complications (i.e. concomitant Type 2 Diabetes, high blood pressure and dyslipidemia (elevated blood cholesterol and triglycerides)).
- DHA is a long chain (22:6, ω3) fatty acid, a fatty acid with pleiotropic actions on cellular function. DHA is used clinically to treat hypertriglyceridemia.

**Figure 1: Study Design**

- Male Ldlr−/− Mice: Western Diet (WD): 22 wks
- Male Ldlr−/− Mice: Maintained on Chow (Ch) 30 weeks

**Figure 2: Food Intake**

<table>
<thead>
<tr>
<th>Gms/day</th>
<th>Calories/day</th>
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<tbody>
<tr>
<td>Chow Cont</td>
<td>WD-Base</td>
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Addition of DHA to WD had no impact on food intake. WD-fed mice consume fewer grams compared to chow fed controls, but there is no difference in calories per day between any group. *p-value ≤ 0.05 compared to chow controls.

**Figure 3: Body and Liver Weights**

- Body Weight
- Liver Weight

WD-feeding induced an increase in both liver and body weight. Addition of DHA did not impact body weight but did decrease liver weight compared to WD + O group.

**Figure 4: Plasma Parameters**

- Plasma Triglycerides
- Plasma Total Cholesterol
- TLR-2 Activation

DHA supplementation decreased plasma AST, ALT, triglycerides, cholesterol, and TLR-2 activation but did not impact blood glucose, plasma leptin or TLR-4 activation (data not shown) when compared to WD + O animals. *p-value ≤ 0.05 compared to chow controls. # p-value ≤ 0.05 compared to WD + O.

**Figure 5: mRNA Expression**

- Timp-1
- Col1a1
- Nox 2
- TLR-4

- Chow Cont | WD-Base | WD + O | WD + DHA | Chow Cont | WD-Base | WD + O | WD + DHA |

A quantitative RT2 Profiler PCR fibrosis array indicates that addition of DHA to diet of WD-fed animals decreased expression of genes associated with hepatic fibrosis. qPCR of candidate genes demonstrate that DHA halts the increase in gene expression of NASH markers, associated with continued WD feeding. *p-value ≤ 0.05 compared to chow controls. # p-value ≤ 0.05 compared to WD + O.

**Figure 6: Hepatic Lipid Content**

- Mole % of Lipid Classes
- Omega-3 & -6 Fatty Acids

WD-feeding induced an increase in hepatic MUFA while decreasing hepatic Omega-3 & -6 fatty acid content. Addition of DHA restored Omega-3 FA content significantly restoring content of hepatic DHA, DPA (22:6,ω3) and EPA (20:5,ω3). *p-value ≤ 0.05 compared to chow controls. # p-value ≤ 0.05 compared to WD + O.

**Figure 7: Liver Histology**

22 weeks of western diet feeding induced fibrosis as evidenced by blue staining in a branching pattern (WD-Base). Extended feeding (50 weeks, WD + O group) worsens fibrosis. Addition of DHA to the WD diet halts increased fibrosis, but does not reverse pre-existing fibrosis.

**Conclusions**

- WD-feeding induces robust NASH at 22 weeks and markers of NASH continue to worsen with continued WD feeding (30 weeks)
- Addition of DHA to WD after pre-established NASH decreases liver fat content, increases hepatic ω3 fatty acid content and halts the worsening on NASH markers. Dietary DHA, however, does not reverse/remove pre-existing fibrosis.