Diet and Non-alcoholic Steatohepatitis (NASH): A Risk Factor for Liver Cancer

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Oregon State University
Corvallis, OR
Outline

• Background on Primary Liver Cancer
• Background on Non-Alcoholic Fatty Liver Disease [NAFLD] and NASH
  – Link between NASH and Liver Cancer
• Prevention of NASH
  – Inflammation
  – Fibrosis
  – Metabolomics
• Is NASH Reversible?
• Wrap Up
Primary Liver Cancer

- **Hepatocellular carcinoma**
  - Most common primary liver cancer; involves hepatic parenchymal cells
  - 5th most common human cancer
  - 3rd most frequent cause of cancer death worldwide
  - http://www.cancer.gov/cancertopics/pdq/treatment/adult-primary-liver/Patient/page1#Keypoint2

- **Cholangiocarcinoma**
  - 2nd most common liver cancer; involves biliary epithelial cells
  - Accounts for ~10% of primary liver cancers
  - J Hepatobiliary Pancreat Sci In press [2014]

- **Chronic liver disease** sets the stage for dis-regulated regeneration of hepatic and biliary epithelia.

- Current treatment options are limited to surgery and drugs (sorafenib)
## Risk Factors for Hepatocellular Carcinoma

<table>
<thead>
<tr>
<th>Established risk factors of hepatocellular carcinoma</th>
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<tr>
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<tr>
<td>Male gender</td>
</tr>
<tr>
<td>Family history of HCC</td>
</tr>
<tr>
<td>Hepatitis B infection Sub-Saharan Africa</td>
</tr>
<tr>
<td>Hepatitis C infection USA &amp; Europe</td>
</tr>
<tr>
<td>Cirrhosis</td>
</tr>
<tr>
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# Risk Factors for Hepatocellular Carcinoma

## Established risk factors of hepatocellular carcinoma

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**Major risk factor of HCC**

*Source: Gastroenterology 142: 1411-1413 [2012]*
# Risk Factors for Hepatocellular Carcinoma

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<th>Likely risk factors</th>
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<tbody>
<tr>
<td>Age</td>
<td>Diabetes (⭐⭐)</td>
</tr>
<tr>
<td>Male gender</td>
<td>Obesity (⭐⭐)</td>
</tr>
<tr>
<td>Family history of HCC</td>
<td>Non-alcoholic fatty liver disease (⭐⭐)</td>
</tr>
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<th>Possible risk factors</th>
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<tr>
<td>Coffee</td>
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<tr>
<td>Micronutrients (e.g. vitamin D, E, selenium)</td>
</tr>
<tr>
<td>Red meat</td>
</tr>
<tr>
<td>White meat (fish, poultry)</td>
</tr>
<tr>
<td>Saturated fat</td>
</tr>
<tr>
<td>N-3 fatty acids</td>
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<tr>
<td>Fructose</td>
</tr>
<tr>
<td>Oral contraceptives</td>
</tr>
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<td>Cholesterol</td>
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Gastroenterology 142: 1411-1413 [2012]
Non-alcoholic Fatty Liver Disease (NAFLD)

• Most common chronic fatty liver disease in patients who consume little or no alcohol.
  – The incidence of NAFLD parallels obesity levels in US.
  – Obese patients: (estimates) 95% have NAFLD
  – NIDDM (T2DM): (estimates) 70% have NAFLD
NAFLD- NASH-Cirrhosis

A spectrum of diseases from benign fatty liver (steatosis) to non-alcoholic steatohepatitis (NASH), i.e., hepatic inflammation.

- Chronic inflammation promotes liver damage and fibrosis (scar).
- 20-30% of patients diagnosed with NAFLD develop NASH
- NASH can progress to cirrhosis, a major risk factor for primary hepatocellular cancer.

- Advanced cirrhosis, resulting from NASH, is predicted to be a major cause for liver transplants by 2020.

World Gastroenterology Organization Global Guidelines-2012
Link Between Non-Alcoholic Fatty Liver Disease (NAFLD) and Primary Hepatocellular Cancer (HCC)

**NASH:**
Non-Alcoholic Steatohepatitis

The Oncologist 15: 14 (2010); Science 332: 1519 (2011)
Gastroenterology 142: 1502 (2012)
Link Between Non-Alcoholic Fatty Liver Disease (NAFLD) and Primary Hepatocellular Cancer (HCC)

6-30% of the General Population Develop NAFLD. Incidence Parallels the Incidence of Obesity & T2DM.

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3-5% General Population Develop NASH. Hepatic Inflammation and Fibrosis.

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3-5% General Population Develop NASH. Hepatic Inflammation and Fibrosis.

10-30% of NASH Patients Develop Hepatic Cirrhosis

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3-5% General Population Develop NASH. Hepatic Inflammation and Fibrosis.

10-30% of NASH Patients Develop Hepatic Cirrhosis

2-4% of NASH Patients Develop HCC

The Oncologist 15: 14 (2010); Science 332: 1519 (2011)
Gastroenterology 142: 1502 (2012)
Hepatic Fibrosis is a Precursor to HCC

Factors Driving NAFLD Progression to NASH:

- Diet (High fat, sucrose, cholesterol)
- Body Fat & Its Distribution
- Insulin Resistance
- Inflammatory factors, like Gut-derived microbial components, e.g., Endotoxin
- Genetics

Visceral Obesity

Excess calories
Saturated fat
Fructose
Lack of satiation

↑ TNF-α
↑ IL-6
↑ CRP
↓ Adiponectin

Insulin resistance
Hepatic and systemic

1

Visceral adipose tissue

1. Excess calories
2. Saturated fat
3. Fructose
4. Lack of satiation

↑ FFA

Altered FA metabolism
De novo lipogenesis

Hepatic steatosis
Triglycerides

FA oxidation

Liver

SFA/MUFA>>>PUFA

ROS/oxidative stress
ER stress

Insulin resistance
Hepatic and systemic

Lipotoxicity

Hepatocyte injury
repair/failure of repair

Inflammation

Steatohepatitis

Fibrosis

Endotoxin

Pathway known to play significant role
Newly recognized pathway of DNL
Area of new investigation

ABC
Inflammatory factor
ABC
Fat

Benign Steatosis \(\rightarrow\) NASH

Benign Steatosis ➔ NASH

1. Excess calories
   - Saturated fat
   - Fructose
   - Lack of satiation

2. Visceral adipose tissue
   - ↑ FFA
   - ↑ TNF-α
   - ↑ IL-6
   - ↑ CRP
   - ↓ Adiponectin
   - Insulin resistance
     - Hepatic and systemic

3. SFAMUFA>>PUFA
   - Hepatic steatosis
   - ↑ Triglycerides
   - ↑ FA oxidation
   - ↑ Plasma VLDL
   - ROS/oxidative stress
   - ER stress

4. Gut-Derived Endotoxin
   - Cell wall gram (-) bacteria

5. Hepatocyte injury
   - Repair/failure of repair

6. Lipotoxicity

7. Inflammation

8. Fibrosis

9. Steatohepatitis

10. Pathway known to play significant role
11. Newly recognized pathway of DNL
12. Area of new investigation
13. ABC: Inflammatory factor
14. ABC: Fat
Benign Steatosis → NASH

Excess calories
↑ Saturated fat
↑ Fructose
Lack of satiation

↑ FFA

↑ TNF-α
↑ IL-6
↑ CRP
↓ Adiponectin

Insulin resistance
Hepatic and systemic

Liver

Pathway known to play significant role
Newly recognized pathway of DNL
Area of new investigation
ABC Inflammatory factor
ABC Fat

Hepatic Damage
Cell Injury & Repair (Fibrosis)

Hepatic steatosis

De novo lipogenesis

↑ Triglycerides

↑ FA oxidation

Altered FA metabolism

↑ Plasma VLDL

Endotoxin

ROS/oxidative stress
ER stress

Lipotoxicity

Hepatocyte injury repair/failure of repair

Inflammation

Steatohepatitis

Fibrosis

Our Focus:
Diet and Non-alcoholic Fatty Liver Disease [NAFLD]

Healthy Liver

Unhealthy Liver

DHA
Prevention of Diet-Induced NAFLD & NASH Using ω3 Fatty Acids

- Can dietary intervention prevent western diet-induced NAFLD/NASH?
  - Western diet (WD) moderately high fat, sucrose & cholesterol
  - ω3 Polyunsaturated fatty acids; ω3 PUFA

Chris Depner, Ph.D.

Eicosapentaenoic Acid
20:5, ω3; EPA

Docosahexaenoic Acid
22:6, ω3; DHA
Rationale for the use of $\omega$3 PUFA to prevent diet-induced NASH.

- $C_{20-22} \omega$3 PUFA (EPA and DHA) are pleiotropic regulators of cell function.

- $C_{20-22} \omega$3 PUFA regulate:
  - membrane function
  - nuclear function
  - cell signaling
  - gene expression
  - metabolism
Rationale for the use of ω3 PUFA to prevent diet-induced NASH.

- **NASH is a chronic inflammatory disease**

- \( \text{C}_{20-22} \omega3 \text{ PUFA are:} \)
  - Anti-inflammatory
  - Inhibit fatty acid synthesis
  - Induce triglyceride catabolism
  - Induce fatty acid oxidation

- Lowers blood triglycerides
  - \( \text{(Lovaza}^{\circledR}, \text{GSK, [EPA + DHA]}) \)
  - Human dose: 1-2% total calories/d

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**Potential to Lower Hepatic Lipid**

**GRAS & Established Hypolipemic Nutrient/Drug**
We developed a mouse model of NASH that recapitulates many of the clinical features of human NAFLD/NASH.

- \(LDLR^{-/-}\) mouse + the “Western Diet”

- Clinical features included:
  - hepatosteatosis
  - ballooning hepatocytes
  - Inflammation
  - oxidative stress
  - fibrosis
Distribution of Calories

Western Diet

- 43% Carbohydrate
  - Sugar > complex CHO
- 42% Fat
  - SFA >>> PUFA
- 15% Protein

Cholesterol: > 300 mg/day

Red Meat
- Processed Meat
- Fried Food (French Fries)
- High-Fat Dairy Products
- Refined Grains
- Sweets & Desserts
- Sugary Beverages
American Heart Assoc. & American Diabetes Assoc. Dietary Recommendations

**Distribution of Calories**

**Western Diet**
- **43% Carbohydrate**
  - Sugar > complex CHO
- **15% Protein**
- **42% Fat**
  - SFA >>> PUFA

**American Heart Assoc. & American Diabetes Assoc. Dietary Recommendations**
- **50% Carbohydrate**
  - Complex CHO >> Sugar
- **15% Protein**
- **<35% Fat**
  - SFA = MUFA = PUFA

**Cholesterol:**
- **>300 mg/day**
  - Red Meat
  - Processed Meat
  - Fried Food (French Fries)
  - High-Fat Dairy Products
  - Refined Grains
  - Sweets & Desserts
  - Sugary Beverages

- **<300 mg/day**
  - Lean Meats (Poultry)
  - Oily Fish (ω3 PUFA)
  - Vegetables
  - Whole Grains
  - Low Fat Dairy Products
  - Fruit
The NASH Model

Lean LDL-R-/- Male Mice

Diets: Chow (Purina 5053) & Western Diet (WD)
WD is supplemented with olive oil, EPA and/or DHA; supplemental fats are 2% energy; all WD are isocaloric.

Obese Mice LDL-R-/-

Overnight Fast

Blood, Urine, Liver

N=8; Chow group 16 weeks

4 WD Groups
1. Olive
2. EPA
3. DHA
4. EPA + DHA
N=8/group 16 weeks

Lean
28 ± 2 g

Obese
42 ± 2 g
Markers of Metabolic Syndrome and NASH

• Anthropometric: body weight & composition (%fat)

• Plasma markers of metabolic syndrome (MetS) and NASH
  – Lipids (cholesterol/TAG) and glucose
  – Hepatic damage (ALT/AST)
  – TNF\(\alpha\), Endotoxin and TLR4 ligands

• Hepatic markers of NASH
  – Histology
  – Gene expression markers: inflammation, oxidative stress, fibrosis
  – Specific nuclear proteins
    • e.g., NF\(\kappa\)B, ChREBP/MLX, PPAR\(\gamma\), Smads, Nrf2
  – Metabolomic analysis (Metabolon, Inc.)

• Urinary markers of oxidative stress: oxidized derivatives of PUFA
  – Isoprostanes: IsoP2, IsoP3, NeuroP4

Inflammation Induced by the Western Diet

Normal Liver

Fatty & Inflamed Liver

Leukocyte Infiltration

H/E Stain
Fibrosis Induced by the Western Diet

Normal Liver

Fatty & Fibrotic Liver
Branching Fibrosis

Trichrome Stain
Key Outcomes

Western Diet + Olive

- Body Weight & Fat Mass
- Hepatosteatosis (SFA/MUFA/Chol)
- Oxidative Stress: NOX2
- Inflammation: CD68/TLR & NFκB-p50
- Fibrosis: proCol1A1, nuclear P-Smad3
  Trichrome stain
Key Outcomes

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<th>Western Diet + Olive</th>
<th>Western Diet + DHA</th>
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<td>Body Weight &amp; Fat Mass</td>
<td>↑</td>
<td>No Change from WD + O</td>
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Key Outcomes

### Western Diet + Olive vs. Western Diet + DHA

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**Inflammation:** DHA = EPA

**Fibrosis:** DHA > EPA
Hepatic Inflammation & NASH

- Controlling hepatic inflammation & fibrosis are key targets for preventing NASH progression.

- Consequence of chronic-inflammation:

  hepatic damage → fibrosis → cirrhosis → hepatocellular cancer

- No FDA-approved therapies for NASH-associated inflammation or fibrosis.
Hepatic Inflammation & NASH

• Sources of inflammatory factors:
  – Adipose tissue
    • Inflammatory cytokines: TNF\(\alpha\), IL6, Leptin
    • Insulin sensitizer: Adiponectin
  – Innate immune cells, e.g., Kupffer & monocytes/macrophage
  – Metabolism: advanced glycation end products derived from glucose
  – Damaged/dead cells; cellular debris
  – Gut: bacterial components,
    • e.g., endotoxin (cell wall component of gram (-) bacteria)

• Mediators of inflammation
  – Toll-like receptors (TLR2, TLR4, TLR9, etc)
  – Inflammatory cytokine and chemokine receptors; cell signaling
  – Inflammatory lipids (oxidized fatty acids and cholesterol)
    • Receptor mediated mechanisms
    • Non-receptor mediated mechanisms
Toll-Like Receptor 4 (TLR4) Plays a Key Role in Diet-Induced Hepatic Inflammation


The Western Diet Induces Hepatic TLR Components

**mRNA Abundance Fold Change**

- **TLR-2**
- **TLR-4**
- **TLR9**
- **CD-14**
- **MD-2**
- **MYD88**

**Groups:***
- NP
- WD + O
- WD + E
- WD + D
- WD + ED

**Comparisons:**
- * P<0.05 versus Chow
- # P<0.05 versus WD + O

**Notes:**
- Required for Endotoxin Activation of TLR4

*(TLR4)*
Nuclear Abundance of $\text{NF}_{\kappa}B$ Subunits

Fold of NP-Fed Mice

- $\text{NF}_{\kappa}B$-p50
- $\text{NF}_{\kappa}B$-p65
- $\text{NF}_{\kappa}B$-p105

Nuclear Abundance of $\text{NF}_{\kappa}B$ Subunits

mRNA Abundance (Precursor of p50)

NP
WD + OO
WD + EPA
WD + DHA
WD + EPA + DHA

Hepatic Inflammation Markers

NP = Chow (Purina 5053)

Key Cellular & Plasma Factors Involved in Hepatic Inflammation

Chemokine
Cell Surface Markers
Inflammatory Cytokines
Inflammasome
Plasma Proteins Made by liver

mRNA Abundance
Fold Change
The Western Diet Induces Hepatic Inflammation

What are the signals?
Does Western Diet Increase Blood Endotoxin?

- **WESTERN DIET**
- **LPS/Endotoxin**
- **CD14**
- **PI**

- **TLR**
- **MyD88**
- **p50**
- **p65**
- **lκB**
- **K+ efflux, ROS lysosomal damage**
- **NLRP3**
- **ASC**
- **pro-caspase-1**
- **caspase-1**
- **pro-IL-1β**
- **pro-IL-18**
- **IL-1β**
- **IL-18**
- **Inflammasome**
- **ATP**
- **P2X7**
- **Pannexin-1**
- **NOX**

**Diagram Details:**
- LPS/Endotoxin binding to CD14 activates the innate immune system.
- Signal 2 leads to the activation of NLRP3 inflammasome.
- NLRP3 inflammasome converts pro-IL-1β and pro-IL-18 to their active forms.
- Activated caspase-1 leads to the release of IL-1β and IL-18.
- Lysosomal damage and K+ efflux are involved in the process.
- NOX is involved in the production of reactive oxygen species (ROS).
- Western diet influences the expression and function of these key players, potentially increasing blood endotoxin levels.
Plasma Endotoxin (LPS)

Western Diet

Plasma Endotoxin (LPS)

* P<0.05 versus Chow
# P< 0.05 versus WD + O
TLRs Function within Membrane Lipid Rafts

- **LPS/Endotoxin**
- **CD14**

**DHA** is assimilated into membrane phospholipids.

DHA disrupts lipid raft structure & TLR4 signaling.
Western Diet Effects on Blood Endotoxin and Hepatic Inflammation
DHA Regulates the Cellular Inflammation at Multiple Levels

WESTERN DIET → DHA → LPS/Endotoxin → CD14 → PI → TLR → MyD88 → NF-κB-p50

DHA Suppresses Nuclear NFκB-p50

K+ efflux, ROS lysosomal damage → NLRP3 inflammasome → caspase-1 → pro-IL-1β → IL-1β

NOX → DHA
DHA Attenuates the Cellular Response to Inflammatory Stimuli!

- **WESTERN DIET**
- **LPS/Endotoxin**
- **CD14**
- **PI**
- **NOX**

DHA does not attenuate plasma Endotoxin (LPS) or TLR4 Agonist.

DHA Suppresses Nuclear NFκB-p50.

DHA suppresses plasma cytokines (IL-1β, IL-8).
The Importance of Controlling NASH & Hepatic Fibrosis

NASH-Fibrosis is a Precursor to Cirrhosis.

Primary hepatocellular carcinoma (HCC) develops in >70% of patients with chronic liver disease, i.e., cirrhosis. [Sanyal et al, The Oncologist 15: 14-22 (2010)]
Fibrosis Induced by the Western Diet
DHA Suppresses WD-Induced Fibrosis

Collagen Subtypes

mRNA Abundance-Fold Change

- Col1A1
- Col1A2
- Col4A1
- Col7A1
- TIMP-1

Chow
WD + O
WD + E
WD + D
WD + ED

TGFβ1

* * # * #
Metabolomic Analysis
[Metabolon, Inc.]

Goal:
To identify metabolites linked to WD-induced hepatic damage.
Metabolomic Analysis of Mouse Liver -Heat Map-

- Total metabolites analyzed: 524
  - Known: 320, Unknown: 204

Known Metabolites Sorted by Fold-Change Relative to Metabolites in Chow-Fed Mice
Metabolomic Analysis of Mouse Liver -Heat Map-

Chow versus WD + Olive

-5.0  0.0  5.0

WD + Olive
WD + EPA
WD + DHA
WD + EPA + DHA

Xenobiotics
Vitamins
Nucleotide
Lipid
Peptide
Energy
Carbohydrate
Amino Acid

Total metabolites analyzed: 524
- Known: 320, Unknown: 204

Known Metabolites Sorted by Fold-Change Relative to Metabolites in Chow-Fed Mice
Metabolites Affected by WD and Reversal by WD + DHA

**LIPIDS**
136 Biochemicals
- Increase: 62
- Decrease: 43
- No Change: 31

**CARBOHYDRATES**
34 Biochemicals
- Decrease: 15
- No Change: 19

**AMINO ACIDS**
78 Biochemicals
- Increase: 40
- Decrease: 27
- No Change: 11

**VITAMINS & COFACTORS**
16 Biochemicals
- Increase: 4
- Decrease: 9
- No Change: 3
Heat Map of Lipid Metabolites

**C_{20-22} \omega 6 PUFA**

**C_{20-22} \omega 3 PUFA**

**C_{18-20} SFA, MUFA & Sphingolipids**

- Palmitoyl Sphingomyelin
- Oleic Acid

- WD + Olive
- WD + EPA
- WD + DHA
- WD + EPA + DHA
Heat Map of Lipid Metabolites

- Saturated (SFA, MUFA)
- N-3 PUFA (ω-3)
- N-6 PUFA (ω-6)
- Pal-Sphingomyelin

Western Diet
- WD + Olive
- WD + EPA
- WD + DHA
- WD + EPA + DHA

C_{18-20} SFA, MUFA & Sphingolipids
C_{20-22} ω-6 PUFA

Bar Graph:
- Fatty Acid Class (μmoles/g protein)
- Ch, Ol, EPA, DHA, EPA + DHA

Box Plots:
- Pal-Sphingomyelin
- Ch, Ol, EPA, DHA, EPA + DHA
Hepatic Sphingomyelin is Associated with Inflammation, Oxidative Stress & Fibrosis

- MCP1 mRNA
- ProCol1a1 mRNA
- TRL4 mRNA
- NOX2 mRNA

$r^2 = 0.7$, $P < 0.0001$
$r^2 = 0.58$, $P < 0.0001$
$r^2 = 0.57$, $P < 0.0001$
$r^2 = 0.47$, $P < 0.0001$
**Linkage Between Hepatic Sphingomyelin & TRL4 Function**

- Sphingomyelin (SM) is a membrane sphingolipid

- SM is also a component of membrane microdomains (lipid rafts); Lipid rafts consists of SM, cholesterol, and phospholipids with saturated fatty acyl chains:
  - *TLR-4 functions within lipid rafts*

- *DHA disrupts optimal lipid raft-dependent clustering of proteins involved in cell signaling.*

Prostaglandin, Leukotrienes & Essential Fatty Acid 82: 159-164 (2010); J Immunol 187: 1529-1535 (2011)
DHA>EPA May be Useful in the Prevention of NASH
Is NASH Reversible?

Kelli A. Lytle, R.D.
Ph.D. Candidate
**Clinical Approach**

- **Manage Diet & Body Weight**
  - Dietary management & exercise to promote weight loss
  - Bariatric surgery

- **Treat co-morbidities associated with NASH:**
  - Obesity
  - T2DM, Insulin Resistance, Metabolic Syndrome (MetS),
  - Hyperlipidemia

- **Drug/Diet Treatments:**
  - Manage blood lipids: triglycerides (fibrates/ω3 PUFA) and cholesterol (statins)
  - Manage blood glucose
    - Glucophage/metformin, Glucagon-like peptide-1 agonist (Dulaglutide/E.Lilly)
    - Thiazolidinediones: PPARγ agonist
  - Anti-oxidants: Vitamin E
  - Hepatic inflammation (no FDA approved clinical therapy)
  - Hepatic fibrosis (no FDA approved clinical therapy)
**Clinical Approach**

- **Manage Diet & Body Weight**
  - Dietary management & exercise to promote weight loss
  - Bariatric surgery

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Can a diet low in sucrose, fat and cholesterol reverse WD-Induced NASH?

Lean \(\text{LDL-R}^-\)-Male Mice, 21 g

- **Western Diet (WD), 16 wks.**

Obese Mice \(\text{LDL-R}^-\)

- **Western Diet, 8 wks.**
- **Chow Diet, 8 wks.**

Chow Diet, 24 wks.

Fasted Overnight Collect Blood and Liver
**Key Outcomes**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Western Diet</th>
<th>Western Diet + DHA</th>
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<tbody>
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What Have We Learned?

• The Western Diet (WD) induces a robust NASH phenotype in *LDLR*\(^{-/-}\) mice

• **DHA > EPA** attenuates WD-induced NASH:
  - *EPA* is anti-inflammatory, but not anti-fibrotic
  - *DHA* is both anti-inflammatory and anti-fibrotic
  - Neither EPA nor DHA regulate body weight or blood levels of glucose or endotoxin

• A diet low in saturated fat, sucrose and cholesterol reverses many (but not all) WD-induced NASH markers.
  - Partial reversal of hepatosteatosis and fibrosis.
  - Plasma parameters are not an accurate measure of hepatic lipid content or fibrosis.
  - Better plasma markers of liver status are required.
A Recommendation

• Obese adults and children are at risk for NASH
  – A diet low in fat, sucrose and cholesterol
  – Diet enriched in DHA

  – Compliance remains a problem with dietary management.
Dietary Management Can Prevent and Reverse NAFLD-NASH.

Add:
DHA @ 2% total Calories

Reduce Dietary:
Sucrose
Saturate/Trans Fat
Cholesterol
Whether dietary management, alone, can reverse cirrhosis or HCC remains an unanswered question.
Personnel

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Research Support

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U.S. Department of Health and Human Services

National Institute of Diabetes and Digestive and Kidney Diseases

DK 43220; DK 094600
Healthy → NASH → Healthy

Questions

DHA

Healthy diet: vegetables, fish

Unhealthy diet: fried food, soda

Obesity

Mice models