NASH
Bench to Bedside

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NonAlcoholic Fatty Liver Disease

• Common
  ~1/4-1/3 US adults

• Outcome highly variable
  Course indolent in most ("winners")
  but deadly in some ("losers")
  Prevalence => significant cause of cirrhosis

• Differentiating "winners" from "losers"
  difficult, but essential
  Health resource utilization realities
  Goals = alleviate suffering,
  prevent premature deaths
Better understanding of NAFLD pathogenesis will:
- permit differentiation of winners and losers
- suggest treatments to improve outcomes
Work at the Bench

Lessons

Bedside

Improve
Diagnosis
Treatment
NonAlcoholic Fatty Liver Disease

NAFL

Hepatocyte Fat

NASH

Hepatocyte Death

Cirrhosis

Stellate Cell Activation
NAFLD Hallmark
Triglyceride Accumulation in Hepatocytes
Why doesn’t NASH occur in everybody who has NAFL?
Lesson #1

All fat is not created equal

- High fat diets
- Peripheral insulin resistance
- Obesity
- Leptin deficiency/resistance

Fat IN

Triglyceride

Fat OUT

- MCD diet
- B-oxidation defects
- Abetalipoproteinemia

Toxic
Lipotoxicity

Mechanisms

• Direct cytotoxicity
  Lipid peroxidation

• Altered lipid signaling
  Nuclear hormone receptors (PPARs, HNF4α)
  Kinase cascades (PI3K)

• Cell stress
  ER (lipid retention)
  Oxidative (lipid metabolism)
  Metabolic (ATP depletion)
# Lesson #1

All fat is not created equal

<table>
<thead>
<tr>
<th>Hi Fat Diet*</th>
<th>MCD Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Increased FA input</td>
<td>• Decreased mitochondrial FA oxidation (less ATP) &amp; lipoprotein secretion (more ER stress)</td>
</tr>
<tr>
<td>• Mitochondrial function relatively preserved (few ROS, more ATP)</td>
<td>• TG synthesis crucial but difficult b/o ATP depletion</td>
</tr>
<tr>
<td>• Efficient &amp; sustained TG synthesis</td>
<td>• More reliance on cyps + peroxisomes for FA metabolism (more ROS)</td>
</tr>
<tr>
<td>• Less reliance on other oxidative metabolism (less ROS) &amp; lipid export (less ER stress)</td>
<td>• More FFA (altered signaling and lipid peroxidation)</td>
</tr>
</tbody>
</table>

*obese, leptin-R

*Hi Fat Diet*: obese, leptin-R

*MCD Diet*: decreased mitochondrial FA oxidation (less ATP) & lipoprotein secretion (more ER stress)
Clinical Predictions
Lesson #1
All fat is not created equal

• Increased “input” of FFA to liver is relatively harmless as long as most of them can be stored as TG

• Problems develop when:
  - there is a chronic demand for increased FFA oxidation, especially by non-mitochondrial routes (microsomes, peroxisomes) and/or
  - excessive FFA accumulate
Clinical Predictions
Lesson #1
All fat is not created equal

• Increased “input” of FFA to liver is relatively harmless as long as most of them can be stored as TG

• Problems develop when:
  - there is a chronic demand for increased FFA oxidation, especially by non-mitochondrial routes (microsomes, peroxisomes)
  - excessive FFA accumulate

Markers for “losers”?
Lesson #2
Success is fragile

Fat IN
脂肪进入

Fat OUT
脂肪出去

Triglyceride
甘油三酯

db/db
db/db

db/db + MCD
db/db + MCD

Worse Steatosis!

图示比较了db/db和db/db + MCD在4周和8周时的Triglycerides含量。MCD处理组的Triglycerides含量显著增加，表明脂肪积累更严重。
Worse Hepatic Steatosis despite less obesity, lower serum FFA & improved diabetes

* P < 0.05
NAFLD
Associated with Metabolic Syndrome

Visceral Obesity
Insulin Resistance
Dyslipidemia

Adipokine-mediated
Chronic Inflammatory State
What happened?

Hepatocytes

DMEM

- Fat accumulation (Oil Red O)
- Viability (cck-8)
- Proliferation (BrdU)
- Apoptosis (Caspase 3/7)

MCD-DMEM
Primary hepatocytes become fatty in MCD medium
1\textsuperscript{0} Hepatocytes Cultured in MCD Medium
Less Viable & More Apoptotic within 24 Hours

![Bar graph showing fold change in cck-8, BrdU, and caspase 3/7 for Reg and MCD conditions.](chart.png)

- cck-8: * (MCD higher than Reg)
- BrdU: MCD higher than Reg
- caspase 3/7: * (MCD significantly higher than Reg)
### Worse NASH in MCD-Fed db/db Mice

#### Lobular Inflammatory Grade

<table>
<thead>
<tr>
<th></th>
<th>4 week</th>
<th>8 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MCD</td>
<td>0.875</td>
<td>1.43</td>
</tr>
</tbody>
</table>

- **0**: no inflammatory foci per 20x field
- **1**: 1-2 inflammatory foci per 20x field
- **2**: 3-4 inflammatory foci per 20x field
- **3**: >4 inflammatory foci per 20x field
Higher Serum Markers of Liver Injury in db/db MCD-Fed Mice

![Graph showing AST and ALT levels in control and MCD-fed mice](image-url)
Clinical Predictions
Lesson #2
Success is fragile

NAFL

Hepatocyte Fat

Overwhelm
safe FFA disposal

Lipotoxicity
↑ ROS
↓ ATP

NASH

Hepatocyte Death
Clinical Predictions

Lesson #2
Success is fragile

NAFL

Hepatocyte Fat

NASH

Hepatocyte Death

Therapeutic targets?
(Adiponectin, Insulin sensitizers)

Overwhelm
safe FFA disposal

Lipotoxicity
↑ ROS
↓ ATP

Therapeutic targets?
(Antioxidants, cytoprotectants)
Why does NASH cause cirrhosis?
No cell is an island

(We get by with a little help from our friends)
Dying Primary Hepatocytes Produce Transforming Growth Factor β

Expression of hepatic growth inhibitor/stellate cell activator

* p < 0.05
Liver Injury Changes HSC Phenotype

Normal Liver

Healthy Hepatocytes

Adipocytic Stellate Cell

Myofibroblastic Stellate Cells

Liver Injury

Injured/Dying Hepatocytes

TGFβ-1

Deposition of Scar Matrix
Loss of Fenestrae
Kupffer Cell Activation

From Scott L. Friedman (2000) JBC 275:2247-2250
More Liver Fibrosis in MCD-Fed db/db Mice

Hydroxyproline Content

- Control
- MCD

8w
MCD diets \( \uparrow \) steatosis & induce steatohepatitis + fibrosis in db/db mice

- Control
- MCD 4 weeks
  - More Fat
- MCD 8 weeks
  - Necroinflammation
  - Fibrosis
Myofibroblastic HSC
Unfriendly to Hepatocytes

Injured/Dying Hepatocytes

Lipid Depleted
Fibroblastic

DGAT1 DGAT2

mRNA (fold change)

TGFB

Adipokines

Adiponectin Resistin

Myofibroblastic Stellate Cells

Injured/Dying Hepatocytes

Modified from SL Friedman JBC (2000) 275:2247-50
## Human Data

Multiple Logistic Regression of Factors Associated with **NASH** Compared to **Matched Controls**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>6.1</td>
<td>2.0-18.9</td>
<td>.001</td>
</tr>
<tr>
<td>(per 5 ug/mL decrease)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>2.0</td>
<td>1.2-3.3</td>
<td>.004</td>
</tr>
<tr>
<td>(per pg/mL increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTNFR2</td>
<td>5.6</td>
<td>1.5-21.4</td>
<td>.01</td>
</tr>
<tr>
<td>(per ng/mL increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.4</td>
<td>1.0-1.9</td>
<td>.03</td>
</tr>
<tr>
<td>(per Unit increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>2.7</td>
<td>1.1-6.9</td>
<td>.04</td>
</tr>
<tr>
<td>(per 0.1 unit increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Hui, et al., Hepatology, 2004*
Myofibroblastic HSC Unfriendly to Hepatocytes

- Myofibroblastic HSC
- Unfriendly to Hepatocytes

Healthy Hepatocytes vs. Injured/Dying Hepatocytes

- Adipocytic Stellate Cells
- Myofibroblastic Stellate Cells

Hepatotrophic Factors
- EGF
- HGF
- IL6

Adipokines
- Adiponectin
- Resistin

Lipid Depleted Fibroblastic

Modified from SL Friedman JBC (2000) 275:2247-50
Clinical Implications
Lesson #3
No cell is an island

Healthy Hepatocytes → Adipocytic Stellate Cell → Trophic Factors

Injured/Dying Hepatocytes → TGFβ-1

Trophic Factors

Deposition of Scar Matrix
Loss of Fenestrae
Kupffer Cell Activation
Why doesn’t everybody with NASH develop cirrhosis?
Net Liver Damage

Hepatocyte Death

Regeneration
Every Cloud Has a Silver Lining
Progenitor Accumulation Increases with Fibrosis Stage

Progenitors in steatosis

Progenitors in cirrhosis

# Ov-6(+) cells/field

p<0.05

Fibrosis Stage
Proximity of Liver Epithelial Progenitors and HSC in Injured Adult Liver

Cell Nuclei
Epithelial progenitors
Stellate Cells

Merged Images
HSC-Derived Factors
Promote Epithelial Progenitor Growth

Cell Cultures

Chol*  HSC*  HSC

Proliferation, apoptosis

*HSC = HSC-8B (Clonal, myofibroblastic)
Chol = Immature bile ductular cell line
Epithelial Growth Increased by Co-Culture with Myofibroblastic HSC

Cell Viability

Cell Proliferation

Chol
Chol with HSC 8B

Chol
Chol with HSC 8B

* *
Blocking Stellate Cell-Derived Growth Factors Reduces Viability of Co-cultured Epithelial Cells

Cell Apoptosis

Shh-Neutralizing Antibodies
Lesson #4
Life is Complex

Stellate Cells are NOT all bad

Myofibroblastic HSC Promote Liver Repair

• Remodel stroma/matrix (PAI-1, collagen, MMPs, TIMPs)
• Debride damaged epithelia
• Produce growth factors for epithelial progenitors (Hepatocyte replacements)
• Reconstruct damaged liver

Injured/Dying Hepatocytes

Myofibroblastic Stellate Cells

Deposition of Scar Matrix

Loss of Fenestrae

Kupffer Cell Activation
Clinical Implications
Lesson #4
Life is Complex

Cirrhosis

What happens to repair if we kill activated HSC?
Thank you
Work at the Bench

Lessons

Bedside

Improve Diagnosis Treatment