Non-alcoholic fatty liver disease

Pathogenesis

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NAFL – steatosis
- mechanisms
- consequences

NASH
When does NAFL become NASH
Why does NAFL become NASH

- multiple factors causing metabolic/ cellular stress and injury
- multiple causes for initiation and perpetuation of hepatic inflammation
- my tentative / provisional theory
• Chronic Disequilibrium

Energy in > energy out

Excess calory
Quality
- n-3 PUFA
- SFA vs MUFA/PUFA
- fructose (corn syrup)
- ....

Physical activity
Basal metabolism
Thermogenesis

Storage capacity in safe depots
Steatosis - mechanisms

Diet
AT Lipolysis

VLDL

β-oxidation

de novo lipogenesis

IR

Glucose

Chronic inflammation

Diet
AT Lipolysis

IR

VLDL
Steatosis
Rapid rise in NAFLD prevalence supports the role of environmental factors

**Fructose consumption as a risk factor for non-alcoholic fatty liver disease**

Xiaosen Ouyang¹,†, Pietro Cirillo¹, Yuri Sautin¹, Shannon McCall², James L. Bruchette², Anna Mae Diehl³, Richard J. Johnson¹, Manal F. Abdelmalek³,*


**Soft drink consumption is associated with fatty liver disease independent of metabolic syndrome**

Ali Abid¹,², Ola Taha², William Nseir², Raymond Farah³, Maria Grosovski⁴, Nimer Assy¹,⁵,*

Journal of Hepatology 51 (2009) 918–924

Sucrose = 50% fructose
High fructose corn syrup = up to 90% fructose
ATP consumption leading to mitochondrial dysfunction and decreased beta-oxidation
Table 2
Dietary constituents in non-alcoholic fatty liver disease (NAFLD) patients with or without metabolic syndrome (MS) and controls and the sources of added sugar during two 7-day periods at the beginning and at the end of the study.

<table>
<thead>
<tr>
<th>Dietary constituents†</th>
<th>NAFLD with MS, n = 29</th>
<th>NAFLD without MS, n = 31</th>
<th>Healthy controls, n = 30</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of excessive soft drinks (&gt;50 g/d of added sugar)*</td>
<td>81%</td>
<td>79%</td>
<td>17%</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of soft drinks per day**</td>
<td>5.4 ± 4.6</td>
<td>4.7 ± 4.7</td>
<td>0.3 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Source of added sugar (g/d)*</td>
<td>80 ± 12</td>
<td>70 ± 8</td>
<td>30 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td>Soft drinks plus juices*</td>
<td>43%</td>
<td>37%</td>
<td>8%</td>
<td>0.001</td>
</tr>
<tr>
<td>Sweetened grains (baked goods) plus sweets and candy (chocolate)</td>
<td>8%</td>
<td>14%</td>
<td>12%</td>
<td>0.5</td>
</tr>
<tr>
<td>Milk and dairy products</td>
<td>5%</td>
<td>4.5%</td>
<td>10%</td>
<td>0.03</td>
</tr>
<tr>
<td>Other®</td>
<td>44%</td>
<td>44.5%</td>
<td>70%</td>
<td>0.01</td>
</tr>
<tr>
<td>Total energy intake (kcal)</td>
<td>2207 ± 618</td>
<td>2164 ± 629</td>
<td>2100 ± 600</td>
<td>0.3</td>
</tr>
</tbody>
</table>

† Percent contribution to dietary carbohydrate from different source of added sugar from regular soft drink (the recommended upper limit for a 2200-kcal diet is 50 g/day).

® Cereals, other beverage (tea, coffee), fresh vegetables, fresh and dried fruits (1 teaspoon of sugar = 4.2 g; 1 ounce = 1/16 pound, 28.349 g).

* p < 0.001.

** p < 0.01 between all NAFLD and controls.
Insulin resistance often considered synonymous with obesity, although....

The most proximal correlate to insulin resistance is fat in the liver

Kumashiro, PNAS 2011
Intrahepatic fat induces insulin resistance:
The fat less mice

Kim et al. *J Biol Chem* 2000;275;8456-60

<table>
<thead>
<tr>
<th>Basal period</th>
<th>Plasma glucose</th>
<th>Plasma insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>μM</em></td>
<td><em>μM</em></td>
<td><em>μM</em></td>
</tr>
<tr>
<td>Wild type</td>
<td>7.0 ± 0.2 <em>μM</em></td>
<td>87 ± 11 <em>μM</em></td>
</tr>
<tr>
<td>Sham-operated fatless</td>
<td>11.1 ± 1.0 <em>μM</em></td>
<td>328 ± 69 <em>μM</em></td>
</tr>
<tr>
<td>Fat-transplanted fatless</td>
<td>7.7 ± 0.4° <em>μM</em></td>
<td>128 ± 31° <em>μM</em></td>
</tr>
</tbody>
</table>

Hepatic insulin sensitivity

% Suppression of basal hepatic glucose production

Fatty liver

Normal liver
Liver controls peripheral glucose and energy metabolism

Increased liver fat

Insulin resistance (i.e. Loss of inhibition by insulin of hepatic glucose production)

Increased endogenous glucose production

Hyperglycemia

Obesity

Chronic beta cell stimulation

Type 2 Diabetes

Liver –to-periphery communication: HEPATOKINES?

METABOLIC FACTORS?

FGF21
AGF
FETUIN
Selenoprotein P
• Liver fat determines hepatic insulin sensitivity but also peripheral insulin sensitivity

• (metabolic/obese) NAFLD predicts T2DM and supports the role of hepatic insulin resistance in the pathology of the disease
The liver in the metabolic syndrome

**Fatty liver**

Altered glucose homeostasis and (hepatic) insulin resistance

**Chronic inflammation** (↑CRP, ↑IL-6, ↑TNF, ↑other acute phase proteins, …)

**Hypercoagulation and hypofibrinolysis** (↑fibrinogen, ↑factor VII, ↑PAI-1 and other coagulation factors)

**Atherogenic dyslipidemia** (↑TG-rich VLDL, ↓HDL-chol, ↑postprandial lipemia, ↑small dense LDL-chol,…)

Maurantoni et al Arch Med Research 2011;42:337-353
Targher et al. New Engl J Med 2010;363;1341-1350

Confirmed in meta-analyses (Sookoian et al.)

Fatty liver a marker of pathological dysfunction of visceral adipose
NAFLD and cardiovascular risk

56,249 patients (33,546 M; 22,703 F): non-obese, mean age: 40

CV mortality increased by 2 in NAFLD population

Message:

• Diagnosis of steatosis
  – Test insulin sensitivity
  – Evaluate cardio vascular risk

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NAFLD without prior CV event

Diabetes?

Yes  No  \(\rightarrow\) FRS

- High >15%
- intermediate 10-15%
- low risk <10% at 10 years

Life style changes
Tailored treatment of individual risk factors
Follow-up

Carotid US or other to better define risk

Life style changes
Follow-up

Lonardo, Metab clin Exp 2015
When does NAFLD becomes NASH?

Steatosis

Glycogen inclusions

hepatocellular injury

inflammation

pericellular fibrosis
WHY does NAFLD become NASH?

Metabolic inflammation
Gut derived factors
BA

hepatocellular injury
pericellular fibrosis

inflammation

“toxic” lipids (ceramides, DAG, cholesterol ...) = NASH a lipotoxic disease
Cellular stress (oxidative, ER, .....)
Mitochondrial dysfunction and failure
Cell death – DAMP – immune and repair processes
How does the disease progress from NAFL to NASH? Who is at risk of progression? How to stop progression?
The multi-hit hypothesis

Overloaded and inflamed adipose tissue
Pro-infl, pro-IR factors, pro-fibrotic factors
- Leptin ↑
- Adiponectin ↓

? High glucose/ insulin
? BA
Toxic lipids
✓ Oxidative stress
✓ Pro-inflam context
✓ TLR4 activation
✓ sCFA
✓ Innate immunity
✓ Gut-derived factors

Hepatocellular injury – chronic inflammation - fibrogenesis

NAFL - steatosis

NASH
BAT stimulation = energy consumption + metabolic regulation

TGR5 stimulation = incretin, anti-inflammatory, anti-fibrotic

FXR agonists, FXR antagonists, TGR5 agonists

Modulation of BA pool

Gut flora, pre/probiotics, bariatric surgery

Brown adipose tissue

- Thermogenesis = fat combustion without ATP production = energy expenditure
- Stimulated by peripheral signals = impaired in obesity
Brown adipose tissue

- Thermogenesis = fat combustion without ATP production = energy expenditure
- Stimulated by peripheral signals = impaired in obesity
- Sends regulatory endocrine signals
Low BAT correlates with
- Obesity
- Diabetes
- Higher steatosis

The presence of brown adipose tissue in adulthood is independently associated with a lower likelihood of NAFLD diagnosed by CT findings.

Yilmaz et al. Aliment Pharmacol Ther 2011; 34: 318-323
Brown Adipose Tissue Activity
(PET-CT with $^{18}$F-FDG)

Upon stimulation ~ combustion of 10 Kg lipids / year

BAT activation
- increased resting metabolic rate
- Whole body lipolysis
- Improvement of glucose homeostasis
The multi-hit hypothesis

NAFL - steatosis

Overloaded and inflamed adipose tissue
Pro-infl, pro-IR factors, pro-fibrotic factors
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? High glucose/ insulin
? BA
? AGEs
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Hepatocellular injury – chronic inflammation - fibrogenesis

NASH
Overloaded and inflamed adipose tissue
Pro-infl, pro-IR factors, pro-fibrotic factors
- Leptin $\uparrow$
- Adiponectin $\downarrow$

The multi-hit hypothesis?

- High glucose/insulin
- BA
- AGEs
- Oxidative stress
- Pro-inflam context
- TLR4 activation
- sCFA
- Innate immunity
- Gut-derived factors

NAFL - steatosis

NASH

20%
Overloaded and inflamed adipose tissue
Pro-infl, pro-IR factors, pro-fibrotic factors
- Leptin ↑
- Adiponectin ↓

Factor x?

NAFL - steatosis

NASH

20%
The adaptive capacity hypothesis?

Inherited & acquired traits

NAFL - steatosis

Overloaded adipose tissue

- Pro-inflammatory, pro-IR factors, pro-fibrotic factors
  - Leptin ↑
  - Adiponectin ↓

MALADAPTATION
- Toxic lipid by-products
- Oxidative stress
- ER stress
- Alteration of innate immunity
- Abnormal tissue repair response

Activation of JNK / NF-κB...

Chronic inflammation, fibrosis and cell damage

? High glucose,
? High insulin
? AGEs
√ Oxidative stress
√ Pro-inflam context
√ TLR4 activation
√ sCFA...

Oxidative stress

NAFL - steatosis = Disease progression

Overloaded adipose tissue

= Disease progression

Pro-infl, pro-IR factors, pro-fibrotic factors

Inherited & acquired traits
NAFL - steatosis

NASH
NAFLD/NASH pathogenesis, and so what?

- Liver controls whole body glucose and energy metabolism
  - HGP, hyperglycemia and type 2 diabetes
- Fatty liver produces cardiovascular risk factors
- NASH a disease caused by lipotoxicity
- Intricate interplay between energy surplus, diet, environment, liver metabolism, adipose tissue, gut, and, at large, defense mechanisms (immune, inflammatory, metabolic) and healing capacities
- Multifactorial disease = multitarget approach = tailored approach
  - diet and life style modification
  - bariatric surgery
  - PPAR α / δ / (γ)
  - BA / FXR / FGF19 / TGR5
  - combined therapy (metab/anti-infl/anti-fib)
NAFLD/NASH pathogenesis, and so what?

• Challenges:
  – Who is at risk of NASH?
  – Who is at risk of progression towards life-threatening complications (cirrhosis, HCC?)
  – Diversity of mechanisms and risk factors
    • DNL versus ROS, mitoch impairment
    • Role of OH?
  – Personalized medicine?
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