Management of lean NASH

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Definition of «Lean» patients with NAFLD

• The presence of non-alcoholic fatty liver disease (NAFLD) in subjects with a BMI within the ethnic-specific cut-off of 25 kg/m² in Caucasians and 23 kg/m² in Asians has been defined as ‘Lean’ NAFLD.

• Asian patients with BMI < 25 should be commonly indicated as “non-obese”
Proportion of individuals with NAFLD stratified by country and obesity status

Younossi&Bugianesi NatRevG&H 2017
How can a lean subject get NAFLD?
Causes of NAFLD in lean subjects

• Environmental
  • BAFLD (Both alcoholic and nonalcoholic fatty liver disease)
  • high-fructose diet, high fat diet
• Metabolically Obese Normal Weight Subjects
• Congenital and acquired lipodistrophy (HIV-HAART)
• Genetic
  • PNPLA 3
  • Congenital defects of metabolism (FHLB, LAL-D)
• Endocrine disorders (PCOS, hypothyroidism, GH deficiency)
• Drug-related (amiodarone, methotrexate, tamoxifen, corticosteroids, others)
• Jejunoileal bypass, starvation, TPN
Causes of NAFLD in lean subjects

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Effects of High and Low Fat Diet on Liver Fat

10 normal subjects treated for 2 weeks in randomized cross-over fashion:

Liver fat

<table>
<thead>
<tr>
<th>High Fat Diet</th>
<th>Low Fat Diet</th>
</tr>
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<tbody>
<tr>
<td>+35%</td>
<td>-21%</td>
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</table>

Fasting serum insulin

<table>
<thead>
<tr>
<th>High Fat Diet</th>
<th>Low Fat Diet</th>
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<tbody>
<tr>
<td>+32%</td>
<td>-20%</td>
</tr>
</tbody>
</table>

Westerbacka J et al, J Clin Endocrinol Metab 2005
The primary dietary sources of fructose are high-fructose corn syrup and sucrose commonly used to sweeten beverages and processed foods.

Intake of soft drinks is 5-fold in NAFLD subjects compared to controls.

The consumption of soft drinks can increase the prevalence of NAFLD independent of the metabolic syndrome.

**Table 1  Soft drink consumption linked with NAFLD**

<table>
<thead>
<tr>
<th>Dietary constituents</th>
<th>Controls $(n = 30)$</th>
<th>NAFLD $(n = 31)$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy intake (kcal)</td>
<td>2200 ± 600</td>
<td>2300 ± 500</td>
<td>0.300</td>
</tr>
<tr>
<td>Added sugar (g/d)</td>
<td>33.6 ± 12.6</td>
<td>75.6 ± 8.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Percent of added sugar from soft drinks</td>
<td>8%</td>
<td>43%</td>
<td>0.001</td>
</tr>
</tbody>
</table>
## Fructose and liver histology
(341 adults, NASH Clinical Research Network)

### Association between fructose consumption and liver histology of NAFLD in different age groups

<table>
<thead>
<tr>
<th></th>
<th>Age &lt; 48 yrs old</th>
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<th>Age &gt; 48 yrs old</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted (Model 1)</td>
<td>Adjusted (Model 2)</td>
<td>Adjusted (Model 1)</td>
<td>Adjusted (Model 2)</td>
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<tr>
<td></td>
<td>OR [95% CI]</td>
<td>p-value</td>
<td>OR [95% CI]</td>
<td>p-value</td>
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<tr>
<td>Steatosis</td>
<td></td>
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<tr>
<td>Fructose consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7 servings</td>
<td></td>
<td></td>
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<tr>
<td>&gt; = 7 servings</td>
<td>1.1 [0.6, 2.0]</td>
<td>0.72</td>
<td>1.0 [0.6, 1.9]</td>
<td>0.95</td>
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<tr>
<td>Lobular inflammation</td>
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<tr>
<td>Fructose consumption</td>
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<tr>
<td>&lt; 7 servings</td>
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</tr>
<tr>
<td>&gt; = 7 servings</td>
<td>0.7 [0.4, 1.3]</td>
<td>0.24</td>
<td>0.9 [0.5, 1.8]</td>
<td>0.83</td>
</tr>
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<tr>
<td>Ballooning</td>
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<tr>
<td>Fructose consumption</td>
<td></td>
<td></td>
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<tr>
<td>&lt; 7 servings</td>
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</tr>
<tr>
<td>&gt; = 7 servings</td>
<td>1.3 [0.7, 2.3]</td>
<td>0.40</td>
<td>1.5 [0.8, 2.8]</td>
<td>0.19</td>
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<tr>
<td>Fibrosis</td>
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</tr>
<tr>
<td>Fructose consumption</td>
<td></td>
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<tr>
<td>&lt; 7 servings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; = 7 servings</td>
<td>2.5 [1.4, 4.4]</td>
<td>0.003</td>
<td>3.2 [1.7, 6.1]</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

*Abdelmalek et al Hepatology 2010*
**De-novo lipogenesis** is increased from excess carbohydrate intake in the form of simple sugars such as fructose and soft drinks sweetened with corn syrup.

De-novo lipogenesis produces only **saturated fatty acids**, that could induce both **inflammation** and **insulin resistance**.

Ingested fructose may **alter the microbiome**, increasing movement of endotoxin into the portal system because of increased permeability of tight junctions.
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• Environmental
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  • PNPLA 3
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BMI is a poor marker of insulin sensitivity

EGIR Study Group
Metabolically healthy obese (MHO) vs Metabolically obese normal weight (MONW)

**MHO subjects:**
- Normal insulin sensitivity
- Low visceral adiposity
- No liver fat
- High HDL cholesterol, low TGC

**MONW subjects:**
- Altered insulin sensitivity
- High fat mass, low lean body mass
- High visceral adiposity
- High liver fat
- High TGC, low HDL cholesterol
- High blood pressure
- High risk for T2DM and CVD
## Risk factors for NAFLD in lean/overweight subjects

<table>
<thead>
<tr>
<th>Authors</th>
<th>Population</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. (Taiwan)(^{18})</td>
<td>Adult (health check-up)</td>
<td>Aged 40 to 60 years, Elevated serum ALT, Triglyceride ≥ 150 mg/dL</td>
</tr>
<tr>
<td>Fu et al. (Taiwan)(^{23})</td>
<td>Adolescents (students)</td>
<td>Increased serum triglyceride, Elevated HOMA index, Elevated serum ALT</td>
</tr>
<tr>
<td>Kim et al. (Korea)(^{29})</td>
<td>Adults (health check-up)</td>
<td>Increased waist circumference, Increased serum triglyceride, Elevated HOMA index</td>
</tr>
<tr>
<td>Das et al. (India)(^{14})</td>
<td>Adults (community-based)</td>
<td>Abdominal obesity, Dysglycemia (fasting plasma glucose &gt; 100 mg/dL or elevated HOMA index), Higher body fat content</td>
</tr>
<tr>
<td>Fan et al. (China)(^{30})</td>
<td>Adults (health check-up, prospective cohort)</td>
<td>At baseline: advanced age, elevated BMI, elevated serum triglyceride and total cholesterol, obesity, and hypertension During FU: weight gain and increase of serum triglyceride</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; BMI, body mass index; FU, follow up; HOMA, Homeostasis Model Assessment.
• NMR imaging: Marked absence of all fat depots in the patient with generalized lipoatrophy, with a lipid-filled liver. The bright color of the muscle is indicative of lipid-filled myocytes.
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Genetic modifiers of NAFLD/NASH

• The most robustly validated association is with a PNPLA3 variant C>G (I148M), associated with increased hepatic triglyceride levels and an increased risk of NASH, HCC and cirrhosis.

• A missense variant in TM6SF2 C>T (E167K), encoding transmembrane 6 superfamily member 2, dissociates NAFLD from CVD.

• The MBOAT C>T variant has an effect on ALD and NAFLD severity.

• The most recent HSD17B13 variant T>TA confers protection from liver damage in NAFLD.
Adipose tissue is inflamed in NAFLD due to obesity but not in NAFLD due to genetic variation in PNPLA3

S. Lallukka • K. Sevastianova • J. Perttilä • A. Hakkarainen • M. Orho-Melander • N. Lundbom • V. M. Olkkonen • H. Yki-Järvinen

- 82 volunteers divided according to on BMI or PNPLA3 genotype
- In obese vs non-obese, adipose tissue gene expression of proinflammatory MCP-1 and CD68 were upregulated, whereas anti-inflammatory (Twist1, ADIPOQ) were downregulated.
- Gene expression of MCP-1, CD68, Twist1 and ADIPOQ similar in PNPLA3-148MM and PNPLA3-148II groups
FAMILIAL HYPOBETALIPOPROTEINEMIA (FHBL)

- Autosomal codominant disorder characterized by apoB less than the fifth percentile and LDL cholesterol usually between 20 and 50mg/dL.
  - Over 60 mutations producing truncations in the apoB gene identified
- Hepatic steatosis in FHBL due to defective export of triglyceride from the liver and is not associated with muscle or adipose tissue insulin resistance
  - Reports of cirrhosis and hepatocellular carcinoma associated with familial hypobetalipoproteinemia (FHBL) suggest that patients should be monitored
- Reportedly, an individual with the apoB-67 mutation died from rapidly progressive hepatocellular carcinoma.

Welty, Curr Opinion in Lipidology 2014.
Dissociation between Intrahepatic Triglyceride Content and Insulin Resistance in Familial Hypobetalipoproteinemia

Fabbrini et al, Gastroenterology. 2010
Lysosomal acid lipase (LAL) deficiency

- Lysosomal Acid Lipase (LAL) enzymatic activity 1-12% Lysosomal accumulation and insufficient cytoplasmic-free cholesterol
- Estimated prevalence of 1:40,000–1:300,000\(^1,2\)
- Onset: early childhood to late adulthood
- Clinical features
  - Hepatomegaly and mildly increased transaminases (100%)
  - Splenomegaly (86%)
  - Chronic liver disease, eventually cirrhosis in 50%
  - Atherosclerosis (type IIb hyperlipoproteinemia, ↓ HDL)

**GENETIC TEST: LIPA gene mutations**

One mutation found: E8SJM (c.894G>A)


**DRIED BLOOD SAMPLE: Lysosomal acid lipase activity**

<0.02 nmol/punch/hr

(reference range 0.37 – 2.30 nmol/punch/hr)


**DIAGNOSIS CONFIRMATION**

5-year-old
ALT 62 IU/L
Tot Chol 332 mg/dL
TG 189 mg/dL
Enzyme replacement treatment: sebelipase-α
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Different metabolic adaptation in Lean NAFLD

Gut microbiota associated with Lean NAFLD
N=538 Caucasian NAFLD, 99 (18%) lean

Chen et al. Hepatology 2019
- Insulin resistance pattern similar to obesity
- Reduced mitochondrial function
- Increased de novo lipogenesis
- ↓ capacity for storing fat in subcutaneous AT
- Lipodystrophy phenotype (genetic/acquired)
- Lower muscle mass
- Higher circulating FFA
- Decreased adiponectin levels
- Lower abundance of *Faecalibacterium* and *Ruminococcus*
- Deficiency in *Lactobacillus*
- No significant qualitative difference between lean and obese
- PNPLA3 [GG]
- CETP
- TM6SF2 C>T
- IFN lambda TT>δG
- PEMT
- High fructose intake
- Intrauterine growth retardation

Younes & Bugianesi; Semin Liver Dis. 2019
How to manage lean subjects diagnosed with NAFLD?
Prevalence of each criterion of the metabolic syndrome in NAFLD patients in relation to BMI

Population-based study: 18.8% with moderate-to-severe NAFLD at US:

- In overweight/obese subjects: 27.7%, in normal BMI: 7.4%
- “lean” NAFLD individuals relatively free from the components of MS

Younossi et al Medicine (Baltimore) 2012

In a tertiary care liver unit “lean” NAFLD less likely associated with MS, but subjects nonetheless insulin resistant

Vos et al Acta Gastroenterol Belg 2011
In patients with NAFLD, metabolically normal individuals are at a lower risk for mortality.
# Longitudinal studies on Lean NAFLD

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leung et al., 2017</td>
<td>Median follow up of 49-months. <strong>Hypertriglyceridemia and higher creatinine</strong> were associated with advanced liver disease in lean. Death and HCC were recorded only in the obese group.</td>
<td>Asian</td>
</tr>
<tr>
<td>Cruz et al., 2014</td>
<td>Despite a better metabolic profile, less insulin resistance and fibrosis, <strong>lean subjects have a higher overall mortality</strong> than patients with NAFLD who are overweight or obese.</td>
<td>Mixed</td>
</tr>
<tr>
<td>Hagstrom et al, 2018</td>
<td>After a median follow-up of 19.9 years, although patients with lean NAFLD showed lower fibrosis, they were <strong>at higher risk for development of severe liver disease compared to patients with NAFLD and a higher BMI</strong>, independent of available confounders.</td>
<td>Caucasian</td>
</tr>
</tbody>
</table>
Do “lean” NAFLD carries the same risk of progression as the fatty liver of obesity?

- A cohort of 1,343 separated into lean and non-lean
- Lean patients were more commonly non-white or Asians, had a significantly lower prevalence of the metabolic syndrome and its components, significantly less steatosis and less advanced fibrosis but similar necroinflammation.
- Over a mean follow-up period of over 11 years mortality was significantly higher in “lean” patients and the Cox model identified “lean” NAFLD (HR 11.8; 95% CI 2.8 - 50.1; p = 0.001) and age as prognostic factors, after adjustment for confounders

Angulo et al, AGA 2013
Do “lean” NAFLD carries the same risk of progression as the fatty liver of obesity?

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Non-Lean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver Events</strong></td>
<td>10 (5%)</td>
<td>91 (9.8%)</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>Cardiovascular events</strong></td>
<td>14 (7%)</td>
<td>123 (13%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

- **1186** Caucasian patients underwent a liver biopsy for clinical suspicion of non-alcoholic fatty liver disease.

- Lean patients (BMI < 25) represented **18.4%** of the cohort (N=218)

- After a median follow-up of **90 months**, both liver and non-liver related events had a significant higher prevalence in the **obese group**.

- Nonetheless, **lean patients demonstrated a progression of NAFLD to decompensated cirrhosis**

Younes et al, AASLD 2018
Conclusions

• Lean patients with NAFLD should be screened for presence of insulin resistance, genetic (PNPLA 3, FHLB, LAL-D) and endocrine disorders (PCOS, hypothyroidism, GH deficiency)

• Pathophysiological mechanisms are not totally understood and may include a dysfunctional adipose tissue, altered body composition, genetic mutations, epigenetic changes occurring early in life and a different pattern of gut microbiota

• Although generally with a more favorable metabolic profile when compared to obese NAFLD, ‘Lean’ NAFLD patients can develop the full spectrum of liver damage

• Data on long-term prognosis of lean patients are insufficient and controversial but suggest that it is not a “benign” disease

• General recommendations include an adoption of a healthy lifestyle, but guidelines do not provide much information as to whether and to what extent prevention and treatment should be adapted in lean patients, given the harder correction of underlying risk factors.
Thank you for your attention!

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Prof. Giorgio Maria Saracco

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