New insights in Non Alcoholic Fatty Liver/
Non Alcoholic Steatohepatitis and
related metabolic diseases

Dott.ssa Elisabetta Bugianesi
Divisione di Gastro-epatologia
Università di Torino
General population

NAFLD 25-30%

NASH 3-10%

HCC Incidence of HCC 0.5-1%/yr

Cirrhosis 2-3%

Sindrome Metabolica

Incidence of HCC 0.5-1%/yr
Insulin resistance

Type 2 diabetes
Cardiovascular disease
Hypertension
Glucose intolerance
Obesity
Dyslipidemia
Fatty liver
Cancer
POCS
Premenopausal ovarian cysts

Biddinger SII, Kahn CR. 2006.
Annu. Rev. Physiol. 68:123–58
• All components of the metabolic syndrome correlate with liver fat content, as determined by $^1$H-MRS.

• Although the prevalence of steatosis increases as a function of obesity, these relationships remain significant even when adjusted for BMI.
Among individuals at baseline, per 1 standard deviation increase in log ALT level, there were increased odds of the development of MetS (odds ratio [OR] 1.21, \( P < .001 \)) and diabetes (OR, 1.48; \( P < .0001 \)) over 20 yrs of f.u.

There was an increased risk of CVD in age/gender adjusted models (hazard ratio, 1.23; \( P < .0001 \)), but this was attenuated in multivariable-adjusted models (hazard ratio 1.05; \( P = .27 \))

Goessling, Gastro 2008
Insulin resistance in the liver impairs the ability of insulin to inhibit glucose production.

**Normal**

- Normal glucose
- Normal insulin action

**Insulin resistance**

- Glucose ↑
- Insulin ↑↑
- Impaired insulin action to inhibit glucose production

- Glucose N↑
- Insulin ↑
A total of 139 men developed new diabetes over 4.9 years of follow-up.

ALT remained a predictor with adjustment for age, BMI, triglycerides, HDL cholesterol, systolic blood pressure, glucose, and alcohol intake

OR: 2.04 [1.16-3.58] for the fourth versus first quartile

Sattar N, Diabetes 2004
LFTs and metabolic diseases

- High ALT is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes in Pima Indians
  
  Vozarova, Diabetes 2002

- In 4401 employees without liver disease or drug treatment (mean age 48 years, BMI 23 kg/m²), the odds ratios of men and women with NAFLD to develop the metabolic syndrome during the follow-up were 4.0 and 11.2 after adjustment for age, alcohol intake, and changes in body weight
  
  Hamaguchi, Ann Intern med 2005

- 7458 nondiabetic men followed for an average 12.8 years, increased serum GT predicted type 2 diabetes independent of BMI
  
  British Regional Heart Study, Diabetes Care 1998

- The prevalence of high ALT is associated with poor metabolic control and obesity grade
  
  Forlani J Clin Endocrinol Invest 2008
NAFLD & Risk of Diabetic Complications

- 2,103 T2DM subjects without overt CVD
- The presence of NAFLD is associated with an increased prevalence of:
  - Chronic Kidney Disease
  - proliferative/laser-treated retinopathy
  - independently of baseline confounding factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate model 1</th>
<th>Multivariate model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-proliferative retinopathy</td>
<td>1.6</td>
<td>1.5</td>
<td>1.19</td>
</tr>
<tr>
<td>OR</td>
<td>1.1–2.3</td>
<td>1.03–2.2</td>
<td>0.8–1.7</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.001</td>
<td>0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>proliferative/laser-treated retinopathy</td>
<td>2.2</td>
<td>2.0</td>
<td>1.75</td>
</tr>
<tr>
<td>OR</td>
<td>1.2–4.2</td>
<td>1.1–4.2</td>
<td>1.1–3.7</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.001</td>
<td>0.001</td>
<td>0.031</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>2.4</td>
<td>2.2</td>
<td>1.87</td>
</tr>
<tr>
<td>OR</td>
<td>1.6–4.7</td>
<td>1.3–4.5</td>
<td>1.3–4.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.001</td>
<td>0.001</td>
<td>0.020</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cohort size: n=2,103
Model 1: adjustment for age, sex, BMI, waist circumference, smoking, LDL-cholesterol, triacylglycerol, HbA1c, diabetes duration and medications use
Model 2: further adjustment for hypertension and chronic kidney disease (for the first and second variable of the table) or for hypertension and proliferative/laser-treated retinopathy (for the last variable of the table)
Prevalence of NASH and cirrhosis in T2DM

• After liver biopsy (n=90), 87% of diabetic patients had NAFLD, with NASH in 62.6% and fibrosis in 37.3%. Prevalence of NASH increased with components of the MS. ALT were higher in NASH, although still within the normal limits.  
  
  Prashanth, J Assoc Physicians India 2009

• After liver biopsy, NASH was found in 25/32 pts with T2DM. Fibrosis was present in 7/32 of them. No biochemical/anthropometric difference to identify NASH  
  
  Gupte, J Gastroenterol Hepatol 2004

• Cirrhosis occur in 25% of pts with NAFLD and DM compared with 10% of those without DM  
  
  Younossi, Clin Gastroenterol Hepatol 2004
The cumulative risk of acute liver failure among veteran patients with and without Type 2 Diabetes

Analysis restricted up to 1997 (before the introduction of troglitazone).

El Serag et al Gastro 2002
Epidemiological evidence of the association bw T2DM and HCC

El Serag et al Clin Gastroenterol Hepatol 2006
Type 2 Diabetes

NAFLD
70-80%

NASH
30-40%

Cirrhosis?

HCC?

13

NAFLD
70-80%

NASH
30-40%

Cirrhosis?

HCC?
Insulin resistance in the liver impairs the ability of insulin to inhibit VLDL production.

**Normal**
- Normal liver
- Normal S-TG
- Normal insulin action

**Insulin resistance**
- Increased liver fat
- Impaired insulin action to inhibit VLDL production
- S-Tg ↑
- HDLchol ↓
- TG ↑
- HDLchol ↓
Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis

- Liver insulin receptor knockout (LIRKO) mouse is a model of pure hepatic insulin resistance.
- Hepatic insulin resistance causes a proatherogenic distribution of serum cholesterol, with an increase in total cholesterol, a 50% decrease in HDL cholesterol and an increase in ApoB.
- When challenged with an atherogenic diet, LIRKO mice rapidly develop extensive atherosclerosis.

Biddinger SB et al, Cell metab 2008
NAFLD and inflammatory markers

100 non-smoking healthy volunteers with (closed bars) and without (open bars) Hepatic Steatosis by US and CT

Targher, Diabet Med 2005
The Hoorn Study:
ALT predicts coronary heart disease

<table>
<thead>
<tr>
<th>Tertile</th>
<th>All-cause mortality, HR (95% CI), 174 events</th>
<th>CVD events, HR (95% CI), 355 events</th>
<th>CHD events, HR (95% CI), 129 events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>0.74 (0.49–1.10)</td>
<td>1.02 (0.78–1.33)</td>
<td>0.94 (0.57–1.53)</td>
</tr>
<tr>
<td>Third</td>
<td>1.30 (0.92–1.83)</td>
<td>1.40 (1.09–1.81)</td>
<td>2.04 (1.35–3.10)</td>
</tr>
</tbody>
</table>

◆ 1439 subjects aged 50-75 at baseline.
◆ Age and sex adjusted HR for subjects in the upper tertile of ALT compared with the first.

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<th>CVD events, HR (95% CI), 355 events</th>
<th>CHD events, HR (95% CI), 129 events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>0.71 (0.48–1.06)</td>
<td>0.99 (0.76–1.20)</td>
<td>0.93 (0.57–1.52)</td>
</tr>
<tr>
<td>Third</td>
<td>1.10 (0.77–1.61)</td>
<td>1.22 (0.94–1.60)</td>
<td>1.88 (1.21–2.92)</td>
</tr>
</tbody>
</table>

◆ HR after adjustment for the MetS and traditional risk factors

Schindhelm Atherosclerosis 2007
NAFLD and endothelial dysfunction

Villanova et al Hepatology 2005
NAFLD and carotid IMT

- After adjustment for traditional risk factors, MetS and HOMA-R

Targher et al Diabetes Care 2006
Coronary alterations in NAFLD

- 317 pts who elective underwent coronary angiography and liver US in the same day
- Factors associated with clinically relevant CAD

Table 2. Binary logistic regression (Dependent: coronary artery disease; Independent: significant variables in Table 1)

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty liver</td>
<td>&lt; 0.001***</td>
<td>8.48 (4.39–16.40)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.002**</td>
<td>2.94 (1.47–5.91)</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.014*</td>
<td>2.31 (1.19–4.48)</td>
</tr>
<tr>
<td>HTN</td>
<td>0.109</td>
<td>1.63 (0.90–2.98)</td>
</tr>
<tr>
<td>LDL</td>
<td>0.102</td>
<td>0.99 (0.98–1.00)</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01.
HTN, hypertension; LDL, low-density lipoproteins.

Mirbagheri et al. Liver Int 2007
LV PCr/ATP ratio in fatty liver

mean±SD
P=0.016; Kruskal-Wallis non-parametric test

Perseghin G et al *Hepatology*, 2008
Cardiovascular outcomes in NAFLD

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Cases</th>
<th>Controls</th>
<th>Follow-up (y)</th>
<th>Cardiovascular Outcome</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matteoni et al³</td>
<td>98</td>
<td>NAFLD</td>
<td>–</td>
<td>8.3</td>
<td>Mortality</td>
<td>11/ –</td>
</tr>
<tr>
<td>Targher et al²⁹</td>
<td>2103</td>
<td>NAFLD + diabetes</td>
<td>–</td>
<td>5</td>
<td>Events</td>
<td>11/ –</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mortality</td>
<td>4/ –</td>
</tr>
<tr>
<td>Ekstedt et al¹⁷</td>
<td>71</td>
<td>NASH</td>
<td>Simple steatosis</td>
<td>13.7</td>
<td>Incident disease</td>
<td>29/ 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mortality</td>
<td>15/ 9</td>
</tr>
<tr>
<td>Adams et al²⁹</td>
<td>420</td>
<td>NAFLD</td>
<td>–</td>
<td>7.6</td>
<td>Mortality</td>
<td>3/ –</td>
</tr>
<tr>
<td>Sanyal et al⁶⁶</td>
<td>152</td>
<td>NASH-related cirrhosis</td>
<td>HCV-related cirrhosis</td>
<td>10</td>
<td>Mortality</td>
<td>5/ 0.7</td>
</tr>
</tbody>
</table>

- **Adults with T2DM and ultrasound evidence of NAFLD had an increased incidence of CV events compared with patients with diabetes mellitus but without NAFLD**
- **In patients with NAFLD histologic severity correlates with CV risk factors and predicts CV outcomes.**

Rubinstein et al Semin Liver Dis 2008
Key concepts in NAFLD/NASH and CVD

- Subjects with NAFLD have a high prevalence of cardiovascular involvement
- The presence of NAFLD is an additional risk factor of CVD, after adjustment for traditional risk factors (diabetes, blood pressure, lipid profile)
- In humans NAFLD is associated with:
  - endothelial dysfunction
  - altered surrogate markers of atherosclerosis
  - altered left ventricular energy metabolism
  - increased expression of mediators of low grade inflammation
- In the long-term, CVD is a common cause of death in NAFLD patients, affecting prognosis more than liver disease itself
Cardiovascular Disease and NAFLD/NASH

Adipose tissue hypertrophy and inflammation

↑ TG, FFA, VLDL
↓ HDL chol
↑ glucose, insulin
↑ CRP
Fibrinogen
PAI-1
↑ oxidative stress
↑ leptin
↓ adiponectin

NAFL

NASH

Overeating
Inactivity
Genetic predisposition

Atherosclerosis
Cardiac lipotoxicity
Energy dysfunction
Impaired perfusion
MetS and NAFLD

- Unknown (worsening IR?)
- CVD risk factors (e.g. glucose, VLDL, CRP, PAI-1, fibrinogen, FVII)
- β-cell failure
- NASH → Cirrhosis
- Type 2 Diabetes
- Cardiovascular disease

Kotronen, Arterioscler Thromb Vasc Biol 2008
Functions of Drosophila’s fat body

- Sensing energy and nutrient availability
- Coordination of metabolic responses
- Coordination of the response to pathogens

Inflammation and metabolic disorders: evolutionary aspects

- Advantage to withstand starvation by storing excess energy as fat
- Excess nutrients provides the ability to mount a more effective immune response toward pathogens.
Grazie per l'attenzione!