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Increased LDL-cholesterol susceptibility to oxidation contributes to oxidative damage in non alcoholic steatohepatitis (NASH).

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Background and aims: The increased lipid peroxidation demonstrated in NASH might be related either to higher circulating levels of pro-oxidants and/or to a peculiar vulnerability of plasma lipids to oxidative stress. Insulin resistance, a common feature in NASH, has been associated with increased oxidative stress. The aims of our study were to establish whether the susceptibility of LDL-cholesterol particles to oxidation is increased in NASH patients and whether this could be related to insulin resistance. **Methods:** We studied 11 non-obese, non diabetic patients with NASH (BMI=26.6±1.6), and 5 matched controls. The following parameters were evaluated: 1) plasma lipid profile 2) total body lipid oxidation by indirect calorimetry 3) plasma LDL-cholesterol vulnerability to oxidation as assessed by the length of the lag-phase during copper-catalyzed LDL oxidative modifications; 4) insulin sensitivity by hyperinsulinemic (1 mU/Kg min) euglycemic clamp. **Results:** The basal lag-phase was significantly shorter in NASH patients compared to controls (49±11 vs. 68±10 min, respectively; p=0.01). Plasma lipid profile was comparable in the two groups, with the exception of slightly higher triglycerides concentration in NASH (117±49 in NASH and 71±11 mg/dL in controls; p=0.05). A slight inverse correlation was found between plasma triglycerides (r=-0.43) and lag phase and FFA concentrations (r=-0.52) and lag phase in NASH patients but not in controls. Total lipid oxidation was 1.13±0.25 and 1.04±0.21 micromol/kg min in NASH and controls, respectively (p=ns) and inversely correlated with the lag-phase in both groups (r=-0.58 and -0.46). Insulin sensitivity was definitely reduced in NASH patients compared to controls (glucose infusion rate = 4.49±1.48 vs. 7.67±0.54 mg/kg min; p<0.01). The lag-phase did not change after the euglycemic hyperinsulinemic clamp in both groups (p=ns) and did not correlate with insulin sensitivity. **Conclusions:** The increased susceptibility of LDL-cholesterol particles contributes to enhanced lipid peroxidation in patients with NASH, independently from lipid levels and lipid oxidation. The LDL oxidizability is not affected by insulin administration and does not appear to be related to insulin resistance.

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GALLSTONE DISEASE (GD) AND RELATED RISK FACTORS IN A LARGE COHORT OF DIABETIC PATIENTS

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Background and Aim. Data concerning the actual prevalence of GD in diabetics remain controversial, mainly as regards the risk factors related to GD formation. Thus, present series was aimed to assess GD prevalence and possibly related risk factors in a large cohort of consecutive diabetics.

Patients and Methods. During a 24 month period 1337 consecutive patients (710 male, 627 female, mean age 63±SD11 and 65±SD11, respectively) with DM were enrolled. Of them, 1235 (92%) had DM type 2 and 102 (8%) type 1. Ultrasound liver scan was done in fasting condition with a 3.5 MHz transducer. GD was defined as the actual presence of stone/s or the lack of gallbladder due to cholecystectomy for gallstones performed at least one year after the diagnosis of DM. Statistical analysis was done by logistic regression analysis.

Results. A total of 332 patients (25%) had GD; in detail 261 had actual stone/s and 71 previous cholecystectomy. The prevalence of GD was higher in female (29 vs 22%, p .003), increased with age (from 13 to 20 and 30% in patients aged ≤ 40, 41-65 and >65 years , respectively, p.001) and BMI (from 24 to 30 % in those with a BMI normal or ≥ 30, respectively, p.001). In addition, GD frequency was significantly higher in patients with family history of GD (31% vs 23%, p.001). An inverse relation was observed between alcohol intake and GD prevalence, which resulted of 28% in those with EtOH g/day ≤ 40 vs 20 and 24% in those assuming 41-80 and > 80 g/day (p .01). Type of diabetes, plasma levels of cholesterol and triglycerides, smoking habit, physical activity, weight reduction in the last year, use of oral contraceptive, parity and menopause were not significantly related to GD. At multivariate analysis sex, age, BMI and family history maintained their statistical significance.

Conclusions. Female sex, increase in BMI and anamnestic evidence of family history for GD, represent independent risk factors for gallstone/s in diabetic patients without difference according to the type of diabetes.

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IRON OVERLOAD, HFE GENE MUTATIONS AND INSULIN RESISTANCE IN NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Background: Insulin resistance is a key feature of NAFLD. Patients with hereditary hemochromatosis, disease characterized by progressive iron overload, due in the large majority of the cases to homozygosity for the C282Y mutation in HFE gene, often have decreased insulin sensitivity and release. **Aims:** to determine whether heterozygosity for the mutations of HFE gene, may confer susceptibility to NAFLD by impairing insulin release/ sensitivity. **Patients, materials and methods:** 134 consecutive patients with clinical and ultrasonographic diagnosis of NAFLD, confirmed by liver biopsy in half of them, 82 with hyperferritinemia. HFE gene mutations were determined by PCR and restriction fragment length polymorphism analysis. **Results:** 1) the prevalence of the C282Y HFE mutation was significantly higher in patients with NAFLD compared to controls (18% vs. 2%; P<0.0001), being the difference more striking in patients with hyperferritinemia (31%) 2) the mild iron overload present in carriers of the C282Y mutation is able to reduce insulin release 3) carriers of the C282Y mutation develop NAFLD despite lower BMI and triglycerides. **Conclusion:** the mild iron overload associated with heterozygosity for the C282Y HFE mutation may confer susceptibility to NAFLD by decreasing insulin release.

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SERUM MARKERS OF FIBROSIS IN NONALCOHOLIC STEATOHEPATITIS (NASH)

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INTRODUCTION: NASH has increasingly been recognised as an important and common form of liver disease over the past 20 years. The strong correlation between the development of fibrosis and the neuroinflammatory activity in the liver is well known and implies a role for inflammatory mediators in fibrogenesis. Non-invasive approaches to assess the presence of liver fibrosis would be helpful to confirm and to quantify the presence of fibrosis.

AIMS & METHODS: The aim was to characterize the fibrosis network in patients with NASH. We assayed TGF beta, metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and laminin in subjects with histologically proven NASH, compared to patients with HCV chronic active hepatitis and patients with well compensated cirrhosis. We studied 19 patients with histological diagnosis of NASH (age: 37±9yrs, M/F:17/2), 23 patients with chronic active hepatitis (CAH) (age:50±9yrs, M/F:15/8) before antiviral treatment and 9 patients with well compensated cirrhosis (age 55±8yrs, M/F:6/3, Child A-B). Eight volunteers (age:31±8yrs, M/F:5/3) served as healthy controls well matched for age, gender and BMI. The fibrosis (Knodel score) was minimal in NASH patients (range 0-1) and mild-severe in CAH (range 1-3).

RESULTS: Table.

	Healthy	NASH	CAH	Cirrhosis
ALT (IU/L)	46 ± 19.7	69.8 ± 44.02	96.77 ± 35.92	60±51
AST (IU/L)	29.5 ± 5.1	39.16 ± 6.4	62.01 ± 22.86	50±43
TGF-beta (ng/ml)	12.15 ±10.03	49.83 ± 18.34*	34.21 ± 22.52*	193.7 ± 181.1*
MMP-1 (ng/ml)	3.68 ± 3.63	6.5 ± 5.06	5.5 ± 15.5	2.21 ± 1.6
MMP-2 (ng/ml)	1264 ± 109	1157 ± 380	1465 ± 399	1445 ± 153
TIMP-1 (ng/ml)	277 ± 48	422 ± 155	1575 ± 394	660.6 ± 328.2
TIMP-2 (ng/ml)	67.07 ± 10.9	149.2 ± 91	113 ± 105.6	170 ± 79
Laminin (ng/ml)	undetectable	141.61 ± 27	124.35 ± 26	150.11 ± 20

* p<0.001 (NASH vs Healthy; CAH vs Healthy; Cirrhosis vs Healthy, CAH, NASH)

CONCLUSIONS: TGF beta is a proinflammatory cytokine that regulates cell growth, and extracellular matrix metabolism. The TGF levels could be used as a marker of fibrosis. Circulating levels of MMPs and TIMPs have been shown to correlate with the development of inflammation and fibrosis. TIMP-1 levels correlates significantly to inflammation rather than fibrosis. MMP-2 levels increase with the amount of fibrosis. In our series we found in NASH and CAH patients similar significant increase of TGF beta levels, while cirrhotics show significantly higher levels if compared to NASH and CAH; TIMP-1 levels increases from normal liver to chronic active hepatitis with a decrease in cirrhotics. The significant increase of TGF beta and TIMPs levels in NASH when compared to healthy controls may represent the progression of fibrosis in these patients; the decrease of TIMP-1 levels in cirrhotics might represent the early stage of cirrhosis or a low grade of inflammation in our series. Patients with NASH, CAH and cirrhosis show similar levels of laminin suggesting that in NASH fibrogenetic events and fibrosis have already started despite histological features. While laminin is an extracellular matrix protein reflecting deposition of fibrosis, our data point out serum TGF-beta and TIMPs as index of "continuous" fibrogenic and inflammation activity in NASH. Their profile may be an useful tool to assess progression of disease.