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Molecular determinants of insulin resistance, cell apoptosis and lipid accumulation in non-alcoholic steatohepatitis

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Abstract *Backgrounds and aims:* Non-alcoholic-steatohepatitis (NASH) is closely related to insulin resistance, but it is unknown whether insulin resistance may be localized in hepatocytes. This study investigates insulin signalling in liver tissue from NASH, and the molecular mechanisms by which insulin-resistance could lead to liver damage (apoptosis). Moreover, to investigate the mechanisms of lipid overload we studied key enzymes in hepatocytes lipid metabolism.

Methods and Results: In liver specimens from 11 patients with NASH and 7 histological normal livers, we measured total and phosphorylated Akt (active form), Bax and Bcl-2 by Western-blot analysis. In addition, we studied AMP-activated protein Kinase and Carnitine-Palmitoyl-Transferase-1 gene expression, key regulators of non-esterified fatty acid synthesis and oxidation, by reverse transcription polymerase chain reaction.

In NASH, phosphorylated Akt was impaired (104.3 ± 10.6 vs 152.6 ± 22.4 AU, $p < 0.002$) and correlated with necroinflammatory score ($r = -0.62$; $p < 0.05$). Bax/Bcl-2 ratio was increased in NASH. Moreover, we observed a decrease of AMP-activated protein Kinase (10.74 ± 6 vs 144.7 ± 41.6 AU, $p < 0.0001$) and Carnitine-Palmitoyl-Transferase-1 gene expression (38.7 ± 14.6 vs 192.1 ± 26.2 AU, $p < 0.0001$), and both were correlated with steatosis score ($r = -0.56$, $p < 0.05$, $r = -0.87$, $p < 0.05$ respectively).

Abbreviations: ACC, Acetyl-CoA Carboxylase; AMP-K, AMP-activated protein Kinase; CPT-1, Carnitine-Palmitoyl-Transferase-1; T2DM, Type 2 Diabetes mellitus; HOMA, homeostatic metabolic assessment; NAFLD, non-alcoholic fatty liver disease; NASH, Non-alcoholic-steatohepatitis; PI3-k, phosphatidyl inositol-3 Kinase; TG, triglycerides.

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Conclusions: Akt, a key molecule of insulin signalling and cell apoptosis is impaired in NASH, suggesting an important role of hepatic insulin resistance in liver failure. Moreover, decreased non-esterified fatty acid oxidation may cause hepatic lipid overload.

Introduction

Non-alcoholic steatohepatitis (NASH), a common liver disease characterized by histological features resembling those associated with alcohol-induced liver injury (hepatic macrovescicular steatosis with lobular and portal inflammation, nuclear glycogenation), occurs in subjects who do not drink significant amounts of alcohol. The clinical implications of NASH are due mostly to its common occurrence in the general population and its potential to progress to cirrhosis and liver failure [1,2]. NASH is the subset of non-alcoholic fatty liver disease (NAFLD) most associated with progressive liver disease and should be differentiated from steatosis, with or without hepatitis, resulting from secondary causes such as surgical and toxic conditions, which have a different pathogenesis [3].

The pathogenesis of NASH is poorly understood. Its frequent association with obesity, type 2 diabetes, dyslipidaemia and the metabolic syndrome has led to the suggestion that insulin resistance could play a major role [4]. To support this hypothesis, several data have been produced to demonstrate that insulin resistance is present in almost all patients with NASH and NASH is a liver manifestation of insulin resistance [5]. However, the different contribution of peripheral vs hepatic insulin resistance in determining liver damage remains to be clarified, as well as the molecular mechanism of hepatic insulin resistance in these patients [6]. To address these issues, we measured Akt protein levels in liver tissue from control subjects and patients with NASH. Akt, in its active phosphorylated form, plays a key role in mediating intracellular insulin actions, thereby regulating metabolism, proliferation, survival and apoptosis in many cell types, including hepatocytes [7,8]. Moreover, to investigate the metabolic pathway involved in liver lipid overload, we also measured the gene expression of AMP-activated protein Kinase (AMPK) and Carnitine-Palmitoyl-Transferase-1 (CPT-1), two enzymes recognized as major regulators of lipid metabolism and non-esterified fatty acids (NEFA) oxidation [9]. AMPK controls lipid metabolism by directing acyl-CoAs towards either oxidative or synthetic pathways through a regulatory effect on several enzymes (Acetyl-CoA Carboxylase, Malonyl-CoA Decarboxylase, CPT-1) [10]. CPT-1 regulates NEFA transport to the mitochondria and represents the rate-limiting step for mitochondrial NEFA beta oxidation [11].

Methods

Eleven patients with a clinicopathological diagnosis of NASH (10 male and 1 female, mean age 40.7 ± 9.5 years) were studied. All patients underwent percutaneous liver biopsy under ultrasound guidance using a modified-Menghini needle; a sample length of the least 15 mm was considered an acceptable specimen. The pathological diagnosis of NASH was based on the criteria of Brunt et al. [12]: steatosis was observed in all the biopsies and was graded 1–3, according to the percentage of fatty infiltration (1 = 0–33%; 2 = 34–66%; 3 = 67–100%). Necroinflammation was graded as 1–3 (1: occasional ballooned hepatocytes and no or very mild inflammation; 2: ballooning of the hepatocytes and mild-to-moderate portal inflammation; 3: intraacinar inflammation and moderate portal inflammation). Fibrosis was graded 0–4 (0: absent; 1: perisinusoidal/pericellular fibrosis; 2: periportal fibrosis; 3: brinding fibrosis; 4: cirrhosis). Patients with significant alcohol consumption (>40 g/wk) and patients with the presence of other causes of liver disease (viral, drugs, toxin, autoimmune, metabolic) were excluded.

In all patients and controls, at the time of liver biopsy, venous blood samples were drawn to determine levels of the following: serum aspartate aminotransferase and alanine aminotransferase, γ-glutamiltransferase, serum insulin and plasma glucose. Classification of diabetes mellitus, impaired glucose tolerance and impaired fasting glycaemia were based on the American Diabetes Association guidelines [13].

Laboratory features of controls and patients are summarized in Table 1 and the histological findings of NASH patients are summarized in Table 2. The control group consisted of 7 patients (3 female and 4 male, mean age 40.1 ± 6 years) undergoing elective abdominal surgery with no history of alcohol abuse and normal-appearing liver histology, normal liver biochemistries and no regular medications. Insulin resistance was calculated by the homeostatic metabolic assessment ($HOMA_{IR}$) method. $HOMA_{IR}$ = fasting serum insulin (μIU/ml) × fasting serum glucose (mmol/ml)/22.5. Informed written consent was obtained from each patient at the time of liver biopsy and the

Table 1 Characteristics and laboratory features of patients with NASH and control subjects

	NASH ($n = 11$)	Control Group ($n = 7$)
Age (yr)	40.7 ± 9.5	40.1 ± 6
Female/Male	1/10	3/4
BMI (Kg/m^2)	28.2 ± 2.7	25.4 ± 0.2
DT2/IGT/IFG/Normal	3/3/0/5	0/0/0/7
Glucose (mg/dl)	128 ± 48*	94.7 ± 5.2
Insulin (μU/ml)	18 ± 9.3*	5.3 ± 1.0
$HOMA_{IR}$	5.3 ± 3.4*	1.23 ± 0.2
AST (IU/L)	48.4 ± 25**	27 ± 4.1
ALT (IU/L)	79 ± 32*	32 ± 7.0
GGT (U/l)	44 ± 20	42 ± 16

Data are means ± SD; *$p < 0.05$ in respect of control subjects; **$p = 0.05$. T2DM: Type 2 Diabetes mellitus; IGT: Impaired Glucose Tolerance; IFG: Impaired Fasting Glucose.