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Original Article

Reduced Lysosomal Acid Lipase Activity in Adult Patients With Non-alcoholic Fatty Liver Disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is characterized by intra-hepatic fat accumulation and mechanisms involved in its pathogenesis are not fully explained. Lysosomal Acid Lipase (LAL) is a key enzyme in lipid metabolism. We investigated its activity in patients with fatty liver.

LAL activity (nmol/spot/h) was measured in 100 adult healthy subjects (HS) and in 240 NAFLD patients. A sub-analysis on 35 patients with biopsy-proven non-alcoholic steatohepatitis (NASH) was performed.

Median LAL activity was 1.15 (0.95–1.72) in HS. It was significantly reduced in NAFLD [0.78 (0.61–1.01), $p < 0.001$ vs. HS]. A further reduction was observed in the subgroup of NASH [0.67 (0.51–0.77), $p < 0.001$ vs. HS]. Patients with LAL activity below median had higher values of serum total cholesterol ($p < 0.05$) and LDL-c ($p < 0.05$), and increased serum liver enzymes (ALT, $p < 0.001$; AST, $p < 0.01$; GGT, $p < 0.01$). At multivariable logistic regression analysis, factors associated with LAL activity below median were ALT (OR: 1.018, 95% CI 1.004–1.032, $p = 0.011$) and metabolic syndrome (OR: 2.551, 95% CI 1.241–5.245, $p = 0.011$), whilst statin use predicted a better LAL function (OR: 0.464, 95% CI 0.248–0.866, $p = 0.016$).

Our findings suggest a strong association between impaired LAL activity and NAFLD. A better knowledge of the role of LAL may provide new insights in NAFLD pathogenesis.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by intra-hepatic fatty acid (lipid) accumulation, affecting a growing number of people worldwide (Vernon et al., 2011; Bellentani et al., 2000). NAFLD includes a wide spectrum of diseases extending from simple fatty liver to non-alcoholic steatohepatitis (NASH) and cirrhosis (Farrell and Larter, 2006), and, before long, will become the main cause of liver failure in the next future (Kemmer et al., 2013).

Many pathophysiological mechanisms have been associated to NAFLD, including insulin resistance (Angelico et al., 2005), dyslipidaemia (Corey et al., 2015), oxidative imbalance (Del Ben et al., 2014a), gut microbiota (Compare et al., 2012) alteration and genetic factors (Del Ben et al., 2014b). Several therapeutic intervention have been proposed (Pastori et al., 2015a; Del Ben et al., 2014c; Angelico et al., 2007; Chalasani et al., 2012).

In the liver, hyperinsulinemia is responsible for impaired mitochondrial oxidation of fatty acids, which consequently accumulate and are then partly metabolised by peroxisomes and microsomes with the production of reactive oxygen species and lipid peroxidation (Del Ben et al., 2014a).

Only a minority of patients with simple steatosis will develop NASH, and no reliable biomarkers of disease progression are available (Sanyal et al., 2015).

Lysosomal Acid Lipase (LAL) is a hydrolase that plays a key role in intra-cellular cholesterol trafficking. Inside the lysosomes, LAL hydrolyses triglycerides and cholesterol esters derived from plasma lipoproteins via LDL-receptor pathway (Fasano et al., 2012). A reduced LAL activity promotes an increased cholesterol ester storage in lysosomes, as observed in two genetic diseases, namely Wolman and Cholesterol Ester Storage Disease (CESD) (Fasano et al., 2012), which are characterized by total or sub-total LAL deficiency (Thelwall et al., 2013; Pisciotta et al., 2009; Fouchier and Defesche, 2013). These conditions are associated with severe liver steatosis and rapid liver failure (Bernstein et al., 2013; Reiner et al., 2014; Reynolds, 2013). Furthermore, LAL supplementation

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in young adults with CESD was associated with an improvement of liver steatosis (Valayannopoulos et al., 2014).

Our hypothesis was that a reduction of LAL activity may contribute to intracellular fatty acid accumulation in adult NAFLD. Thus, we measured the activity of LAL in a cohort of adult patients affected by NAFLD, and we investigated factors associated with reduced LAL activity.

2. Methods

2.1. Study Design

The study was performed in 240 consecutive patients with ultrasonography (US) evidence of fatty liver, referring to the Day Service of Internal Medicine of the Policlinico Umberto I University Hospital in Rome.

Inclusion criteria were: no history of excessive alcohol consumption defined as a mean daily intake of alcohol > 20 g; no history of Hepatitis C–B viruses infection with negative tests for the presence of hepatitis B surface antigen and antibody to hepatitis C virus; no history for other chronic liver diseases; and no therapy with drugs known to promote liver steatosis (e.g., amiodarone). Subjects underwent routine biochemical evaluation including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), fasting total and HDL-cholesterol, triglycerides, glucose and insulin. Waist circumference, height and weight were recorded and body mass index (BMI) was calculated.

The homeostasis model of insulin resistance (HOMA-IR) was used as a measure of IR (Matthews et al., 1985). Metabolic syndrome was diagnosed according to the ATP III modified criteria (Anon., 2001). The presence of diabetes and arterial hypertension was defined according to international guidelines (Authors et al., 2013; Mancia et al., 2013).

All patients provided signed informed consent before the study. The study was approved by the local ethical board of Sapienza University of Rome (ref. n° 2277/2011), and conducted according to the ethical principles embodied in the Declaration of Helsinki. Funding source: none.

2.2. Ultrasonography Evaluation of Fatty Liver

Liver US scanning was performed to assess the degree of steatosis. All US were performed by the same operator who was blinded to laboratory values using a GE Vivid S6 apparatus equipped with a convex 3.5 MHz probe. Liver steatosis was defined according to Hamaguchi criteria based on the presence of abnormally intense, high level echoes arising from the hepatic parenchyma, liver–kidney difference in echo amplitude, echo penetration into deep portion of the liver and clarity of liver blood vessel structure (Saverymuttu et al., 1986; Hamaguchi et al., 2007).

2.3. Liver Biopsy

Percutaneous liver biopsy was performed under US guide in 35 of the above fatty liver patients with clinical suspicion of NASH by their treating hepatologists. The decision to perform the biopsy was individualized and based on a persistent elevation of serum alanine aminotransferase levels (>1.5 upper normal values) for more than 6 months and the presence of bright liver at US scan. A single operator performed ultrasound-guided liver biopsies. Pathologist who examined biopsies specimen was blinded to patients' identity or clinical information. NASH diagnosis was defined using standard criteria (Sanyal et al., 2011). All patients who underwent liver biopsy satisfied histological criteria for NASH.

2.4. Lysosomal Acid Lipase Activity Assay

All blood samples were taken after a 12-hour fast. LAL-activity was dosed with dried blood spot (DBS) technique using the inhibitors Lalistat 2. Ethylene-diamine-tetra acetic acid (EDTA) blood, obtained by venepuncture, was spotted on to filter paper (Whatman grade 903 Schleicher & Schuell) and allowed to dry overnight at room temperature. Samples were stored double-bagged with desiccant at −20 °C and analysed within 2 weeks of storage. Uninhibited and inhibited with Lalistat 2 activities were dosed. LAL activity was determined by subtracting activity in the inhibited reaction from uninhibited reaction (total lipase) and expressed as nmol/spot/h of 4 MU (methylumbelliferone) (Hamilton et al., 2012). DBS tests were performed in Bambino Gesù Hospital in Rome. Physicians analysing LAL activity were unaware of clinical and biochemical characteristics of any enrolled patient. Inter and intra-assay variations were 2.4% and 2.3%, respectively.

To establish a normal value of LAL activity in adults, we performed DBS tests in 100 normal weight healthy subjects (HS), matched for age and sex with NAFLD patients. HS were not taking any drug or supplement before the blood sample collection, had no ultrasound evidence of fatty liver disease and did not suffer for any acute or chronic disease.

2.5. Statistical Analysis

Distribution of continuous variables was tested using a Kolmogorov–Smirnov test. Data are expressed as the mean ± standard deviation for normally distributed variables and as median followed by the 25th and 75th percentiles in parenthesis for non-normally distributed data. Group comparisons were performed by unpaired Student's t-test and by Mann–Whitney or Kruskal–Wallis test for non-normally distributed variables.

Proportions and categorical variables were tested by the χ^2 test or by the 2-tailed Fisher's exact.

For the analyses, we divided the cohort according to the median value of LAL activity. We performed a multivariable logistic regression analysis with LAL activity below median as dependent variable. After testing for collinearity, the following covariates were used for the model: female gender, body mass index, alanine aminotransferase (ALT), statin therapy, anti-hypertensive drug, triglycerides, gamma-glutamyl transpeptidase (γ-gt), and metabolic syndrome. Moreover, a multivariable logistic regression analysis was performed to evaluate the independent predictors of the presence of NASH after controlling for gender, age, platelets, metabolic syndrome, homeostasis model assessment-insulin resistance (HOMA-IR), serum total cholesterol, triglycerides, γ-GT, ALT and LAL activity below median. All tests are two-tailed, and a $p < 0.05$ was considered as cut-off for statistical significance. Statistical analysis was performed by using the SPSS statistical software version 20.0 for Windows (SPSS, Inc., Chicago. Illinois).

3. Results

3.1. LAL Activity in Healthy Subjects

Median blood LAL activity in 100 HS was 1.15 (IQR 0.95–1.72) nmol/spot/h; no difference between males (n = 55) and females (n = 45) [1.08 (0.94–1.70) vs. 1.17 (0.96–1.74) p = 0.486] was found. HS group had mean age of 53.0 ± 11.3 years; LAL activity was not correlated with age (rs = −0.53, p = 0.590).

3.2. Analysis of LAL Activity in NAFLD Patients

Clinical and biochemical characteristics of 240 NAFLD patients are listed in Table 1. Mean age was 55.4 ± 11.0 years; 60.4% were men.

LAL activity was significantly reduced in patients with NAFLD, as compared to those without [1.15 (0.94–1.72) vs. 0.78 (0.61–1.01) nmol/spot/h, $p < 0.001$].

To investigate factors associated with reduced LAL activity, we divided NAFLD patients in two groups according to the median value of LAL (0.78 nmol/spot/h, Table 2).