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Influence of insulin resistance in obese patients on elevated serum alanine aminotransferase

D.A. DE LUIS, R. ALLER*, O. IZAOLA, M. GONZALEZ SAGRADO, R. CONDE, J.M. GONZALEZ HERNANDEZ*

*Institute of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid; *Digestive Dept. Hospital Clinico Universitario – Valladolid (Spain)*

Abstract. – *Background and Objective:* Insulin resistance has been associated with fat liver and non-alcoholic fatty liver disease. The aim of our study was to study the influence of insulin resistance in obese patients on elevated serum alanine aminotransferase.

***Research Methods:* A population of 91 obesity non diabetic outpatients was analyzed in a cross sectional study. HOMA-IR was calculated as indicator of insulin resistance.**

***Results:* The mean age was 39.2 ± 16.7 years and the mean BMI 34.4 ± 5.2. Patients were classified as group I (control, n = 74) when serum (Alanine Aminotransferase) ALT activity was normal or group II (NAFLD, n = 17) when serum ALT activity was greater than the upper limit of normal reference laboratory (≥ 43 UI/L). Waist circumference, fat mass and hip to waist ratio were higher in group II. Insulin (13.5 ± 7.8 mUI/L vs 24.9 ± 16.7 mUI/L; $p < 0.05$), HOMA-IR (1.9 ± 1.1 vs 3.9 ± 2.8), and triglycerides levels (115.1 ± 66.8 mg/dl vs 153.2 ± 71.2 mg/dl; $p < 0.05$) were higher in group II than group I. In the logistic regresion analysis with a dependent dicotomic variable (ALT; group I and group II), the HOMA-IR remained in the model, with an Odd's Ratio to develop ALT > 43 U/L of 2.18 (CI:95%: 1.12-4.2) with each 1 unit of HOMA-IR adjusted by age, sex, weight, and dietary intake.**

***Conclusion:* Insulin resistance in obese patients is associated with ALT activity. Further study is needed to evaluate histological changes and new treatments in these patients.**

Key Words:

Alanine aminotransferase, Determinants, Insulin resistance, Nonalcoholic fatty liver disease, Obesity.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common liver disease characterized by elevated serum aminotransferase levels, hepatomegaly and accumulation of fat in liver accompanied by inflammation and necrosis resembling alcoholic hepatitis in the absence of heavy alcohol consumption[1]. Although not all patients with NAFLD are obese, obesity is considered the most important risk factor.

There are a lot of reasons for the association of overweight with NAFLD. In different series, waist to hip circumference ratio was correlated with degree of steatosis on liver biopsy[2]. Insulin resistance has been associated with fat liver and NAFLD, too[3]. Furthermore, none of these studies examined whether dietary intake and/or insulin resistance in obese patients could account for the association of NAFLD and obesity.

The association with insulin resistance and obesity has also suggested to some that NAFLD should be considered part of the metabolic syndrome with hyperlipidemia, glucose intolerance, hypertension, and increased waist circumference[4].

The aim of our study was to study the influence of insulin resistance in obese patients with elevated serum alanine aminotransferase as an indicator of NAFLD.

Subjects and Methods

Subjects

A population of 91 obesity outpatients was analyzed in a cross sectional study. The exclusion criteria were hepatitis B, C, cytomegalovirus, Epstein Barr infections, nonorgan-specific autoantibodies, alcohol consumption, diabetes mellitus, intolerance fasting glucose, medication (blood-pressure lowering medication and statins) and hereditary defects (iron and copper storage

Corresponding Author: Daniel A de Luis, MD; e-mail: dadluis@yahoo.es

diseases and alpha 1-antitrypsin deficiency). The following variables were specifically recorded: age, smoking habit, weight, and body mass index (BMI). These patients were studied in a Nutrition Clinic Unit. The study was approved by an institutional Ethics Committee.

Procedure

All patients with a 2 weeks weight-stabilization period before recruitment were enrolled. Weight, blood pressure, basal glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides blood levels were measured.

Patients were classified as group I (control; n = 74) when serum ALT activity was normal or group II (NAFLD; n = 17) when serum ALT activity was greater than the upper limit of normal reference laboratory (≥ 43 UI/L).

Assays

Alanine amino transferase and aspartate aminotransferase activities were determined by enzymatic colorimetric assay Hitachi 917 (Roche Diagnostics, Geneve, Switzerland).

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values[5]. Cortisol was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany.

Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged.

Anthropometric Measurements

Body weight was measured to an accuracy of 0.1 kg and body mass index computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Bipolar body electrical bioimpedance was used to determine body composition[6]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat and fat-free mass.

Dietary Intake

Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference[7]. Drinking and smoking habit were recorded as dicotomic variables.

Statistical Analysis

The results were expressed as mean ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, unpaired Student's-t test and ANOVA test. Non-parametric variables were analyzed with the Mann Whitney U test and K-Kruskal tests. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. Correlation analyze was realized with Pearson and Spearman tests. A logistic model was used to study the dependent variable (ALT as a dicotomic variable, group I: < 42 UI/L and group II ≥ 43 UI/L). A p-value under 0.05 was considered statistically significant. Values are mean ± SD.

Results

Ninety one patients gave informed consent and were enrolled in the study. The mean age was 39.2 ± 16.7 years and the mean BMI 34.4 ± 5.2. Baseline characteristics of patients were presented in Table I.

Table I. Clinical and epidemiological characteristics of study population.

Characteristics	Values
Age (years)	39.2 ± 16.7
Sex (male/female)	20/71
BMI (kg/m^2)	34.4 ± 5.2
Systolic BP (mmHg)	136 ± 15
Diastolic BP (mmHg)	79 ± 11
Glucose (mmol/dl)	3.9 ± 1.2
Total Cholesterol (mg/dl)	211 ± 43.8
LDL-Cholesterol (mg/dl)	130.5 ± 44.7
HDL-Cholesterol (mg/dl)	54 ± 14.5
Insulin (UI/L)	15.7 ± 10.7
HOMA-IR	2.6 ± 2.2

BP: Blood Pressure. HOMA: Homeostasis model assessment [glucose 0 min (mmol/L) × insulin 0 min (mU/ml)/22.5].

Table III. Cardiovascular risk factors by ALT.

Characteristics	ALT	
	(< 42 UI/L) (n = 74)	(≥ 43) (n = 17)
Systolic BP (mmHg)	132.3 ± 13.1	136.7 ± 10
Diastolic BP (mmHg)	80.3 ± 9	77.3 ± 8.6
Glucose (mg/dl)	92.1 ± 8.3	96.6 ± 5.1
Total Ch. (mg/dl)	203.8 ± 33.6	207.7 ± 34.5
LDL Ch. (mg/dl)	119.6 ± 53.2	131.8 ± 41.8
HDL Ch. (mg/dl)	54.9 ± 10.3	45.2 ± 15.7*
Triglycerides (mg/dl)	115.1 ± 66.8	153.2 ± 71.2*
Insulin (mUI/L)	13.5 ± 7.8	24.9 ± 16.7*
HOMA-IR	1.9 ± 1.1	3.9 ± 2.8*

*($p < 0.05$) BP: Blood Pressure. Ch.: cholesterol. HOMA: Homeostasis model assessment [glucose 0 min (mmol/L) × insulin 0 min (mU/ml)/22.5].

All subjects were weight stable during the 2 weeks period preceding the study (body weight change, 0.3 ± 0.1 kg). Anthropometric measurements showed an average waist circumference (109.2 ± 14.7 cm), waist-to hip ratio (0.94 ± 0.9), and average weight (92.2 ± 17 kg). Tetrapolar body electrical bioimpedance showed the next data; fat free mass (49.8 ± 14.4 kg) and fat mass (40.1 ± 12.7 kg).

Serial assessment of nutritional intake with 3 days written food records showed a caloric intake of 1673 ± 554 kcal/day, a carbohydrate intake of 168.63 ± 70.6 g/day, a fat intake of 73.7 ± 29.2 g/day and a protein intake of 80.1 ± 22.9 g/day. Polyunsaturated fatty acid intake was 6.6 ± 3.3 g/day, monounsaturated fatty acid intake was 34.9 ± 13 g/day and saturated fatty acid intake was 20.2 ± 10.6 g/day.

Table II. Anthropometric characteristics by ALT group.

Characteristics	ALT	
	(< 42 UI/L) (n = 74)	(≥ 43) (n = 17)
Weight (kg)	89.2 ± 14	101.5 ± 26*
BMI (kg/m^2)	34.1 ± 4	36.2 ± 6
Fat free mass (kg)	48.2 ± 11	47.2 ± 12.1
Fat mass (kg)	25.8 ± 9.8	36.7 ± 11.6*
Waist circunference	105.8 ± 13.7	114.4 ± 17.3*
Waist to hip ratio	0.9 ± 0.11	0.95 ± 0.1*

BMI: Body Mass Index.
*($p < 0.05$).

Anthropometric variables by ALT groups are shown in Table II. Waist circumference, fat mass, hip to waist ratio were higher in group II.

Cardiovascular risk factors variables by ALT groups are shown in Table III. Insulin, HOMA-IR levels and triglycerides levels were higher in group II. HDL cholesterol were higher in group I and group II.

Caloric intake and saturated fatty acids intake was higher in group II (Table IV). No differences were detected among other intakes.

Correlation analysis showed a significant correlation among ALT levels and the independent variables (weight (r = 0.37; $p < 0.05$), waist circumference (r = 0.24; $p < 0.05$), waist-hip ratio (r

Table IV. Dietary intake and habits.

Characteristics	ALT	
	(< 42 UI/L) (n = 74)	(≥ 43) (n = 17)
Energy (kcal/d)	1561 ± 508	1906 ± 593*
Carbohydrate (g/d)	154.9 ± 69	189 ± 85
Fat (g/d)	69.8 ± 25.8	88.1 ± 26.1
Cholesterol (mg/d)	355 ± 144	483 ± 219
Polyunsaturated fat (g/d)	6.2 ± 25	7.7 ± 3.5
Monounsaturated fat (g/d)	33.4 ± 10.2	39.4 ± 11.7
Saturated fat (g/d)	19.1 ± 10.3	29.2 ± 11.8*
Protein (g/d)	77.2 ± 21.5	88.7 ± 23.5
% Smoking habit	7.7	7.5
Hs. Aerobic exercise per week	0.8 ± 2.1	0.5 ± 1.6

*($p < 0.05$).

= 0.2; $p < 0.05$), saturated fatty acid intake (r = 0.23; $p < 0.05$), triglycerides (r = 0.2; $p < 0.05$), HDL cholesterol (r = -0.3; $p < 0.05$), insulin (r = 0.39; $p < 0.05$), and HOMA-IR (r = 0.52; $p < 0.05$).

HOMA-IR was correlated with (weight (r = 0.7; $p < 0.05$), waist circumference (r = 0.5; $p < 0.05$), waist-hip ratio (r = 0.3; $p < 0.05$), fat mass (r = 0.5; $p < 0.05$) and HDL cholesterol (r = -0.3; $p < 0.05$).

In the logistic regression analysis with a dependent dichotomic variable (ALT < 42 U/L (group I) and ALT ≥ 43 U/L (group II), the HOMA-IR remained in the model, with an Odds ratio to develop ALT > 43 U/L of 2.18 (CI:95%: 1.12-4.2) with each 1 unit of HOMA-IR adjusted by age, sex, BMI, and dietary intake.

Discussion

The present study demonstrates that insulin resistance determined with HOMA model is associated with elevated serum alanine aminotransferase (ALT), irrespective of weight, body mass index and dietary intake.

Insulin resistance was measured by the homeostasis model assessment method, this method correlates closely with other test, such as the euglycemic glucose clamp[5]. Patients with diabetes or fasting altered glycaemia were excluded from the study, because the homeostasis model is not reliable with marked hyperglycemia. In univariant analysis, ALT levels were associated with weight, waist circumference, triglycerides, HDL cholesterol, total caloric amount and saturated fatty acids dietary intakes. However in logistic analysis adjusted by other variables only insulin resistance remained in the model. Marchesini et al[8] demonstrated a closely correlation between insulin resistance (HOMA) and NAFLD, too. Other authors have been detected this relation using the clamp technique[3,9,10] with results supporting our conclusions.

Body mass index, fat mass and waist to hip ratio were not independently associated with ALT levels, suggesting that obesity and splanchnic fat distribution might also be effects of insulin resistance, rather than being directly involved in the etiology of fatty liver. The finding of NAFLD in lean patients without diabetes mellitus, reinforces the proposal that insulin resistance is the main key in NAFLD, rather than the degree of generalized adiposity alone [3,10].

A limitation of our study was the use of elevated serum ALT activity and exclusion criterias as indicator of NAFLD, without a histologic diagnosis. However, excluding persons with the most common other causes of liver injury, we believe that most of the remaining patients with elevated ALT activity had NAFLD[11]. Another limitation was the inability to evaluate the severity of liver injury, as would be possible in histologic studies. However, liver biopsy is not feasible in our population which participants are asymptomatic.

The nature of the connection between insulin resistance and hepatic steatosis remains unclear[12]. In obese patients, the primary abnormality may be genetically induced insulin resistance, with a secondary increase of serum triglyceride levels due to enhance of peripheral lipolysis. The resulting hepatic supply of fatty acids and insulin may increase triglyceride deposition in the liver[13]. This fatty acid deposition increases substrates for oxidative stress. Acylation stimulating protein (ASP) may play role in the pathogenesis of the NAFLD. Yesilova et al[14] has detected significantly higher levels of ASP in NAFLD patients than controls.

Our results have therapeutic implications. An insulin-sensitizing drugs or a weight-reducing nutritional regimen might break the link between insulin resistance and hepatic steatosis. For example, a high fat diet leads to the progression of NAFLD in an obese rat model[15]. Marchesini et al[16] have treated these patients with metformin, this drug reduced mean transaminase concentrations, insulin resistance and liver volume. In other study, acarbose attenuated NAFLD progression in an experimental model of NAFLD in rats[17]. Two drugs used in obese patients, orlistat and sibutramine[17-19], have shown that drug-induced weight losses result in reduction of insulin resitance and improvements in biochemical markers of NAFLD.

In conclusion, insulin resistance in obese patients is associated with ALT activity. Further study is needed to evaluated histological changes and new treatments in these patients.

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