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Role of insulin resistance and adipocytokines on serum alanine aminotransferase in obese patients with type 2 diabetes mellitus

D.A. DE LUIS, R. ALLER, O. IZAOLA, M. GONZALEZ SAGRADO, R. CONDE, B. DE LA FUENTE

Institute of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid, Valladolid, Spain

Abstract. – BACKGROUND AND OBJECTIVE: The aim of our study was to study the association of insulin resistance expressed by HOMA and adipokines in obese type 2 diabetic patients with or without hyper-transaminasemia.

SUBJECTS AND METHODS: A population of 72 obese patients with type 2 diabetes mellitus was analyzed. HOMA-IR was calculated as indicator of insulin-resistance. Adipocytokines blood levels were measured.

RESULTS: Patients were classified as group I (n=37) when serum ALT activity was normal or group II (NAFLD patients: n=35) when serum ALT activity was greater than the median value of the group (≥ 28 UI/L). In NAFLD group, BMI, weight, fat mass, waist to hip ratio, waist circumference, triglycerides, HOMA and insulin levels were higher than control group.

In the logistic regression analysis with a dependent variable (ALT) and the statistical univariant variables as independent variables, the HOMA-IR remained in the model, with an Odd's ratio of 1.21 (CI:95%: 1.11-1.35) to have a high ALT level with each 1 unit of HOMA-IR adjusted by age, sex, weight, and dietary intake.

CONCLUSIONS: Some metabolic parameters are associated with elevated ALT in female obese patients. However, adjusted by other variables, only insulin resistance remained associated.

Key Words:
Adipocytokines, Alanine aminotransferase, Diabetes mellitus, Insulin resistance, Non-alcoholic fatty liver disease, Obesity.

Introduction

Non-alcoholic fat 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]. The natural history of NAFLD is not well defined; obesity is considered the most important risk factor.

There are a lot of reasons for the association of overweight with NAFLD. In different studies, 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[3-4]. The association with insulin resistance and obesity has also suggested that NAFLD should be considered part of the metabolic syndrome with hyperlipidemia, glucose intolerance, hypertension, and obesity[5]. This implies that the increased mortality rate among NAFLD patients is partly due to these associations.

Adipose tissue secrets several bioactive proteins or adipokines, that regulate the metabolism. These adipokines include leptin, resistin, adiponectin and tumor necrosis factor alpha (TNF-α). Leptin suppresses food intake and increases energy expenditure by enhancing thermogenesis and metabolic rate. Recent reports suggest that leptin contributes to atherosclerosis and cardiovascular disease in obese patients[6]. Adiponectin is an adipocyte-derived collagen like protein identified through an extensive search of adipose tissue. Hypoadiponectinemia increased risk of coronary artery disease togheter with the presence of multiple risk factors, indicating that adiponectin is a key factor of the metabolic syndrome[5]. Resistin is a 12.5 KD, cysteine-rich protein indentified by screening for the genes that are induced during the differentiation of the adipocytes with a questionable role in human obesity[7]. TNF alpha and interleukin 6 are increased in most animal and human models with obesity and insulin resistance[8]. Some studies have demonstrated an association of ALT with low levels of adiponectin and insulin resistance, too[9]. However, this type of studies on liver mark-

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

ers and insulin resistance has not included the adipocytokines, which are important since these are known to relate insulin resistance and adipose tissue. Insulin resistance was measured by the homeostasis model assessment method because this method correlates with euglycemic glucose clamp[10] and it is an easy method to be use in clinical practice.

The aim of our study was to study the association of insulin resistance expressed by HOMA and adipokines in obese type 2 diabetic patients with or without hyper-transaminasemia.

Subjects and Methods

Subjects

A population of 72 naïve obese patients with type 2 diabetes mellitus was analyzed in a cross sectional study. The exclusion criteria was hepatitis B, C, cytomegalovirus, Epstein Barr infections, nonorgan-specific autoantibodies, alcohol consumption, hereditary defects (iron and copper storage diseases and alpha 1-antitrypsin deficiency) as well as the use of sulphonylureas, thiazolidinediones, metformin, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, drinking and/or smoking habit. These patients were studied in a Nutrition Clinic Unit. The study was approved by the institutional Ethics Committee of our University.

Procedure

Weight, height, body mass index (BMI), blood pressure, basal glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, C reactive protein, triglycerides and adipocytokines (leptin, resistin, adiponectin, interleukin-6 and TNF-alpha) blood levels were measured.

Assays

Serum total cholesterol and triglyceride concentrations were determined by automated 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, CA, USA). 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[10], with a normal value of (2-14 UI/L) and analytical sensitivity 0.5 UI/L.

CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensivity 0.5 mg/dl.

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

Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Minneapolis, MN, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml. Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.246 ng/ml and a normal range of 865-21424 ng/ml. TNF alpha was measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of TNF-alpha was (0.5-15.6 pg/ml).

Anthropometric Measurements

Body weight was measured to an accuracy of 0.01 Kg and height on meters, body mass index (BMI) computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences were measured and waist-to hip ratio (WHR) were measured, too. Bipolar body electrical bioimpedance was used to determine body composition (fat mass and fat free mass)[11]. 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.

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

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 dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used validated as reference[12]. Physical activity was assayed by a validated questionnaire fulfilled by the patients.

Statistical Analysis

The results were expressed as average ± 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 with Bonferroni corrections as needed. Nonparametric variables were analyzed with the Mann Whitney U test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. Correlation analysis was realized with Pearson and Spearman tests. A logistic regression model was used to study the dependent variable (ALT) (group 1 vs group 2). A p value ≤ 0.05 was considered statistically significant.

Results

Seventy two patients (28 males and 44 females) gave informed consent and were enrolled in the study. The mean age was 57.5±15.7 years and the mean BMI 37.8±6.4.

Anthropometric measurements showed an average waist circumference (115.4±14.1 cm), waist-to hip ratio (0.95±0.9), and average weight (95.3±17.3 kg). Tetrapolar body electrical bioimpedance showed the next data: fat free mass (51.4±14.1 kg) and fat mass (51.4±14.3 kg). Serial assessment of nutritional intake with 3 days written food records showed a caloric intake of 1634±521 kcal/day, a carbohydrate intake of 164.9±62.6 g/day, a fat intake of 70.7±31.6 g/day and a protein intake of 82.1±23.2 g/day.

Patients were classified as group I (n=37, 15 males and 22 females) when serum ALT activity was normal or group II (NAFLD patients; n=35, 13 males and 22 females) when serum ALT activity was greater than the median value of the group (≥ 28 UI/L).

Table I shows the differences in anthropometric variables. In NAFLD group, BMI, weight, fat mass, waist to hip ratio and waist circumference were higher than control group.

Table II shows the differences in classic cardiovascular risk factors. In NAFLD group, triglycerides, HOMA and insulin levels were higher than control group. Caloric intake and physical activity were similar in both groups (Table III).

Table IV shows the blood adipocytokines levels. Adipocytokines levels were similar in both groups.

In the logistic regression analysis with a dicotomic dependent variable (ALT, group 2 vs group 1) and the statistical univariant variables as independent variables, the HOMA-IR remained in the model, with an Odd's ratio of 1.21 (CI:95%: 1.11-1.35) to have a high ALT level (group 2) with each 1 unit of HOMA-IR adjusted by age, sex, weight, and dietary intake.

Discussion

The present study demonstrates that anthropometric parameters, triglyceride levels and higher levels of HOMA are associated to higher values

Table I. Anthropometric characteristics.

Characteristics	ALT (Group 1) (n = 37)	ALT (Group 2) (n = 35)
Weight (kg)	91.4 ± 16.3	99.3 ± 17.4*
BMI (kg/m^2)	37.9 ± 6.4	39.6 ± 6.4*
Fat mass (kg)	45.1 ± 9.4	58.3 ± 15.1*
Waist circumference	114.6 ± 14.4	116.2 ± 13.9*
Waist to hip ratio	0.94 ± 0.07	0.97 ± 0.08*

BMI: Body mass index. ($p < 0.05$), significant differences between groups.

Table II. Cardiovascular risk factors.

Characteristics	ALT (Group 1) (n = 37)	ALT (Group 2) (n = 35)
Systolic BP (mmHg)	135.1 ± 15.9	139.8 ± 15.6
Diastolic BP(mmHg)	84.6 ± 8.5	86.4 ± 10.2
Glucose (mg/dl)	126.4 ± 21.7	134.3 ± 26.8
Total ch. (mg/dl)	215.8 ± 41	212.4 ± 42
LDL ch. (mg/dl)	140.5 ± 39.8	137.8 ± 35.6
HDL ch. (mg/dl)	53.5 ± 13.1	50.3 ± 12.2
Triglycerides(mg/dl)	125.9 ± 54.5	172.3 ± 70.4*
Insulin (mUI/L)	18.8 ± 12.1	28.8 ± 14.7*
CRP (mg/dl)	8.1 ± 5.8	6.2 ± 4.6
HOMA-IR	5.8 ± 3.6	10.1 ± 6.7*

CRP: c reactive protein. HOMA-IR (homeostasis model assessment –insulin resistance) BP: blood pressure. ($p < 0.05$), significant differences between groups.

of serum alanine aminotransferase (ALT). However, in a logistic regression only HOMA remained in the model as a predictor to have high levels of ALT.

Insulin resistance was measured by the HOMA method; this method correlates with euglycemic glucose clamp[10]. In univariant analysis, patients with high ALT levels had better metabolic and anthropometric profile. However in logistic regression model adjusted by other variables only insulin resistance remained in the model. Previously, Marchesini et al[13] have demonstrated a closely correlation between insulin resistance (HOMA) and NAFLD. Other authors have been detected this relation using the clamp technique[14-16] with results supporting our conclusions. Insulin resistance has a key role in the development of hepatic steatosis and, potentially, steatohepatitis. Obesity and type 2 diabetes, conditions associated with peripheral insulin resistance, are frequently observed in patients with NAFLD. Resistance to the action of insulin results in important changes in lipid metabolism. These include enhanced peripheral lipolysis, increased triglyceride synthesis, and increased hepatic uptake of fatty acids. Each of these contribute to the accumulation of hepatocellular triglyceride[14], which in turn results in a preferential shift from carbohydrate to fatty acids beta-oxidation, an occurrence that has been demonstrated in patients with insulin resistance.

In our study, BMI 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[15].

The reasons for the association between ALT and serum levels of leptin, adiponectin, are not clear in other studies[16-17]. However, physiological

Table III. Dietary intakes and habits.

Characteristics	ALT (Group 1) (n = 37)	ALT (Group 2) (n = 35)
Energy (kcal/d)	1576 ± 507	1685 ± 396
Carbohydrate(g/d)	163.4 ± 67.2	166.2 ± 54.3
Fat (g/d)	69.5 ± 26.7	75.4 ± 22.5
Cholesterol (mg/d)	215.8 ± 39.3	212.4 ± 39.6
Protein (g/d)	82.8 ± 26.1	81.4 ± 20.6
Hs. Aerobic exercise per week	0.8 ± 2.0	0.9 ± 1.8

No statistical differences.

Table IV. Circulating adipocytokines by ALT groups.

Characteristics	ALT (Group 1) (n = 37)	ALT (Group 2) (n = 35)
IL 6 (pg/ml)	2.63 ± 4.6	2.46 ± 3.8
TNF-œ (pg/ml)	4.4 ± 2.9	5.6 ± 4.4
Adiponectin (ng/ml)	31.2 ± 27.3	29.7 ± 28.6
Resistin (ng/ml)	3.7 ± 1.9	3.4 ±1.7
Leptin(ng/ml)	93.3 ± 87.1	80.7 ± 78.2

IL-6: interleukin 6. No statistical differences.

explanations are possible. Adiponectin has been found to improve hepatic steatosis[18] and leptin has been shown to be associated with NAFLD[19]. Adiponectin inhibits liver TNF alpha expression and also inhibits expression of several cytokines in hepatic stellate cells[20]. In interpreting these differences, a number of points need to be kept in mind. First, the lack of association in multivariant analysis between adiponectin and ALT in our study may be adiponectin is a subrogate variable of insulin resistance. Second, the type of patients included in the studies are different, one study has been designed[21] with only men without overweight, perhaps these healthy subjects have a preliminary influence of adipocytokines and our sample (with females and males with and average BMI of 37) has a more important influence of insulin resistance than in a healthy population.

Conclusions

Anthropometric parameters, triglyceride levels, insulin resistance and HOMA are associated with elevated serum alanine aminotransferase (ALT) in naive obese patients with type 2 diabetes mellitus. However, in a logistic regression model only insulin resistance remained in the model. Further studies are needed to evaluate this complex interaction.

Conflict of Interest

None declared.

References

1) Ludwig J, Viggiano TR, McGill DB, Oh BJ. Non-alcoholic steatohepatitis: Mayo Clinic experiencies with a hitherto unnamed disease. Mayo Clinic Proc 1980; 55: 434-438.
2) Kral JG, Schaffner F, Pierson RN, Wang J. Body fat topography as an independent predictor of fatty liver. Metabolism 1993; 42: 548-551.
3) Chitturi S, Abeygunasekera S, Farell GC, Holmes-Walker J, Hui M, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002; 35: 373-379.
4) Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, Kral JG. Liver pathology and the metabolic syndrome X in severe obesity. J Clin Endocrinol Metab 1999; 84: 1513-1517.
5) Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T, Matsuzawa Y; Osaka CAD Study Group. Coronary artery disease. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003; 23: 85-89.
6) Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature 1999; 401: 73-76.
7) De Luis DA, Gonzalez Sagrado M, COnde R, Aller R, Izaola O, de la Fuente B, Castrillón JL, Romero E. Relation of resistin levels with cardiovasular risk factors and insulin resistance in non-diabetes obese patients. Diabetes Res Clin Pract 2010; 89: 110-114.
8) Matsuzawa Y. Adipocytokines: Emerging therapeutic targets. Curr Atheroscler Rep 2005; 7: 58-62.
9) Vosarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002; 51: 1889-1895.
10) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.

11) Pichard C, Slosman D, Hirschel B, Kyle U. Bioimpedance analysis in patients: an improved method for nutritional follow up. Clin Res 1993; 41: 53ª.

12) Mataix J, Mañas M. Tablas de composición de alimentos españoles. Ed: University of Granada, 2003.

13) Marchesini G, Brizi M, Morselli-Labate A, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of non-alcoholic fatty liver disease with insulin resistance. Am J Med 1999; 107: 450-455.

14) Sanyal AJ, Campbell Sargent C, Mirshashi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA. Non-alcoholic steatohepatitis: association of insulin resistance and mitocohondrial abnormalities. Gastroenterology 2001; 120: 1183-1192.

15) Rashid M, Roberts EA. Non-alcoholic steatohepatitis in children. J Pediatr Gastroenterol Nutr 2000; 30: 48-53.

16) de Luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Conde R, Bellido D. Influence of insulin resistance and adipocytokines on elevated serum alanine aminotransferase in obese patients. Arch Med Res 2008; 39: 10-114.

17) Aller R, de Luis DA, Fernandez L, Calle F, Velayos B, Olcoz JL, Izaola O, Gonzalez Sagrado M, Conde R, Gonzalez JM. Infleuce of insulin resistance and adipokines in the grade of steatosis of non-alcoholic fatty liver disease. Dig Dis Sci 2008; 53; 1088-1092.

18) Xu A, Wang Y, Keshaw H, Xu A, Wang Y, Keshaw H. The fat derived hormone adiponectin alleviates alcoholic and non-alcoholic fatty liver diseases in mice. J Clin Invest 2003; 112: 91-100.

19) Lee DH, Ha HM, Kim JH, Christiani DC, Gross MD, Steffes M, Blomhoff R. Gamma glutamyltransferase and diabetes-a year follow up study. Diabetologia 2003; 46: 359-364.

20) Pagano C, Soardoo G, Esposito W, Fallo F, Basan L, Donnini D, Federspil G. Plasma adiponectin is decreased in non-alcoholic fatty liver disease. Eur J Endocrinol 2005; 152: 113-118.

21) Kazumi T, Kawaguchi A, Hirano T, Yoshino G. Serum alanine aminotransferase is associated with serum adiponectin, C reactive protein and apolipoprotein B in young Healthy Men. Horm Res Metab 2006; 38: 119-124.