Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1566–1570

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Hyperinsulinemia predicts hepatic fat content in healthy individuals with normal transaminase concentrations

Diego Ardigò[a,c], Filippo Numeroso[a], Silvia Valtueña[a], Laura Franzini[a], Pier Marco Piatti[b], Lucilla Monti[b], Roberto Delsignore[a], Gerald M. Reaven[c], Ivana Zavaroni[a,*]

[a]*Department of Internal Medicine and Biomedical Sciences, University of Parma, Parma 43100, Italy*
[b]*Metabolic and Cardiovascular Rehabilitation Unit, IRCCS San Raffaele Hospital, Milan 20132, Italy*
[c]*Falk Cardiovascular Research Center, School of Medicine, Stanford University, Stanford, CA 94305, USA*

Received 18 April 2005; accepted 27 May 2005

Abstract

Although the prevalence of insulin resistance (IR) and compensatory hyperinsulinemia (CH) is increased in patients with nonalcoholic fatty liver disease, the role of IR/CH in regulation of hepatic fat content in healthy volunteers with normal concentrations of alanine transaminase (ALT) has not been defined. To address this issue, hepatic fat content was quantified by ultrasound in 69 (30 men, 39 women) healthy individuals, without known risk factors for liver disease and with plasma ALT concentrations of less than 30 U/L. Experimental variables quantified included body mass index, waist circumference, systolic and diastolic blood pressures, and fasting plasma glucose, fasting plasma insulin (FPI), and lipid concentrations. Subjects were classified as having no (55%), mild (27%), or moderate to severe (18%) hepatic steatosis on the basis of the ultrasound results. Statistically significant ($P < .05$-$.001$) correlations (Spearman ρ values) existed between liver fat content and ALT (0.26), body mass index (0.52), waist circumference (0.50), systolic blood pressure (0.28), diastolic blood pressure (0.27), fasting plasma glucose (0.47), FPI (0.56), triglycerides (0.30), and high-density lipoprotein cholesterol (−0.35). Multivariate general discriminant analysis and multiple linear regression analysis indicated that FPI was the only independent predictor ($P < .001$) of both liver fat content and ALT concentrations. Fasting plasma insulin (a surrogate estimate of IR/CH) predicts hepatic fat content and ALT in healthy volunteers with normal transaminase concentrations, independently of the other anthropometric and metabolic variables measured.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is estimated to be the most common cause of chronic liver disease in developed countries, with prevalence rates ranging from 15% to 39% in general population studies [1]. Although neither the exact etiology of NAFLD nor the factors responsible for its progression are completely understood, there is substantial evidence of a strong relationship between NAFLD and the presence of metabolic abnormalities typically associated with insulin resistance (IR) syndrome [2-8].

Because most studies examining the relationship between NAFLD and IR have been performed in patients with clinical evidence of liver disease [9,10], there is relatively little information concerning a possible link between hepatic fat content and IR/hyperinsulinemia in apparently healthy individuals as defined by normal transaminase concentrations. The most common laboratory abnormality in patients with NAFLD is an increase in aminotransferase activity, particularly alanine transaminase (ALT), but the degree of enzyme elevation is not marked [2]. Furthermore, there is evidence that increases in hepatic fat content can occur in individuals without manifest liver disease, whose ALT concentrations are within the normal range [11,12]. Thus, it seemed important to examine the role that IR/hyperinsulinemia might play in regulation of hepatic fat content in apparently healthy individuals with normal values for liver transaminase concentrations. The present study was initiated to address this issue and was based on the hypothesis that there would be a significant correlation between IR (assessed by the plasma insulin levels) and both hepatic fat content and ALT concentrations in apparently healthy individuals with ALT concentrations less than 30 U/L.

* Corresponding author.

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.05.027

2. Materials and methods

The experimental population consisted of 69 individuals (39 women and 30 men) who volunteered to have an echographic study of their liver. They were recruited during a follow-up examination of participants in the Barilla Study of apparently healthy individuals, followed since 1981 to assess the impact of IR on human disease. To qualify, volunteers had to be free of a history or clinical evidence of diabetes, cardiovascular, or liver disease, and had to have a daily alcohol intake of less than 30 g for men and less than 20 g for women (calculated by a frequency questionnaire of the usual intake of spirits, wine, or beer).

Presence of hepatitis B or C virus infection was excluded by negative blood test for markers of infection (HBsAg, anti-HBsAg, anti-HBcAg, anti-HCV) or blood donation within the last 6 months. Finally, to qualify for inclusion, the concentrations of aspartate transaminase (AST) and ALT had to be less than 40 and 30 U/L, respectively.

A single trained individual, unaware of the results of the anthropometric and metabolic results, performed the echographic examinations of the liver. The presence of steatosis was assessed on the basis of sonographic findings of (1) increased echogenicity of the echotexture (bright liver), (2) increased liver echotexture compared with the kidneys, (3) vascular blurring, and (4) deep-echo attenuation [13]. Volunteers were classified as having no steatosis if they had a normal hepatic echo pattern and their kidney was brighter than their liver. Subjects were considered to have mild steatosis if there was a slight increase in fine echoes in liver parenchyma, with normal visualization of the diaphragm and intrahepatic vessel borders. Moderate to severe steatosis was said to be present if the magnitude of the increase in fine echoes in the liver parenchyma was accentuated, associated with greater impairment of visualization of the intrahepatic vessels and diaphragm, and lack of visualization of the posterior right lobe of the liver [13].

Table 1
Relationship between clinical characteristics and degree of hepatic steatosis

Variables	Degree of steatosis			P trend[a]	
	Absent	Mild	Moderate to severe	ρ	P
Subjects (n [%])	38 (55)	19 (27)	12 (18)	–	–
Sex (M/F)	13:25	11:8	6:6	0.165	NS
Age (y)	60 ± 1	64 ± 1	59 ± 2	0.068	NS
BMI (kg/m^2)	25.5 ± 0.4	28.7 ± 0.8	30.0 ± 1.3	0.524	<.001
Waist (cm)	90 ± 1	98 ± 2	103 ± 3	0.504	<.001
SBP (mm Hg)	131 ± 3	146 ± 4	139 ± 5	0.281	<.05
DBP (mm Hg)	84 ± 1	89 ± 2	91 ± 3	0.267	<.05
ALT (U/L)	19 ± 1	19 ± 1	23 ± 1	0.259	<.05
AST (U/L)	22 ± 1	21 ± 1	22 ± 1	0.056	NS

Values are expressed as mean ± SEM. M indicates male; F, female; NS, not significant; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[a] Based on Spearman correlation.

Table 2
Relationship between the metabolic variables and degree of hepatic steatosis

Variables	Degree of steatosis			P trend[a]	
	Absent	Mild	Moderate to severe	ρ	P
FPG	90 ± 1	95 ± 2	102 ± 3	0.471	<.001
FPI (μmol/mL)	7 ± 1	10 ± 1	18 ± 3	0.560	<.001
TC	228 ± 8	219 ± 9	217 ± 11	−0.070	NS
HDL-C	65 ± 2	58 ± 3	53 ± 2	−0.349	<.005
TG	83 ± 9	101 ± 12	109 ± 13	0.301	<.05
LDL-C	146 ± 7	141 ± 8	142 ± 10	−0.010	NS

Values are expressed as mean ± SEM and are in milligrams per deciliter unless otherwise noted.

[a] Based on Spearman correlation.

Height, weight, waist circumference (WC), and blood pressure were measured by physical examination. Waist circumference was assessed following the National Institutes of Health guidelines for obesity evaluation [14]. Body mass index (BMI) was calculated as weight (kilograms) divided by the square of height (meters). Blood pressure was measured after the patient had been quietly seated for at least 5 minutes and until the last 2 measurements of systolic blood pressure and diastolic blood pressure varied less than 5 mm Hg. The mean of these last 2 values was used as experimental variable. Blood was drawn after an overnight fast for determination of plasma concentrations of glucose (fasting plasma glucose [FPG]), insulin (fasting plasma insulin [FPI]), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), and transaminases (AST and ALT). FPG, FPI, TG, TC, and HDL-C were determined as described previously [15], and low-density lipoprotein cholesterol (LDL-C) values were calculated using the formula of Friedewald [16].

Mean values of the experimental variables were compared among the 3 groups of subjects (no steatosis, mild, and moderate to severe steatosis) using the Spearman correlation test to assess the presence of significant trends of change as a function of the degree of hepatic fat. To determine which of the variables were independent predictors of hepatic steatosis, a stepwise general discriminant analysis (GDA) model was created using, as the dependent variable, the degree of steatosis and, as independent, all the variables that significantly changed as a function of steatosis degree in the univariate analysis. Furthermore, to determine the independent predictors of the ALT values, a stepwise multiple linear regression analysis was performed using the ALT concentrations as the dependent variable and the same independent variables of the GDA.

Results are expressed in the text and in the tables as mean ± SEM. The P value for significance was set to .05, and statistical analyses were performed by using SPSS statistical software for Windows, version 12.0 (SPSS Inc, Chicago, Ill).

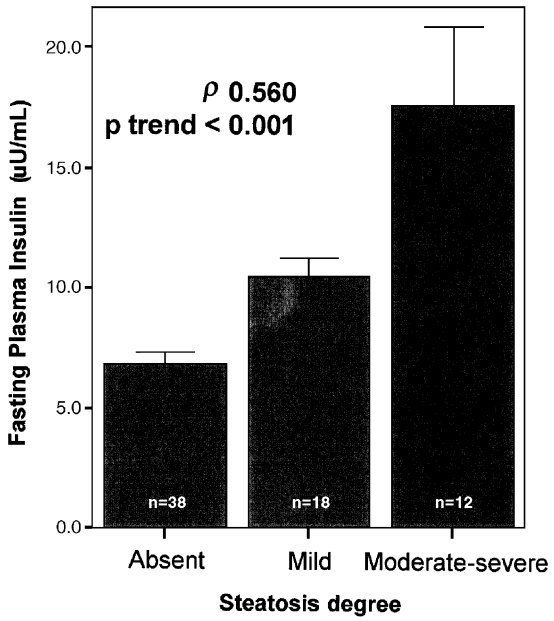

Fig. 1. Relationship between FPI concentration (a surrogate marker of IR/hyperinsulinemia) and degree of hepatic steatosis. *P* trend calculation is based on the Spearman correlation coefficient (ρ).

The protocol has been approved by the Ethics Review Committee of the University of Parma. All subjects gave written informed consent to the study prior enrollment.

3. Results

Table 1 presents the clinical characteristics of the experimental population, subdivided into 3 groups on the basis of their degree of steatosis. These data indicate that 31 (45%) of these apparently healthy individuals had some degree of steatosis, which was considered to be moderate or severe in 12 (18%) of them. It can be seen that, although the 3 groups were of similar age, the greater the BMI and the larger the WC, the more severe the degree of steatosis. In addition, there was a statistically significant trend for both blood pressure and ALT concentrations to increase in parallel to the amount of hepatic fat. However, there was no significant relationship between the estimates of hepatic fat and AST concentration.

Table 2 presents the metabolic characteristics of the 3 experimental groups and demonstrates that there were statistically significant relationships between FPG, FPI, HDL-C, and TG, and hepatic fat content. In contrast, neither TC nor LDL-C concentrations varied as a function of degree of steatosis. These findings show that the metabolic variables that usually cluster with IR/hyperinsulinemia [17,18] were also associated with degree of hepatic steatosis. To pursue this issue further, we compared mean FPI concentrations as a surrogate estimate of IR [19,20] in the 3 experimental groups. These results are shown in Fig. 1, and it is quite clear that the greater the degree of hepatic steatosis, the higher the FPI concentration ($P < .001$).

To evaluate the factors that might be independent predictors of hepatic fat content, a stepwise GDA was performed using, as dependent variable, the amount of hepatic fat and, as independent variables, age, BMI, or WC, and all the metabolic variables in Table 2 that were significantly associated with hepatic fat content. The results of the GDA analysis are seen in Table 3 and indicate that FPI was the only independent predictor of degree of hepatic steatosis.

As shown in Table 1, ALT concentrations were significantly associated with hepatic fat content, whereas AST concentrations were not. Multivariate regression analysis

Table 3
Stepwise multivariate GDA to assess the independent predictors of degree of hepatic steatosis

Step	Independent variable	F	*P*
1	FPI	16.369	<.001

The GDA was performed using the echographic degree of liver steatosis as dependent variable and age, BMI or WC, and FPI, FPG, TG, and HDL-C concentrations as independent variables.

Table 4
Multivariate linear regression analysis to assess the independent predictors of ALT concentrations

Step	Independent variable	β coefficient	*P*	R^2
1	FPI	.448	<.001	0.20

The multivariate linear regression analysis was performed using ALT concentrations as the dependent variable and age, BMI or WC, FPI, FPG, TG, and HDL-C concentrations as the independent variables.

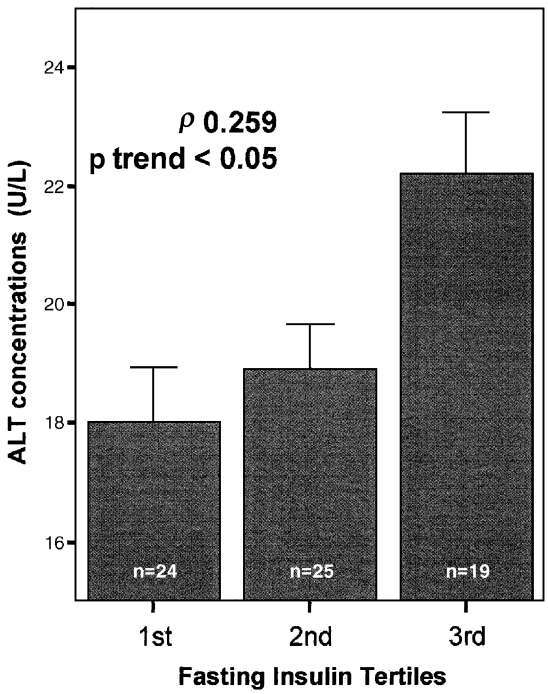

Fig. 2. Relationship between FPI tertiles and ALT concentrations. *P* trend calculation is based on the Spearman correlation coefficient (ρ).

was performed to see if any of the variables shown to be associated with increased hepatic fat content were also independent predictors of ALT concentrations. The results of this analysis are shown in Table 4, and similar to the findings in regards to hepatic fat content, FPI concentration was the only independent predictor of ALT concentration.

The relationship between FPI and ALT concentrations was further explored in Fig. 2 by comparing the ALT concentrations as a function of tertile of FPI. These results are displayed in Fig. 2 and demonstrate that ALT concentration increases progressively in parallel with tertile of FPI.

4. Discussion

The results presented indicate that the higher the FPI concentration is in apparently healthy individuals, the greater will be the amount of fat in the liver and the higher the ALT concentrations. As such, they support the hypothesis that the untoward effects of IR/hyperinsulinemia on hepatic fat content can be observed in individuals without clinical evidence of any liver abnormality. To put these findings into context, several issues must be addressed. Most fundamentally, care has been taken to exclude individuals with a history of diabetes or cardiovascular or liver disease, and to only include volunteers with a daily alcohol intake of less than 30 g in men and less than 20 g in women; negative blood tests for HBsAg, anti-HBsAg, anti-HBcAg, and anti-HCV; and with normal ALT (<30 U/L) and AST (<40 U/L) concentrations. Based on these criteria, it seems highly unlikely that any of the participants in this study had any cause other than IR/hyperinsulinemia to account for the evidence of hepatic steatosis.

Secondly, we have used the FPI concentration as a surrogate estimate of IR, and there is evidence that this is a reasonable choice [19,20]. However, in addition to providing a surrogate estimate of IR, the compensatory increase in plasma insulin concentration also serves the purpose of preventing frank hyperglycemia from developing in insulin-resistant individuals [17,18]. Unfortunately, the IR/hyperinsulinemia is associated with an increase in hepatic TG synthesis [21] and the resultant accumulation of fat in the liver and the higher ALT concentrations. Thus, it seems most likely that IR, per se, is not responsible for the adverse consequence noted in this study, but it is the philanthropic effort on the part of the pancreatic beta cell to overcome the defect in insulin action that is the guilty party. This separation of the relative roles of IR vs compensatory hyperinsulinemia is similar to the ability of hyperinsulinemia in insulin-resistant individuals to increase renal sodium retention, decrease renal uric acid clearance, and stimulate testosterone secretion by the ovary [18].

The third issue that must be addressed is the manner in which ultrasound was used to identify those with and without evidence of hepatic steatosis, as well as the validity of the separation of those with increased hepatic fat content into 2 broad categories: mild steatosis vs moderate to severe steatosis. Although tissue obtained by liver biopsy would provide the most accurate way to quantify hepatic fat content, the fact that our study population had no evidence, either by history or transaminase values, of liver disease, biopsy was not a viable alternative. There are other noninvasive approaches we could have used, that is, magnetic resonance imaging, computed tomography, or nuclear magnetic resonance, but they are more complicated and expensive than is ultrasound, and it is not clear that they are more closely correlated with hepatic fat than estimates obtained by echocardiography. For example, evidence has been summarized pointing out that there is very good sensitivity (80%-90%) and specificity (85%-95%) when the ability of ultrasound to detect hepatic steatosis is compared with histologic analysis [15], and the accuracy to demonstrate increased fat in the liver approaches 100% when used in patients with echographic features of moderate or severe steatosis [22]. Finally, the relationship between ultrasound estimates of hepatic fat and the amount quantified by liver biopsy is essentially as good as is seen when computed tomography and magnetic resonance imaging are used as the imaging technique [15,23,24].

Based on the considerations discussed previously, we would argue that the relationship we observed between FPI concentrations and hepatic fat content is unlikely the result of our reliance on the echographic quantification of fat in the liver. The presence of a statistically significant relationship between FPI and ALT concentrations lends further credence to the view that IR/hyperinsulinemia leads to an increase in hepatic fat content.

In conclusion, IR/compensatory hyperinsulinemia and the metabolic abnormalities related to these defects have been shown to be associated with both hepatic fat content and ALT concentrations in apparently healthy individuals with normal liver function tests. Statistical analysis of the relationship observed indicate that IR/hyperinsulinemia was the only independent predictor of both hepatic fat content and ALT, consistent with the existence of a causal relationship between the abnormalities in insulin metabolism and hepatic steatosis in apparently healthy individuals without a clinical diagnosis of NAFLD. This finding does not imply that measurement of plasma insulin concentration is a useful way to identify patients with NAFLD but does suggest that liver function should be evaluated in persons with the metabolic abnormalities and clinical syndromes associated with IR/hyperinsulinemia, that is, the IR syndrome [17,18].

Acknowledgment

The study was supported by a grant from Italian Ministry of University and Scientific Research (COFIN MIUR 2001–protocol no. 2001065732_003).

We thank Dr Luigi Ippolito, MD (biochemical analysis laboratory), and Dr Gianfranco Elia, MD (Division of Infectious Diseases), from Azienda Ospedaliera–Universitaria di Parma (Parma Hospital), Parma, Italy.

References

[1] Younossi ZM, Diehl AM, Ong JP. Nonalcoholic fatty liver disease: an agenda for clinical research. Hepatology 2002;35:746-52.

[2] Sanyal AJ, American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002; 123:1705-25.

[3] Angelico F, Del Ben M, Conti R, Francioso S, Feole K, Maccioni D, et al. Non-alcoholic fatty liver syndrome: a hepatic consequence of common metabolic diseases. J Gastroenterol Hepatol 2003;18:588-94.

[4] Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease. A feature of metabolic syndrome. Diabetes 2001;50:1844-50.

[5] Cortez-Pinto H, Camilo ME, Baptista A, De Oliveira AG, De Moura MC. Non-alcoholic fatty liver: another feature of the metabolic syndrome? Clin Nutr 1999;18:353-8.

[6] Silverman JF, O'Brien KF, Long S, Leggett N, Khazanie PG, Pories WJ, et al. Liver pathology in morbidly obese patients with and without diabetes. Am J Gastroenterol 1990;85:1349-55.

[7] Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, et al. Liver pathology and the metabolic syndrome X in severe obesity. J Clin Endocrinol Metab 1999;84:1513-7.

[8] Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. Gastroenterology 2002;122:1649-57.

[9] Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107:450-5.

[10] Loguercio C, De Simone T, D'Auria MV, de Sio I, Federico A, Tuccillo C, et al. Non-alcoholic fatty liver disease: a multicentre clinical study by the Italian Association for the Study of the Liver. Dig Liver Dis 2004;36:398-405.

[11] Clark JM, Diehl AM. Defining nonalcoholic fatty liver disease: implications for epidemiologic studies. Gastroenterology 2003;124: 248-50.

[12] Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003;37: 1286-92.

[13] Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in non-alcoholic fatty liver disease. Gastroenterology 2002;123:745-50.

[14] The practical guide identification, evaluation, and treatment of overweight and obesity in adults. Bethesda (Md): National Institutes of Health; 2000 (NIH publication no. 00-4084).

[15] Zavaroni I, Bonini L, Gasparini P, Barilli AL, Zuccarelli A, Dall'Aglio E, et al. Hyperinsulinemia in a normal population as a predictor of non–insulin-dependent diabetes mellitus, hypertension and coronary heart disease: the Barilla Factory revisited. Metabolism 1999;48:989-94.

[16] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[17] Reaven GM. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.

[18] Reaven GM. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am 2004;33:283-303.

[19] Olefsky J, Farquhar JW, Reaven GM. Relationship between fasting plasma insulin level and resistance to insulin-mediated glucose uptake in normal and diabetic subjects. Diabetes 1973;22:507-13.

[20] Yeni-Komshian H, Carantoni M, Abbasi F, Reaven GM. Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy, nondiabetic volunteers. Diabetes Care 2000;23:171-5.

[21] Olefsky JM, Farquhar JW, Reaven GM. Reappraisal of the role of insulin in hypertriglyceridemia. Am J Med 1974;57:551-60.

[22] Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary? Eur J Gastroenerol Hepatol 2003;15:539-43.

[23] Mathiesen UL, Franzen LE, Aselius H, Resjo M, Jacobsson L, Foberg U, et al. Increased liver echogenicity in ultrasound reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. Dig Liver Dis 2002;34:516-22.

[24] Sandford NL, Walsh P, Matis C, Baddelsey H, Powell LW. Is ultrasound useful in assessment of diffuse parenchymal liver disease? Gastroenterology 1985;89:186-91.