

Original

Effect of two different hypocaloric diets in transaminases and insulin resistance in nonalcoholic fatty liver disease and obese patients

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Abstract

Objective: The aim of our study was to examine the changes in hypertransaminasemia after weight reduction in obese patients with and without NAFLD and the relation with insulin resistance.

Research Methods: A population of 162 obese patients was randomly allocated to two groups: a) diet I (low fat) and b) diet II (low carbohydrate), dieting along 3 months. Patients were classified as group I (n=112) when serum ALT activity was normal or group II (NAFLD, n=30) when serum ALT activity was (≥ 43 UI/L).

Results: In control group with diet I, BMI, weight, fat mass, waist to hip ratio, waist circumference, systolic pressure, total cholesterol, LDL cholesterol, HOMA and insulin levels decreased. In NAFLD group with diet I improved the same parameters and glucose, triglycerides, ALT, AST, gamma-glutamyl transferase levels, too. In control group with diet II, BMI, weight, fat mass, waist to hip ratio, waist circumference, systolic pressure, total cholesterol, LDL cholesterol, HOMA and insulin levels decreased. In NAFLD group with diet II improved the same parameters and glucose, triglycerides, ALT and gamma-glutamyl transferase levels, without statistical changes in AST.

Conclusion: We showed that weight reduction secondary to two hypocaloric diets was associated with improvement in hypertransaminasemia and insulin resistance in NAFLD patients.

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Key words: *Insulin resistance. Low carbohydrate hypocaloric diet. Low fat hypocaloric diet. Nonalcoholic fatty liver disease. Obesity.*

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EFECTO DE DOS DIETAS HIPOCALÓRICAS EN LOS NIVELES DE TRANSAMINASAS Y RESISTENCIA A LA INSULINA EN PACIENTES OBESOS CON HÍGADO GRASO NO ALCOHÓLICO

Resumen

Objetivo: El objetivo de nuestro trabajo fue evaluar los cambios en las transaminasas tras la pérdida de peso en pacientes obesos con y sin esteatohepatitis no alcohólica (EHNA) y su relación con la resistencia a la insulina

Material y métodos: Se aleatorizó una muestra de 162 pacientes obesos en dos grupos: a) dieta I (baja en grasa) y b) dieta II (baja en hidratos de carbono), siguiendo al dieta durante 3 meses. Los pacientes se clasificaron como grupo I (n=112), con unos niveles de ALT normales y grupo II (EHNA, n=30) con unos niveles de ALT superiores a 43 UI/L.

Resultados: En el grupo I con la dieta I disminuyó el IMC, peso, masa grasa, índice cintura cadera, circunferencia cintura, presión sistólica, colesterol LDL, colesterol total, HOMA e insulina. En el grupo NASH con la dieta I disminuyeron los mismos parámetros además de la glucosa, triglicéridos, ALT, AST y gamaGT. En el grupo control con la dieta II disminuyó el IMC, peso, masa grasa, índice cintura cadera, circunferencia de la cintura, presión sistólica, colesterol total, LDL colesterol, HOMA e insulina. En el grupo NASH con la dieta II disminuyeron los mismos parámetros junto a la glucosa, triglicéridos, ALT y gama GT sin cambios en los niveles de ALST.

Conclusión: La pérdida de peso con dos dietas hipocalóricas diferentes mejora los niveles de transaminasas y la resistencia a la insulina en pacientes con NASH.

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Palabras clave: *Resistencia a la insulina. Dieta baja en hidratos de carbono. Dieta baja en grasa. Esteatohepatitis no alcohólica.*

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 consumption¹. 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 obesity with NAFLD. In different series, abdominal fat was correlated with degree of steatosis on liver biopsy². Insulin resistance has been associated with fat liver and NAFLD, too^{3,4}. No proven treatment for patients with NAFLD is currently available. Weight reduction with diet changes are usually recommended as the first step in the treatment of patients with this condition. Achieving and maintaining weight reduction may improve NAFLD, but the results of several reports are inconsistent⁵⁻⁹. Other study¹⁰ has demonstrated an improvement in hypertransaminasemia and insulin resistance in NAFLD patients after a hypocaloric diet.

The aim of our study was to examine the changes in hypertransaminasemia after weight reduction in obese patients with and without NAFLD and the relation with insulin resistance, secondary to two hypocaloric diets.

Subjects and methods

Subjects

A population of 162 obesity outpatients was analyzed (134 patients in group I as obese individuals with a BMI ≥ 30 and 28 patients in group II as patients with NAFLD). In group I, the inclusion criteria was BMI ≥ 30 and in group II BMI ≥ 30 and ALT ≥ 43 UI/L. The exclusion criteria in both groups were, alcohol consumption, medication (blood-pressure lowering medication and statins) assessed by direct questions to the patients. And diabetes mellitus, intolerance fasting glucose, hepatitis B, C, cytomegalovirus, Epstein Barr infections, nonorgan-specific autoantibodies and hereditary defects (iron and copper storage diseases and alpha 1-antitrypsin deficiency), assessed by biochemical tests. The following variables were specifically recorded: age, smoking habit, weight, waist circumference, body mass index (BMI). The study was approved by our institutional ethic committee.

Procedure

Patients were classified as group I (obese patients; n=134) when serum ALT activity was normal or group II (NAFLD patients; n=28) when serum

ALT activity was greater than the upper limit of normal reference laboratory (≥ 43 UI/L).

Patients were randomly allocated to two groups during three months: a) diet I (low fat) 1500 kcal/day, 53% carbohydrates, 20% proteins, 27% fats) and b) diet II (low carbohydrate: 1507 kcal/day, 38% carbohydrates, 26% proteins, 36% fats). Subject food intakes were recorded at baseline and after dietary advice after 3 months with 3 days written food records.

Weight, blood pressure, basal glucose, transaminases, insulin, HOMA, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides blood levels were measured at baseline time and after 3 months.

Assays

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 values¹¹.

Alanine amino transferase, aspartate aminotransferase activity, bilirubin and gamma-glutamyl transferase were determined by enzymatic colorimetric assay Hitachi 917 (Roche Diagnostics, Geneva, 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.

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 (BMI) computed as body weight/(height²). 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 composition¹². 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 (Dietsource, Novartis, Geneva, Switzerland), incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analysed with a computer-based data evaluation system. National composition food tables were used as reference¹³. Physical activity remained unchanged during the follow up period.

Statistical analysis

Sample size was calculated to detect differences over 5 UI/L on transaminases levels with 90% power and 5% significance (n=75, in each diet group). 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, paired Student's-t test. Non-parametric variables were analyzed with the W-Wilcoxon test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A p-value under 0.05 was considered statistically significant.

Results

One hundred and sixty two patients gave informed consent and were enrolled in the study. All subjects were weight stable during the 2 weeks period preceding the study (body weight change, 0.19±0.11 kg;p=0.7).Patients with both diets reached the dietary recommendations.

Table 1 shows the differences in anthropometric variables. In control group with diet I (age 46.9±17.3

years, 17 males and 51 females), BMI, weight, fat mass, waist to hip ratio and waist circumference decreased. In NAFLD group with diet I (age 45.5±14.9 years, 4 males and 11 females), BMI, weight, fat mass, waist to hip ratio and waist circumference decreased, too. After dietary intervention an average of 4 kg has been loss in both groups. All anthropometric parameters were higher in NAFLD group than control group with diet I.

In control group with diet II (age 45.8±14.3 years, 13 males and 53 females), BMI, weight, fat mass, waist to hip ratio and waist circumference decreased. In NAFLD group with diet II (age 46.8±15.9 years, 3 males and 11 females), BMI, weight, fat mass, waist to hip ratio and waist circumference decreased, too. After dietary intervention an average of 4 kg has been loss in both groups. All anthropometric parameters were higher in NAFLD group than control group with diet II (table I).

Table II shows the differences in classic cardiovascular risk factors. In control group with diet I, systolic pressure, total cholesterol, LDL cholesterol, HOMA and insulin levels decreased. In NAFLD group, systolic pressure, glucose, total cholesterol, LDL cholesterol, triglycerides, insulin and HOMA decreased. Systolic blood pressure, glucose, triglycerides, insulin and HOMA were higher in NAFLD group than control group. In control group with diet II, systolic pressure, total cholesterol, LDL cholesterol, HOMA and insulin levels decreased. In NAFLD group, systolic pressure, glucose, total cholesterol, LDL cholesterol, triglycerides, insulin and HOMA decreased. Glucose, total cholesterol, triglycerides, insulin and HOMA were higher in NAFLD group than control group.

In NAFLD group, alanine aminotransferase, aspartate aminotransferase, and gamaglutamine transferase improved with diet type I. With diet type II, alanine aminotransferase and gamaglutamine transferase improved. However aspartate aminotransferase remained unchanged. Alanine amino-

Table I
Anthropometric response in control group and non alcoholic fatty liver disease group

Characteristics	(group I) (n = 68)		(group II) (n = 15)		(group I) (n 66)		(group II) (n = 13)	
	Basal	3 months	Basal	3 months	Basal	3 months	Basal	3 months
	BMI	35.6±6.7	34.2±6.6*	36.3±3.7 [§]	34.9±3.7 [§]	35.6±7.2	34.1±7.1*	38.2±5.8 [§]
Weight (kg)	92.0±17.9	88.3±17.5*	101.2±17 [§]	97.3±17.1 [§]	92.0±17.9	89.9±17.2*	103.3±22.2 [§]	98.3±21.4 [§]
FM (kg)	40.1±12.7	37.6±12.9*	42.5±12.6 [§]	39.3±12.3 [§]	39.5±10.9	36.4±10.4*	42.4±12 [§]	40.3±12.8 [§]
WC	107.1±15.6	102.9±15.8*	112.2±9.3 [§]	108.6±10.8 [§]	107.1±13.7	101.5±13.9*	116.1±15 [§]	109.1±15.4 [§]
WHR	0.89±0.07	0.88±0.07*	0.93±0.1 [¶]	0.92±0.2*, [¶]	0.90±0.08	0.88±0.09*	0.94±0.1 [¶]	0.91±0.2 [§]

BMI: Body mass index.FM: fat mass. WC: waist circumference. WHR: Waist to hip ratio. * (p<0.05) with basal values in each group. [§] (p<0.05) among values of group I and II.

Table II
Cardiovascular risk factors response in control and non alcoholic fatty liver disease group

Characteristics	(group I) (n = 68)		(group II) (n = 15)		(group I) (n 66)		(group II) (n = 13)	
	Basal	3 months	Basal	3 months	Basal	3 months	Basal	3 months
	Systolic BP (mmHg)	138.8±17.1	120.1±25.7*	140.4±10.5	126.1±11.1*	144.4±19.9	122.6±27*	140±3.5
Diastolic BP(mmHg)	84.4±8.1	81.8±6.5	85.1±7.5	82.0±7.5	80.6±11.1	79.5±16.6	84.6±4.9	85.0±5.0
Glucose (mg/dl)	95.9±11.4	93.5±11.1	119.1±10 [†]	98.0±16.4* [†]	99.1±21.4	96.5±15.8*	07.6±15 [†]	121±22.5* [†]
Total ch. (mg/dl)	205.9±37.4	193.2±34.3*	208.9±28.5	197.4±40*	194.7±39.5	183.1±35.8*	205±51	199.4±40* [†]
LDL ch. (mg/dl)	120.8±36.9	115.1±33.2*	129.0±12.2	104.7±14.4*	116.8±36.1	104±33.1*	141±40.1 [†]	23±49.4* [†]
HDL ch. (mg/dl)	53.9±13.2	51.7±12.2	55.6±10.4	54.0±9.1	57.8±13.2	56.5±12.2	50.3±14.5	49.6±9.1
TG (mg/dl)	126.8±52.8	117.7±49.3	201.8±63.4 [†]	126.5±50* [†]	110.4±52.8	114.3±39.3	156±66.4 [†]	37.3±66* [†]
Insulin(mUI/L)	16.2±8.3	14.4±7.1*	36.4±16.9 [†]	13.5±7.4*	14.4±8.1	11.7±5.8*	28.8±14 [†]	16.3±8.4*
HOMA	3.95±2.3	3.37±1.6*	12.1±4.6 [†]	3.1±1.8* [†]	3.46±2.1	2.71±1.33*	8.1±9.1 [†]	4.2±2.4*

CH: cholesterol. HOMA: homeostatic model assessment. TG: triglycerides. * (p<0.05) with basal values in each group. ** (p<0.05) among values of group I and II.

Table III
Liver function response in control group and non alcoholic fatty liver disease group

Characteristics	(group I) (n = 68)		(group II) (n = 15)		(group I) (n 66)		(group II) (n = 13)	
	Basal	3 months	Basal	3 months	Basal	3 months	Basal	3 months
	ALT (UI/L)	22.6±7.8	20.7±8.1	68.5±22.3 [†]	33.9±14.4* [†]	19.6±4.8	18.8±4.7	53.1±11.2 [†]
AST (UI/L)	19.8±4.5	19.3±4.5	42.4±13.1 [†]	24.2±6.9* [†]	20.5 ±7.8	19.2±8.1	30.5±6.2 [†]	28.7±7.8 [†]
BT (mg/dl)	0.55±0.2	0.56±0.3	0.6±0.4	0.6±0.3	0.6±0.3	0.59±0.3	0.6±0.4	0.5±0.3
GGT (UI/L)	22.3±18.3	20.6±24.4	50.0±10.8 [†]	32.1±14.9* [†]	19.3±13.3	20.1±20.7	55.1±46 [†]	45.7±38* [†]

ALT: alanine aminotransferase. AST: aspartate aminotransferase. BT: bilirubin. GGT: gamaglutamine transferasa. BMI: Body mass index.WC: waist circumference. WHR: Waist to hip ratio. * (p<0.05) with basal values in each group. [†](p<0.05) among values of group I and II.

transferase, aspartate aminotransferase and gamaglutamine transferase levels were higher in NAFLD group than control group. These enzymes remained unchanged in control group after treatment.

Discussion

In this study, we found that weight reduction secondary to a low fat diet was associated with ALT and AST improvement and only ALT improvement with a low carbohydrate diet in NAFLD patients.

We showed that 4% weight reduction for three months was associated with transaminases improvement in obese patients with NAFLD. Hence, based on our data, a 4-5% reduction in body weight could be recommended as an initial therapeutic target in patients with NAFLD to improve liver function. ALT and AST levels improved with low fat diet, this data indicate a main role of the dietary fat in the physiology of NFALD¹⁴. Furthermore, in our study, 4% of

weight reduction was associated with decrease of anthropometric parameters, glucose, total cholesterol, LDL-cholesterol, HOMA and insulin. These data indicate that achieving and maintaining 5% weight reduction will improve not only liver function but also several other components of the metabolic syndrome, for instance insulin resistance and it is not related with the type of hypocaloric diet. Implications of weight loss on lowering the risk of type 2 diabetes mellitus in such patients is an important goal demonstrated in previous studies¹⁵.

In our design, insulin resistance was measured by the homeostasis model assessment method, this method correlates closely with other test, such as the euglycemic glucose clamp¹⁶. Marchesini et al¹⁷ have demonstrated a closely correlation between insulin resistance (HOMA) and NAFLD. Other authors have been detected this relation using the clamp technique¹⁸ with results supporting our conclusions.

A limitation of our study was the use of elevated serum ALT activity and exclusion criteria as indicator of NAFLD, without a histological 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 NAFLD¹⁹. Another limitation was the inability to evaluate the severity of liver injury, as would be possible in histological studies. However, liver biopsy is not feasible in our population which participants are asymptomatic and ethic problems could be reached.

The nature of the connection between insulin resistance and hepatic steatosis remains unclear. 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 liver²⁰. This fatty acid deposition increases substrates for oxidative stress. Acylation stimulating protein (ASP) may play role in the pathogenesis of the NAFLD²¹.

Our results have therapeutic implications. A weight-reducing nutritional regimen might break the link between insulin resistance and hepatic steatosis and a diet with low fat could improve transaminases better than other hypocaloric diets. For example, a high fat diet leads to the progression of NAFLD in an obese rat model^{14,22}. Marchesini et al²³ 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 rats²⁴. Two drugs used in obese patients, orlistat and sibutramine^{25,26}, have shown that drug-induced weight losses result in reduction of insulin resistance and improvements in biochemical markers of NAFLD.

In conclusion, we showed that weight reduction secondary to two different hypocaloric diets was associated with improvement in hipertransaminasemia and insulin resistance in NAFLD patients. A low fat diet could decrease more transaminase levels than a low carbohydrate diet.

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