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Effect of a high monounsaturated vs high polyunsaturated fat hypocaloric diets in nonalcoholic fatty liver disease

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Abstract. – OBJECTIVE: Hyperaminotransferase is an important problem in obese patients. We decide to examine the changes in hyperaminotransferasemia after weight reduction in obese patients with and without NAFLD secondary to a high monounsaturated fat vs. a high polyunsaturated fat hypocaloric diets.

PATIENTS AND METHODS: A population of 306 obese patients was randomly allocated to two groups: Diet M (high monounsaturated fat hypocaloric diet) and Diet P (high polyunsaturated fat hypocaloric diet). Patients were classified as group I (obese subjects; n=262) when serum ALT activity was normal or group II (NAFLD patients; n=44) when serum ALT activity was (≥ 43 UI/L).

RESULTS: In NAFLD group with diet M, BMI, weight, fat mass, waist circumference, systolic blood pressure, total cholesterol, LDL cholesterol), insulin and HOMA-R decreased. In NAFLD group with diet P, BMI, weight, fat mass, waist circumference, systolic blood pressure, total cholesterol, LDL cholesterol), insulin and HOMA-R decreased, too. In NAFLD group, alanine aminotransferase [(diet M) –20.3±19.2 UI/L vs. (diet P) –14.2±20.1 UI/L], aspartate aminotransferase [(diet M) –11.3±12.2 UI/L vs. (diet P) –11.1±10.1 UI/L], and gammaglutamyl transferase [(diet M) –18.1±12.2 UI/L vs. (diet P) –10.9±20.1 UI/L] improved with both diets.

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

Key Words:

Insulin resistance, High monunsaturated fat hypocaloric diet, High polyunsaturated fat hypocaloric diet, Nonalcoholic fatty liver disease, Obesity.

Introduction

The rising incidence of obesity is associated with many obesity-related health complications including diabetes mellitus type 2, cardiovascular disease, hypertension, hyperlipidemia and nonalcoholic fatty liver disease (NAFLD). NAFLD is defined as the accumulation of lipids, primarily in the form of triacylglycerols in individuals who do not consume significant amounts of alcohol and in whom other known causes of steatosis, such as certain toxins and drugs, have been excluded[1]. The spectrum of NAFLD includes simple fatty liver, non-alcoholic steatohepatitis (NASH), cirrhosis post NASH, hepatocellular carcinoma and advanced liver disease, which leads to liver related death[2].

Although not all patients with NAFLD are obese, obesity is considered the most important risk factor. Insulin resistance is present in and is a significant predictor of NAFLD in most patients[3], even a percentage of patients who are not overweight[4-5]. NAFLD is a multifactorial disease that involves a complex relation of diet and genetics. A cornerstone of the management strategy in such patients is the use of diet to decrease body weight, and improve all metabolic parameters. Achieving and maintaining weight reduction may improve NAFLD, but the results of several reports are variables[6-10]. However, other studies[11,12] have demonstrated an improvement in hypertransaminasemia and insulin resistance in NAFLD patients after different hypocaloric diets. Perhaps the distribution of macronutrients and type of dietary fat, considering previous studies, may influence secondary transaminases changes after weight loss. Despite the fact that weight loss and dietary changes are recommended as primary treatment for NAFLD, no specific guidelines exist pertaining to diet.

Considering the evidence that dietary changes play a role in metabolic response of patients with NAFLD. We decide to examine the changes in aminotransferases levels after weight reduction in obese subjects with and without NAFLD and the relation with insulin resistance, secondary to

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an enriched monounsaturated fat vs. an enriched polyunsaturated fat hypocaloric diets in obese subjects.

Patients and Methods

Subjects

A population of 306 obese subjects was analyzed (262 patients in group I as obese participants with a BMI ≥ 30 and 44 patients in group II as patients with NAFLD). In group I, the inclusion criteria was BMI ≥ 30 and in group II BMI ≥ 30 and alanine amino transferase (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 subjects; n=262) when serum ALT activity was normal or group II (NAFLD patients; n=44) when serum ALT activity was greater than the upper limit of normal reference laboratory (≥ 43 UI/L). Patients were randomly allocated to one of two diets for a period of three months. Diet M (enriched monounsaturated fat hypocaloric diet) consisted in a diet of 1342 kcal with the next distribution of percentage of macronutrients; 46.6% of carbohydrates, 34.1% of lipids and 19.2% of proteins. The distribution of fats was; 21.7% of saturated fats, 67.5% of monounsaturated fats and 10.8% of polyunsaturated fats. Diet P (enriched polyunsaturated (PUFAs) fat hypocaloric diet) consisted in a diet of 1459 kcal, 45.7% of carbohydrates, 34.4% of lipids and 19.9% of proteins). The distribution of fats was; 21.8% of saturated fats, 55.5% of monounsaturated fats and 22.7% of polyunsaturated fats (7 g per day of w-6 fatty acids, 2 g per day of w-3 fatty acids and a ratio w6/w3 of 3.5) The exercise program consisted of an aerobic exercise at least 3 times per week (60 min each).

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

Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, 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 analyzer 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-IR) was calculated using these values[13].

Alanine amino transferase, aspartate aminotransferase activity, bilirrubin and gammaglutamyl transferase were determined by enzymatic colorimetric assay Hitachi 917 (Roche Diagnostics, Basel, Switzerland).

Anthropometric Measurements

Body weight was measured to an accuracy of 0.1 kg and 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 to derive waist-to hip ratio (WHR) were measured, too. Bipolar body electrical bioimpedance was used to determine body composition[14]. 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. The same investigator measured patients and controls. Precautions taken to insure valid BIA measurements were: no alcohol within 24 hours of taking the test, no exercise or food for four hours before taking the test.

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 (Dietsource, Novartis, Geneve, Switzerland), 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[15]. Physical activity remained unchanged during the follow up period.

Statistical Analysis

Sample size was calculated to detect differences over 10 UI/L on aminotransferases levels with 90% power and 5% significance (n=150, 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

Three hundred and six patients gave informed consent and were enrolled in the study. The mean age was 49.3±16.7 years and their mean BMI 36.5±5.4, with 86 males (28.1%) and 220 females (71.9%).

In the 154 subjects (128 *group I with ALT < 43 UI/L* and 26 group II with NAFLD) treated with diet M, basal assessment of nutritional intake with a 3 days written food record showed a calorie intake of 1923.2±589.6 kcal/day, a carbohydrate intake of 212.1±72.1 g/day (44.2% calories), a fat intake of 77.4±35.2 g/day (36.3% calories) and a protein intake of 87.6±32.7 g/day (19.5% calories). During the intervention, these patients reached the dietary recommendations of diet M; 1439.1±298.2 calories (44.4% carbohydrates, 34.5% lipids and 21.1% proteins). The distribution of dietary fats was; 20.1% saturated fats, 68.2% monounsaturated fats and 11.7 % polyunsaturated fats.

In the 152 subjects (134 *group I with ALT<43 UI/L* and 18 group II with NAFLD treated with diet P, basal assessment of nutritional intake with a 3 days written food record showed a calorie intake of 1823.9±489.1 kcal/day, a carbohydrate intake of 199.8±51.3 g/day (43.9% calories), a fat intake of 75.8±17.2 g/day (36.2% calories) and a protein intake of 84.8±18.1 g/day (19.9% calories). During the intervention, these patients reached the recommendations of diet; 1471.2±543.3 calories (44.6% carbohydrates, 34.4% lipids and 21.0% proteins). The distribution of dietary fats was; 20.2% saturated fats, a 53.6% monounsaturated fats and a 24.2% polyunsaturated fats (6.8 g per day of w-6 fatty acids, 2.4 g per day of w-3 fatty acids and a ratio w6/w3 of 2.8).

Table I shows the differences in anthropometric variables. In control group with diet M (age 49.2±17.3 years, 37 males and 91 females), BMI (–1.7±1.4 kg/m^2), weight (–4.3±3.7 kg), fat mass (–3.5±3.3 kg) and waist circumference (–4.6±4.3 cm) decreased. In NAFLD group with diet M (age 49.5±12.9 years, 7 males and 19 females), BMI (–1.6±2.3 kg/m^2), weight (–4.9±3.6 kg/m^2), fat mass (–3.0±3.6 kg) and waist circumference (–3.6±2.7 cm) decreased, too. After dietary intervention an average of 4.3±2.2 kg has been loss in both groups. All anthropometric parameters (pre- and post- intervention diet) were higher in NAFLD group than control group with diet M.

In control group with diet P (age 48.8±12.3 years, 37 males and 97 females), BMI (–2.1±1.3 kg/m^2), weight (–3.2±3.1 kg), fat mass (–3.5±3.1 kg) and waist circumference (–3.2±2.2 cm) decreased. In NAFLD group with diet P (age 48.9±12.9 years, 5 males and 13 females), BMI (–1.8±1.4 kg/m^2), weight (–1.7±1.4 kg/m^2), fat mass (–4.8±2.4 kg/m^2) and waist circumference (–4.3±1.2 cm) decreased, too. After dietary intervention an average of 4.0±1.9 kg has been loss in both groups. All anthropometric parameters were higher in NAFLD group than control group with diet P (Table I).

Table II shows the differences in classic cardiovascular risk factors. In control group with diet M, systolic blood pressure (–4.2±1.7 mmHg), total cholesterol (–9.9±8.4 mg/dl), LDL cholesterol (–5.7±7.4 mg/dl), HOMA-R (–0.53±1.2 units) and insulin (–1.6±1.1 UI/L) levels decreased. In NAFLD group, systolic blood pressure (–5.7±2.2 mmHg), total cholesterol (–10.1±2.2 mg/dl), LDL cholesterol (–16.1±4.2 mg/dl), insulin (–2.91±2.3 units) and HOMA-IR (–0.81±1.5 units) decreased. Triglycerides, insulin and HOMA-IR were higher in NAFLD group than control group.

Table I. Summary of CAPE effects, all effects are significant ($p \leq 0.05$).

Characteristics	Diet M				Diet P			
	Group I (n = 128)		Group II (n = 26)		Group I (n = 134)		Group II (n = 18)	
	Basal	3 months	Basal	3 months	Basal	3 months	Basal	3 months
BMI	37.6 ± 6.3	35.9 ± 5.9*	39.4 ± 5.4§	37.7 ± 4.2*,§	38.2 ± 6.2	35.8 ± 7.1*	38.9 ± 5.3§	37.1 ± 6.2*,§
Weight (kg)	94.9 ± 17.7	90.6 ± 16.3*	110.6 ± 21.6§	105.7 ± 20.5*,§	93.6 ± 17.9	90.4 ± 17.2*	101.5 ± 21.2§	96.8 ± 20.4*,§
FM (kg)	40.9 ± 12.5	37.4 ± 11.5*	43.1 ± 12.6§	40.1 ± 12.0*,§	38.5 ± 10.9	35.0 ± 10.4*	41.4 ± 11.1§	36.4 ± 10.8*,§
WC	112.7 ± 13.6	108.1 ± 13.2*	118.4 ± 13.3§	114.6 ± 12.8*,§	111.1 ± 13.1	108.0 ± 12.9*	116.8 ± 10.8§	112.5 ± 11.4*,§
WHR	0.89 ± 0.07	0.88 ± 0.06	0.93 ± 0.1§	0.92 ± 0.1§	0.90 ± 0.08	0.89 ± 0.09	0.94 ± 0.1§	0.92 ± 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	Diet M				Diet P			
	Group I (n = 128)		Group II (n = 26)		Group I (n = 134)		Group II (n = 18)	
	Basal	3 months	Basal	3 months	Basal	3 months	Basal	3 months
Systolic BP (mmHg)	127.4 ± 15.1	123.1 ± 13.7*	126.8 ± 16.1	121.1 ± 10.1*	127.4 ± 13.9	123.3 ± 11.1*	128.0 ± 3.4	120.2 ± 4.1*
Diastolic BP(mmHg)	80.7 ± 8.1	78.4 ± 8.5	83.7 ± 9.9	81.0 ± 7.2	81.6 ± 8.1	79.2 ± 7.6	82.4 ± 7.9	81.0 ± 5.0
Glucose (mg/dl)	101.4 ± 11.4	99.7 ± 10.1	100.9 ± 11.1	99.7 ± 9.1	103.1 ± 21.4	98.7 ± 15.5	104.6 ± 19.9	100.2 ± 12.5
Total ch. (mg/dl)	210.8 ± 39.4	200.9 ± 35.6*	200.4 ± 43.1	190.4 ± 31.8*	200.8 ± 40.5	191.1 ± 25.8*	210.7 ± 40.1	191.4 ± 31.5*
LDL ch. (mg/dl)	130.8 ± 34.2	125.1 ± 31.5*	120.3 ± 42.2	104.7 ± 14.4*	124.8 ± 26.1	116 ± 23.1*	146.9 ± 38.1	130.3 ± 29.4*
HDL ch. (mg/dl)	55.6 ± 10.1	52.7 ± 10.1	49.3 ± 10.4	50.0 ± 9.1	50.6 ± 11.2	50.9 ± 10.2	50.3 ± 12.5	49.6 ± 10.1
TG (mg/dl)	116.3 ± 45.8	115.9 ± 51.3	158.8 ± 43.4§	159.7 ± 50§	132.4 ± 64.8	124.3 ± 31.3	155 ± 65.4§	144.3 ± 66.1*,§
Insulin(mUI/L)	11.4 ± 6.3	9.8 ± 4.9*	19.8 ± 13.3§	16.9 ± 12.4*,§	12.4 ± 9.1	11.6 ± 7.9*	19.2 ± 10.1§	13.8 ± 6.4*,§
HOMA	2.84 ± 2.0	2.35 ± 1.2*	5.21 ± 3.5§	4.40 ± 3.3*,§	3.31 ± 2.8	2.71 ± 1.23*	4.18 ± 1.1§	3.11 ± 1.4*,§

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	Diet M				Diet P			
	Group I (n = 128)		Group II (n = 26)		Group I (n = 134)		Group II (n = 18)	
	Basal	3 months	Basal	3 months	Basal	3 months	Basal	3 months
ALT (UI/L)	21.9 ± 7.8	22.8 ± 13.1	71.5 ± 33.3[§]	51.9 ± 36.4*,[§]	22.6 ± 7.8	21.8 ± 4.7	63.6 ± 10.2[§]	49.6 ± 7.8*,[§]
AST (UI/L)	21.5 ± 5.1	22.4 ± 4.5	45.8 ± 27.1[§]	34.6 ± 20.9*,[§]	21.7 ± 5.8	32.7 ± 9.1	46.1 ± 24.2[§]	35.2 ± 12.8*,[§]
BT (mg/dl)	0.54 ± 0.3	0.56 ± 0.3	0.61 ± 0.4	0.60 ± 0.3	0.60 ± 0.2	0.59 ± 0.3	0.61 ± 0.4	0.59 ± 0.3
GGT (UI/L)	30.7 ± 27.3	27.2 ± 26.8	75.7 ± 20.8[§]	47.6 ± 34.9*,[§]	32.7 ± 30.3	29.7 ± 20.1	46.1 ± 14.2[§]	35.3 ± 12.6*,[§]

ALT: alanine aminotransferase. AST: aspartate aminotransferase. BT: bilirubin. GGT: gammaglutamine transferase. * ($p < 0.05$) with basal values in each group. [§] ($p < 0.05$) among values of group I and II.

In control group with diet P, systolic blood pressure (–4.1±2.2 mg/dl), total cholesterol (–9.7±10.2 mg/dl), LDL cholesterol (–8.1±13.2 mg/dl), HOMA-IR (–0.61±1.3 units) and insulin (–0.91±3.2 units) levels decreased. In NAFLD group, systolic blood pressure (–8.1±9.2 mmHg), total cholesterol (–9.3±7.2 mg/dl), LDL cholesterol (–16.3±11.2 mg/dl), insulin (–5.4±3.2 UI/L) and HOMA-IR (–1.07±1.5 units) decreased. Triglycerides, insulin and HOMA-IR levels were higher in NAFLD group than control group.

In NAFLD group, levels of alanine aminotransferase [(diet M) –20.3±19.2 UI/L vs. (diet P) –14.2±20.1 UI/L], aspartate aminotransferase [(diet M) –11.3±12.2 UI/L vs. (diet P) –11.1±10.1 UI/L], and gammaglutamyl transferase [(diet M) –18.1±12.2 UI/L vs. (diet P) –10.9±20.1 UI/L] improved with both diets. Alanine aminotransferase, aspartate aminotransferase and gammaglutamyl 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 two different enriched fat hypocaloric diets was associated with a decrease of ALT, AST and GGT levels in NAFLD patients. After both dietary intervention, obese subjects and NAFLD patients showed a significant decrease of weight, fat mass, systolic blood pressure, total cholesterol, LDL cholesterol, insulin and HOMA-R.

We showed that a modest weight reduction for three months was associated with a aminotransferases improvement in obese subjects with NAFLD. Hence, based on our data, a 5% reduction in body weight could be recommended as an initial therapeutic target in patients with NAFLD. Furthermore, in our study, 5% of weight reduction was associated with decrease of anthropometric parameters, 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 fat enriched hypocaloric diet[16].

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 clamp[17]. Some Authors[18] have demonstrated a closely correlation between insulin resistance (HOMA) and NAFLD. Also, other authors have been detected this relation using the clamp technique[19] with results supporting our conclusions. 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 liver[20] and this fatty acid deposition increases substrates for oxidative stress.

In most patients, inappropriate diet is thought to lead to chronically elevated glucose, insulin resistance and free fatty acids (FFAs). Both, excessive fat intake and carbohydrate intake could play a role in increasing blood glucose, insulin resistance and FFAs[21]. Therefore, especially in the presence of insulin resistance, in which the flux of FFAs from adipose is not suppressed by insulin, elevated rate of lipogenesis may be a significant source of accumulated triacylglycerol in the liver. Despite the fact that weight loss is the main intervention to these patients. Very few studies of the effects of different diets on NAFLD have been performed[6-12]. Monounsaturated fatty acids (MUFAs) are a class of fatty acids that are found in foods such as olive oils and nuts. The beneficial effects of MUFAs on cardiovascular risk factors and blood lipid profiles have been extensively studied[22]. As we can see in our diet M, an increase in the intake of MUFAs, particularly as a replacement for SFAs may be beneficial for NAFLD patients. Polyunsaturated fatty acids (PUFAs) are a class of fatty acids that include n-3 and n-6 fatty acids. PUFAs have been shown to decrease cardiovascular risk factors[23]. Replacement of PUFAs with alpha-linolenic acid improved insulin resistance and lowered cholesterol concentrations[24]. One study found that 1 g fish oil/day for 12 months decreased blood triacylglycerol concentrations, liver enzymes and steatosis in NFALD[25].

A criticism to our study 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. Moreover, a limitation 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 NAFLD[20].

Our results have therapeutic implications. In a recent systematic review[26], lifestyle modifications leading to weight reduction and/or increased physical activity reduced liver fat and improve insulin sensitivity. As our data shows, the weight loss secondary to two hypocaloric diets enriched in MUFAs or PUFAs improve lipid profile, insulin resistance and liver enzymes in NAFLD patients. Therefore, other potential interventions as the next drugs; metformin[27], acarbose[28], orlistat[29] and sibutramine[30] could be use in a second step to treat NFALD patients.

Conclusions

We showed that weight reduction secondary to two different fat enriched hypocaloric diets was associated with improvement in aminotransferases levels, lipid profile and insulin resistance in NAFLD patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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