Prevalence and factors associated with the presence of nonalcoholic fatty liver disease in an adult population in Spain

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Background/aims **The prevalence of nonalcoholic fatty liver disease (NAFLD) is unknown in Spain. The purpose of detecting NAFLD patients is to determine the associated factors and prevent its evolution to more severe forms. The aim of this study is to determine the prevalence and factors associated with NAFLD.**

Methods **This is a multicentre, cross-sectional, populational study. Individuals between 15 and 85 years of age were randomly selected from 25 primary healthcare centres in the province of Barcelona, Spain. Clinical histories were reviewed, and anamnesis, physical examination, blood analysis and hepatic echography were performed. Individuals with an alcohol intake greater than 30 g/day in men and greater than 20 g/day in women or with known liver disease were excluded.**

Results **Seven hundred and sixty-six individuals with a mean age of 53 ± 14 years (range 17–83, 42.2% men) were included in the study. One hundred and ninety-eight individuals presented NAFLD with echographic criteria (prevalence 25.8, 33.4% men and 20.3% women $P < 0.001$). On multivariate analysis, the following were associated with NAFLD: male sex [odds ratio (OR): 2.34, 95% confidence interval (95% CI): 1.57–3.49], age (OR: 1.04 per year, 95% CI: 1.02–1.05), metabolic syndrome (OR: 2.19, 95% CI: 1.29–3.72), insulin resistance (OR: 6.00, 95% CI: 3.43–10.5) and alanine aminotransferase (OR: 4.21, 95% CI: 2.23–7.95). Of the individuals who consumed alcohol, 29.4% consumed alcohol within the inclusion criteria, with a mean of 9.17 ± 6.75 standard beverage units per week. Moderate alcohol intake was not related to NAFLD, although a possible protector effect was found with the quantity consumed among the drinkers who did not consume excessive amounts of alcohol (OR: 0.93 per standard beverage units, 95% CI: 0.88–0.98).**

Conclusion **NAFLD prevalence in our population is very high. Male sex, age, metabolic syndrome, insulin resistance and alanine aminotransferase are the factors associated with NAFLD. Furthermore, studies should be carried out with respect to the controversial effect of alcohol on NAFLD.** ***Eur J Gastroenterol Hepatol*** **22:24–32 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Nonalcoholic fatty liver disease (NAFLD) consists of the accumulation of fat in the hepatocytoes of patients who do not consume toxic quantities of alcohol [1]. This disease covers a wide spectrum of lesions from simple hepatic steatosis to steatohepatitis with a variable grade of fibrosis until hepatic cirrhosis to, in some cases, hepatocarcinoma [2]. This disease is very prevalent in Western countries, and is currently considered one of the most common liver diseases [3]. Through various epidemiological populational studies (analytical, histological and echographical studies) carried out to date, it has been calculated that between 20 and 30% of the adult populations in these countries present NAFLD [4–9]. In contrast, in Eastern countries, in which the disease is infrequent, increasingly more studies are reporting a rise in its prevalence, thereby showing a change in customs towards an ever more Westernized society (changes in

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dietetic habits, less physical activity and an increase in obesity) [10,11]. The factors most frequently associated with NAFLD are overweight, obesity, diabetes, dyslipemia and metabolic syndrome (MS) [12]. Among these, obesity is of note because of its increasing prevalence in Western societies, beginning in adolescence and being deemed a significant health problem in these countries. In contrast, NAFLD is considered the hepatic component of MS [13], which consists of central obesity, hyperglycaemia, hypertriglyceridaemia, low high-density lipoprotein (HDL) levels and arterial hypertension [14].

In general, few populational epidemiological studies have been carried out to evaluate the prevalence of NAFLD, and to date none have been carried out in Spain. Thus, the objectives of this study were to determine in a general population the prevalence of NAFLD using echographic criteria and to evaluate the factors associated with it.

Methods

Study design

The protocol of the main characteristics of the study has been published earlier [15]. This was a transversal, multicentre study with a populational base of adult individuals ascribed to 25 primary healthcare centres in the area of Barcelonès Nord i Maresme (Catalonia, Spain).

The individuals were randomly selected from the population assigned through the primary healthcare information system (SIAP), a system used in our healthcare centres, which is equivalent to the municipal census. Participant selection was stratified only by the proportion of people assigned to each of the centres participating in the study to ensure that each centre was appropriately represented. The study was carried out between February 2007 and June 2008.

Inclusion criteria

An adult population between 15 and 85 years of age provided written informed consent to participate in the study.

Exclusion criteria

The exclusion criteria were alcohol intake greater than 30 g/day in men and greater than 20 g/day in women, patients with known chronic liver disease, the presence of the hepatitis B virus surface antigen or hepatitis C virus antibodies, individuals with incapacitating diseases or cognitive deterioration, institutionalized patients or those with no fixed address in any of the centres participating in the study.

Procedure

The clinical histories of all the participants were reviewed, and the participants were submitted to anamnesis, a physical examination, and a complete blood analysis. Abdominal echography was performed within a maximum period of 3 months after the consultation.

In the anamnesis, alcohol intake focused on the years of intake, type of beverages and the quantity consumed being expressed in standard drink units (SDU), differentiating consumption during the week from that of the weekend (teetotallers were considered persons who did not currently drink alcohol and had not drunk alcohol in the past). SDU was defined as 10 g of alcohol.

The analytical determination included a complete blood analysis, basal glycaemia, lipid profile [total cholesterol, HDL, low-density lipoprotein (LDL), triglycerides], liver profile [aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT)], and hepatitis B virus surface antigens and hepatitis C virus antibodies. The cutoff values of the biochemical variables were considered according to the usual parameters of the reference laboratory of the participating centres, and are unique for all of the centres. The baseline insulin level was determined by immunochemoluminiscence, defining insulin resistance (IR) with the homeostasis model assessment (HOMA) method{[glycaemia (mmol/l) × insulinemia (mU/l)]/22.5}and IR was considered with HOMA at least 3.8.

Diagnosis of MS was performed using the American criteria of the ATP-III NCEP [16], which includes abdominal perimeter greater than 102 cm in men and greater than 88 cm in women, arterial hypertension ($\geq 135/\geq 85$ mmHg), basal glycaemia at least 110 mg/dl, HDLc less than 50 mg/dl in women and less than 40 mg/dl in men and triglycerides at least 150 mg/dl. A patient must present three or more of these components to be considered as having MS.

The diagnosis of NAFLD was achieved by abdominal echography according to standard criteria [17]. Given the extension of the population reference area in this study, the echographies were undertaken in each of the four radiology reference centres available. A total of four radiologists participated, and among them a consensus was arrived at of the criteria for the diagnosis of NAFLD. None were aware of either the clinical or analytical results. The apparatus used for performing the echographies was identical in each of the centres. The criteria for the diagnosis of NAFLD included an increase in hepatic echogenicity using renal echogenicity as a reference, the presence of enhancement and a lack of differentiation of periportal and bile duct walls reinforcement because of great hyperechogenicity of the parenchyma. The grade of involvement was standardized using a semiquantitative scale of the grade of hepatic enhancement.

This study was approved by the Clinical Investigation Ethics Committees of the Fundación IDIAP Jordi Gol and the Instituto de Salud Carlos III.

Statistical analysis

The description of the data was carried out using frequency tables and their respective percentages (categorical variables) and the mean and the standard deviation (continuous variables). The prevalence of NAFLD was computed with raw scores and standardized by the Spanish [18] and European [19] age and sex distribution. The prevalence of NAFLD was compared in each category of the categorical variables using the χ^2 and Fisher's exact tests, and the means of the continuous variables among the individuals with and without NAFLD using the Student's *t*-test and the Mann–Whitney nonparametric tests.

Logistic regression was performed with NAFLD as a dependent variable and adjusted for potential confounders to determine the factors independently associated with the presence of NAFLD. The likelihood ratio test was used to test whether the terms of interaction included in the models were statistically significant. Seven patients who did not currently consume alcohol but who had done so in the past were excluded from the analysis in which alcohol was considered. Receiver operating characteristic curves were used to determine the best cutoff of ALT to discriminate patients with and without NAFLD. All the comparisons were carried out at a bilateral level with a significance of 5%.

Results

There was 40% participation in the study, and this was lower among the young and men. Initially, 773 patients were included, of whom seven were excluded because one had a clinical history of alcoholism, five were hepatitis C virus-positive and one was hepatitis B virus-positive.

Finally, 766 individuals were included: 323 men (42.2%) and 443 women (57.8%) with a mean age of 53 ± 14 years with a range between 17 and 83 years. Of these, 198 patients presented NAFLD by echographic criteria, representing a prevalence of 25.8%; 33.4% in men and 20.3% in women ($P < 0.001$). Table 1 shows the prevalence by age and sex strata, standardized by the Spanish and European population. Table 2 describes the sample and the differences between the individuals with or without NAFLD. Two hundred and twenty-five patients (29.4%) consumed alcohol within the limits established in the inclusion criteria, with a mean current intake of 9.17 ± 6.75 SDU per week. The prevalences of other factors potentially associated with NAFLD were as follows: 25.2% patients with obesity (BMI $\geq 30\,kg/m^2$), 44.0% overweight (BMI between 25 and $30\,kg/m^2$), 19.8% with diabetes (glycaemia $\geq 110\,mg/dl$), 42.2% with hypertension (≥ 130 or $\geq 85\,mmHg$), 22.1% with low HDLc ($< 40\,mg/dl$ in men and $< 50\,mg/dl$ in women), 21.2% with hypertriglyceridaemia ($\geq 150\,mg/dl$), 8.0% with high ALT levels, 12.0% with high GGT values, 12.4% with IR and 13.5% with MS.

Table 1 Age and sex sample distribution and its NAFLD prevalence and standardized NAFLD prevalence by the Spanish and European age and sex distribution

	Sex					
	Male		Female		Total	
Age (years)	*n*	Prev. (%)	*n*	Prev. (%)	*n*	Prev. (%)
<40	83	16.9	82	8.5	165	12.7
40–60	125	37.6	206	16.5	331	24.5
Above 60	115	40.9	155	31.6	270	35.6
Total	323	33.4	443	20.3	766	25.8
Total (standardized by the Spanish age and sex distribution)						23.0
Total (standardized by the European age and sex distribution)						22.3

NAFLD, nonalcoholic fatty liver disease; Prev., NAFLD prevalence.

Risk factors for the presence of NAFLD according to univariate analysis

Table 3 shows the results of the individual analysis of the risk factors associated with the presence of NAFLD according to the logistic regression model adjusted for age, sex and radiologist. Positive significant association was found in male sex, age, obesity, arterial hypertension, diabetes, HDLc, triglycerides, ALT, GGT, IR and MS. Neither total cholesterol nor LDLc achieved statistical significance.

On stratifying this analysis by alcohol intake (never/current), the effect of age and male sex was greater among the individuals with moderate alcohol consumption, whereas overweight, obesity, arterial hypertension, diabetes, HDLc, triglycerides, transaminases, IR and MS were more strongly associated with NAFLD among the teetotallers. Nonetheless, the interaction of these variables with never/current drinker did not achieve statistical significance, except for IR.

Risk factors for the presence of NAFLD on multivariate analysis

Table 4 shows the variables significantly associated with NAFLD on multivariate analysis adjusted by radiologists: male sex [odds ratio (OR): 2.34, 95% confidence interval (95% CI): 1.57–3.49], age (OR: 1.04 per year, 95%CI: 1.02–1.05), MS (OR: 2.19, 95% CI: 1.29–3.72), IR (OR: 6.00, 95% CI: 3.43–10.5) and elevated ALT (OR: 4.21, 95% CI: 2.23–7.95).

When MS was substituted by its components in this model, all were associated with risk except HDLc, with none of the remaining factors predominating in determining the risk of NAFLD (Table 5).

Table 2 Sample characteristics regarding presence of NAFLD

	NAFLD						
	Yes		No		Total		
	n	%	*n*	%	*n*	%	*P* value*
Sex							<0.0005
Female	90	20.3	353	79.7	443	57.8	
Male	108	33.4	215	66.6	323	42.2	
Age (years)	Mean	SD	Mean	SD	Mean	SD	
All	57.97	12.61	51.21	14.39	52.96	14.26	<0.0005
Categorized							<0.0005
<40	21	12.7	144	87.3	165	21.5	
40–60	81	24.5	250	75.5	331	43.2	
≥ 60	96	35.6	174	64.4	270	35.2	
Education							0.001
No school	38	36.5	66	63.5	104	13.7	
Primary school	107	28.8	265	71.2	372	48.9	
Secondary/high school	35	17.0	171	83.0	206	27.1	
University	17	21.5	62	78.5	79	10.4	
Alcohol consumption[a]							0.006
Never (teetotallers	122	22.8	412	77.2	534	70.4	
Current	73	32.4	152	67.6	225	29.6	
Years drinking alcohol[a]	Mean	SD	Mean	SD	Mean	SD	
Current consumers	30.03	12.92	21.45	14.37	24.26	14.45	<0.0005
Current SDU consumed per week[a]	Mean	SD	Mean	SD	Mean	SD	
Current consumers	8.78	5.97	9.36	7.10	9.17	6.75	0.551
Tobacco smoking							0.011
Never smoker	106	25.0	318	75.0	424	55.4	
Former smoker	53	34.6	100	65.4	153	20.0	
Current smoker	39	20.6	150	79.4	189	24.7	
Obesity							<0.0005
Infraweight/average (BMI<25 kg/m^2)	22	9.3	214	90.7	236	30.8	
Overweight (25 ≤ BMI<30 kg/m^2)	87	25.8	250	74.2	337	44.0	
Obese (BMI ≥ 30 kg/m^2)	89	46.1	104	53.9	193	25.2	
Central obesity							<0.0005
No	94	19.7	383	80.3	477	62.4	
Yes [waist circumference ≥ 102 cm (men), 88 cm (women)]	103	35.9	184	64.1	287	37.6%	
Hypertension (direct measurement)							<0.0005
No (systolic pressure<130 mmHg and diastolic pressure<85 mmHg)	81	18.3	361	81.7	442	57.8	
Yes	116	35.9	207	64.1	323	42.2	
Diabetes (direct measurement)							<0.0005
No	119	20.1	473	79.9	592	80.2	
Yes (glyceamia ≥ 110 mg/dl)	77	52.7	69	47.3	146	19.8	
Total cholesterol (direct measurement)							0.579
Low	80	27.7	209	72.3	289	39.2	
High (>200 mg/dl)	116	25.8	333	74.2	449	60.8	
HDL (direct measurement)							0.003
High	129	23.8	414	76.2	543	77.9	
Low (<40 mg/dl men, <50 mg/dl women)	55	35.7	99	64.3	154	22.1	
Triglycerides (direct measurement)							<0.0005
Low	119	21.2	443	78.8	562	78.8	
High (≥ 150 mg/dl)	69	45.7	82	54.3	151	21.2	
ASAT (direct measurement)							0.036
Normal	180	25.9	514	74.1	694	95.5	
High (≥ 35 U/l men, ≥ 31 U/l women)	14	42.4	19	57.6	33	4.5	
ALAT (direct measurement)							<0.0005
Normal	162	23.9	515	76.1	677	92.0	
High (≥ 45 U/l men, ≥ 34 U/l women)	34	57.6	25	42.4	59	8.0	
GGT (direct measurement)							<0.0005
Normal	154	24.0	489	76.0	643	88.0	
High (≥ 56 U/l men, ≥ 39 U/l women)	40	45.5	48	54.5	88	12.0	
Insuline resistance							<0.0005
No	132	20.7	505	79.3	637	87.6	
Yes (homeaostasis model assessment ≥ 3.8)	61	67.8	29	32.2	90	12.4	
Metabolic syndrome (NCEP criteria)							<0.0005
No	136	21.9	486	78.1	622	86.5	
Yes	52	53.6	45	46.4	97	13.5	

ALAT, alanineaminotransferase; ASAT, aspartateaminotransferase; GGT, γ-glutamyl transferase; HDL, high-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; SD, standard deviation; SDU, standard drink units of alcohol.

[a]Seven former alcohol consumers excluded.

*P value for a χ^2 test (categorical variable) and t-test (continuous variable). Fisher's exact test and rank sum tests have been also performed for categorical and continuous variables, respectively, leading to similar P values.

Table 3 Association between NAFLD and potential risk factors

	Overall ($n=766$)[a]			Teetotallers ($n=534$)			Current alcohol consumers ($n=225$)		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Sex (ref Female)									
Male	2.21	1.56–3.14	<0.0005	1.70	1.07–2.69	0.024	3.06	1.44–6.50	0.004
Age (years)	1.04	1.03–1.06	<0.0005	1.04	1.02–1.05	<0.0005	1.05	1.02–1.08	<0.0005
Categorized (ref <40)									
40–60	2.71	1.58–4.65	<0.0005	1.90	1.01–3.58	0.047	6.61	1.85–23.6	0.004
≥ 60	4.56	2.64–7.87	<0.0005	3.42	1.80–6.48	<0.0005	9.84	2.72–35.6	<0.0005
Education (ref No school)									
Primary school	0.99	0.60–1.62	0.965	0.81	0.46–1.42	0.465	1.86	0.56–6.20	0.310
Secondary/high school	0.69	0.37–1.28	0.236	0.47	0.22–1.00	0.051	1.55	0.40–5.97	0.522
University	1.01	0.47–2.18	0.975	0.98	0.36–2.68	0.968	1.60	0.37–7.01	0.530
Alcohol consumption (ref Never)[b]									
Current	1.33	0.89–1.99	0.171						
Years drinking alcohol[b]							1.01	0.98–1.03	0.711
Current standard drink units of alcohol consumed per week[b]							0.94	0.89–0.99	0.016
Tobacco smoking (ref Never)									
Former smoker	1.56	0.98–2.47	0.060	1.51	0.79–2.87	0.212	1.33	0.62–2.88	0.467
Current smoker	1.00	0.63–1.60	0.988	1.02	0.56–1.86	0.944	0.76	0.33–1.73	0.509
Obesity [ref Infraweight/average (BMI<25 kg/m^2]									
Overweight (25 ≤ BMI<30 kg/m^2)	2.19	1.29–3.74	0.004	3.44	1.63–7.22	0.001	1.12	0.49–2.55	0.788
Obese (BMI ≥ 30 kg/m^2)	6.78	3.88–11.8	<0.0005	10.5	4.95–22.4	<0.0005	3.67	1.39–9.67	0.009
Central obesity (ref No									
Yes [waist circumference ≥ 102 cm (men), 88 cm (women)]	2.97	2.00–4.43	<0.0005	3.64	2.21–6.01	<0.0005	2.27	1.12–4.59	0.023
Hypertension (direct measurement) [ref No (systolic pressure<130 mmHg and diastolic pressure<85 mmHg)]									
Yes	2.04	1.41–2.94	<0.0005	2.12	1.34–3.35	0.001	2.07	1.08–3.95	0.028
Diabetes (direct measurement) (ref No)									
Yes (glycaemia ≥ 110 mg/dl)	3.22	2.12–4.89	<0.0005	4.75	2.84–7.95	<0.0005	1.59	0.74–3.43	0.236
Total cholesterol (direct measurement) (ref Low)									
High (>200 mg/dl)	0.94	0.65–1.35	0.739	1.07	0.68–1.68	0.785	0.72	0.37–1.37	0.315
HDL (direct measurement) (ref High)									
Low (<40 mg/dl men, <50 mg/dl women)	1.84	1.20–2.82	0.005	2.66	1.59–4.44	<0.0005	0.86	0.36–2.09	0.746
Triglycerides (direct measurement) (ref Low)									
High (≥ 150 mg/dl)	3.36	2.22–5.07	<0.0005	4.48	2.68–7.51	<0.0005	1.88	0.92–3.84	0.083
AST (direct measurement) (ref Normal)									
High (≥ 35 U/l men, ≥ 31 U/l women)	1.91	0.89–4.09	0.096	2.66	1.04–6.82	0.042	1.16	0.29–4.53	0.835
ALT (direct measurement) (ref Normal)									
High (≥ 45 U/l men, ≥ 34 U/l women)	4.93	2.72–8.95	<0.0005	6.51	3.06–13.8	<0.0005	2.75	0.99–7.68	0.053
GGT (direct measurement) (ref Normal)									
High (≥ 56 U/l men, ≥ 39 U/l women)	2.99	1.82–4.91	<0.0005	3.25	1.76–5.99	<0.0005	3.47	1.38–8.71	0.008
Insuline resistance (ref No)									
Yes (homeaostasis model assessment ≥ 3.8)	8.18	4.82–13.9	<0.0005	14.3	7.15–28.6	<0.0005	2.90	1.18–7.11	0.020
Metabolic syndrome (NCEP criteria) (ref No)									
Yes	3.61	2.23–5.84	<0.0005	5.32	2.96–9.57	<0.0005	1.83	0.73–4.60	0.196

Logistic regression models adjusted by age, sex and radiologist and stratified by never/current alcohol consumption.
ALT, alanineaminotransferase; AST, aspartateaminotransferase; CI, confidence interval; HDL, high-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio.
[a]Seven former alcohol consumers included.
[b]Seven former alcohol consumers excluded.

Table 4 Association between NAFLD and potential risk factors

	OR[a]	95% CI	*P* value
Men	2.34	1.57–3.49	<0.0005
Age (per year)	1.04	1.02–1.05	<0.0005
Metabolic syndrome (NCEP criteria)	2.19	1.29–3.72	0.004
Insuline resistance (homeaostasis model assessment ≥ 3.8)	6.00	3.43–10.5	<0.0005
ALT High (≥ 45 U/l men, ≥ 34 U/l women)	4.21	2.23–7.95	<0.0005

Multivariate logistic regression models adjusted by radiologist.
ALT, alanineaminotransferase; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio.
[a]On the basis of 186 NAFLD and 522 non-NAFLD individuals.

There did not seem to be any effect when the variable never/current drinker was added to the models in Tables 4 and 5. However, the amount of alcohol intake appeared as a variable with a slight protector effect (OR: 0.93 for SDU, 95% CI: 0.88–0.98) when it was included in the models restricted to drinkers (Table 6). The years of alcohol intake did not show any effect. The introduction of amount or duration of intake in the multivariate models in drinkers did not substantially modify the risk of the remaining factors, but on stratification of never/current drinker, greater effects were observed for age and sex among moderate alcohol consumers, with higher effects

for MS (or its components), IR and ALT among the teetotallers (Table 6).

Discussion

The prevalence of NAFLD is not well known and is possibly underestimated, as a large proportion of the patients are asymptomatic, present discrete biological alterations and do not undergo liver biopsy. The latter is the best test to diagnose liver diseases, and, in the case of NAFLD, it allows simple steatosis to be distinguished from steatohepatitis [20]. However, carrying out such biopsies in this type of study is not viable because of the difficulty and cost. Therefore, populational studies such as this use hepatic echograpy for the diagnosis of NAFLD. The diagnostic value of abdominal echography in NAFLD has been estimated to have a sensitivity greater than 80% and a specificity greater than 90% [21]. Thus, in this study based on the diagnosis of NAFLD by abdominal echography, the prevalence of this disease was 25.8%, which is in agreement with the percentages referred to in recent years of between 20 and 30% in Western countries.

NFALD was significantly more prevalent in men compared with women. Initially, epidemiologic studies on NAFLD showed a higher prevalence among women [1,22]. However, recent studies have reported a greater prevalence in men [23–25]. The results of these studies may, nonetheless, be biased because of the inclusion criteria used, especially those based on alterations in transaminases, a parameter that, as commented on later, a significant percentage of patients with NAFLD present as normal [8].

In our study, age, especially of above 60 years, was an independent risk factor for NAFLD. In contrast, in Italian and Taiwanese studies, an age of above 66 years

Table 5 Association between NAFLD and potential risk factors using metabolic syndrome components

	OR[a]	95% CI	*P* value
Men	2.28	1.44–3.62	<0.0005
Age (per year)	1.03	1.01–1.05	0.001
Central obesity [waist circumference ≥ 102 cm (men), 88 cm (women)]	1.94	1.22–3.06	0.005
Hypertension (systolic pressure ≥ 130 mmHg or diastolic pressure ≥ 85 mmHg)	2.04	1.32–3.14	0.001
Diabetes (glyceamia ≥ 110 mg/dl)	1.76	1.07–2.88	0.026
Low HDL (<40 mg/dl men, <50 mg/dl women)	1.14	0.69–1.88	0.603
High triglycerides (≥ 150 mg/dl)	1.71	1.06–2.78	0.029
Insuline resistance (homeaostasis model assessment ≥ 3.8)	4.39	2.43–7.92	<0.0005
High ALT (≥ 45 U/l men, ≥ 34 U/l women)	3.76	1.93–7.35	<0.0005

Multivariate logistic regression models adjusted by radiologist.
ALT, alanineaminotransferase; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio.
[a]On the basis of 182 NAFLD and 504 non-NAFLD individuals.

Table 6 Association between NAFLD and potential risk factors

Model	Variable	OR	95% CI	*P* value
1 (*n*=701)	Men	2.07	1.34–3.18	0.001
	Age (per year)	1.04	1.02–1.05	<0.0005
	Metabolic syndrome (NCEP criteria)	2.37	1.38–4.07	0.002
	Insuline resistance (HOMA ≥ 3.8)	5.94	3.38–10.4	<0.0005
	ALT High (≥ 45 U/l men, ≥ 34 U/l women)	3.93	2.07–7.45	<0.0005
	Current drinker[a]	1.44	0.91–2.27	0.118
2a Teetotallers only (*n*=491)	Men	1.85	1.07–3.21	0.027
	Age (per year)	1.04	1.02–1.06	<0.0005
	Metabolic syndrome (NCEP criteria)	2.89	1.49–5.61	0.002
	Insuline resistance (HOMA ≥ 3.8)	9.50	4.57–19.8	<0.0005
	ALT High (≥ 45 U/l men, ≥ 34 U/l women)	5.27	2.31–12.0	<0.0005
2b Current drinkers only (*n*=210)	Men	2.90	1.33–6.31	0.007
	Age (per year)	1.04	1.01–1.07	0.005
	Metabolic syndrome (NCEP criteria)	1.46	0.54–3.95	0.452
	Insuline resistance (HOMA ≥ 3.8)	2.46	0.95–6.35	0.063
	ALT High (≥ 45 U/l men, ≥ 34 U/l women)	2.50	0.87–7.19	0.089
3 Current drinkers only (*n*=210)	Men	4.08	1.77–9.40	0.001
	Age (per year)	1.05	1.02–1.08	0.001
	Metabolic syndrome (NCEP criteria)	1.41	0.51–3.92	0.512
	Insuline resistance (HOMA ≥ 3.8)	2.32	0.88–6.13	0.091
	ALT High (≥ 45 U/l men, ≥ 34 U/l women)	3.03	1.03–8.87	0.044
	Current SDU consumed per week (per unit)	0.93	0.88–0.98	0.017
4 Current drinkers only (*n*=208)	Men	3.88	1.65–9.10	0.002
	Age (per year)	1.05	1.01–1.09	0.011
	Metabolic syndrome (NCEP criteria)	1.40	0.50–3.89	0.520
	Insuline resistance (HOMA ≥ 3.8)	2.27	0.86–6.04	0.099
	ALT High (≥ 45 U/l men, ≥ 34 U/l women)	3.00	1.02–8.83	0.046
	Current SDU consumed per week (per unit)	0.93	0.88–0.99	0.017
	Years drinking alcohol (per year)	1.01	0.97–1.04	0.753

Multivariate logistic regression models adjusted by radiologist with different alcohol consumption variables or individual's characteristics
ALT, alanineaminotransferase; CI, confidence interval; HOMA, homeaostasis model assessment; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; SDU, standard drink units of alcohol.
[a]Seven former alcohol consumers excluded.

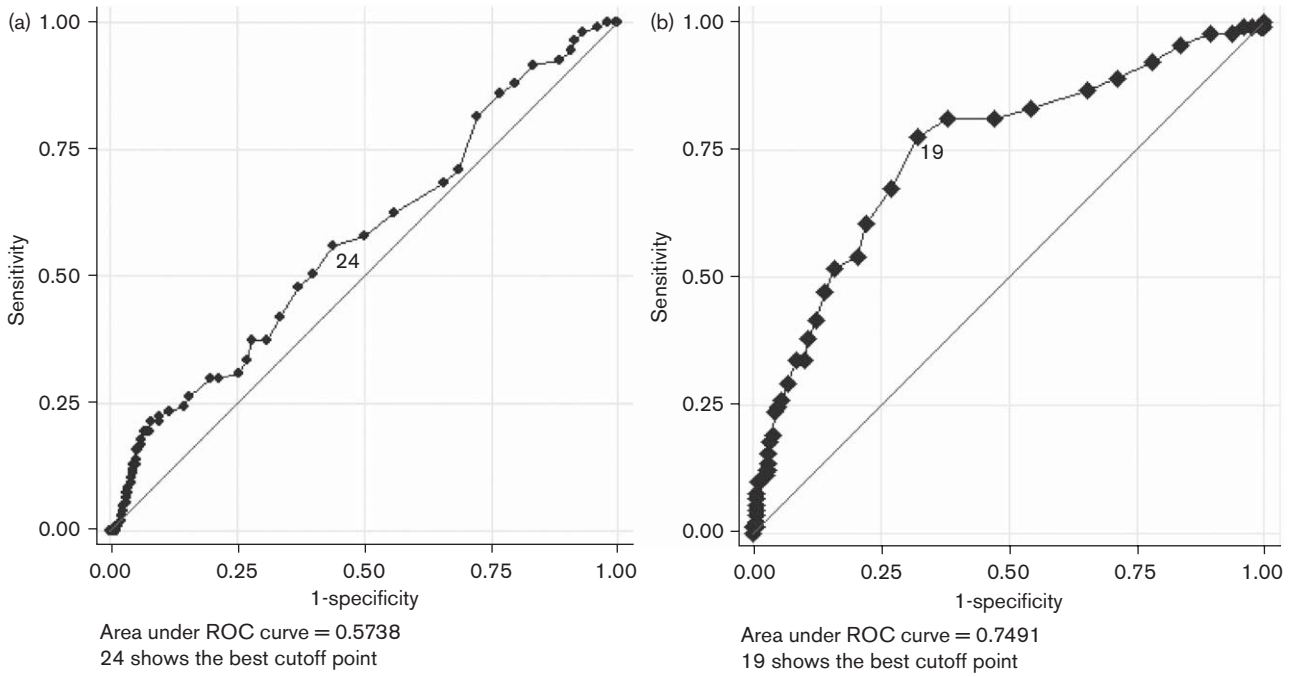
and 65 years, respectively, was inversely associated with the presence of NAFLD, leading those investigators to suggest that age may have a protector effect for NAFLD [8,11]. In other studies, age was not associated with the presence of NAFLD [9]. Given the protector effect found by some investigators, we analysed the effect of age adjusted for sex in persons aged more than 65 years (OR = 1.00) and more than 70 years (OR = 1.11) and found that it was not significant. Thus, we cannot support the contention of a protector effect of age above 65 years.

In this study, ALT (< 45 U/l men and < 34 U/l women), AST (< 35 U/l men and < 31 U/l women) and GGT (11–55 U/l men and 7–38 U/l women) were significantly elevated in individuals with NAFLD. As AST and GGT are highly correlated with ALT, the variable with the greatest effect, ALT was the variable introduced into the analysis. Thus, an elevation in ALT was present in 8.0% and was an independent risk factor for NAFLD on multivariate analysis. Some authors have reported similar results [11]. However, of these, only 57.6% (34/59) had NAFLD. These percentages also coincide with those reported by other authors [8,11,26]. This is important, as we hypothesized that patients with an increase in transaminases may present NAFLD, yet, as observed in this study, almost half of the patients with NAFLD had normal transaminase values. This has important clinical repercussions, as shown in studies based on liver biopsy in which patients with NAFLD and normal transaminases present severe lesions [27,28]. This is why several authors have proposed that the value of normality of ALT be redefined to improve its sensitivity [8,26,29,30]. To this end, we performed a receiver operating characteristic curve (Fig. 1) and the values with the best discrimination were 24 U/l in men and 19 U/l in women, with areas under the curve of 57 and 75%, respectively.

IR was a powerful and constant independent risk factor for NAFLD. In this study, the close relationship between NAFLD and the different components of MS support the already-described theory that NAFLD is the hepatic component of MS [31]. IR is the most constant pathogenic factor in patients with liver disease because of fat deposits, and produces an increase in free fatty acids in skeletal muscle, decreases glucose uptake and inhibits glucogenesis in the hepatocytes and stimulates glucogenolysis and gluconeogenesis. Moreover, IR causes a lesser release of triglycerides outside the hepatocytes. The accumulation of triglycerides also contributes to an increase in IR [32].

We analysed MS according to the criteria of the ATPIII-NCEP [16]. In our study, 53.6% of the patients with MS had NAFLD, similar to the percentages found in other series [25,33]. In addition, on multivariate

Fig. 1



Receiver operating characteristic (ROC) curves for men (a) and women (b) for the accuracy of alanine aminotransferase levels in discriminating nonalcoholic fatty liver disease. The selected cutoff points have a sensitivity and specificity of 56 and 56% (men) and 78 and 68% (women).

logistic regression, MS was an independent risk factor of NAFLD. These results agree with those from other studies [22], which have shown that MS is an important risk factor for NAFLD. On analysing the different components of MS mutually adjusted in the multivariate analysis, all were independent risk factors for NAFLD except for HDLc, with OR values between 1.7 and 2, and with none predominating on determination of risk.

One important finding in this study was the high percentage of individuals with obesity (25.2%), a value that rises to 69.2% if we add the overweight individuals. Of these, 46.1% obese and 25.8% overweight patients present NAFLD, and these factors are strongly associated with the disease (OR = 2.19 and 6.78 for overweight and obesity, respectively, compared with normal weight). An important value is abdominal obesity, which was present in 37.6% of the study participants and was also associated with NAFLD (OR = 2.97). Despite general obesity being more strongly associated with NAFLD than central obesity, the latter was included in the model, as it is included in the definition of MS. The two were not included at the same time because of the high correlation between them.

An important aspect of this study was to ensure that the patients did not consume quantities of alcohol considered toxic. The criteria used to define excessive alcohol intake was 30 g/day for men and 20 g/day for women, which are the criteria used in most studies undertaken in recent years. In addition to alcohol consumption, we reviewed the clinical histories of the individuals to determine a possible history of elevated alcohol intake, and in doubtful cases alcohol intake was evaluated by laboratory tests, mean corpuscular volume of erythrocytes and GGT.

Of the participants, 29.4% consumed alcohol within the limits established, with a mean intake of 9.17 SDU per week. Being a teetotaller did not have any effect on NAFLD. This may be affected by the high correlation between this variable and sex. In this study, 83% of the women were teetotallers whereas only 51% of the men did not drink. On stratifying the multivariate analysis by sex, being a teetotaller was a protector factor among men (OR: 0.58, 95% CI: 0.33–1.00) and did not affect the women (OR: 0.93, 95% CI: 0.40–2.20), with the coefficients of the remaining factors studied remaining relatively unaltered.

Paradoxically, among the drinkers, the SDUs consumed showed a protector effect in the men (OR: 0.93, 95% CI: 0.88–0.99) and not in the women (OR: 0.98, 95% CI: 0.72–1.34). These results should be interpreted with caution, as an important limitation of our study is the lack of knowledge of the historical alcohol consumption of these patients, that is, patients who drank more in the past but now drink little by their own free will or because of the diagnosis of some disease related to alcohol intake. These considerations were also taken into account in another study [34], and when these patients were excluded the results were not modified. Our study evaluated whether the protector effect observed for the SDUs among the drinkers was reproduced in those drinking up to 7 SDUs/week and those who drank more than this quantity. No significant results were found, probably because of the reduction in the power produced by the reduction in the sample and the range of the variable studied.

The years of alcohol intake did not show any effect or stratification by sex and/or age, even on excluding the SDUs from the models. Seven persons who had drunk in the past (with no known history of alcoholism) but did not presently do were excluded from the analysis of alcohol to avoid potential contamination of never/current drinker categories. The inclusion of these individuals in the models did not alter any of the results.

Other studies have reported a lower probability of presenting NAFLD among moderate alcohol drinkers, although the protector effect found in being a teetotaller, at least among men, should be emphasized. Thus, Bedogni *et al.* [8] found that the percentage of teetotallers was significantly higher in the patients with NAFLD (48%) than in the individuals with normal liver (31%), supporting the hypothesis by Dixon *et al.* [35] in which moderate alcohol intake is associated with a lower risk of NAFLD.

One limitation of the study was the participation rate of 40%. Those least willing to participate were young people and men. Standardizing for the Spanish population age and sex distribution NAFLD prevalence diminishes from 25.8 to 23.0%, which can be considered the selection bias because of nonresponse. Another limitation is the diagnosis of NAFLD by abdominal echography. Despite echography being a good diagnostic method, it is not useful for diagnosing liver fat when its presence represents less than 30% of the liver volume [4,5,8,9,15,36], or in cases of morbid obesity [37].

In conclusion, NAFLD is very prevalent in our region. Age, male sex, MS, IR and ALT levels are the risk factors most associated with NAFLD. In view of our results, in relation to alcohol intake, abstention is recommended, contrary to the suggestion made in some studies that moderate alcohol intake may have a protector effect. Further studies are required to confirm these possible effects of alcohol.

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Conflicts of interest: the authors declare that they have no competing interests.

References

1 Ludwig J, Viggiano TR, McGuill DB, Ott BGJ. Nonalcoholic steatohepatitis. Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clinic Proc* 1980; **55**:434–438.
2 Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW. Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histologic scoring system for NAFLD. *Hepatology* 2005; **41**:1313–1321.
3 Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol.* 2003; **98**:960–967.
4 Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology.* 2003; **37**:1202–1219.
5 Bedogni G, Bellentani S. Fatty liver: how frequent is it and why? *Ann Hepatol* 2004; **3**:63–65.
6 Bellentani S, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, *et al*. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**:112–117.
7 Lee JY, Kim KM, Lee SG, Yu E, Lim YS, Lee HC, *et al*. Prevalence and risk factors of non-alcoholic fatty liver disease in potential living liver donors in Korea: a review of 589 consecutive liver biopsies in a single center. *J Hepatol* 2007; **47**:239–244.
8 Bedogni G, Miglioli L, Masutti F, Tribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos Nutrition and Liver Study. *Hepatology* 2005; **42**:44–52.
9 Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, Oren R. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int* 2006; **26**:856–863.
10 Fan JG, Saibara T, Chitturi S, Kim BI, Sung JJ, Chutaputti A. Asia-Pacific Working Party for NAFLD. *J Gastroenterol Hepatol* 2007; **22**:794–800.
11 Chen CH, Huang MH, Yang JC, Nien CK, Yang CC, Yeh YH, Yueh SK. Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of Taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. *J Clin Gastroenterol* 2006; **40**:745–752.
12 Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**:1221–1331.
13 Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianese E, Lenzi M, *et al*. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**:1844–1850.
14 Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005 **365**:1415–1428.
15 Caballeria LL, Auladell MA, Torán P, Miranda D, Aznar J, Pera G, *et al*. Prevalence and factors associated with the presence of non alcoholic fatty liver disease in an apparently healthy adult population in primary care units. *BMC Gastroenterology* 2007; **7**:41.
16 Executive summary of the third report of the National Cholesterol Education (NCEP). Expert Panel on Detection, Evaluation, and Treatment of High Cholesterol in Adults (Adults Treatment Panel III). *JAMA.* 2001; **285**:2486–2497.
17 Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology.* 2002; **123**:1705–1725.
18 INE: Instituto Nacional de Estadistica. http://www.ine.es/jaxi/tabla.do?path=/t20/e245/p04/provi/10/&file=00000003.PX&type=pcaxis&L=0 [last accessed on 15 June 2009].
19 Ahmad OB, Boschi-Pinto C, Lopez AD, Murray CJL, Lozano R, Inoue M. Age standardization of rates: a new WHO standard. GPE Discussion Paper Series: No.31. EIP/GPE/EBD. World Health Organization. Available at http://www.who.int/healthinfo/paper31.pdf [last access 15thJun2009].
20 Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, *et al*. Sampling variability of the liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**:1898–1906.
21 Joseph AE, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. *Clin Radiol* 1991; **43**:26–31.
22 Falck-Yttr Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* 2001; **21**:17–26.
23 Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, *et al*. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**:179–187.
24 Harrison SA, Torgerson S, Hayashi PH. The natural history of NAFLD: a clinical histopathologic study. *Am J Gastroenterol* 2003; **98**:2042–2047.
25 Marchesini G, Bugianesi E, Forlani G, Cerelli F, Lenzi M, Manini R, *et al*. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**:917–923.
26 Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, *et al*. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology* 2004; **40**:1387–1395.
27 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**:1286–1292.
28 Park JW, Jeong G, Kim SJ, Kim MK, Park SM. Predictors reflecting the pathological severity of non-alcoholic fatty liver disease: comprehensive study of clinical and immunohistochemical findings in younger Asian patients. *J Gastroenterol Hepatol* 2007; **22**:491–497.
29 Elkstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**:865–873.
30 Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, *et al*. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Int Med* 2002; **137**:1–10.
31 Loria P, Lonardo A, Carulli N. Should NAFLD be renamed? *Dig Dis* 2005; **23**:72–82.
32 Charlton M, Angulo P, Chalasani N, Merriman M, Viker K, Charatcharoen-witthaya P, *et al*. Low circulating levels of dehidroepiandrosterone in histolologically advanced nonalcoholic fatty liver disease. *Hepatology* 2008; **47**:482–492.
33 Radu C, Grigorescu M, Crisan D, Lupsor M, Constantin D, Dina L. Prevalence and associated risk factors of non-alcoholic fatty liver disease in hospitalized patients. *J Gastrointestin Liver Dis* 2008; **17**:255–260.
34 Dunn W, Xu R, Schwimmer JB. Modest wine drinking and decreased prevalence of suspected nonalcoholic fatty liver disease. *Hepatology* 2008; **47**:1947–1954.
35 Dixon JB, Bhathal PS, O'Brian PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; **121**:91–100.
36 Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, *et al*. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**:745–750.
37 Mottin CC, Moretto M, Padoin AV, Swarowsky AM, Toneto MG, Glock L, Repetto G. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. *Obes Surg* 2004; **14**:635–637.