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Risk factors differentially associated with non-alcoholic fatty liver disease in males and females with metabolic syndrome

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ABSTRACT

Background and aims: non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in western countries. This study aimed to investigate putative risk factors differentially related with NAFLD in obese males and females diagnosed with metabolic syndrome (MetS), stratified using the non-invasive hepatic steatosis index (HSI).

Methods and results: a cross-sectional analysis of the PREDIMED Plus study was performed of 278 participants with MetS (141 males and 137 females) of the Navarra-Nutrition node. Subjects were categorized by HSI tertiles and gender. Baseline clinical, biochemical variants and adherence to a Mediterranean diet and physical activity were evaluated. Multivariate analyses showed that females had 4.54 more units of HSI (95% CI: 3.41 to 5.68) than males. Both sexes showed increased levels of triglycerides, TG/HDL cholesterol ratio and triglyceride glucose index across the HSI tertiles. Physical activity exhibited a negative statistical association with HSI (males: $r = -0.19$, $p = 0.025$; females: $r = -0.18$, $p = 0.031$). The amount of visceral fat showed a positive association with HSI in both sexes (males: $r = 0.64$, $p < 0.001$; females: $r = 0.46$, $p < 0.001$). Adherence to the Mediterranean diet was lower in those subjects with higher HSI values (males: $r = -0.18$, $p = 0.032$; females $r= -0.19$, $p = 0.027$).

Conclusion: females had a poor liver status, suggesting gender differences related to NAFLD. Adherence to a Mediterranean diet and physical activity were associated with beneficial effects on cardiovascular disease features. Thus, reducing the risk of hepatic steatosis in subjects with MetS and obesity.

Keywords: Non-alcoholic fatty liver disease. Abdominal obesity. Metabolic syndrome. Visceral adipose tissue. Sex.

INTRODUCTION

Epidemiological data indicate that almost 25% of the adult population suffer from non-alcoholic fatty liver disease (NAFLD) (1). This pathology encompasses a broad spectrum of liver diseases characterized by an excess accumulation of intrahepatic fat (2). Early stage disease starts with hepatic steatosis that could develop to steatohepatitis (inflammation and/or fibrosis), progressing to cirrhosis and to hepatocellular carcinoma in late-stage disease (2). Several clinical studies have shown that the increased prevalence of NAFLD is closely related to central obesity, metabolic syndrome (MetS) features and associated co-morbidities (1,3,4). NAFLD prevalence varies according to age, sex, race and ethnicity, with a higher incidence in Hispanic males (5). In females, physiological and biological changes occur after the menopause, which could promote metabolic disorders and increase the risk of developing NAFLD (6). Nowadays, a liver biopsy is the gold standard for diagnosis. However, it is an invasive and expensive technique that can cause medical complications and sampling errors (2). In this sense, several alternative methods and non-invasive approaches have been proposed for early diagnosis of NAFLD in the clinical practice (7,8). The hepatic steatosis index (HSI) is a non-invasive liver marker, which has been previously validated (9). Moreover, it could be useful for early detection of hepatic steatosis (8). In this context, the objective of this study was to determine if males and females diagnosed with MetS have different risk factors such as a sedentary lifestyle, unhealthy dietary patterns and other characteristics associated with NAFLD, stratified by the HSI.

METHODS

Study population

This study analyzed data from the Navarra-Nutrition center of the PREDIMED Plus study of people with a risk of NAFLD diagnosed by the HSI at baseline (8). Briefly, PREDIMED Plus is a multicenter randomized trial with the main objective of determining the effect of a dietary intervention for the prevention of cardiovascular disease (CVD). This is based on a hypocaloric Mediterranean diet pattern, physical activity and behavioral support *versus* advice on a Mediterranean diet pattern without caloric restriction (10). Males between 55 and 75 years and females between 60 and

75 years, with a body mass index (BMI) ≥ 27 and < 40 kg/m^2, who met at least three criteria for MetS were recruited (11). Exclusion criteria included patients with a diagnosis of cirrhosis or liver failure, with inflammatory bowel disease and/or the consumption of immunosuppressive or cytotoxic drugs, among others (10). A total of 422 participants entered the pre-inclusion period, two cases did not meet the inclusion criteria and 89 subjects were excluded prior to randomization. Three hundred and thirty-one subjects were finally recruited. For the present study, 50 subjects were excluded because they had a high alcohol intake (males ≥ 30 g/d and females ≥ 20 g/d) and three participants did have the clinical data for the calculation of HSI. Two hundred and seventy-eight participants were included in the analysis and individuals were stratified according to HSI tertiles. This study was performed in compliance with the ethical principles of the Declaration of Helsinki. Participants who agreed to participate in the study signed an informed consent. This clinical trial is registered with the number ISRCTN89898870.

Evaluation of sociodemographic, anthropometric, body composition and biochemical characteristics

Sociodemographic data were collected via a general questionnaire developed by the study, as well as anthropometric measurements such as weight, height and circumferences that were performed according to the protocol established by the project (http://medpreventiva.es/QufSWn). The determinations of body composition were evaluated by DEXA (Lunar iDXA™, software version 6.0, Madison, WI, USA). Blood pressure measurements were performed with a calibrated instrument and a blood pressure monitor (Omron HEM-705CP, The Netherlands) following a revised protocol. With regard to the biochemical variables, total cholesterol, HDL-c, triglycerides (TG), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), mean corpuscular volume (MCV) and albumin were analyzed. LDL-c was calculated using the Friedewald formula (12). In addition, the triglyceride glucose index (TyG) was evaluated as a marker of insulin resistance, which was defined as the natural logarithm of TG and glucose (13).

Assessment of NAFLD risk

The estimation of hepatic steatosis was performed by calculating the HSI, using the formula: HSI = 8 * ALT / AST + body mass index (BMI) + 2 (if type 2 diabetes mellitus) + 2 (if female) (9). The evaluation of liver fibrosis was performed via the NAFLD fibrosis score formula = -1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose (IFG) / diabetes (yes = 1, no = 0) + 0.99 × AST / ALT ratio - 0.013 × platelets (× 10^9/l) - 0.66 × albumin (g/dl) (14).

Dietary intake and physical activity assessment

The evaluation of dietary intake was carried out by applying a semi-quantitative 143-item food frequency questionnaire, which had been previously validated (15,16). Adherence to the Mediterranean diet was evaluated based on a nine-point scale proposed by Trichopoulou et al. (17,18). Physical activity was determined by the short version of the REGICOR questionnaire (19).

Statistical analysis

Data were analyzed according to HSI tertiles stratified by gender. For the descriptive analyses, qualitative variables were presented as n (%) and continuous variables were expressed as the mean and standard deviation (SD). The gender differences within each tertile of HSI were determined using the Student's t-test. The 2 x 2 factorial analysis of the variables were evaluated using the two criteria ANOVA test (sex x HSI tertile). The correlations between the HSI and the main risk factors associated with NAFLD were assessed using the Pearson's coefficient for continuous variables or the Spearman's rank test for discrete variables. Likewise, a multiple linear regression was applied to determine the association between HSI and sex adjusted for several confounding factors (age, physical activity, waist circumference, smoking, total energy and alcohol consumption). Two-sided p-values less than 0.05 were considered as statistically significant. Data were analyzed using the STATA statistical package version 12.0 (StataCorp, College Station TX, USA).

RESULTS

The analysis included a total of 278 participants, 137 females (49.3%) and 141 males (50.7%). The baseline characteristics of the subjects separated by HSI and gender tertiles (Table 1) indicated that females were older than males (p_{sexo} < 0.001). In addition, males had a higher alcohol consumption and were more likely to be smokers in all HSI groups. The BMI increases among tertiles, with a significant difference between males and females (Table 1). Moreover, males were more physically active compared to females. Furthermore, there were differences between HSI groups with regard to physical activity. The TyG index showed a significant upward linear trend across HSI tertiles. With regard to the variables of body composition, the amount of visceral fat and total body fat increased as the HSI increased. Furthermore, there were differences between genders within each HSI tertiles, indicating that men had a higher concentration of visceral fat and females had a greater proportion of total fat. On the other hand, the two-way ANOVA analysis identified differences between the sexes with regard to the concentration of plasma lipids (Table 1). There were significant differences among the HSI tertiles with regard to the levels of HDL-c (p = 0.027), TG (p < 0.001) and TG/cholesterol HDL ratio (p < 0.001). Regarding liver function parameters, no differences were found between the levels of AST, albumin and NAFLD fibrosis score. Nevertheless, there was a significant increase in ALT in all HSI groups. In addition, the results showed differences in hematological variables (platelet count and MCV) between the sexes. In relation to dietary variables (Table 2), males had a higher energy intake compared to females but this difference was not statistically significant between the groups. However, the consumption of vegetable foods was different in males and females (Table 2). Meanwhile, the adherence to the Mediterranean diet decreased in both sexes among HSI tertiles (p = 0.012). There was a positive correlation between the HSI and risk factors related to NAFLD in both sexes (Fig. 1) such as waist circumference (Fig. 1A) and visceral fat (Fig. 1B). Moreover, there was a negative relationship between adherence to the Mediterranean diet (males: r = -0.18, p = 0.032; females: r = -0.19, p = 0.027) (Fig. 1C) and physical activity (males: r = -0.19, p = 0.025; females: r = -0.18, p = 0.031) (Fig. 1D). The linear regression analysis adjusted for confounding factors (Table 3) and showed that females had a 4.54 fold (95% CI: 3.41-5.68) higher risk to have higher HSI values than males.

DISCUSSION

This study suggests that females have a higher risk of liver steatosis than males in older subjects diagnosed with obesity and MetS. This finding is consistent with several clinical studies (1,20,21). The prevalence of NAFLD is higher in young males compared to females of the same age (5). However, this trend reverses between 50-60 years of age, probably due to the decrease in sex steroid hormones after the menopause in women (6,21,22). This aspect is relevant in the menopausal status (21). Estradiol levels decrease, thus promoting a reduction of the fatty acid oxidation capacity, which increases hepatic lipogenesis contributing to the accumulation of lipids in the hepatocytes (6,21,22). Similarly, lower estrogen levels affect choline biosynthesis, inhibiting the export of triglycerides from the liver (6). In this sense, Ryu et al. (23) reported higher rates of NAFLD in perimenopausal and postmenopausal compared to premenopausal women. On the other hand, our analyses indicate that both sexes have increased markers related to insulin resistance and lipid disruption. This interpretation should be highlighted as the study population were obese and had MetS, and these entities are closely related to the activation of several inflammatory processes and oxidative stress involved in NAFLD pathogenesis (24,25). In this context, Navarro-González et al. (13) observed that values of the TyG index > 8.43 in males and > 8.19 in females have a risk to develop diabetes mellitus (DM) (13). In our study, males and females have a higher cardiometabolic risk (triglycerides, TG/ HDL cholesterol ratio) across the HSI groups. In fact, the increase in platelet levels could be associated with NAFLD as an inflammatory response to the liver damage (26). However, our results only showed differences between the sexes. In accordance with our results, Fangs et al. (27) showed a positive association between platelet count and MetS, regardless of hepatic steatosis. The NAFLD fibrosis score (14) has been validated in subjects diagnosed with NAFLD by liver biopsy (14). In this study, values for the detection of liver fibrosis are not shown. Lower estrogen levels in post-menopausal females induce changes in the distribution of body fat, especially abdominal fat, increasing the risk to develop NAFLD (21,28). Visceral adipose tissue drains into the portal circulation to the liver, increasing the amount of free fatty acid (FFA) as a substrate for hepatic

lipoprotein metabolism and glucose production (29). According to our data, visceral fat and waist circumference had a positive association with HSI in both sexes. Furthermore, males had more visceral fat than females. Menopausal women with obesity could have a higher prevalence of NAFLD and metabolic disorders (30). Nevertheless, these results could be related to differences in body composition between the sexes (21,31). Our findings corroborate the results obtained by Nielsen et al. (29); their study found that visceral fat mass was associated with an increase in the fraction of liver FFA. Moreover, sex and visceral fat were significant predictors of FFA delivery to the liver from adipose tissue lipolysis. Hence, the differences in body fat distribution between the sexes might not only be influenced by hormonal status, but also could be determined by the interaction of genetic and epigenetic factors, among others (31).

Emerging data suggest that a Mediterranean diet might have beneficial effects on several features associated with NAFLD. The Mediterranean diet is characterized by bioactive compounds with anti-inflammatory and antioxidant effects (32,33), improving the lipid and glucose profile (34). These substances also favor liver function and reduce hepatic steatosis (35). The analysis of dietary variables showed that the level of adherence to the Mediterranean diet is inversely associated with HSI. Our results are in agreement with a study reported by Kontogianni et al. (36) that determined a negative association between the Mediterranean diet and the severity of the degree of hepatic steatosis. Furthermore, physical activity had an inverse relationship with hepatic steatosis (HSI). In fact, it has been suggested that physical activity exerts beneficial effects on patients with NAFLD (37) and might improve metabolic disorders (38).

A strength of this study is the large number of participants, variables and non-invasive liver markers included. This allowed a comparison of several risk factors between older males and females diagnosed with obesity and MetS, stratified by HSI within the PREDIMED Plus study. However, this study has some limitations. Firstly, the causal relationship cannot be determined due to the cross-sectional design. Secondly, the design of the PREDIMED Plus study considers CVD as the main outcome. However, MetS and obesity (inclusion criteria) are key factors associated with NAFLD. Thirdly,

the identification of patients at risk of NAFLD was assessed by non-invasive diagnostic tools, as the diagnosis is not possible due to the lack of biopsy or liver ultrasound. This may underestimate the prevalence of NAFLD. However, the HSI has been previously validated, showing an adequate sensitivity for the diagnosis of NAFLD (8,9). Finally, our results focused on Caucasian older adult participants. Therefore, these results may not be generalized to other populations.

CONCLUSION

Females between 60 and 75 years of age have an increased risk of liver steatosis compared to males, suggesting that gender and metabolic disorders associated with NAFLD play a putative role in disease progression in elderly adult individuals with obesity and MetS. On the other hand, adherence to a Mediterranean diet and physical activity could have a protective effect on NAFLD in both sexes. These findings reinforce the importance of preventing NAFLD and co-morbidities by adhering to healthy dietary patterns and lifestyle changes in both sexes. However, a better understanding of the differential mechanisms of gender and lifestyle in the pathogenesis of NAFLD in older adults with obesity and MetS is required.

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Table 1. Baseline characteristics of males and female diagnosed with metabolic syndrome according to hepatic steatosis index (HSI)

Variables	*Hepatic steatosis index (HSI)* T1		T2		T3		Sex	HSI tertiles	*p*
	Males	Females	Males	Females	Males	Females			
	(n = 47)	(n = 46)	(n = 47)	(n = 46)	(n = 47)	(n = 45)			
Age (years)	64.5 (6.0)	67.6 (4.0)†	64.9 (5.4)	67.7 (3.7)†	63.7 (5.2)	66.5 (4.0)†	< 0.001	0.210	0.977
BMI (kg/m^2)	29.3 (1.3)	29.6 (1.9)	31.4 (1.7)	32.1 (2.3)	34.1 (3.0)	36.4 (2.7)‡	< 0.001	< 0.001	0.006
Physical activity (MET-h/weeks)	76.0 (63.0)	43.1 (32.7)†	57.2 (43.7)	49.8 (38.2)	49.3 (39.7)	29.9 (27.0)†	< 0.001	0.005	0.124
Smoking status, n (%)									
Never	15 (31.9)	31 (67.4)†	10 (21.3)	34 (73.9)‡	6 (12.8)	28 (62.2)‡			
Former	26 (55.3)	10 (21.7)	26 (55.3)	9 (19.6)	36 (76.6)	14 (31.1)			
Current	6 (12.8)	5 (10.9)	11 (23.4)	3 (6.5)	5 (10.6)	3 (6.7)			
Systolic blood pressure (mmHg)	142.2 (14.7)	139.2 (17.0)	142.7 (18.0)	142.9 (17.0)	142.4 (14.4)	139.9 (13.3)	0.355	0.633	0.772
Diastolic blood pressure	86.5 (8.4)	82.7 (10.1)	88.1 (9.7)	84.0 (7.7)*	87.3 (8.3)	87.2 (7.7)	0.011	0.118	0.22

(mmHg)									9
Triglyceride glucose index (TyG)	8.7 (0.5)	8.8 (0.3)	8.9 (0.6)	8.9 (0.5)	9.2 (0.6)	9.1 (0.5)	0.543	< 0.001	0.511
Alcohol consumption (g/d)	8.1 (7.4)	1.8 (3.4)‡	11.7 (9.2)	1.6 (2.6)‡	11.4 (10.1)	1.8 (2.9)‡	< 0.001	0.151	0.102
Body composition§									
Total fat (kg)	26.8 (5.0)	31.0 (4.3)‡	30.9 (4.4)	34.7 (4.9)‡	37.7 (8.4)	42.7 (6.3)†	< 0.001	< 0.001	0.818
Visceral fat (Kg)	2.2 (0.7)	1.5 (0.6)‡	2.7 (0.6)	1.8 (0.4)‡	3.4 (0.8)	2.2 (0.6)‡	< 0.001	< 0.001	0.012
Lipid parameters									
Total cholesterol (mg/dl)	182.9 (32.8)	208.8 (34.6)‡	200.4 (35.4)	214.5 (36.8)	191.6 (37.1)	206.8 (38.1)	< 0.001	0.076	0.467
LDL-c (mg/dl)	117.2 (29.7)	132.9 (31.9)*	128.3 (33.7)	136.2 (33.6)	116.9 (34.9)	131.0 (32.8)	0.002	0.185	0.707
HDL-c (mg/dl)	43.9 (10.7)	49.4 (7.8)†	44.6 (9.6)	50.7 (11.2)†	41.2 (9.0)	46.7 (8.8)†	< 0.001	0.027	0.970
Triglycerides (mg/dl)	115.7 (52.2)	132.4 (42.1)	147.7 (80.0)	142.2 (54.0)	171.1 (85.3)	152.4 (52.4)	0.747	< 0.001	0.160
TG/ cholesterol HDL ratio	2.9 (1.8)	2.8 (1.1)	3.7 (2.6)	3.0 (1.6)	4.5 (2.7)	3.5 (1.8)*	0.020	< 0.001	0.307

Hepatic function

ALT (U/l)	20.3 (7.5)	17.6 (7.3)	31.8 (33.5)	24.1 (9.6)	38.0 (20.8)	30.4 (15.3)*	0.007	< 0.001	0.560
AST (U/l)	22.2 (6.8)	19.9 (5.0)	26.3 (25.2)	22.2 (7.3)	25.0 (8.7)	22.5 (8.7)	0.047	0.162	0.879
Albumin (g/dl)	4.2 (1.4)	4.2 (0.8)	4.3 (0.8)	4.3 (0.3)	4.3 (1.3)	4.2 (1.2)	0.863	0.774	0.923
MCV (fl)	90.6 (5.2)	89.7 (7.2)	92.4 (4.3)	90.5 (4.1)*	91.6 (3.6)	86.4 (13.9)*	0.002	0.070	0.106
Platelet (10^3/µl)	221.7 (56.2)	238.6 (60.7)	214.1 (55.5)	243.8 (48.6)†	216.3 (45.1)	254.5 (69.3)†	< 0.001	0.714	0.438
NAFLD fibrosis score	-0.7 (1.3)	-0.9 (1.1)	-0.7 (1.1)	-0.7 (0.9)	-0.5 (1.2)	-0.5 (1.2)	0.498	0.257	0.736

Data are expressed as the mean (SD), *p < 0.05; †p < 0.01 y ‡p < 0.001; p interaction between sex and HSI tertiles. §Available data, total fat (n = 222); visceral fat (n = 213). BMI: body mass index; MET: metabolic equivalent; TyG: triglyceride glucose index; LDL-c: low density lipoprotein cholesterol; HDL-c: high density lipoprotein cholesterol; TG: triglycerides; TG/HDL: triglycerides/ cholesterol ratio; ALT: alanine aminotransferase; AST: aspartate aminotransferase; MCV: mean corpuscular volume.

Table 2. Dietary parameters of males and females diagnosed with metabolic syndrome according to the hepatic steatosis index (HSI)

	Hepatic steatosis index (HSI)								
	T1		T2		T3				
Variables	Males	Females	Males	Females	Males	Females	Sex	HSI tertiles	*p*
	(n = 47)	(n = 46)	(n = 47)	(n = 46)	(n = 47)	(n = 45)			
Energy intake (kcal/d)	2,592.2 (572.7)	2,480.1 (534.0)	2,675.8 (456.7)	2,416.7 (515.1)*	2,555.3 (553.1)	2,520.8 (536.4)	0.034	0.991	0.343
Carbohydrates (g/d)	290.7 (82.9)	277.3 (67.9)	291.9 (62.6)	271.1 (81.1)	274.9 (78.7)	280.2 (71.4)	0.284	0.837	0.474
Proteins (g/d)	102.6 (23.9)	99.8 (22.3)	104.3 (22.0)	101.7 (23.4)	99.2 (26.3)	107.1 (18.3)	0.761	0.803	0.195
Lipids (g/d)	107.0 (30.3)	106.6 (32.0)	112.1 (23.5)	101.6 (22.8)*	108.8 (28.4)	106.6 (29.1)	0.191	0.968	0.421
Vegetable food group (g/d)[§]	750.5 (276.9)	820.8 (286.8)	787.6 (310.9)	820.3 (371.3)	652.1 (261.6)	783.6 (277.8)*	0.031	0.123	0.528
Animal food group (g/d)[¶]	250.6 (82.4)	241.5 (68.7)	244.4 (64.8)	238.3 (61.8)	238.0 (93.3)	267.1 (57.7)	0.595	0.575	0.141
Mediterranean diet adherence (0-9)	4.9 (1.6)	4.6 (1.4)	4.7 (1.7)	4.2 (1.6)	4.1 (1.5)	4.0 (1.4)	0.138	0.012	0.736

Data are expressed as the mean (SD), *p < 0.05; †p < 0.01 y ‡p < 0.001; p interaction between sex and HSI tertiles. §Vegetable food groups: vegetables and fruits. ¶Animal food group: total meat, fish and seafood.

Table 3. Multiple regression analysis exploring the association between gender and HSI as a dependent variable in subjects with metabolic syndrome

	Males	Females	p value
HSI		β (95% CI)	
N	141	137	
Crude	0 Ref.	2.19 (1.07-3.32)	< 0.001
Model 1	0 Ref.	2.68 (1.52-3.84)	< 0.001
Model 2	0 Ref.	4.54 (3.41-5.68)	< 0.001

Model 1: adjusted for age. Model 2: model 1 + physical activity (MET-h/week), waist circumference (cm), smoking habits (smoker, non-smoker, former smoker), total energy intake (Kcal/d) and alcohol intake (g/d). HIS: hepatic steatosis index; β: coefficient beta; CI: confidence interval.

Fig. 1. Correlation analysis of the principal risk factors related to NAFLD in males and females diagnosed with metabolic syndrome.

A

B

C

D