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## Original article

## Gender-specific differences in clinical and metabolic variables associated with NAFLD in a Mexican pediatric population



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## ABSTRACT

**Introduction and Objectives:** Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children and it is more prevalent in Hispanic males. The gender differences can be explained by body fat distribution, lifestyle, or sex hormone metabolism. We evaluated anthropometric and metabolic differences by gender in children with and without NAFLD.

**Methods:** We included 194 participants (eutrophic, overweight, and individuals with obesity). The presence of NAFLD was determined using ultrasonography, and we evaluated the association between this disease with metabolic and anthropometric variables by gender.

**Results:** The mean age was  $10.64 \pm 2.54$  years. The frequency of NAFLD in boys was 24.51% and in girls was 11.96% (OR = 2.39; 95%CI = 1.10–5.19;  $p = 0.025$ ). For girls, NAFLD was significantly associated with triglycerides ( $p = 0.012$ ), homeostatic model assessment of insulin resistance (HOMA-IR) ( $p = 0.048$ ), and the visceral adiposity index (VAI) ( $p = 0.024$ ). The variables related to NAFLD in a gender-specific manner were body mass index (BMI) ( $p = 0.001$ ), waist circumference (WC) ( $p < 0.001$ ), HDL cholesterol ( $p = 0.021$ ), alanine aminotransferase (ALT) ( $p < 0.001$ ), and aspartate aminotransferase (AST) ( $p = 0.002$ ).

**Conclusions:** In our study NAFLD is more frequent in boys, only ALT, and no other clinical or metabolic variables, were associated with NAFLD in these patients. HOMA-IR, VAI, triglyceride levels, and ALT were associated with NAFLD only in girls. The ALT cut-off points for the development of NAFLD in our study were 28.5 U/L in females and 27.5 U/L in males. Our findings showed that NAFLD should be intentionally screened in patients with obesity, particularly in boys.

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## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined by the presence of steatosis in more than 5% of hepatocytes in patients with no history of alcohol consumption, no pharmacological treatment, and no congenital disease [1,2]. NAFLD is the most frequent type

of liver disease in the pediatric population, with an overall prevalence of 7.6% and a cumulative prevalence of 34.2% in children with obesity [3]. Epidemiological studies of NAFLD in Mexico show a prevalence of 17.05% in asymptomatic adults and 12.6% in children with a body mass index (BMI) >85th percentile (overweight and obesity) [4,5].

Cross-sectional studies on humans and animal models have reported a higher prevalence of NAFLD in the male gender. In the pediatric population with obesity, the distribution by sex is 35.3% in males and 21.8% in females [3]. Central obesity, insulin resistance, dyslipidemia, Hispanic ethnicity, and the genetic variants COL13A1, ADIPOQ, SAMM50, and PNPLA3 in Mexican Americans

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and the Mexican-Mestizo population are the variables that are most frequently associated with NAFLD [6–9].

Although a higher prevalence of NAFLD has been reported in boys, gender-specific characteristics of this disorder have rarely been explored. It has been proposed that the gender-specific differences observed in the prevalence of NAFLD may result from the possible protective role of estrogens, which decrease the risk for this disorder among females. Recent findings on the physiopathology of NAFLD, evaluated from a gender-specific perspective, have indicated that lipid metabolism and body fat distribution may play a relevant role [10,11].

Pediatric patients are not frequently screened for NAFLD, and thus, it is rarely identified, primarily because of the complexity of the diagnosis [2,12]. This study aimed to evaluate the gender-specific differences in the clinical and metabolic variables in a pediatric population with NAFLD.

## 2. Methods

### 2.1. Study design

A cross-sectional comparative study was performed. Eutrophic, overweight, and individuals with obesity and ranging in age from 6–18 years were consecutively included from January 2015 to September 2016. Patients with overweight/obesity from the Childhood Obesity Clinic of the Hospital General de Mexico were invited to participate, whereas eutrophic children were recruited from surrounding schools. All patients included in this study were born and raised in Mexico City and the suburban area. The local ethics committee approved the protocol.

The participants were classified into the following groups, according to the Centers for Disease Control and Prevention guidelines: eutrophic (BMI <85th percentile); overweight (BMI ≥85th and <95th percentile); and obesity (BMI ≥95th percentile).

Patients with obesity that had a genetic or endocrine origin; those with a previously known liver disease, diabetes or other endocrinopathies; patients receiving a pharmacological treatment that was able to modify lipid or carbohydrate metabolism; and those who reported alcohol consumption were excluded.

Informed consent and assent were obtained from the participants and their parent/legal guardian. All sensitive patient data were protected in compliance with the Health Insurance Portability and Accountability Act (HIPAA). A complete medical record was documented for each participant.

The NAFLD diagnosis was determined by a liver ultrasound that was performed by an expert ultrasonographer.

### 2.2. Anthropometric and metabolic evaluation

The clinical variables assessed were age, gender, weight, height, BMI, waist circumference (WC), and sexual development stage.

A mechanical scale and a stadiometer were used to evaluate weight and height. The measuring location for WC was the midway point between the last costal cartilage and the anterosuperior iliac crest. Pediatricians and pediatric endocrinologists appraised the sexual status, according to the Tanner scale [13,14]. Arterial blood pressure was measured from the right brachial artery using a manual sphygmomanometer and special-sized pressure cuffs according to the patient's age, and the measurement was taken twice per patient, with the patient in a sitting position.

We calculated the BMI according to Quetelet's equation [15]. Additionally, using the homeostatic model assessment of insulin resistance (HOMA-IR), we calculated the insulin resistance index [16].

A gender-specific visceral adiposity index (VAI) was calculated using a formula that was previously proposed by our group for

pediatric populations, as follows:

$$\text{Female VAI} = \text{WC}/(-0.018[\text{BMI}^2] + 3.3[\text{BMI}] + 7.86) \\ \times (\text{Triglycerides}/0.91) \times (1.42/\text{HDL}); \text{ and}$$

$$\text{Male VAI} = \text{WC}/(-0.023[\text{BMI}^2] + 3.83[\text{BMI}] + 0.65) \\ \times (\text{Triglycerides}/0.76) \times (1.37/\text{HDL}).$$

Metabolic variables were evaluated by obtaining venous blood after the patients had fasted for 12 h. Enzymatic methods were performed to measure glucose, aminotransferases, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides, using available commercial kits. Insulin was determined through chemiluminescence analysis. Normal values were considered according to the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and the Endocrine Society clinical practice guidelines for NAFLD and pediatric obesity, respectively (alanine aminotransferase (ALT) <25 U/L in boys and <22 U/L for girls, HDL <40 mg/dL, and triglycerides in patients 0–9 years <100 mg/dL and 10–19 years <130 mg/dL) [17,18].

### 2.3. Ultrasonography

The NAFLD diagnosis was performed using a B mode ultrasound and a 1–4 mHz curve transducer (Siemens Acuson S2000, Mountain View, CA, USA), according a standardized protocol, by an expert ultrasonographer who was blinded to the clinical and metabolic conditions [19,20]. The kappa coefficient for intra-observer concordance was 0.90. The steatosis condition was categorized as present/not present, according to the hepatic tissue's echogenicity, in contrast to the adjunct right kidney's parenchyma.

### 2.4. Statistical analyses

Descriptive and inferential statistics for numerical variables were reported as the mean and standard deviation (SD), and the percentages were used for categorical variables.

The inferential statistical analyses included a two-factor analysis of variance (ANOVA) for all anthropometric and metabolic variables, considering the presence of NAFLD and gender as factor variables, and we ensured that the test assumptions were met. The effect size was calculated using Cohen's *f* and was considered to be as follows: >0.10 small; >0.25 medium; and >0.40 large [21,22].

Chi-squared tests and Fisher's exact test were used to assess categorical variables. Binary logistic regression evaluated the association between NAFLD and BMI-Pc, ALT, aspartate aminotransferase (AST), WC by gender, and triglycerides by sex [23].

The most relevant variables related to NAFLD were evaluated using the receiver operating characteristics (ROC) curve analysis, according to gender. The cut-off points, sensitivity, and specificity were obtained for each variable [24,25].

All analyses were performed using the statistical software IBM SPSS Statistics version 22.0 (IBM, Armonk, NY, USA). A *p* value of <0.05 was considered to be statistically significant.

## 3. Results

### 3.1. Descriptive statistics

A total of 194 patients (102 males and 92 females) were recruited: 44 eutrophic, 38 overweight, and 112 with obesity. The mean age of the participants was  $10.64 \pm 2.54$  years.

The overall NAFLD frequency in our study was 18.55%; it was 24.51% in boys and 11.96% in girls, which was significantly different (OR = 2.39; 95%CI = 1.10–5.19;  $p = 0.025$ ).

In the obesity group, the frequency was 29.46%: 32.8% in males and 24.4% in females (OR = 1.51; 95%CI = 0.65–3.54;  $p = 0.340$ ). We found that 91.7% of the patients with a NAFLD were obese.

The participants' demographic and anthropometric characteristics (by gender) are shown in Table 1.

### 3.2. Inferential statistics

Differences in anthropometric and metabolic variables by gender and the presence of NAFLD were assessed using two-factor ANOVAs.

We found statistically significant differences between gender and the presence of NAFLD for the following variables: triglycerides ( $F(1,185) = 6.618$ ,  $p = 0.012$ ,  $f = 0.0895$ ); insulin ( $F(1,174) = 6.197$ ,  $p = 0.021$ ,  $f = 0.087$ ); HOMA-IR ( $F(1,174) = 4.28$ ,  $p = 0.048$ ,  $f = 0.0895$ ), and VAI ( $F(1,185) = 5.47$ ,  $p = 0.02$ ,  $f = 0.0959$ ). The combined effect of the presence of NAFLD and gender was also evaluated for all significant ANOVAs. The mean triglyceride concentration in males with and without NAFLD and in females with and without NAFLD were  $132.32 \pm 68.14$ ,  $120.85 \pm 68.08$ ,  $210.18 \pm 73.26$ , and  $126.38 \pm 74.13$  mg/dL, respectively. When comparing differences in triglyceride concentrations among the male and female patients with and without this disease, we found that the mean triglyceride concentration in female NAFLD patients was 77.86 mg/dL higher than that in males. Similarly, in the group without NAFLD, the triglyceride levels were higher in females compared to males, with a mean difference of 5.53 mg/dL. The difference between the differences was 70.993 (95%CI, 15.966–126.02,  $p = 0.012$ ).

For insulin concentrations, the mean levels in males with and without NAFLD and in females with and without this disorder were  $15.00 \pm 5.69$ ,  $13.11 \pm 6.75$ ,  $14.16 \pm 12.08$ , and  $14.92 \pm 8.43$  UI/L, respectively. In a mean comparison by gender and the presence of NAFLD, male NAFLD patients had higher insulin concentrations compared to females, with a mean difference of 0.84 UI/L, whereas among patients without NAFLD, females had higher insulin concentrations, with a mean difference of 1.81 UI/L. The difference between the differences was 7.357 (95%CI, 1.145–13.569,  $p = 0.021$ ).

The mean HOMA-IR levels in males with and without NAFLD and in females with and without NAFLD were  $3.24 \pm 1.20$ ,  $2.56 \pm 1.69$ ,

$5.20 \pm 2.70$ , and  $3.04 \pm 1.99$ , respectively. In a mean comparison by gender and the presence of NAFLD, in patients with NAFLD, females had a higher mean HOMA-IR level compared to males, with a mean difference of 1.96 points. Similarly, in patients without NAFLD, the HOMA-IR level was also higher in females than in males, with a smaller mean difference of 0.48 points. The difference between the differences was 1.471 (95%CI, 0.013–2.928,  $p = 0.048$ ).

For VAI scores, the mean values in males with and without NAFLD and in females with and without NAFLD were  $2.87 \pm 1.97$ ,  $2.57 \pm 1.89$ ,  $4.30 \pm 1.75$ , and  $2.38 \pm 1.66$ , respectively. In a mean comparison by gender and the presence of NAFLD, in NAFLD patients, the VAI scores were higher in females compared to males, with a mean difference of 1.43 points. Similarly, among the participants without NAFLD, females had higher VAI scores than males, with a mean difference of 0.19 points. The difference between the differences was 1.62 (95%CI, 0.215–3.024,  $p = 0.024$ ) (Table 1 and Fig. 1).

For the variables that did not demonstrate significant interactions in factorial ANOVAs, the main effects were evaluated. The following significant main effects from NAFLD were observed: for BMI-Percentile (Pc) the mean was  $97.48 \pm 2.01$  for patients with NAFLD vs.  $83.87 \pm 22.52$  for those without NAFLD ( $p = 0.001$ ); for WC, the mean was  $86.82 \pm 12.73$  with NAFLD vs.  $77.43 \pm 13.47$  without NAFLD ( $p < 0.001$ ); for HDL cholesterol, the mean was  $37.53 \pm 6.93$  with NAFLD vs.  $41.54 \pm 10.21$  without NAFLD ( $p = 0.018$ ); for ALT, the mean was  $48.28 \pm 21.98$  with NAFLD vs.  $22.22 \pm 9.81$  without NAFLD ( $p < 0.001$ ); and for AST, the mean was  $36.14 \pm 10.55$  with NAFLD vs.  $27.22 \pm 6.58$  without NAFLD ( $p = 0.002$ ; Table 1 and Fig. 1).

A binomial logistic regression was performed to ascertain the effects of BMI percentile, ALT, WC by gender, and triglycerides by gender on the likelihood that participants would have NAFLD. The logistic regression model was statistically significant ( $\chi^2(6) = 79.47$ ,  $p < 0.0001$ ). The model explained 54.9% (Nagelkerke's  $R^2$ ) of the variance in NAFLD and correctly classified 81.2% of the cases. The sensitivity of the model was 79%, and specificity was 90%; the positive predictive value was 53%, and the negative predictive value was 97%. The model was then internally validated using the ROC curve. The AUC value was 0.922 (95%CI = 0.877–0.966,  $p < 0.0001$ ). The only statistically significant variable was ALT (Table 2).

With the aim of evaluating the accuracy of the variables associated with NAFLD by gender, we performed receiver-operating characteristics (ROC) curve discrimination. For female participants

**Table 1**  
Clinical and metabolic variables associated to NAFLD by gender in Mexican children.

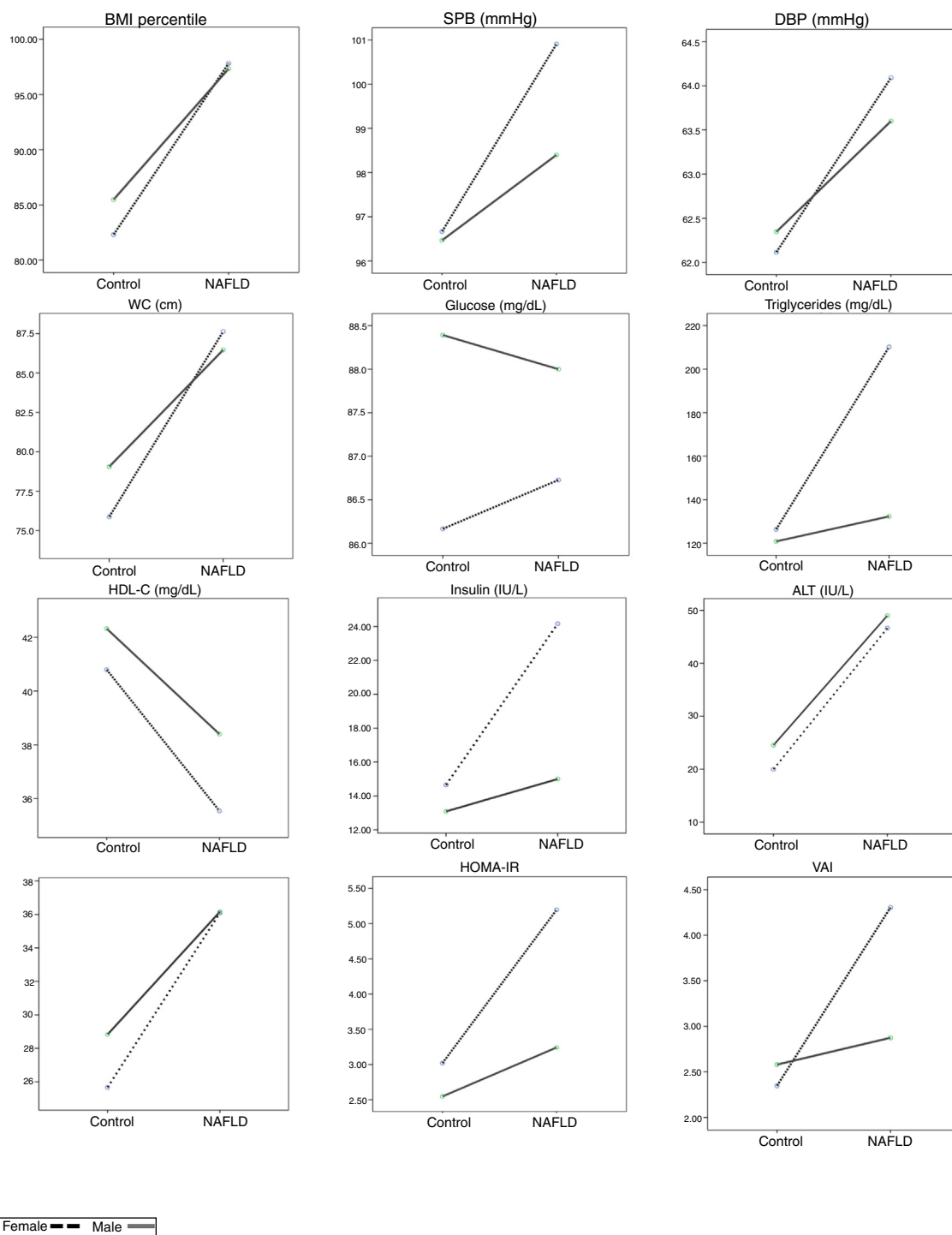
Variables	Male		Female		Gender	NAFLD	Gender and NAFLD
	NAFLD $n = 25$	No NAFLD $n = 77$	NAFLD $n = 11$	No NAFLD $n = 81$			
Age (years)	11.08 ± 2.04	10.26 ± 2.47	10.83 ± 3.27	10.85 ± 2.64	0.736	0.423	0.406
BMI (percentile)	97.33 ± 2.19	85.85 ± 22.49	97.82 ± 1.56	81.72 ± 23.75	0.661	<b>0.001</b>	0.579
SBP (mmHg)	99.17 ± 8.66	96.59 ± 8.92	100.91 ± 9.44	96.54 ± 8.83	0.493	0.083	0.461
DBP (mmHg)	63.75 ± 6.25	62.03 ± 6.72	64.09 ± 7.36	61.91 ± 6.92	0.925	0.171	0.781
WC (cm)	86.82 ± 12.73	77.57 ± 13.66	86.46 ± 14.51	75.89 ± 11.89	0.670	<b>&lt;0.001</b>	0.388
Glucose (mg/dL)	88.00 ± 5.88	88.38 ± 5.83	86.73 ± 7.17	86.23 ± 11.59	0.325	0.972	0.800
Triglycerides (mg/dL)	132.32 ± 68.14	120.85 ± 68.08	210.18 ± 73.26	126.38 ± 74.13	<b>0.003*</b>	<b>0.001*</b>	<b>0.012</b>
Total-C (mg/dL)	158.64 ± 26.64	163.62 ± 31.14	171.27 ± 30.24	154.86 ± 30.59	0.747	0.342	0.076
HDL-C (mg/dL)	38.40 ± 6.72	42.61 ± 12.76	35.55 ± 7.30	40.78 ± 8.54	0.247	<b>0.021</b>	0.801
ALT (IU/L)	49.0 ± 24.50	24.49 ± 10.94	46.64 ± 15.67	22.50 ± 24.57	0.173	<b>&lt;0.001**</b>	0.662
AST (IU/L)	36.16 ± 11.41	28.83 ± 7.10	36.09 ± 8.80	27.43 ± 16.66	0.768	<b>0.002*</b>	0.789
Insulin (IU/L)	15.00 ± 5.69	13.11 ± 6.75	14.16 ± 12.08	14.92 ± 8.43	<b>0.001*</b>	<b>0.001*</b>	<b>0.021</b>
HOMA-IR	3.24 ± 1.20	2.56 ± 1.69	5.20 ± 2.70	3.04 ± 1.99	<b>0.001*</b>	<b>&lt;0.001*</b>	<b>0.048</b>
VAI	2.87 ± 1.97	2.57 ± 1.89	4.30 ± 1.75	2.38 ± 1.66	0.083	<b>0.002*</b>	<b>0.024</b>

Values expressed as means ± standard deviation.

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; C: cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HOMA-IR: homeostatic model assessment; VAI: visceral adiposity index.

Effect size ( $f$ ) = \*0.10–0.24; \*\*0.25–0.39.

Bold values are  $p$  values < 0.05.



**Fig. 1.** Clinical and metabolic variables adjusted by gender and NAFLD condition in Mexican children.

On X axis NAFLD condition is expressed. The values on Y axis express the estimated marginal means.

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; Chol.: cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HOMA-IR: homeostatic model assessment – insulin resistance; VAI: visceral adiposity index.

Female: - - - ; Male: — .

with NAFLD, we found a significant AUC value for ALT, AST, WC, HOMA-IR, triglycerides, and VAI values. Based on these results, and by calculating the Youden index, we obtained the cut-off points for these variables in males and females. In females, the optimal cut-off points were as follows: ALT, 28.5 U/L (sensitivity 100%, specificity 89.2%); AST, 27.5 U/L (sensitivity 90.90%, specificity 62.20%); WC, 79.5 cm (sensitivity 90.90%, specificity 64.90%); HOMA-IR, 2.08 (sensitivity 100%, specificity 44.6%); triglycerides, 128 mg/dL

(sensitivity 81.8%, specificity 69%); and VAI score, 2.33 (sensitivity 100%, specificity 63.5%).

In male patients, the only significant AUC value was found for ALT concentrations, and the cut-off point was 27.5 U/L (sensitivity 82.60%, specificity 80.60%; Fig. 2).

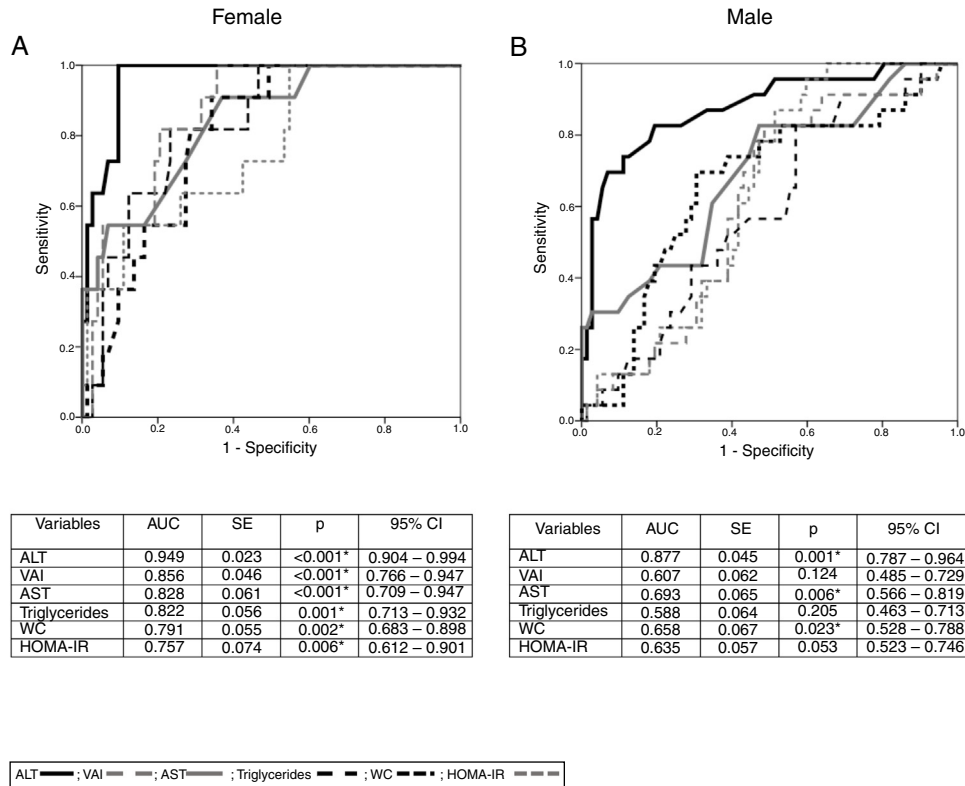
The presence of metabolic syndrome (MS) in females with and without NAFLD was statistically significant, as shown by Fisher's exact test (OR = 9.019; 95%CI = 2.30–35.30;  $p = 0.002$ ). However, in

**Table 2**

Logistic regression predicting likelihood of NAFLD in Mexican children based on BMI, ALT, WC by gender and triglycerides by gender.

Variable	B	SE	p	OR	95%CI	
					Lower bound	Upper bound
BMI (percentile)	0.148	0.094	0.116	1.159	0.964	1.394
ALT (IU/L)	0.100	0.020	<b>&lt;0.001</b>	1.106	1.062	1.151
WC by gender	0.014	0.013	0.281	1.014	0.988	1.041
Triglycerides by gender	−0.008	0.006	0.230	0.992	0.980	1.005

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; WC: waist circumference; SE: standard error; OR: Odds Ratio; CI: confidence interval. Bold values are p values <0.05.

**Fig. 2.** ROC curves for NAFLD by gender and ALT, AST, WC, VAI, HOMA-IR and triglycerides.

(A) Figure and table for female ROC curve. (B) Figure and table for male ROC curve.

NAFLD: non-alcoholic fatty liver disease; ALT: alanine aminotransferase; AST: aspartate aminotransferase; WC: waist circumference; VAI: visceral adiposity index; HOMA-IR: homeostatic model assessment – insulin resistance; AUC: area under curve; SE: standard error; CI: confidence interval; \* $p < 0.05$ .

ALT: — ; VAI: — — ; AST: — — — ; triglycerides: — — — ; WC: — — — — ; HOMA-IR: — — — — .

males with and without NAFLD, there was no significant difference (OR = 1.125; 95%CI = 0.36–3.51;  $p = 0.84$ ).

#### 4. Discussion

Our study results revealed gender-specific differences in metabolic and anthropometric variables related to NAFLD. Moreover, we found evidence that the variables that were positively associated with insulin resistance, such as insulinemia, HOMA-IR, triglyceride concentration, and VAI scores, were also associated with NAFLD in a gender-specific manner. For example, VAI, HOMA-IR, and triglycerides were significantly higher in female patients with NAFLD compared to males. However, in the regression analysis, the only variable that significantly predicted the presence of NAFLD was ALT, which increased the risk of NAFLD by 10% for each unit increment for both genders.

Mexico currently has one of the highest proportions of people with obesity, and consistent with the prevalence of NAFLD, the frequency of NAFLD in our obesity group was 29.46%. The

development of NAFLD requires a complex interaction of multifactorial features such as genetic susceptibility and environmental factors. Ramos-López et al. analyzed the pattern of dietary intake in a Mexican population with and without liver disease, and they found that both have a diet high in fat and cholesterol that lacks polyunsaturated fatty acids and some micronutrients with antioxidant properties that have been associated with the development of liver damage in the general population. The previous statements could explain the relationship between pediatric patients and the development of NAFLD through the influence of genetic variants of risk, a sedentary lifestyle, and the Mexican obesogenic diet [26].

In our study, the frequency of NAFLD by gender was higher in boys compared to girls, with a 2:1 ratio. Previous studies that were included in an epidemiological meta-analysis of NAFLD showed results similar to ours. Although the diagnostic methodologies used in the studies included in this meta-analysis were heterogeneous, the most frequently used method for diagnosing NAFLD was ultrasonography [3,11,27].

NAFLD is associated with various anthropometric and metabolic factors, such as high BMI, ethnic group (with a higher prevalence

in Asian and Hispanic patients), male gender, insulin resistance, and dyslipidemia. BMI has also been related to increased visceral and profound subcutaneous adiposity, which are also considered to be risk factors for NAFLD [28]. In our study, univariate analyses performed by gender and the presence of NAFLD showed that in males, the only variables that were significantly related to NAFLD were ALT and those that indirectly reflect visceral adiposity (BMI and WC). However, in females, NAFLD was also associated with a higher BMI, WC, and ALT, as well as higher insulin, HOMA-IR, and VAI. Although in previous studies the variables involved in NAFLD and insulin resistance are frequently reported, we found that there is a gender-specific association between these variables and the female population [2,6,7].

Our results also showed a significant association between NAFLD and the BMI-Pc, WC, HDL-cholesterol, and ALT concentration, with no differences observed between the genders. This finding is in accordance with that of Lonardo et al. and Singh et al., who reported that BMI, WC, waist-to-height ratio, and waist-to-hip ratio are gender-independent predicting factors for NAFLD in adults [29,30].

ROC curve discrimination analysis by gender showed a significant association between NAFLD and AST, ALT, triglycerides, HOMA-IR, insulin, and VAI only in girls. In boys, the only variable that behaved as a possible diagnostic biomarker was ALT, confirming the gender-specific behavior. The means for ALT in both genders from our study are aligned with the cut-off points that are recommended by the SAFETY study and the Council for the Treatment of Child Obesity published by the Endocrine Society [18,31].

In the last two decades, researchers worldwide have described NAFLD as a condition with sexual dimorphism. Given the complex multifactorial pathophysiology of NAFLD, research on the sexual dimorphism inherent to this disease may lead us to a better understanding of the pathophysiological processes that are involved [11,29].

Women tend to have more subcutaneous adipose tissue and higher leptin levels, which, combined with estrogen production, protects them from the accumulation of fatty tissue in the visceral compartment. In contrast, the distribution of adipose tissue in males is primarily visceral, a condition that is associated with insulin resistance and a greater influx of free fatty acids into the portal venous system, thereby promoting NAFLD [10,11,32]. In our study, we did not observe an association between NAFLD and any of the factors related to insulin resistance in boys.

The influence of sex steroids on metabolic disturbances remains an unanswered question because hyperandrogenic women as well as men with androgen deficiency tend to develop abdominal obesity, insulin resistance, type 2 diabetes, and NAFLD, and they have a higher cardiovascular risk [33].

Moreover, women with polycystic ovarian syndrome (PCOS) have an increased prevalence of NAFLD compared to age and BMI-paired controls (OR=2.54; 95%CI=2.19–2.95), and NAFLD-PCOS women have an androgen bioavailability that increases independently of BMI and insulin resistance [34–36]. Conversely, androgen deficiency in men has been related to NAFLD, independent of other known risk factors such as insulin resistance and visceral adiposity [37]. These studies show that female androgen excess and male androgen deficiency have similar metabolic phenotypes, showing the complexity of androgens' role in metabolism.

The variables in our study that were associated with NAFLD in females are consistent with those observed in previous reports, and these include insulin resistance, hypertriglyceridemia, and visceral adiposity (evaluated as VAI and WC in our study). Given that we did not find an association between these variables and NAFLD in males, we believe that it is possible that the pathophysiological mechanisms in this population may differ from those involved in insulin resistance.

For lipid metabolism, studies performed in humans and in animal models have shown an increased ability in females to metabolize free fatty acids by lipolysis. However, males can have prolonged *de novo* lipogenesis, which may condition them for a longer period of free fatty acid exposure and their associated damage. These changes could help us to understand why men are more likely to develop NAFLD [38].

A study performed in the United Kingdom evaluated fatty acid metabolism according to gender, and it showed that the beta-oxidation during fasting in men is less efficient compared to women, and it also has longer lasting *de novo* lipogenesis. Both phenomena may promote esterification and storage of free fatty acids in the liver, and consequently, stimulate the development of NAFLD in the male population [38].

Experimental models have often shown that androgens and estrogens are balanced in the modulation of hepatic lipid metabolism. Zhang et al. suggested that the combined administration of selective androgen and estrogen receptor modulators could be a potential treatment for patients with NAFLD [39]. Considering that there is a fluctuation of estrogens or androgens in teenagers, we attempted to evaluate the effect of these hormones by pubertal stage; however, there were no differences between pubertal and prepubertal patients.

The Metabolic syndrome (MS) is a critical factor that is related to NAFLD, to the extent that NAFLD has been considered by many to be the hepatic manifestation of this syndrome [40,41]. Concerning the association of this disorder with MS, it is relevant to say that in our study, a statistically significant relationship was observed between NAFLD and MS in females but not in males. The frequency of NAFLD in patients with fewer than three criteria for MS in our study was 63.9%. These results are clinically relevant, considering that in children with obesity, the presence of MS is believed to be the most important risk factor for the development of other comorbidities [42,43]. Our results contribute to the evidence that supports considering each component of the MS as a risk factor for NAFLD, particularly hypertriglyceridemia (>110 mg/dL) [44]. Srinivas et al. studied, from a gender perspective, the association of NAFLD with MS component risk factors in an urban cohort, and they found that the components related to this disorder in women were hyperglycemia and hypertriglyceridemia, whereas in men, only BMI was related (used as a criterion for MS instead of WC) [27]. In our study, the only risk component among males was the indirect measure of adiposity (WC), while in females, it was low HDL levels and hypertriglyceridemia.

This study has some limitations. The most relevant limitation is that the diagnosis of NAFLD was not performed using the gold standard (liver biopsy), because of the inherent risks related to the procedure and ethical issues. We evaluated NAFLD using ultrasonography because of accessibility in our population.

According to our study, ALT, AST, WC, triglycerides, HOMA-IR, and VAI were only related to NAFLD in the female population. Logistic regression showed that only ALT was predictive of NAFLD in both genders. The ALT cut-off points for the development of NAFLD in our study were 28.5 U/L (sensitivity 100%, specificity 89.2%) in females and 27.5 U/L (82.60% sensitivity, 80.60% specificity) in males. Although ALT was the only predictor for NAFLD in boys, it does not have the same diagnostic efficiency as it does in females.

## 5. Conclusions

We demonstrated that, although NAFLD is more frequent in boys, only ALT, and no other clinical or metabolic variables, were associated with NAFLD in these patients. HOMA-IR, VAI, triglycerides levels, and ALT were associated with NAFLD only in girls.



The ALT cut-off points for the development of NAFLD in our study were 28.5 U/L in females and 27.5 U/L in males. Our results suggest that we may consider each MS criterion individually as a risk factor for developing NAFLD. Our findings showed that NAFLD should be strongly suspected and that patients with obesity should be intentionally screened for NAFLD, particularly boys.

Further research is required using non-invasive methods and biomarkers that can help us to predict NAFLD in children and teenagers.

#### Abbreviations

NAFLD	non-alcoholic fatty liver disease
HOMA-IR	homeostatic model assessment of insulin resistance
VAI	visceral adiposity index
BMI	body mass index
WC	waist circumference
HDL	high density lipoprotein
ALT	alanine aminotransferase
AST	aspartate aminotransferase
HIPAA	Health Insurance Portability and Accountability Act
LDL	low density lipoprotein
SD	standard deviation
ANOVA	analysis of variance
ROC	receiver operating characteristics
AUC	area under curve
PCOS	polycystic ovarian syndrome
FAI	free androgen index

#### Ethics

Informed consent and assent were obtained from the participants and their parent/legal guardian. All sensitive patient data were protected in compliance with the Health Insurance Portability and Accountability Act (HIPAA).

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#### Author contributions

VOE and GNN conceived the idea and evaluate the patients. VOE, GNN, LAJC and EG contributed to the interpretation of the results.

GHMJ, QG and CCS contribute to patients recruitment and collected the data.

HRA performed the ultrasonographic evaluation.

LAJC and LSE performed the statistical analysis of the data.

All authors discussed the results and contribute to the final manuscript.

#### Conflict of interest

The authors have no conflicts of interest to declare.

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#### References

[1] AIKhter SA. Paediatric non-alcoholic fatty liver disease: an overview. *Obes Rev* 2015;16:393–405.

- [2] Temple JL, Cordero P, Li J, Nguyen V, Oben JA. A guide to non-alcoholic fatty liver disease in childhood and adolescence. *Int J Mol Sci* 2016;17:947.
- [3] Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PLOS ONE* 2015;10:e0140908.
- [4] Lizarde-Cervera J, Laparra DIB, Chavez-Tapia NC, Ostos MER, Esquivel MU. Prevalence of NAFLD and metabolic syndrome in asymptomatic subjects. *Rev Gastroenterol Mex* 2006;71:453–9.
- [5] Flores J, Gómez R, Rodríguez G, Morán S. P0223 prevalence of nonalcoholic steatohepatitis (NASH) in Mexican children of an elementary school. *J Pediatr Gastroenterol Nutr* 2004;39:S143.
- [6] Uppal V, Mansoor S, Furuya KN. Pediatric nonalcoholic fatty liver disease. *Curr Gastroenterol Rep* 2016;18:24.
- [7] Benedict M, Zhang X. Non-alcoholic fatty liver disease: an expanded review. *World J Hepatol* 2017;9:715–32.
- [8] Hernaez R, McLean J, Lazo M, Brancati FL, Hirschhorn JN, Borecki IB, et al. Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol* 2013;11:1183–90.
- [9] Larrieta-Carrasco E, Flores YN, Macías-Kauffer LR, Ramírez-Palacios P, Quiñero M, Ramírez-Salazar EG, et al. Genetic variants in COL13A1, ADIPOQ and SAMM50, in addition to the PNPLA3 gene, confer susceptibility to elevated transaminase levels in an admixed Mexican population. *Exp Mol Pathol* 2018;104:50–8.
- [10] Lee YH, Kim SH, Kim SN, Kwon HJ, Oh JY, Jung YS. Sex-specific metabolic interactions between liver and adipose tissue in MCD diet-induced non-alcoholic fatty liver disease. *Oncotarget* 2016;7:46959–71.
- [11] Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Lonardo A. NAFLD as a sexual dimorphic disease: role of gender and reproductive status in the development and progression of nonalcoholic fatty liver disease and inherent cardiovascular risk. *Adv Ther* 2017;34:1291–326.
- [12] AlShaalan R, Aljiffry M, Al-Busafi S, Metrakos P, Hassanain M. Nonalcoholic fatty liver disease: noninvasive methods of diagnosing hepatic steatosis. *Saudi J Gastroenterol* 2015;21:64–70.
- [13] Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970;45:13–23.
- [14] Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291–303.
- [15] Khosla T, Lowe Cr. Indices of obesity derived from body weight and height. *Br J Prev Soc Med* 1967;21:122–8.
- [16] Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998;21:2191–2.
- [17] Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the expert committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr* 2017;64:319–34.
- [18] Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, et al. Pediatric obesity – assessment, treatment and prevention: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2017;102:709–57.
- [19] Lapadat AM, Jianu IR, Ungureanu BS, Florescu LM, Gheonea DI, Sovaila S, et al. Non-invasive imaging techniques in assessing non-alcoholic fatty liver disease: a current status of available methods. *J Med Life* 2017;10:19–26.
- [20] Angulo P. Nonalcoholic fatty liver disease. *N Eng J Med* 2002;346:1221–31.
- [21] Alin A, Kurt S. Testing non-additivity (interaction) in two-way ANOVA tables with no replication. *Stat Methods Med Res* 2006;15:63–85.
- [22] Selya AS, Rose JS, Dierker LC, Hedeker D, Mermelstein RJ. A practical guide to calculating Cohen's  $f(2)$ , a measure of local effect size, from PROC MIXED. *Front Psychol* 2012;3:111.
- [23] Preisser JS, Das K, Benecha H, Stamm JW. Logistic regression for dichotomized counts. *Stat Methods Med Res* 2016;25:3038–56.
- [24] Kaufmann J, Werner C, Brunner E. Nonparametric methods for analyzing the accuracy of diagnostic tests with multiple readers. *Stat Methods Med Res* 2005;14:129–46.
- [25] Bantis LE, Nakas CT, Reiser B. Construction of confidence regions in the ROC space after the estimation of the optimal Youden index-based cut-off point. *Biometrics* 2014;70:212–23.
- [26] Ramos-López O, Román S, Ojeda-Granados C, Sepúlveda-Villegas M, Martínez-López E, Torres-Valadez R, et al. Patrón de ingesta alimentaria y actividad física en pacientes hepátopatías en el Occidente de México. *Rev Endocrinol Nutr* 2013;21:7–15.
- [27] Srinivas M, Srinivasan V, Mohan MB, Varghese J, Venkataraman J. A study of gender-wise risk association between fatty liver and metabolic syndrome components (Asia-Pacific criteria) in a South Indian urban cohort. *Indian J Gastroenterol* 2015;34:38–42.
- [28] Nazare JA, Smith JD, Borel AL, Haffner SM, Balkau B, Ross R, et al. Ethnic influences on the relations between abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: the International Study of Prediction of Intra-Abdominal Adiposity and Its Relationship With Cardiometabolic Risk/Intra-Abdominal Adiposity. *Am J Clin Nutr* 2012;96:714–26.
- [29] Lonardo A, Trande P. Are there any sex differences in fatty liver? A study of glucose metabolism and body fat distribution. *J Gastroenterol Hepatol* 2000;15:775–82.

- [30] Singh A, Parida S, Narayan J, Nath P, Padhi PK, Meher C, et al. Simple anthropometric indices are useful for predicting non-alcoholic fatty liver disease (NAFLD) in Asian Indians. *J Clin Exp Hepatol* 2017;7:310–5.
- [31] Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010;138:1357–64.
- [32] Ayonrinde OT, Olynyk JK, Beilin LJ, Mori TA, Pennell CE, de Klerk N, et al. Gender-specific differences in adipose distribution and adipocytokines influence adolescent nonalcoholic fatty liver disease. *Hepatology* 2011;53:800–9.
- [33] Schiffer L, Kempegowda P, Arlt W, O'Reilly MW. Mechanisms in endocrinology: the sexually dimorphic role of androgens in human metabolic disease. *Eur J Endocrinol* 2017;177:R125–43.
- [34] Rocha ALL, Faria LC, Guimaraes TCM, Moreira GV, Candido AL, Couto CA, et al. Non-alcoholic fatty liver disease in women with polycystic ovary syndrome: systematic review and meta-analysis. *J Endocrinol Investig* 2017;40:1279–88.
- [35] Condorelli RA, Calogero AE, Di Mauro M, Mongioi' LM, Cannarella R, Rosta G, et al. Androgen excess and metabolic disorders in women with PCOS: beyond the body mass index. *J Endocrinol Investig* 2018;41:383–8.
- [36] Cai J, Wu CH, Zhang Y, Wang YY, Xu WD, Lin TC, et al. High-free androgen index is associated with increased risk of non-alcoholic fatty liver disease in women with polycystic ovary syndrome, independent of obesity and insulin resistance. *Int J Obes (Lond)* 2017;41:1341–7.
- [37] Kim S, Kwon H, Park JH, Cho B, Kim D, Oh SW, et al. *BMC Gastroenterol* 2012;12:69.
- [38] Pramfalk C, Pavlides M, Banerjee R, McNeil CA, Neubauer S, Karpe F, et al. Sex-specific differences in hepatic fat oxidation and synthesis may explain the higher propensity for NAFLD in men. *J Clin Endocrinol Metab* 2015;100:4425–33.
- [39] Zhang H, Liu Y, Wang L, Li Z, Zhang H, Wu J, et al. Differential effects of estrogen/androgen on the prevention of nonalcoholic fatty liver disease in the male rat. *J Lipid Res* 2013;54:345–57.
- [40] Saad V, Wicklow B, Wittmeier K, Hay J, MacIntosh A, Venugopal N, et al. A clinically relevant method to screen for hepatic steatosis in overweight adolescents: a cross sectional study. *BMC Pediatr* 2015;15:151.
- [41] Mikolasevic I, Milic S, Turk Wensveen T, Grgic I, Jakopcic I, Stimac D, et al. Nonalcoholic fatty liver disease – a multisystem disease? *World J Gastroenterol* 2016;22:9488–505.
- [42] Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation* 2008;118:277–83.
- [43] Kelishadi R, Cook SR, Adibi A, Faghihimani Z, Ghatrehsamani S, Beihaghi A, et al. *Diabetol Metab Syndr* 2009;1:29.
- [44] Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988–1994. *Arch Pediatr Adolesc Med* 2003;157:821–7.