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Is Homeostasis Model Assessment for Insulin Resistance >2.5 a distinguished criteria for metabolic dysfunction-associated fatty liver disease identification?

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ABSTRACT – Background – Insulin resistance (IR), assessed by different criteria, is an important factor in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). More recently with the characterization of this metabolic dysfunction-associated fatty liver disease (MAFLD), one of the proposed criteria for this diagnosis has been the determination of the homeostasis model assessment-insulin resistance (HOMA-IR). **Objective** – The purpose of this study was to evaluate the relationship of HOMA-IR>2.5 with clinical, metabolic, biochemical and histological data obtained in non-diabetic patients diagnosed with NAFLD by liver biopsy. **Methods** – Cross-sectional, retrospective study was carried out with data from 174 adult individuals of both genders with non-diabetics NAFLD, without obvious signs of portal hypertension. The body mass index (BMI) was classified according to the World Health Organization (1998), and the metabolic syndrome by the criteria of NCEP-ATP-III. Biochemical tests were evaluated using an automated method and insulinemia through immunofluorometric assay. Histological findings were classified according to Kleiner et al. (2005). **Results** – The mean age of the studied population was 53.6±11.2 years, with 60.3% being female. The average BMI was 30.3 kg/m^2 and 75.9% of the patients had increased waist circumference. Among evaluated metabolic parameters, there was a higher prevalence of metabolic syndrome (MS) in patients with HOMA-IR>2.5, with no statistical difference in relation to BMI between studied groups. Values of liver enzymes and serum ferritin were significantly higher in patients with this marker of IR, who had a higher prevalence of non-alcoholic steatohepatitis (NASH) and advanced liver fibrosis. In the multivariate analysis, the clinical diagnosis of MS, hyperferritinemia and the presence of NASH in the liver biopsy were the factors independently associated with the presence of altered HOMA-IR. **Conclusion** – HOMA-IR values >2.5 identify patients with NAFLD with distinct clinical and metabolic characteristics and with a greater potential for disease progression, which validates this parameter in the identification of patients with MAFLD.

Keywords – Non-alcoholic fatty liver disease; metabolic dysfunction-associated fatty liver disease; non-alcoholic steatohepatitis; insulin resistance; metabolic syndrome.

INTRODUCTION

Recently, it was proposed to modify the concept and the denomination of NAFLD for metabolic dysfunction-associated fatty liver disease (MAFLD), in which metabolic parameters are used for this liver disease diagnosis, in contrast to the previous situation, in which the diagnosis was made by excluding other etiologies[1,2]. These changes are important, as we are dealing with a growing health problem, that is considered nowadays as the main cause of chronic liver disease in the world[3].

The necessary criteria for the MAFLD diagnosis are those considered to be risk factors and which may influence the disease's natural history, such as obesity/overweight, changes in blood glucose and metabolic syndrome (MS) parameters frequently found in these patients[4,5]. Excess weight has been associated with a higher prevalence of NAFLD and progression of liver fibrosis, alone or when associated with changes in glycemic levels, an association that also increases the risk of developing hepatocellular carcinoma (HCC)[6-8].

Regardless of obesity, changes in blood glucose and the presence of type 2 diabetes mellitus (T2DM) are important in relation to the disease's severity, as they increase the risk of progressing to cirrhosis and the appearance of HCC[7-9]. On the other hand, it was shown in a cohort study with 19 years of follow-up that mortality from all causes was 24 times higher in patients with NAFLD with components of MS than individuals with NAFLD without the presence of MS[10].

In addition to these criteria, IR, calculated from the homeostatic model, with HOMA-IR >2.5 was introduced as a risk factor for MAFLD in thin patients or those with normal body mass index

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(BMI), when it is associated with another risk factor, such as one of the criteria of MS or diagnosis of pre-diabetes or even with high plasma high-sensitivity C-reactive protein level[1,2].

In this study, we aimed to assess the importance of a cutoff value of HOMA-IR >2.5 in the differentiation of non-diabetic patients with histological diagnosis of NAFLD in relation to clinical, biochemical and histological parameters, in order to validate the importance of such cutoff value in the characterization of the MAFLD and its severity.

METHODS

Retrospective study, with data collection from medical records of individuals with NAFLD treated at the outpatient clinic of liver disease of the Gastroenterology Section of Universidade Federal de São Paulo (UNIFESP) in the period between 2014 to 2019. The sample consisted of patients of both genders with NAFLD confirmed by histological analysis of liver tissue.

Exclusion criteria were considered: patients with a previous or current diagnosis of T2DM; history of alcohol ingestion >140 g per week for men and >70 g per week for women; debilitated patients with impaired nutritional status or the presence of any type of neoplasia including hepatocellular carcinoma. Those individuals with positive serological markers for hepatitis B or C virus or other recognized etiologies in liver biopsy, using insulin-sensitizing or iron replacement drugs or hepatotoxic drugs and patients with portal hypertension detected by clinical, radiological and/or endoscopic criteria were also excluded[11].

The following demographic and clinical variables were assessed in this study: age, gender, presence of metabolic comorbid conditions, like altered blood glucose (glucose >99 mg/dL and <125 mg/dL), systemic arterial hypertension, dyslipidemia and MS according to ATP III criteria[12]. Weight and height were evaluated to determine the BMI obtained using the formula [BMI = weight (kg) / height (m^2)] using the World Health Organization classification to assess nutritional status according to BMI[13]. Waist circumference (WC) was measured with the patient standing, between the last rib and the iliac crest, at its smallest perimeter and at the end of a normal expiration[14].

The serum activity of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and serum levels of total cholesterol (TC), HDL-cholesterol, triglycerides (TG), LDL-cholesterol, and blood glucose were obtained using an automated colorimetric method in Cobas Mira, Roche, Switzerland. Serum ferritin was determined by chemiluminescence, with upper normal limits of up to 400 ng/mL for men and 150 ng/mL for women. The insulin values were determined by an immunofluorometric assay (Perkin Elmer BR-CS), which enabled the determination of HOMA-IR index, calculated using the formula: HOMA-IR = [glucose (nmol/L) * insulin (μU/mL) / 22.5] and the presence of IR was characterized by HOMA-IR >2.5[15].

Histological evaluation of liver tissue fragments obtained by percutaneous biopsy was performed according to the criteria of Kleiner et al.[16].

In the statistical analysis, the categorical variables were described as absolute and relative frequencies. The numerical variables were described by mean and standard deviation. The other non-normal continuous variables were described by quartiles. For univariate analysis, comparing groups with and without IR, we used the chi-square test for categorical variables and the Student's "*t*" test for independent samples or Mann-Whitney U test for normal or non-normal continuous variables, respectively. For the identification of variables independently associated with HOMA-IR >2.5, a logistic regression model was designed. The correlation analysis between histological, clinical and biochemical parameters was performed according to Pearson's relationship coefficient (with normal distribution) or Spearman's (without normal distribution). All tests were two-tailed and the results with $P<0.05$ were considered significant. All analyzes were performed using SPSS software (IBM SPSS Statistics for Windows, version 24.0. Armonk, NY: IBM Corp.) This study was approved by the Research Ethics Committee from UNIFESP, under decision number 1.662.400.

RESULTS

The calculation of sample size aimed the obtainment of a 60% prevalence of NAFLD cases with HOMA-IR >2.5, with a sampling error of 7% and a 95% confidence interval, according to previous published data[4]. From 202 selected consecutive medical records, 28 ended up being excluded because they did not present values of biochemical tests studied at the time of biopsy that were diagnosed with diabetes mellitus or with detectable portal hypertension. Thus, 174 patients remained, with a mean age of 53.6±11.2 years old, 39.7% male, with an average BMI of 30.3 kg/m^2. In this same table it is observed that, except for the presence of MS, significantly more prevalent among patients with IR, none of the other demographic and nutritional parameters studied was able to differentiate the group of patients with and without IR, measured by HOMA-IR. In the analysis of biochemical data, in addition to fasting insulin values and, obviously, HOMA-IR, there were also significant differences between groups in the values of enzymes AST, ALT and GGT and ferritin in absolute and categorized values (TABLE 1).

As to morphological variables observed in liver histology, there was a higher frequency of ballooning, steatohepatitis and advanced fibrosis in patients with elevated HOMA-IR (TABLE 2).

The logistic regression analysis of factors that were significantly associated in the univariate analysis shows that the presence of IR, characterized from the values of HOMA-IR >2.5, was independently associated with elevated serum ferritin levels, presence of NASH in liver biopsy and MS assessed by ATP-III criteria (TABLE 3).

DISCUSSION

IR plays a central role in the installation of steatosis and appears to be important in the progression of NAFLD to more advanced forms of the disease, which makes it an important pathophysiological mechanism of NAFLD[17-19]. Although IR is defined based on more complex tests such as a hyperinsulinemic-euglycemic clamp, its evaluation by HOMA-IR has been more used, due to the simplicity of its determination and good correlation with glycemic clamp in non-diabetic patients[20,21], in addition, the intrasubject coefficients of variation of current methods are around 7.7% to 10.3%[22]. Another point to be addressed is the cutoff value of HOMA-IR used in the definition of IR. Although the value used here was proposed by a group of experts from the European Association for the Study of the Liver for the characterization of MAFLD[1,2], it must be remembered that there is a huge variation

TABLE 1. Distribution of demographic, nutritional, metabolic and laboratory variables in the sample, according to the presence (HOMA-IR >2.5) or absence of insulin resistance (HOMA-IR <2.5).

Variable	Total (n=174)	HOMA IR <2.5 (n=61)	HOMA-IR ≥2.5 (n=113)	*P*-value
Age (mean ± SD)	53.6±11.2	54.0±11.1	53.3±11.3	0.719
Male	69 (39.7)	22 (36.1)	47 (41.6)	0.477
BMI (Mean ± SD)	30.3±4.5	29.8±4.6	30.6±4.5	0.273
BMI ≥25	153 (87.9)	51 (83.6)	102 (90.3)	0.198
Increased WC	132 (75.9)	43 (70.5)	89 (78.8)	0.224
Arterial hypertension	119 (68.4)	44 (72.1)	75 (66.4)	0.436
Metabolic syndrome	133 (76.4)	40 (65.6)	93 (82.3)	0.013
Hypercholesterolemia	69 (39.7)	23 (37.7)	46 (40.7)	0.699
High LDL-c	66 (37.9)	21 (34.4)	45 (39.8)	0.484
Low HDL-c	89 (51.1)	27 (44.3)	62 (54.9)	0.182
Hypertriglyceridemia	81 (46.6)	26 (42.6)	55 (48.7)	0.445
Hyperglycemia	90 (51.7)	26 (42.6)	64 (56.6)	0.078
AST	29 (23–42)	28 (21–36)	33 (24–44)	0.008
ALT	38 (27–61)	35 (24–46)	42 (28–74)	0.023
GGT	51 (32–92)	40 (24–86)	57 (36–102)	0.009
Ferritin	195 (109–393)	137 (78–326)	264 (119–404)	0.010
Elevated ferritin	74 (42.5)	17 (27.9)	57 (50.4)	0.004
Basal insulin	13 (7–20)	6 (4–8)	17 (14–24)	<0.001
HOMA-IR	3.3 (1.8–5.0)	1.5 (1.0–1.9)	4.3 (3.4–6.3)	<0.001

Data presented as n (%), unless specified. Values corrected for gender according to ATP-III. SD: standard deviation; BMI: body mass index. WC: waist circumference. LDL: low-density lipoprotein; HDL: high-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HOMA-IR: homeostasis model assessment-insulin resistance.

TABLE 2. Characterization of studied groups according to the presence (HOMA-IR ≥2.5) or absence of insulin resistance (HOMA-IR <2.5) and histological variables obtained through percutaneous liver biopsy and classified according to Kleiner et al.[16].

Variables	Total (174)	HOMA-IR <2.5	HOMA-IR >2.5	*P*-value
		No (61)	Yes (113)	
Steatosis (0/1/2/3)		2/45/48/5	0/39/53/8	0.457
Inflammation (0/1/2/3)		15/40/37/8	13/35/45/7	0.990
Ballooning (0/1/2)		34/35/31	19/38/53	0.008
NASH	127 (73.0)	35 (57.4)	92 (81.4)	0.001
Fibrosis (any grade)	100 (57.5)	24 (39.3)	76 (67.3)	<0.001
Degree of fibrosis				
0	74 (42.5)	37 (60.7)	37 (32.7)	0.006
1	46 (26.4)	13 (21.3)	33 (29.2)	
2	26 (14.9)	7 (11.5)	19 (16.8)	
3	19 (10.9)	2 (3.3)	17 (15.0)	
4	9 (5.2)	2 (3.3)	7 (6.2)	
Advanced fibrosis (grades 3–4)	28 (16.1)	4 (6.6)	24 (21.2)	0.012
Siderosis	32 (18.4)	7 (11.5)	25 (22.1)	0.091

HOMA-IR: homeostasis model assessment-insulin resistance; NASH: non-alcoholic steatohepatitis.

TABLE 3. Multivariate analysis by logistic regression to identify factors independently associated with insulin resistance (HOMA-IR ≥2.5) in studied patients.

Variables	OR	95%CI	*P*-value
Elevated ferritin (yes/no)	2.401	1.238–4.657	0.010
NASH (present/absent)	2.633	1.313–5.279	0.006
GGT (each unit)	1.004	1.000–1.008	0.076
Metabolic syndrome (present/absent)	2.221	1.048–4.704	0.037
ALT (each unit)	1.002	0.994–1.011	0.591
Advanced fibrosis (present/absent)	1.866	0.739–4.708	0.187

OR: odds ratio; HOMA-IR: homeostasis model assessment-insulin resistance; NASH: non-alcoholic steatohepatitis; GGT: gamma glutamyl transferase; ALT: alanine aminotransferase. N=174.

in the dependence of the studied population and employed methodology[23]. Although not unanimous[24], HOMA-IR with a cutoff value of 2.5 has been used in the diagnosis of NAFLD in several previous studies[25-27], in addition, this cutoff coincides with or is close to those observed in Brazil in patients with NAFLD and in the characterization of IR in the general population[18,19].

Patients with T2DM were excluded from the evaluation because it is already a MAFLD criteria and because different values can be obtained, especially in advanced diabetics due to the interference of functional exhaustion of beta cells[24,28]. Cirrhotic patients with portal hypertension, in turn, present hyperinsulinemia due to pancreatic hypersecretion, decreased hepatic insulin degradation and increased glucagon levels and, therefore, were also excluded from the sample[29,30]. Although IR is considered almost universal in NAFLD, about one-third of Brazilian NAFLD patients have HOMA-IR values below the established cutoff[4,18]. Thus, we decided to study the value of HOMA-IR in our patients diagnosed with NAFLD confirmed by biopsy and its relationship with clinical, metabolic and histological parameters.

From the definitions and criteria, we selected a population mainly composed of women, with an average age of 55 years and an average BMI of 30.5 kg/m^2. MS and visceral obesity were observed in 80% of cases, and more than three-quarters of patients had NASH and some degree of fibrosis in just over half of the patients. These histological data are comparable to those obtained in the Brazilian multi-center study[4] carried out in reference services, which found almost 60% of NASH and liver fibrosis in 46% of these patients. These data certainly reflect the bias in the referral of patients to the disease's reference centers and in the selection of patients for liver biopsy[31].

In this study, when patients were separated according to the presence or absence of IR, the fact that there are no differences in BMI is noteworthy, since in the general population there is a relationship between BMI and HOMA-IR[25], and in NAFLD patients between the degree of steatosis and HOMA-IR[24,25,30]. However, in these NAFLD patients BMI seemed not to be directly associated with IR; is relation had been challenged, in contrast to the significant correlation. Corroborating these data, in a study with 138 morbidly obese individuals, only 34% of them had high HOMA-IR values, with no correlation between this parameter and the obesity degree[32]. Also, studies in thin patients with NAFLD demonstrate that these patients have a lower frequency of MS, but show higher levels of HOMA-IR and plasma TG when compared with obese patients with NAFLD[30].

The mean values of liver enzyme activity were similar to those described in other studies. Elevated levels of GGT activity have been linked to the presence of IR in several studies in NAFLD, associating these changes with excessive deposition of visceral fat, steatosis and fibrosis[33,34].

Serum hyperferritinemia is frequently seen in patients with NAFLD. In our study, it was possible to verify the presence of hyperferritinemia in 50% of individuals with IR compared to only 28% in NAFLD patients with HOMA-IR <2.5 and this parameter remained present as a factor independently related to the presence of IR. Such a result is consistent with findings of Brudevold et al.[35] that in patients without iron overload, hyperferritinemia was associated with the presence of steatosis and NAFLD. The association between hyperferritinemia and IR was observed by several other authors, having been considered a marker of oxidative stress for NAFLD and related to the presence of IR, glucose intolerance and progression of liver disease[31,35,36]. In addition, elevated serum ferritin has been associated with increased histological activity, being an independent predictor for advanced fibrosis in patients with NAFLD[37]. Considering the presence of siderosis as any degree of iron deposition detected in the biopsy, regardless of its location, we observed a significant correlation between ferritinemia and siderosis (rS=0.420, P<0.001, data not shown), but it should be noted that only 18% of patients had detectable iron in liver histology and there was no relationship between IR and iron deposition in liver tissue.

As expected, our study showed the well-known relationship between MS and IR. Studies confirm the role of IR in the pathophysiology of MS[38,39]. IR is increasingly recognized as a key factor linking MS and NAFLD, being associated with increased circulating levels of free fatty acids (FFA) and excessive accumulation of FFA in liver tissue[40]. The contribution of MS to NAFLD involves different factors such as IR, central obesity, inflammation and oxidative stress[41]. Although most individuals with NAFLD have IR, only a portion of those with NAFLD exhibit complete MS.

The observed relationship between IR and histological signs of progressive liver disease (NASH and fibrosis) was expected since IR is considered a disease progression factor. Experimental data have highlighted high levels of glucose and insulinemia would stimulate the release of connective tissue growth factor and that hyperinsulinemia could directly induce oxidative stress and stimulate the proliferation of hepatic stellate cells, resulting in the progression of fibrosis[42,43].

The inclusion of cirrhotic patients can be discussed as a confounding factor in this study, since, regardless of the etiology, there is a high prevalence of IR in cirrhosis when compared to control subjects or with less advanced fibrosis. It is known that the lower glucose uptake in the splanchnic region and, especially, in the skeletal muscle is associated with IR in these patients. In addition, reduced liver function, or port-systemic shunts, could contribute to increasing insulinemia and stimulating fibrogenesis[44,45]. In addition to the small participation of these patients in the sample (only 5%), the exclusion of patients with clinically detectable portal hypertension, as previously mentioned, would minimize the impact of the presence of these cirrhotic patients in the analysis.

The analyzed histological parameter that was independently associated with IR was steatohepatitis. Indeed, IR tends to be more frequent in patients with NASH[18,26,46,47] and in a study with 212 patients with NAFLD with elevated enzymes, it was observed that disease progression was associated with IR and more pronounced steatosis and weight gain above 5 kg during follow-up[48]. In another study in individuals with severe obesity (BMI ≥40 kg/m^2) with NAFLD it was observed that HOMA-IR was an independent factor in predicting NASH in this population[23]. Besides interrelated in NAFLD, individually, the three factors that were related to the presence of IR are also factors associated with the progression of NAFLD, as observed in meta-analyzes and logistic regression studies[49,50].

CONCLUSION

Thus HOMA-IR values >2.5 identify patients with NAFLD with distinct clinical and metabolic characteristics and with a greater potential for progression of liver disease, which validates its use in identifying patients with MAFLD.

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Authors' contribution

Barreto BFM: conceptualization; data curation; funding acquisition; methodology; project administration; writing original draft; writing review and editing. Punaro GR: writing, review and editing. Elias MC: data curation; formal analysis; investigation; methodology; writing review and editing. Parise ER: conceptualization; data curation; formal analysis; funding acquisition; supervision; writing review and editing.

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Barreto BFM, Punaro GR, Elias MC, Parise ER. O modelo de avaliação da homeostase para resistência à insulina >2,5 é um critério distinto para a identificação da doença hepática gordurosa associada à disfunção metabólica? Arq Gastroenterol. 2022;59(3):402-7.

RESUMO – Contexto – A resistência à insulina (RI), avaliada por diferentes critérios, é um fator importante na patogênese da doença hepática gordurosa não alcoólica (DHGNA). Mas, recentemente, com a caracterização desta disfunção metabólica associada com a doença hepática gordurosa (DGH), um dos critérios propostos para este diagnóstico tem sido a determinação do modelo de avaliação da homeostase-resistência à insulina (HOMA-IR). **Objetivo –** O objetivo deste estudo foi avaliar a relação do HOMA-IR> 2,5 com dados clínicos, metabólicos, bioquímicos e histológicos obtidos em pacientes não diabéticos diagnosticados com DHGNA por biópsia hepática. **Métodos** – Estudo transversal, retrospectivo, com dados de 174 indivíduos adultos de ambos os sexos com DHGNA não-diabética, sem sinais evidentes de hipertensão portal. O índice de massa corporal (IMC) foi classificado de acordo com a Organização Mundial da Saúde (1998) e a síndrome metabólica pelos critérios do NCEP-ATP-III. Os exames bioquímicos foram avaliados pelo método automatizado e a insulinemia por imunofluorometria. Os achados histológicos foram classificados de acordo com Kleiner et al. (2005). **Resultados –** A média de idade da população estudada foi de 53,6±11,2 anos, sendo 60,3% do sexo feminino. O IMC médio foi de 30,3 kg/m^2 e 75,9% dos pacientes apresentaram circunferência da cintura aumentada. Entre os parâmetros metabólicos avaliados, houve maior prevalência de síndrome metabólica (SM) em pacientes com HOMA-IR >2,5, sem diferença estatística em relação ao IMC entre os grupos estudados. Os valores das enzimas hepáticas e da ferritina sérica foram significativamente maiores nos pacientes com este marcador de RI, que apresentaram maior prevalência de esteato-hepatite não alcoólica (EHNA) e fibrose hepática avançada. Na análise multivariada, o diagnóstico clínico de SM, hiperferritinemia e a presença de EHNA na biópsia hepática foram os fatores independentemente associados à presença de HOMA-IR alterado. **Conclusão –** Valores de HOMA-IR >2,5 identificam pacientes com DHGNA com características clínicas e metabólicas distintas e com maior potencial de progressão da doença, o que valida esse parâmetro na identificação de pacientes com DHG.

Palavras-chave – Doença hepática gordurosa não alcoólica; doença hepática gordurosa associada à disfunção metabólica; esteatohepatite não alcoólica; resistência à insulina; síndrome metabólica.

REFERENCES

1. Eslam M, Sanyal AJ, George J, International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology. 2020;158:1999-2014. doi: 10.1053/j.gastro.2019.11.312
2. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, George J, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73:202-9. doi: 10.1016/j.jhep.2020.03.039
3. Younossi ZM, Stepanova M, Younossi Y, Golabi P, Mishra A, Rafiq N, et al. Epidemiology of chronic liver diseases in the USA in the past three decades. 2020;69:564-8. doi: 10.1136/gutjnl-2019-318813
4. Cotrim H, Parise E, Oliveira C, Leite N, Martinelli A, Galizzi J, et al. Nonalcoholic fatty liver disease in Brazil: clinical and histological profile. Ann Hepatol. 2011;10:33-7.
5. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73-84. doi: 10.1002/hep.28431
6. Wong VW-S, Wong GL-H, Choi PC-L, Chan AW-H, Li MK-P; Chan H-Y, et al. Disease progression of non-alcoholic fatty liver disease: A prospective study with paired liver biopsies at 3 years. Gut 2010,59:969- 974. doi: 10.1136/gut.2009.205088
7. Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol. 2013;59:550-6. doi: 10.1016/j.jhep.2013.04.027
8. Marengo A, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. Annu Rev Med. 2016;67:103-17. doi: 10.1146/annurev-med-090514-013832
9. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. Natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology. 2005;129:113-21. doi: 10.1053/j.gastro.2005.04.014
10. Golabi P., Otgonsuren M., Avila D.L., Sayiner M., Rafiq N., Younossi ZM. Components of metabolic syndrome increase the risk of mortality in nonalcoholic fatty liver disease (NAFLD). Medicine. 2018;97(13):e0214. doi: 10.1097/MD.0000000000010214
11. Turco L, Garcia-Tsao G. Portal Hypertension: Pathogenesis and Diagnosis. Clin Liver Dis. 2019;23:573-87. doi: 10.1016/j.cld.2019.07.007
12. The Third Report of the National Cholesterol Education Program (NECP). Expert Panel on Detection. Evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001;285:2486-97. doi: 10.1001/jama.285.19.2486
13. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation, Geneva, 3-5 Jun 1997. Geneva: World Health Organization, 1998.

14. Wang J, Thornton JC, Bari S, Williamson B, Gallagher D, Heymsfield SB, et al. Comparisons of waist circumferences measured at 4 sites. Am J Clin Nutr. 2003;77:379-84. Doi: 10.1093/ajcn/77.2.379
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-19. doi: 10.1007/ BF00280883
16. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313-21. doi: 10.1002/hep.20701
17. Khan RS, Bril F, Cusi K, Newsome PN. Modulation of Insulin Resistance in Nonalcoholic Fatty Liver Disease. Hepatology. 2019,70:711-24. doi: 10.1002/hep.30429
18. Salgado AL, Carvalho L, Oliveira AC, Santos VN, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arq Gastroenterol. 2010;47:165-9. doi: 10.1590/S0004- 28032010000200009
19. Aller R, Luis DA, Fernandez L, Calle F, Velayos B, Olcoz JL, et al. Influence of insulin resistance and adipokines in the grade of steatosis of nonalcoholic fatty liver disease. Dig Dis Sci. 2007,53:1088-92. doi: 10.1007/s10620-007- 9981-3
20. Lansang MC, Williams GH, Carroll JS. Correlation between the glucose clamp technique and the homeostasis model assessment in hypertension. Am J Hypertens. 2001;14:51-3. doi: 10.1016/S0895-7061(00)01229-2
21. Geloneze B, Repetto EM, Geloneze SR, Tambascia MA, Ermetice MN. The threshold value for insulin resistance (HOMA-IR) in an admixtured population IR in the Brazilian Metabolic Syndrome Study. Diabetes Res Clin Prac. 2006;72:219-20. doi: 10.1016/j.diabres.2005.10.017
22. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27:1487-95. doi: 10.2337/diacare.27.6.1487
23. Seki Y, Kakizaki S, Horiguchi N, Hashizume H, Tojima H, Yamazaki Y, et al. Prevalence of nonalcoholic steatohepatitis in Japanese patients with morbid obesity undergoing bariatric surgery. J Gastroenterol. 2016;51:281-9. doi: 10.1007/s00535-015-1114-8
24. Isokuortti E, Zhou Y, Peltonen M, Bugianesi E, Clement K, Bonnefont- Rousselot D, et al. Use of HOMA-IR to diagnose non-alcoholic fatty liver disease: a population-based and inter-laboratory study. Diabetologia. 2017;60:1873-82. doi: 10.1007/s00125-017-4340-1
25. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med. 1999;107:450-5. doi: 10.1016/S0002-9343(99)00271-5
26. Suresh S, Rajanbabu B, Veetil VM, Hussain A, Veetil JN. A study on the altered glycemic and lipid parameters and prevalence of insulin resistance in nonalcoholic fatty liver disease. J Family Med Prim Care. 2018;7:93-7. doi: 10.4103/jfmpc.jfmpc_352_16
27. Ju DY, Choe YG, Cho YK, Shin DS, Yoo SH, Yim SH, et al. The influence of waist circumference on insulin resistance and nonalcoholic fatty liver disease in apparently healthy Korean adults. Clin Mol Hepatol. 2013;19:140-7. doi: 10.3350/cmh.2013.19.2.140
28. Kahn SE. Beta cell failure: causes and consequences. Int J Clin Pract. 2001;123:13-8.
29. Marchesini G, Pacini G, Bianchi GP, Patrono D, Cobelli C. Glucose disposal, b-cell secretion, and hepatic insulin extraction in cirrhosis. a minimal model assessment. Gastroenterology. 1990;99:1715-22. doi: 10.1016/0016-5085(90)90478-J
30. Gonzalez-Cantero J, Martin-Rodriguez JL, Gonzalez-Cantero A, Arrebola JP, Gonzalez-Calvin JL. Insulin resistance in lean and overweight non- diabetic Caucasian adults: Study of its relationship with liver triglyceride content, waist circumference and BMI. PLoS One. 2018;13:e0192663. doi: 10.1371/journal.pone.0192663
31. Manousou P, Kalambokis G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, et al. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. Liver Int. 2011;31:730-9. doi:10.1111/j.1478-3231.2011.02488.x
32. Perugini RA, Quarfordt SH, Baker S, Czerniach DR, Litwin DEM, Kelly JJ. Metabolic characterization of nondiabetic severely obese patients undergoing roux-em-Y gastric bypass: preoperative classification predicts the effects of gastric bypass on insulin-glucose homeostasis. J Gastrointest Surg. 2007;11:1083-90. doi: 10.1007/s11605- 007-0158-3
33. Hossain IA, Rahman Shah MM, Rahman MK, Ali L. Gamma glutamyl transferase is an independent determinant for the association of insulin resistance with nonalcoholic fatty liver disease in Bangladeshi adults. Diabetes Metab Syndr. 2016;10:S25-9. doi: 10.1016/j.dsx.2015.09.005
34. Lee MY, Koh SB, Koh JH, Nam SM, Shin JY, Shin YG, et al. Relationship between gamma-glutamyltransferase and metabolic syndrome in a Korean population. Diab Med. 2008;25:469-75. doi: 10.1111/j.1464-5491.2008.02415.x
35. Brudevold R, Hole T, Hammerstrøm J. Hyperferritinemia is associated with insulin resistance and fatty liver in patients without iron overload. PLoS One. 2008;3:e3547. doi: 10.1371/journal.pone.0003547
36. Zimmermann A, Zimmermann T, Schattenberg J, Pöttgen S, Lotz J, Rossmann H, et al. Alterations in lipid, carbohydrate and iron metabolism in patients with non-alcoholic steatohepatitis (NASH) and metabolic syndrome. Eur J Intern Med. 2011;22:305-10. doi: 10.1016/j.ejim.2011.01.011
37. Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology. 2012;55:77-85. doi: 10.1002/hep.24706
38. Reaven GM. Banting lecture 1988: role of insulin resistance in human disease. Diabetes. 1988;37:1595-607. doi: 10.2337/diabetes.37.12.1595
39. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365:1415-28. doi: 10.1016/S0140-6736(05)66378-7
40. Donnelly KI, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted with lipoproteins inpatients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343-51. doi: 10.1172/JCI23621
41. Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link? Mol Cell Endocrinol. 2015;418:55-65. doi: 10.1016/j.mce.2015.02.018
42. Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, et al. High glucose and hyperinsulinemia stimulate connective tissue. growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001;34:738-44. doi: 10.1053/jhep.2001.28055
43. Choudhury J, Sanyal AJ. Insulin resistance and the pathogenesis of nonalcoholic fatty liver disease. Clin Liver Dis. 2004;8:575-94. doi: 10.1016/j.cld.2004.04.006
44. Imano E, Kanda T, Nakatani Y, Motomura M, Arai K, Matsuhisa M, et al. Impaired splanchnic and peripheral glucose uptake in liver cirrhosis. J Hepatol. 1999;31:469-73. doi: 10.1016/S0168- 8278(99)80039-7
45. Kawaguchi T, Taniguchi E, Itou M, Sakata M, Sumie S, Sata M. Insulin resistance and chronic liver disease. World J Hepatol. 2011;3:99-107. doi: 10.4254/wjh.v3.i5.99
46. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA. 2015;313:2263-73. doi: 10.4254/wjh.v3.i5.99
47. Bugianesi E, Moscatiello S, Ciaravella MF, Marchesini G. Insulin resistance in nonalcoholic fatty liver disease. Curr Pharm Des. 2010;16:1941-51. doi: 10.2174/138161210791208875
48. Ekstedt M, Franzen LE, Mathiesen UL, Orelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006;44:865-73 doi: 10.1002/hep.21327
49. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43:617-49. doi: 10.3109/07853890.2010.518623
50. Barros RK, Cotrim HP, Daltro CH, Oliveira YA. Hyperferritinemia in patients with nonalcoholic fatty liver disease. Rev Assoc Med Bras. 2017:63;284-9. doi: 10.1590/1806-9282.63.03.284.

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