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PNPLA3 gene polymorphism and red meat consumption increased fibrosis risk in NASH biopsy-proven patients under medical follow-up in a Tertiary Center in Southwest Brazil

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HIGLIGHTS

- An increase in NAFLD in populations with higher consumption of red meat, processed and cooked at high temperatures has been observed.
- SNPs in genes are potentially involved in oxidative stress, lipogenesis de novo, and IR have an important role in the development and progression of NAFLD.
- PNPLA3 gene polymorphism has been implicated in susceptibility to NAFLD and liver fibrosis.
- The present study shows that high red meat intake and PNPLA3 gene polymorphism seem to synergistically affect NAFLD and liver fibrosis.

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ABSTRACT – Background – Recent studies show an increase in nonalcoholic fatty liver disease (NAFLD) in populations with higher consumption of red meat, processed and cooked at high temperatures. On the other hand, the single nucleotide polymorphism rs738409 in the *Patatin-like phospholipase domain containing 3* (*PNPLA3*) gene has been implicated in susceptibility to NAFLD and liver fibrosis. However, the synergistic effect between red meat consumption and the *PNPLA3* gene polymorphism in NAFLD has not yet been evaluated. **Objective –** To evaluate the association between the presence of the polymorphism in the *PNPLA3* gene and the consumption of macronutrients, including meat consumption and its cooking method among NAFLD patients. **Methods –** This was a cross-sectional study with 91 patients diagnosed with NAFLD by liver biopsy with genotyping for the polymorphism in the *PNPLA3* gene were included. The consumption of calories and macronutrients was verified using the semi-quantitative food frequency questionnaire and the specific questionnaire on meat consumption. *PNPLA3* gene polymorphism was analyzed by real-time polymerase chain reaction (RT-PCR) and anthropometric evaluation was realized. **Results –** The mean BMI was 32.38±4.58 kg/m² and the waist circumference was 107±10 cm. On liver biopsy, 42% of patients had significant fibrosis (F≥2). The odds ratio of F≥2 was 2.12 for the GG group and 1.54 for the CG group, compared to the CC group. The mean caloric intake was 1170±463.20 kcal/d. The odds ratio in the CC group concerning high red meat consumption in comparison to low consumption was 1.33. For white meat, the odds ratio was 0.8 when comparing high and low intake, also in the CC group. **Conclusion –** High red meat intake and *PNPLA3* gene polymorphism seem to synergistically affect NAFLD and liver fibrosis, requiring confirmation in a larger number of patients and in different populations.

Keywords – Non-alcoholic fatty liver disease; single nucleotide polymorphism; *Patatin-like phospholipase domain-containing 3*; diet; red meat.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is considered the most common chronic liver disease and affects about 25% of the world's population. The number of cases of NAFLD has been increasing over the years due to the spread of metabolic risk factors such as obesity, sedentary lifestyle, type II diabetes mellitus (T2DM), and dyslipidemia[1,2]. NAFLD encompasses a large spectrum of the disease since simple steatosis, steatohepatitis (NASH), and a risk of progression to cirrhosis or even hepatocellular carcinoma (HCC)[3]. Recently, the term Metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed as a more appropriate term to describe liver disease associated with known metabolic dysfunctions[4].

For NAFLD or MAFLD individuals, the dietary recommendations are daily energy calorie restriction and a restriction of foods with harmful components such as processed foods, fructose-rich beverages, high intake of saturated fat, trans fat, and simple carbohydrates[5].

Recently, Zelber-Sagi et al. observed an association between the consumption of red and processed meat and meat cooked at high temperatures for a long time with insulin resistance (IR)[6]. They also demonstrated a higher prevalence of IR in individuals with a high intake of, heterocyclic aromatic amines (AHAs), considered to be one of the initiators of carcinogenic processes, are found on the surface of charred meat AHAs in meats that have undergone prolonged, cooking at high temperatures (>150 °C) or in meats that have been exposed directly to the flames of a fire. It is assumed that these effects are found more in red meats due to their high concentration of heme iron[7,8].

Although meat is an important source of protein, iron, zinc, vitamin B12, and other nutrients, mainly red meat has saturated fat, cholesterol, nitrate, and nitrite that are associated with IR, abdominal obesity, Metabolic Syndrome (MtS), and oxidative stress, relevant to the development of NAFLD. The iron contained in meat can causes cellular stress that decreases the effect of insulin, increasing the risk for T2DM. Nitrite and nitrate are used for the preservation of processed meat. These substances are converted into nitrosamines that are linked with IR and T2DM[9,10].

Single nucleotide polymorphisms (SNPs) in genes are potentially involved in oxidative stress, lipogenesis *de novo*, and IR have an important role in the development and progression of NAFLD[11,12]. Polymorphism in the *Patatin-like phospholipase domain containing 3* (*PNPLA3*) gene has an important impact on the susceptibility of NAFLD. The I148M variant of *PNPLA3* replaces isoleucine with methionine at codon position 148 by changing the C nucleotide to G (rs738409 c.444 C>G, p. I148M). Adiponutrin (ADPN) variant I148M has the intracellular function of regulating lipid flux in hepatocytes, belonging to a group of enzymes that metabolize lipids. *PNPLA3* has several enzymatic functions, including phospholipase activity, triacylglycerol hydrolase activity, and acyl-CoA-dependent or acyl-CoA-independent lipogenic transacetylase activities[13,14]. Study realized by Mazo et al. demonstrated that the CG and GG genotypes showed an increased risk for NAFLD compared to the CC genotype. When comparing NASH patients with the GG genotype, they showed a higher serum aspartate aminotransferase (AST) level and a high frequency of significant fibrosis[15]. However, in the other hand, there is evidence that individuals with the *PNPLA3* polymorphism respond more sensitively to changes in lifestyle, being important to study the genetic and dietary influence on NAFLD[12]. Therefore, this study aimed to evaluate the qualitative and quantitative association between meat intake and the presence of *PNPLA3* polymorphism in Brazilian individuals with NASH biopsy-proven.

METHODS

Patients

Biopsy-proven NASH patients whose had already done PNPLA3 polymorphims in a previous study[15] and were in follow-up at the the Hepatology Outpatient Clinic at the *Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo* (HC-FMUSP), São Paulo, Brazil, were invited by telephone to attend a specialized consultation, including anthropometric assessment, semi-quantitative Food Frequency Questionnaire (FFQ) and detailed meat consumption. Blood tests used in the analysis were from medical records near to the liver biopsy.

The exclusion criteria were the presence of any other chronic liver disease, excessive alcohol intake (<20–30 g/day), and calorie intake below or above the acceptable range for men 800–4.000 kcal/day and for women 500 to 3.500 kcal/day.

Nutritional evaluation

The analysis of food intake was based on a 24-hour dietary recall and the FFQ. Data were calculated with the software Avanutri 4.0 (Avanutri, Rio de Janeiro, Brazil). The FFQ includes different meat types with specified serving sizes. We calculated daily meat consumption in grams (g) per day (d) for each subject. Meat types were categorized as previously described[6]. Meat consumption was based on the specific questionnaire about meat consumption and cooking method. The average daily consumption of red meat and white meat (chicken and fish) was calculated from the frequency and amount in grams.

Anthropometric

Participants' weight and height were measured using a digital scale, where the individual stood barefoot, heels together, back straight, head positioned at a 90° angle to the neck. Body mass index (BMI) was calculated and classified according to the World Health Organization classification (WHO, 2000)[16] for adults aged 18 to 60 years and the Pan American Health Organization (PAHO, 2002) classification for the elderly[17]. Waist circumference was measured at the waist region is considered the midpoint between the last rib and the iliac crest, using a flexible, inelastic tape measure, with an accuracy of 0.1 cm.

DNA extraction and genotyping

Genomic DNA was extracted from the 15 mL blood sample from each patient. Polymorphism in the *PNPLA3* gene (rs738409) was assessed. Genomic DNA was isolated from 200 µL of blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). DNA quantification was determined by spectrophotometry (GeneQuant DNA/RNA Calculator, Pharmacia, LKC Biotechnology, Uppsala, Sweden).

Polymorphism was genotyped by real-time polymerase chain reaction (RT-PCR), using specific primers and labeled probes that recognize each of the possible genotypes. RT-PCR was performed using the commercial StepOne Plus kit, Applied Biosystems (Applied Biosystems Foster, California, USA) according to the manufacturer's recommendations. Allelic discrimination was performed by measuring allele-specific fluorescence on the Opticon 2 detection system (MJ Research, Waltham, MA, USA). Random samples were confirmed by direct genotyping to assess concordant results.

Liver histology

Liver histology was classified according to the NASH Clinical Research Network[18]: steatosis (0–3), inflammation (0–3), and hepatocyte ballooning (0–2). The histological fibrosis scale varies from F0 to F4 and is classified as follows: F0: no fibrosis; F1: portal fibrosis without septa; F2: portal fibrosis with few septa; F3: fibrosis/bridge septa between the central and portal veins; and F4: cirrhosis[18]. NASH was diagnosed with at least grade 1 for steatosis, ballooning, and lobular inflammation. NASH was defined when NAS≥4, and significant fibrosis stage F≥2[19].

Statistical analysis

Data were described as the median standard deviation for variables with normal distribution. For parametric tests, Student's *t*-test was used, for non-parametric tests, Mann-Withney's U test, according to the variable distribution (normal or not) and homogeneity of variance. Categorical variables were compared by the chi-square test or Fisher's exact test. The descriptive analysis of qualitative data was done using proportions, with a confidence interval of 95%. The significance level adopted was 5%, and descriptive levels lower than this value (P<0.05) were considered significant. To estimate the magnitude of the association between NAFLD polymorphism and meat consumption, we used the relative risk estimation – odds ratio (OR). The patients were divided into three groups according to the polymorphism: *PNPLA3* gene allele CC with 27 patients, GG with 17 patients, and finally the CG allele with 47 patients.

RESULTS

The study sample consisted of 91 patients with biopsy-proven NASH. Females comprised the majority, 78% of the cohort. The mean age of the partici-

pants was 64±9 years (range: 31 and 79 years) and the mean BMI was 32.38±4.58 kg/m^2 and the waist circumference was 107±10 cm. T2DM, dyslipidemia, and hypertension occurred in 74%, 70%, and 76% of the patients, respectively. Liver biopsy showed significant fibrosis (≥F2) in 42% of patients (TABLE 1).

TABLE 1. Clinical and demographic characteristics regarding frequency according to the number obtained for each variable.

Variable	n	Mean ± SD or %
Gender (female) %	91	78
Age (years)	91	64±9
BMI (kg/m^2)	91	32.4±4.6
Waist circumference (cm)	91	107±10
Type II diabetes mellitus (%)	91	74
Dyslipidemia (%)	91	70
Systemic arterial hypertension (%)	91	76
Mild steatosis (%)	91	66
Significant steatosis (%)	91	34
Absent or mild lobular inflammation 0/1 (%)	87	48
Significant lobular inflammation 2/3 (%)	87	47
Ballooning 1 (%)	89	37
Ballooning 2 (%)	89	60
Mild fibrosis 0/1 (%)	88	55
Significant fibrosis 2/4(%)	88	42

BMI: body mass index.

The mean total caloric intake was low 1.170±463.20 kcal/day and the mean macronutrients intake was 54.8±12.1%, for carbohydrates, 19.7±7.7% for protein, and 25.5±9.7% for the fat of the total energy value.

The mean saturated, polyunsaturated, and monounsaturated fat was 10.2±7.2 g, 4.7±4.5 g, and 22.1±134.4 g, respectively. The mean cholesterol intake was 166.7±135.2 mg (TABLE 2).

TABLE 2. Characteristics of daily caloric intake, macronutrients, fat, and fiber.

Variable	n	Mean ± SD
Daily caloric intake (<2000 kcal/d)	91	1170±463.2
Carbohydrate intake (<60%)	91	54.8±12.1
Protein Intake (>10%)	91	19.7±7.7
Lipid intake (<35%)	91	25.5±9.7
Saturated fat (%)	91	10.2±7.2
Polyunsaturated fat (%)	91	4.7±4.5
monounsaturated fat (%)	91	22.1±134.4
Cholesterol (<300 mg)	91	166.7±135.2
Fibers (>14g)	91	10.4±5.4

When comparing meat consumption by groups, we observe that there is a higher consumption of chicken and fish compared to meat consumption in general (TABLE 3).

TABLE 3. Mean meat intake distributed by type.

	Type of meat	n	Mean ± SD
Red meat consumption	Grilled steak (g)	91	62±54
	Pan-seared steak (g)	91	42±55
	Meat cooked in sauce (g)	91	50±54
	Hamburguer (g)	90	16±29
	Minced meat without sauce (g)	90	28±34
White meat consumption	Grilled chicken (g)	90	88±77
	Roast chicken (g)	90	63±62
	Chicken cooked in sauce (g)	90	54±58
	Baked fish (g)	91	39±102
	Grilled fish (g)	91	34±78
	Fish cooked in sauce (g)	91	60±74

Weekly meat consumption was categorized as a little (less than the median) or a lot (greater than or equal to the median) since the median consumption of red meat was 35 g and white meat was 60 g. When comparing the risk factors between the *PNPLA3* polymorphism and the consumption of red and white meat, we observed a significant OR for fibrosis of 2.12 (95%CI, 0.59–7.57) for the *PNPLA3* allele GG group and low meat consumption and 1.54 (95%CI, 0.56–4.21) for the CG group and low meat consumption compared to the *PNPLA3* allele CC group and low meat consumption. In contrast, those with the *PNPLA3* CC allele and high red meat consumption had a significant OR for fibrosis of 1.33 (95%CI, 0.56–3.17) relative to the *PNPLA3* CC allele group and low meat consumption. Conversely, the *PNPLA3* CC allele group and consumption of a lot of white meat did not show a significant relationship with fibrosis. Estimates of OR with their approximate confidence intervals (95% confidence interval) are shown in TABLE 4.

TABLE 4. Odds ratios calculated for patients with *PNPLA3* (CC) and low consumption of red and white meat.

Patients with	Limits of CI (95%)		
	OD	inferior	superior
PNPLA3 (GG) + low meat	2.12	0.59	7.57
PNPLA3 (CG) + low meat	1.54	0.56	4.21
PNPLA3 (CC) + lots of red meat ≥35g	1.33	0.56	3.17
PNPLA3 (CC) + lots of white meat ≥60g	0.80	0.35	2.92

OD: odds ratio; CI: confidence interval.

DISCUSSION

Our study demonstrates in a small cohort of NASH biopsy-proven patients that the risk to develop significant fibrosis increases in the presence of the G allele of the *PNPLA-3* polymorphism (GG or CG) and high red meat intake comparing with the C allele and low red meat intake. These findings can suggest a synergistic effect on the risk of developing NASH wirth significant fibrosis (≥ F2) in patients under medical follow-up in a Tertiary Center in Southwest Brazil.

In the present study, probably because the patients were followed-up in a tertiary hospital, there was a high prevalence of metabolic risk factors above 70% (74% were diabetics; 70% with dyslipidemia, 76% hypertensive), not to mention obesity (mean BMI 32.38±4.58 kg/m^2) and visceral fat (waist circumference 107±10 cm). Recent studies have shown in patients with NAFLD, a prevalence of 70 to 90% of dyslipidemia, hypertension in 60%, and T2DM in 40 to 60%, rates lower than those found in our study. This high prevalence of metabolic factors in this selected population from a tertiary care hospital may reflect the severity of fibrosis demonstrated in this sample, mainly because of the known relationship between cardio-metabolic risk factors and pro-inflammatory and pro-fibrotic pathways existing in NAFLD. Furthermore, in the studies mentioned above, 70% of the individuals with NAFLD were obese, which was not different from our sample that presented a mean BMI of 32.38±4.58, considered as grade I obesity[20-22].

Dietary intervention with calorie restriction and quality similar to Mediterranean diet (MedDiet) has benefits for patients with NAFLD. The caloric intake of patients with NAFLD is high in some studies[23,24]. A German study showed that patients with NAFLD had a mean total caloric intake of 2.739 kcal (range 1.009–5.941) compared to 2.173 kcal (1.199–4.320) in the control group (P<0.001)[23]. In the present study, we observed that calorie intake was low (1.170±463.20 kcal/day) compared to other studies as shown above. However, we remember that this population had already been followed for more than 3 years with a multidisciplinary team, including dietary monitoring, which may generate a bias in the interpretation of the low-calorie intake sample. On the other hand, regarding the quality of the diet and macronutrients of these patients, it was observed that the mean carbohydrate intake was 54.8±12.1%, 19.7±7.7% protein, and 25.5±9.7% total fat of the total energy value (TEV), with 10% unsatured fat and 22% monounsaturated fat. Qualitatively, the diet of the patients in the present study did not differ from an Italian study, which showed that the MedDiet with adjustment of 1.400 to 1.600 kcal/day, 50–60% carbohydrate, 15–20% protein, 30% mono- and polyunsaturated fats, and less than 10% saturated fat for 6 months, reduced weight (83 to 78 kg), BMI (31 to 29 kg/m^2), waist circumference (108 to 102 cm), and significant improvements in lipid profile, such as reduction of triglycerides and LDL cholesterol and FLI index [Fatty Liver Index (a noninvasive method that assesses liver fat)][25]. The MedDiet is indicated by many studies, but there are some barriers such as socioeconomic differences and various geographical regions where access to some foods are more difficult and expensive. The diet should be individualized, and culturally adjusted, and ensure the availability of foods[26]. Therefore, calorie restriction and a more lipid-quality adaptation are beneficial for NAFLD and MtS.

Although the patients demonstrated a lower caloric intake than in other studies of NAFLD, they on the other hand had a high intake of red meat, which had a positive relationship with the risk of fibrosis. It has also been shown that a high intake of white meat portends a lower chance of significant fibrosis.The relationship between high meat intake and NAFLD was evaluated in a study in southern China, where the semi-quantitative FFQ was administered to 1.594 individuals. They were evaluated into four groups according to the amount of meat consumed. The group with the highest meat consumption was associated with risk for NAFLD in treatment-weighted inverse probability analysis[27].

Recently Ivancovsky-Wajcman et al. demonstrated that higher consumption of red and processed meat are associated with changes in ALT and with a higher presence of fibrosis in NAFLD patients[28]. Furthermore, in another multiethnic cohort study of 2974 cases of NAFLD, the association of high red meat consumption with the disease was positive, both corroborating our findings[29].

Red meat consumption may increase the risk of chronic diseases due to the formation of advanced glycation end products (AGEs) during the cooking process, especially at high temperatures (>150°C) and for prolonged periods, which increase oxidative stress and stimulate inflammation. The increase in serum levels of AGEs by exogenous factors showed a relationship between IR and individuals with NAFLD[26].

In the present study, about 40% of patients had significant fibrosis (≥F2) that tended to be associated with higher red meat consumption. Similary, in Iranian a study, the consumption of red and white meats, refined grains, and soft drinks was associated with a higher chance for fibrosis analyzed by three-day food recalls[30].

About the genetic study of the *PNPLA3* gene polymorphism, our study obtained the highest number of patients with the CG allele, and there is an increased risk for fibrosis in the presence of the mutant G. In a meta-analysis study that associated polymorphism and susceptibility to NAFLD, it showed OR values of 2.76 between GG × CC alleles and 1.57 between GC × CC alleles, similar to our study[31].

However, although studies were confirming the association between red meat consumption, NAFLD, and fibrosis, and the presence of the G allele of the *PNPLA3* gene and a higher chance of NAFLD and fibrosis, this study suggesting that the association of the environmental factor (higher intake of red meat) and the presence of the *PNPLA3* polymorphism may have a synergistic effect, even without statistical significance due to the sample size.

On the other hand, Seko et al. demonstrated a correlation between reduced liver stiffness with body weight loss in the CG and GG genotype after one year of dietary treatment. While the G allele is known to aggravate the disease, it may be a factor that shows a better response in lifestyle change treatments and bariatric surgery for liver fat reduction[32]. It is suggested that the *PNPLA3* 148M variant promotes a significant suppression of peripheral lipolysis after the ketogenic diet, enhancing the antilipolytic effect of insulin and consequently a further improvement in insulin sensitivity[33]. This proves again that this disease is complex and that its treatment needs to be individualized.

The limitations of the present study refer mainly to being a small sample, from a tertiary hospital with a higher number of cases of significant fibrosis. In addition, although this is a cross-sectional study, the patients had been being followed-up in the service with a multidisciplinary team for a long time, which may be a bias in assessing the quantity and quality of food in these patients.

Therefore, we conclude that high red meat intake and *PNPLA3* polymorphism seem to have a synergistic effect on NASH and liver fibrosis. There is a need for confirmation in a larger number of patients and in different populations. If this synergism is confirmed, we can recommend reducing red meat consumption in people with *PNPLA3* polymorphism.

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Authors' contribution

Yoshimura SM: collected the data and wrote the manuscript. Duarte SMB: collected the data. Stefano JT: wrote and helped with the revision of the manuscript. Pinho JRR: polymorphism analyses. Mazo DFC: polymorphism analyses. Oliveira CP: study design, wrote, and helped with the revision of the manuscript.

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Yoshimura SM, Duarte SMB, Stefano JT, Mazo DFC, Pinho JRR, Oliveira CP. Polimorfismo no gene *PNPLA3* e o consumo de carne vermelha aumentam o risco de fibrose em pacientes com DHGNA comprovado por biópsia em acompanhamento médico em um centro terciário no Sudoeste do Brasil. Arq Gastroenterol. 2023;60(1):98-105.

RESUMO – Contexto – Estudos recentes mostram um aumento da doença hepática gordurosa não alcoólica (DHGNA) em populações com maior consumo de carne vermelha, processada e cozida em altas temperaturas. Por outro lado, o polimorfismo rs738409 no gene *Patatin-like fosfolipase contendo 3* (*PNPLA3*) tem sido implicado na suscetibilidade à DHGNA e fibrose hepática. No entanto, o efeito sinérgico entre o consumo de carne vermelha e o polimorfismo no gene *PNPLA3* na DHGNA ainda não foi avaliado. **Objetivo –** Avaliar a associação entre a presença do polimorfismo no gene *PNPLA3* e o consumo de macronutrientes, incluindo o consumo de carne e seu modo de cozimento em pacientes com DHGNA. **Métodos –** Realizamos um estudo transversal com 91 pacientes diagnosticados com DHGNA por biópsia hepática e genotipados para o polimorfismo no gene *PNPLA3*. O consumo de calorias e macronutrientes foi verificado por meio do questionário de frequência alimentar semi-quantitativo (QFA) e do questionário específico sobre consumo de carnes. O polimorfismo no gene *PNPLA3* foi analisado por reação em cadeia da polimerase em tempo real (RT-PCR) e a avaliação antropométrica foi realizada. **Resultados –** O **índice de massa corporal** médio foi de 32,38±4,58 kg/m² e a circunferência da cintura foi de 107±10 cm. Na biópsia hepática, 42% dos pacientes apresentavam fibrose significativa (F≥2). O *odds ratio* de F≥2 foi de 2,12 para o grupo GG e 1,54 para o grupo GC, comparado ao grupo CC. A ingestão calórica média foi de 1.170±463,20 kcal/d. O *odds ratio* para alto consumo de carne vermelha no grupo CC em comparação ao baixo consumo foi de 1,33. Para a carne branca, este valor foi de 0,8 ao comparar o alto e o baixo consumo, também no grupo CC. **Conclusão –** A alta ingestão de carne vermelha e o polimorfismo no gene *PNPLA3* parecem afetar sinergicamente a DHGNA e a fibrose hepática, necessitando de confirmação em maior número de pacientes e em diferentes populações.

Palavras-chave – Doença hepática gordurosa não alcoólica; polimorfismo de nucleotídeo único; *Patatin-like phospholipase domain-containing 3*; dieta; carne vermelha.

REFERÊNCIAS

1. Mendez-Sanchez N, Arrese M, Gadano A, Oliveira CP, Fassio E, Arab JP, et al. The Latin American Association for the Study of the Liver (ALEH) position statement on the redefinition of fatty liver disease. Lancet Gastroenterol Hepatol. 2021;6:65-72.
2. Samji NS, Verma R, Satapathy SK. Magnitude of Nonalcoholic Fatty Liver Disease: Western Perspective. J Clin Exp Hepatol. 2019;9:497-505.
3. Sharma M, Mitnala S, Vishnubhotla RK, Mukherjee R, Reddy DN, Rao PN. The Riddle of Nonalcoholic Fatty Liver Disease: Progression From Nonalcoholic Fatty Liver to Nonalcoholic Steatohepatitis. J Clin Exp Hepatol. 2015;5:147-58.
4. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73:202-9.
5. Akhlaghi M, Ghasemi-Nasab M, Riasatian M. Mediterranean diet for patients with non-alcoholic fatty liver disease, a systematic review and meta-analysis of observational and clinical investigations. J Diabetes Metab Disord. 2020;19:575-84.
6. Zelber-Sagi S, Ivancovsky-Wajcman D, Fliss Isakov N, Webb M, Orenstein D, Shibolet O, et al. High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance. J Hepatol. 2018;68:1239-46.
7. Turesky RJ. Mechanistic Evidence for Red Meat and Processed Meat Intake and Cancer Risk: A Follow-up on the International Agency for Research on Cancer Evaluation of 2015. CHIMIA. 2018;72:718.
8. Boada LD, Henríquez-Hernández LA, Luzardo OP. The impact of red and processed meat consumption on cancer and other health outcomes: Epidemiological evidences. Food Chem Toxicol. 2016;92:236-44.
9. Hydes TJ, Ravi S, Loomba R, E Gray M. Evidence-based clinical advice for nutrition and dietary weight loss strategies for the management of NAFLD and NASH. Clin Mol Hepatol. 2020;26:383-400.
10. Zelber-Sagi S, Salomone F, Mlynarsky L. The Mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: Evidence and plausible mechanisms. Liver Int. 2017;37:936-49.
11. Mana MF, Parisi MCR, Correa-Giannella ML, Neto AM, Yamanaka A, Cunha-Silva M, et al. Non-Alcoholic Fatty Liver Disease in Long-Term Type 2 Diabetes: Role of rs738409 PNPLA3 and rs499765 FGF21 Polymorphisms and Serum Biomarkers. Molecules. 2022;27:3193.
12. Nishioji K, Mochizuki N, Kobayashi M, Kamaguchi M, Sumida Y, Nishimura T, et al. The Impact of PNPLA3 rs738409 Genetic Polymorphism and Weight Gain ≥10 kg after Age 20 on Non-Alcoholic Fatty Liver Disease in Non-Obese Japanese Individuals. PLoS One. 2015;10:e0140427.
13. Kumari M, Schoiswohl G, Chitraju C, Paar M, Cornaciu I, Rangrez AY, et al. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. Cell Metab. 2012;15:691-702.
14. Winberg ME, Motlagh MK, Stenkula KG, Holm C, Jones HA. Adiponutrin: a multimeric plasma protein. Biochem Biophys Res Commun. 2014;446:1114-9.
15. Mazo DF, Malta FM, Stefano JT, Salles APM, Gomes-Gouvea MS, Nastri ACS, et al. Validation of PNPLA3 polymorphisms as risk factor for NAFLD and liver fibrosis in an admixed population. Ann Hepatol. 2019;18:466-71.
16. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253.
17. Encuesta Multicentrica salud beinestar y envejecimiento (SABE) em América Latina el Caribe: Informe Preliminar. In: XXXVI Reunión del Comité asesor de investigaciones em Salud; 9-11 jun 2001; Kingston, Jamaica: OPAS, 2002. 2002.
18. 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.
19. Tavaglione F, Jamialahmadi O, De Vincentis A, Qadri S, Mowlaei ME, Mancina RM, et al. Development and Validation of a Score for Fibrotic Nonalcoholic Steatohepatitis. Clin Gastroenterol Hepatol. 20221:S1542-3565(22)00385-8. doi: 10.1016/j.cgh.2022.03.044..
20. Giraldi L, Miele L, Aleksovska K, Manca F, Leoncini E, Biolato M, et al. Mediterranean diet and the prevention of non-alcoholic fatty liver disease: results from a case-control study. Eur Rev Med Pharmacol Sci. 2020;24:7391-8.
21. Cariou B, Byrne CD, Loomba R, Sanyal AJ. Nonalcoholic fatty liver disease as a metabolic disease in humans: A literature review. Diabetes Obes Metab. 2021;23:1069-83.
22. Naik A, Košir R, Rozman D. Genomic aspects of NAFLD pathogenesis. Genomics. 2013;102:84-95.

23. Wehmeyer MH, Zyriax BC, Jagemann B, Roth E, Windler E, Schulze Zur Wiesch J, et al. Nonalcoholic fatty liver disease is associated with excessive calorie intake rather than a distinctive dietary pattern. Medicine (Baltimore). 2016;95:e3887.
24. Ricci G, Canducci E, Pasini V, Rossi A, Bersani G, Ricci E, et al. Nutrient intake in Italian obese patients: relationships with insulin resistance and markers of non-alcoholic fatty liver disease. Nutrition. 2011;27:672-6.
25. Abenavoli L, Greco M, Milic N, Accattato F, Foti D, Gulletta E, et al. Effect of Mediterranean Diet and Antioxidant Formulation in Non-Alcoholic Fatty Liver Disease: A Randomized Study. Nutrients. 2017;9:870.
26. Zelber-Sagi S. Dietary Treatment for NAFLD: New Clinical and Epidemiological Evidence and Updated Recommendations. Semin Liver Dis. 2021;41:248-62.
27. Peng H, Xie X, Pan X, Zheng J, Zeng Y, Cai X, et al. Association of meat consumption with NAFLD risk and liver-related biochemical indexes in older Chinese: a cross-sectional study. BMC Gastroenterology. 2021;21:221.
28. Ivancovsky-Wajcman D, Fliss-Isakov N, Grinshpan LS, Salomone F, Lazarus JV, Webb M, et al. High Meat Consumption Is Prospectively Associated with the Risk of Non-Alcoholic Fatty Liver Disease and Presumed Significant Fibrosis. Nutrients. 2022;14:3533.
29. Noureddin M, Zelber-Sagi S, Wilkens LR, Porcel J, Boushey CJ, Le Marchand L, et al. Diet Associations With Nonalcoholic Fatty Liver Disease in an Ethnically Diverse Population: The Multiethnic Cohort. Hepatology. 2020;71:1940-52.
30. Soleimani D, Ranjbar G, Rezvani R, Goshayeshi L, Razmpour F, Nematy M. Dietary patterns in relation to hepatic fibrosis among patients with nonalcoholic fatty liver disease. Diabetes Metab Syndr Obes. 2019; 12:315-24.
31. Dai G, Liu P, Li X, Zhou X, He S. Association between PNPLA3 rs738409 polymorphism and nonalcoholic fatty liver disease (NAFLD) susceptibility and severity: A meta-analysis. Medicine (Baltimore). 2019;98:e14324.
32. Seko Y, Yamaguchi K, Tochiki N, Yano K, Takahashi A, Okishio S, et al. The Effect of Genetic Polymorphism in Response to Body Weight Reduction in Japanese Patients with Nonalcoholic Fatty Liver Disease. Genes (Basel). 2021;12:628.
33. Wang J-Z, Cao H-X, Chen J-N, Pan Q. PNPLA3 rs738409 underlies treatment response in nonalcoholic fatty liver disease. World J Clin Cases. 2018;6:167-75.