

# Impact of current diet at the risk of non-alcoholic fatty liver disease (NAFLD)

Sebastião Mauro Bezerra **DUARTE**<sup>1</sup>, José Tadeu **STEFANO**<sup>2</sup>, Denise Siqueira **VANNI**<sup>3</sup>, Flair José **CARRILHO**<sup>1,3</sup> and Claudia Pinto Marques Souza de **OLIVEIRA**<sup>1,2</sup>

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**ABSTRACT** – The nonalcoholic fatty liver disease (NAFLD) affects approximately 20%–30% of general population and is even more prevalent among obese individuals. The risk factors mainly associated with NAFLD are diseases related to the metabolic syndrome, genetics and environment. In this review, we provide a literature compilation evaluating the evidence behind dietary components, including calories intake, fat, protein, fibers and carbohydrate, especially fructose which could be a trigger to development and progression of the NAFLD. In fact, it has been demonstrated that diet is an important factor for the development of NAFLD and its association is complex and extends beyond total energy intake.

**HEADINGS** – Non-alcoholic fatty liver disease. Energy intake. Dietary fats. Dietary carbohydrates. Fructose.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common forms of liver disease primarily related to the progressive increase of obesity in the world. Initially, it was considered to be a liver disease with benign course, however, it is currently known to be a complex disease which involves environmental factors and genetic predisposition<sup>(1)</sup>. NAFLD ranges from simple Steatosis to non-alcoholic steatohepatitis (NASH) that can have different degrees of fibrosis and progress to liver cirrhosis and hepatocellular carcinoma (HCC) in patients with no history of alcoholism<sup>(2)</sup>. Sedentary lifestyle, inadequate intake of foods with high fat and fructose consumption, as well as obesity, metabolic syndrome (MtS), type 2 diabetes mellitus (T2DM), hormonal status and genetic background have been described as responsible for the development of NAFLD<sup>(3)</sup>.

Due to increasing rates of obesity, NAFLD has become more prevalent in all populations, especially in the Western world and has been defined by specialists as the disease of the modern world<sup>(4)</sup>. NAFLD is highly prevalent in the United States and around the world. However, this prevalence differs significantly according to the diagnostic method used and the population studied. As rates of obesity, T2DM and MtS continue to increase, NAFLD can significantly affect health care and the development of long-term complications such as cirrhosis and HCC in the next few years<sup>(5)</sup>.

## DIET, NUTRITION INTAKE AND NAFLD

Balanced and healthy nutrition is one of the main determinants of nutritional status that can prevent many diseases, such as NAFLD.

In fact, an inadequate diet, with a high concentration of saturated fat and cholesterol, simple carbohydrates and xenobiotics, or external contaminants, which lacks in vitamins and fibers, is an important factor for the development of NAFLD. Zelber-Sagi et al. demonstrated that excess caloric consumption, unhealthy diet, sedentary lifestyle leading to obesity and related comorbidities and weight gain per se, even a modest weight gain of 3–5 kg, are leading risk factors for NAFLD, regardless of baseline body mass index (BMI)<sup>(6)</sup>.

Research studies, in order to identify factors related to the development of NAFLD and progression to NASH have discovered that endogenous and exogenous factors [biological origin, synthetic (industrial) or environmental] act as hepatotoxins, including food and water<sup>(7,8)</sup>. Diet is an important factor in the development of NAFLD and this relationship is complex and extends beyond total energy intake. NAFLD patients shown higher energy intake, significantly lower protein and carbohydrate and higher total fat, higher intake of saturated fat, and n-6 consumption was identified compared to controls<sup>(9-11)</sup>.

## CALORIES AND FAT

Studies have analyzed the association between liver fat content and calorie and fat intake<sup>(12-14)</sup>. It has been demonstrated that patients with NAFLD have a significantly higher daily overall calories intake whereas the general dietary composition displays only moderate deviations when compared to healthy controls<sup>(9,15)</sup>.

Moreover, epidemiological studies have demonstrated that NASH patients have a diet richer in saturated fat and cholesterol and poorer in polyunsaturated fatty acids (PUFA) when compared to healthy controls with same age, gender and BMI<sup>(11,16)</sup>.

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<sup>1</sup> Universidade de São Paulo, Faculdade de Medicina, São Paulo, SP, Brasil. <sup>2</sup> Universidade de São Paulo, Hospital das Clínicas, Laboratório de Gastroenterologia Clínica e Experimental (LIM-07) do Departamento de Gastroenterologia da FMUSP, São Paulo, SP, Brasil. <sup>3</sup> Universidade de São Paulo, Hospital das Clínicas, Divisão de Gastroenterologia e Hepatologia Clínica e Departamento de Gastroenterologia da FMUSP, São Paulo, SP, Brasil.

Corresponding author: Claudia Pinto Marques de Souza de Oliveira. E-mail: cpm@usp.br

A recent study of Solon-Biet et al. has revealed that a high fat diet induces hepatic steatosis quickly in ad libitum-fed mice<sup>(8)</sup>.

In obese humans, a diet containing about 55% of fat increases the amount of intrahepatic triglycerides (TG) by 35% and the fasting insulin levels are augmented, regardless of body weight<sup>(17)</sup>.

Despite the fact that excessive consumption of saturated fatty acids promotes stress in the endoplasmic reticulum and hepatocyte injury, severe restriction may not be beneficial for patients with NAFLD as previously shown by Zivkovic et al. study. They analyzed dietary patterns with different percentages of total and saturated fat and concluded that, although there was a reduction of low-density lipoprotein cholesterol (LDL), hypolipidic diets also reduced high-density lipoprotein cholesterol levels and increased serum TG and, in individuals with higher percentages of body fat and with insulin resistance (IR), these changes were much more significant after ingestion of very low amounts of total and saturated fats<sup>(18)</sup>.

On the other hand, monounsaturated fatty acids, mainly olive oil, oleaginous (nuts, almonds) and avocado, can be very beneficial for patients with NAFLD as they lower the levels of LDL cholesterol and TG. Diets that range between 20% and 40% of the total caloric value in the form of monounsaturated fats have been shown to be beneficial in NAFLD patients, increasing the oxidation of other fatty acids (by the activation of peroxisome proliferator-activated receptors gamma and alpha) and by reduction of lipogenesis (reducing the activation of the sterol regulatory element binding protein)<sup>(19)</sup>.

Low-calorie diets (800 to 1.000 kcal) were used in an attempt to promote faster and meaningful weight loss to for a promotion adipose tissue metabolism. A calorie diet derived from low carbohydrate supply (based on 10% of total calorie in one induction phase and then a gradual increase to 34%), was associated with weight reduction when compared to a diet with low lipid content. However, this weight loss was not sustained, and the patient showed difficulty in maintaining his weight in the long term<sup>(20)</sup>. More evidence suggests that limiting carbohydrate intake to less than 20 g/day as well as maintaining a caloric deficit of 30%, both were beneficial in achieving improvements in NAFLD Activity Score<sup>(21)</sup>, hepatic lipids<sup>(22)</sup> and serum hepatic enzymes<sup>(23)</sup>. Therefore, a caloric deficit of 500-750 kcal/day is an appropriate therapeutic intervention for NAFLD. It is suggested that women should eat 1200 kcal/day and men 1500 kcal/day and a slightly higher amount of 1500 kcal/day for women and 1800 kcal/day for men might be considered, with adjustments based on their physical activity<sup>(15)</sup>.

## CARBOHYDRATE

During the last decade, dietary habits have evolved to more consumption of sweetened and fatty foods<sup>(24)</sup>. There are substantial evidences which demonstrates that the implication of increased consumption of sugars, specially fructose (sweetened beverage intake), is related to a higher risk of developing T2DM, MtS, NAFLD and cardiovascular (CV) diseases<sup>(25)</sup>.

Furthermore, excessive carbohydrate intake may be harmful in patients with NAFLD, and high carbohydrates intake appear to be associated with inflammation and disease progression<sup>(26)</sup>. In fact, low-carbohydrate diets with less than 45% carbohydrates of their total kcal composition show positive results concerning weight loss, reduction of intrahepatic TG content and improvement of metabolic parameters among obese individual. However, in an

animal model study the maintenance of low carbohydrate diets for extended period stimulated the development of NAFLD and promoted glucose intolerance<sup>(27)</sup>. In this year, Sekkarie et al. evaluated various dietary patterns which were not limited exclusively to reduced added sugars. Such diets turned out to be protective to development and progression of NAFLD<sup>(28)</sup>.

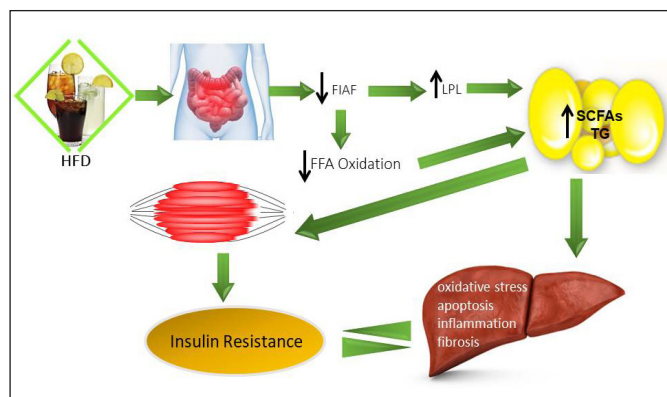
High glycemic index (GI) diets are associated with increased risk of obesity, T2DM, Hyperlipidemia and non-alcoholic fatty liver. High GI may be a good dietary marker of the effect of IR on NAFLD which confirms the importance of choosing a low-GI diet as a tool to prevent NAFLD induced by IR<sup>(29)</sup>.

In the class of carbohydrates, fructose is perhaps the one that is most related to the progression of NAFLD<sup>(30,31)</sup>. In the 60s, high fructose corn syrup was inserted in the food industry as a substitute of sugar and the intake increased<sup>(32)</sup>. Over time, fructose was identified as a sugar affecting lipid metabolism by augmenting plasma TG and free fatty acids (FFA). Recently, a cross-sectional study with obese children and adolescents with NAFLD demonstrated that fructose consumption is positively and independently associated with the prevalence of NASH, as diagnosed using NAFLD Activity Score and the fatty liver progression algorithm<sup>(33)</sup>. Another large-scale study with NAFLD patients analyzed the role of overconsumption of fructose-containing diet in the development of this disease. In this study, the authors describe that high daily fructose-containing diet is associated with a higher hepatic fibrosis stage in younger and elderly patients. Surprisingly, this diet was also related to a lower steatosis grade in the older group of patients<sup>(34)</sup>. Volynets et al. have observed that lifestyle intervention focusing on lower fructose intake leads to reduction in hepatic reperfusion and in transaminases levels<sup>(35)</sup>. These studies reinforce fructose consumption as an important preventable risk factor that may lead to advanced NAFLD.

It is known that high sugar consumption upregulates the transport of fructose through the GLUT5 transporter, increasing fructokinase levels in the liver, independently, of energy intake excess<sup>(7)</sup>. Other studies have demonstrated that fructose can also increase TG levels, lead to *de novo* synthesis of fatty acids, hyperuricemia, hyperferritinemia, and IR increasing the risk of NAFLD<sup>(36,37)</sup>. A study conducted by Sullivan et al. investigated the absorption of fructose in lean children, obese children, and obese children with biopsy proven NAFLD and realized that children with NAFLD may be absorbing and metabolizing fructose more effectively than lean subjects, which could contribute to the pathophysiology of NAFLD. Whether this fact may be related to up-regulation of GLUT5 and fructokinase by previous fructose exposure or whether this is due to genetic/ethnic differences has yet to be determined<sup>(38)</sup>.

On the other hand, high fructose consumption promotes gut inflammation followed by increasing endotoxin release, epithelial dysfunction, and reduction of tight junction proteins independently of the fat content in the diet and energy intake<sup>(39)</sup>. These data illustrate the high impact of nutritional fructose on the intestinal barrier function. An hypothesis exists whether fructose could cause dysbiosis and increase intestinal permeability and endotoxins in the blood<sup>(40)</sup>. Studies have shown a relation between NAFLD-microbiome and diet<sup>(33,41,42)</sup>, leading to intestinal dysbiosis, a scenario that is related to risk factors inducing the development and progression of liver diseases<sup>(43,44)</sup>. A proposed mechanism to explain the relation of a high fructose diet and altered intestinal microbiota leading to increased body fat is the intestinal suppression provoked by fasting induced adipose factor (FIAP). This protein is produced by

enterocytes and its function is to inhibit lipoprotein lipase (LPL)<sup>(45)</sup>, which when activated, increases the absorption of FFA and TG. These actions are regulated by FIAF. Conclusively, by suppressing FIAF, LPL becomes more active, triggering a greater uptake of short-chain fatty acids and TG; occurs a reduction of oxidation of FFA and augmentation of systemic and peripheral IR. All factors cited contribute to the development of MtS and to the accumulation of FFA in hepatocytes<sup>(18,46,47)</sup>, leading to several clinically relevant conditions, including NASH and cirrhosis<sup>(48)</sup> (FIGURE 1). On the other hand, a healthy gut microbiota is maintained by gut bacteria fermentation of non-digestible carbohydrates, this function being also responsible for energy production<sup>(49)</sup>. Moreover, there is evidence that many substances present in fruits, such as flavanols, epicatechin, vitamin C and other antioxidants may also protect against fructose-induced MtS<sup>(50,51)</sup>.



**FIGURE 1.** Fructose mechanisms mediating development and progression of non-alcoholic fatty liver disease (NAFLD).

HFD: high-fructose diet; FIAF: fasting induced adipose factor; LPL: lipoprotein lipase; FFA: free fatty acids; SCFAs: short-chain fatty acids; TG: triglycerides.

## FIBERS

Fibers can be classified according to their solubility, being the soluble ones represented by pectin (fruits) and gums (oats, barley and legumes like beans, chickpeas, lentils) and insoluble fibers being represented by cellulose (wheat), hemicellulose (grains) and lignin (vegetables)<sup>(52)</sup>.

A study evaluating dietary patterns in patients with NASH revealed that these patients consumed less carbohydrate, more fat and less fibers than healthy controls<sup>(10)</sup>. A review of the therapeutic effects of dietary fibers, especially derived from whole grains, demonstrated the benefits of its consumption and the reduction of comorbidities associated with MtS and NAFLD. In addition to reduction of liver fat, fibers derived from whole grains could also reduce inflammation<sup>(53,54)</sup>.

## FISH OIL

A recent research has suggested that PUFA administration improves plasma lipid profile and may be useful in the treatment of NAFLD<sup>(55)</sup>. The PUFA's precursor is  $\alpha$ -linoleic acid (ALA) and its metabolites are, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These acids are detected in fish that synthesize PUFA from ingestion of marine plants. Some studies have shown that PUFA have a protective role in the development and progression of

NAFLD<sup>(56,57)</sup>. EPA and DHA are able to prevent and reverse liver disease in animal models of obesity<sup>(56)</sup> and in humans, obesity and NAFLD, are negatively associated with the long-chain omega-3 fatty acid<sup>(58,59)</sup>.

Early interest in PUFA supplements as possible therapy for NASH can be hypothesized considering the close association between MtS, CV disease, fatty liver and reports of CV benefits of omega-3-rich foods<sup>(60,61)</sup>. A meta-analysis presented by Parker et al. showed that dietary  $\omega$ -3 PUFA supplements ameliorated the hepatic steatosis and liver injury in adult NAFLD patients<sup>(62)</sup>. Yuan et al. demonstrated that supplementation of  $\omega$ -3 PUFA reduced steatosis and hepatic inflammation in animal models with hepatic TG accumulation, inflammation and fibrogenesis induced by chronic high-fat diet<sup>(63)</sup>. Our group have performed a controlled randomized trial evaluating the PUFA supplementation and demonstrated significant impact on lipid profile in NASH patients by increasing plasma  $\omega$ -3 PUFA levels, decreasing levels of the potentially pro-inflammatory  $\omega$ -6 arachidonic acid (AA), and decreasing serum TG levels. Nonetheless, no significant improvement in NAFLD Activity Score was verified<sup>(64)</sup>.

Noticeable heterogeneity was observed in the trials including dose, formulation and time of PUFA supplementation for the treatment of NAFLD. None of the studies have shown improvement in key prognostic histological features such as fibrosis. One study using biopsy as the measure of fat content reported no change in steatosis after 12 months of synthetic EPA supplementation (up to 2.700 mg/day) compared to placebo treated subjects<sup>(65)</sup>. In contrast, another trial has reported significant hepatic fat reduction after 15–18 months of 4.000 mg/day of a synthetic mixture of EPA and DHA supplementation compared to placebo<sup>(66)</sup>. Similarly, Argo et al. have demonstrated that 12 months supplementation of 3.000 mg/day of a fish oil derived mixture of EPA and DHA *versus* soybean oil placebo, reduced liver fat in magnetic resonance imaging. However, the effect was significantly less evident in patients who lost a modest amount of weight during the study period<sup>(67)</sup>. While there appears to be greater evidence that regular consumption of PUFA has metabolic benefit, the effect of additional oily fish or fish oil supplementation in NAFLD is uncertain and current optimal dose is still unknown. Even though some studies have shown consistent improvement in liver fat content, recent trials have not shown a significant benefit in liver histology or fibrosis.

Several therapeutic approaches are used in the treatment of NAFLD and NASH but it is confirmed that the most effective treatment is lifestyle change such as regular physical activity and weight reduction<sup>(68-70)</sup>, however, very fast weight loss up to 1.6 kg per week may aggravate the inflammatory status of individuals with NAFLD<sup>(71)</sup>. In this case, the most important is moderate weight loss and mainly prioritize the correct choice of dietary nutrients. Although low-calorie diets are effective approaches to improve NAFLD<sup>(23,72)</sup>, it has been shown that the dietary pattern of Mediterranean countries has been the best strategy for NAFLD management<sup>(73,74)</sup>. In the following chapters, we will describe some dietary patterns such as protein diet, vegetarian diet and Mediterranean diet, as well as some foods like coffee and dark chocolate as beneficial components in NAFLD.

## PROTEIN DIET

There are few studies which demonstrate any relation between protein intake and NAFLD. Little is known about the effect of

the quantity, quality and composition of dietary proteins on the development and treatment of NAFLD. Whereas protein deficiency malnutrition can cause steatosis, and excessive protein intake can lead to glomerular sclerosis, hypertension in intrarenal capillaries as well as malfunction of the kidneys in individuals vulnerable to kidney disease<sup>(18)</sup>. Limited evidence suggests that a high protein diet could be effective to treat NAFLD because of increase in energy expenditure and hepatic lipid oxidation, as liver catabolism of ingested amino acids is an intense energy process<sup>(75)</sup>. In obese sedentary women, short-term protein supplementation was advantageous for hepatic steatosis and lipid profile<sup>(76)</sup>. Soy protein has also been successfully implicated in this same clinical scenario, however the functional properties of soybeans related to the amino acid profile, polyunsaturated fatty acids and isoflavonoids were the focus on study, not the protein intake<sup>(77)</sup>. As a matter of fact, soy protein has been advocated as ideal for NAFLD patients based on animal studies. Clinical evaluations are insufficient in most protocols, however weight loss tends to be prominent<sup>(78)</sup>.

There are recent evidences that a high protein, low calorie diet is associated with improvement of lipid profile, glucose homeostasis and liver enzymes<sup>(23)</sup> and findings are consistent with the well-established principle of calorie restriction in the management of MtS components and liver histology<sup>(79,80)</sup>.

Studies comparing different diets for NASH treatment are still scarce. In the largest randomized trial up until now, 170 overweight adults were enrolled and followed for a period of six months. They were divided in three groups of diet: high in protein, low in fat and low in carbohydrate content. Results were equivalent among groups which demonstrated: reductions in intrahepatic fat, alanine aminotransferase (ALT), visceral adiposity and total weight as well as changes in whole body insulin sensitivity<sup>(22)</sup>.

A recent study showed that whey protein supplementation (60 g/day) for 30 days, without energy restriction, decreased intrahepatic lipids. Despite stability of BMI, body fat mass had diminished. Additional metabolic advantages were identified in plasma lipids levels, however it was not seen in glucose homeostasis markers<sup>(76)</sup>. Two short-term protocols with protein supplementation showed also improvement in hepatic fat content with a stable body weight<sup>(81,82)</sup>.

Ideal dietary intake is intended to guarantee both moderate caloric restriction and adequate protein intake. According to European Association for the Study of the Liver (EASL) guidelines, although good quality data are lacking, particular attention must be paid to the protein intake needed to maintain muscle mass, because of the potential risk of exacerbating sarcopenia during weight loss interventions<sup>(83)</sup>.

### VEGETARIAN DIET

The benefits of a vegetarian healthy diet have been reported over the last few years, but there are still few *randomized* controlled trials<sup>(84,85)</sup>. Recently, evidence has shown that the consumption of vegetables and fruits protects against metabolic diseases such as T2DM<sup>(86)</sup>, CV disease<sup>(87)</sup> and NAFLD<sup>(88)</sup>. These aliments are rich in fiber and antioxidant vitamins and their caloric density is low, which favors a higher daily energy consumption, fastly promoting satiety which is sustained for a longer time. All of these facilitates the management of weight loss<sup>(89,90)</sup>.

A meta-analysis conducted by Yang Y et al. have demonstrated that the consumption of vegetables, but not fruit, was associated with lower risk of HCC<sup>(91)</sup>. These different effects of vegetables and

fruit might be explained by differences in their nutrient compositions. Fruits contain more calories and antioxidants, such as vitamin A, C, E and carotenoids. Whether vegetables not only provide dietary fiber and vitamins A and E, but they are also sources of phytochemicals (folate, tocopherols, and carotenoids) which have shown anti-tumor activities in different diseases<sup>(92,93)</sup>.

Patients with NAFLD are advised to adhere to several dietary recommendations such as avoiding simple carbohydrates, saturated fats, and sweetened drinks as well as consuming diets rich in fruits and vegetables<sup>(70,94,95)</sup>. Studies have demonstrated that due to a high content of fiber, phytochemicals, and antioxidants in fruits and vegetables, the high intake of these food groups showed protective effects on NAFLD. Phytochemicals and antioxidants are anti-inflammatory compounds and can prevent developing hepatic steatosis and maintaining blood glucose, insulin and free fatty acids within laboratory reference ranges<sup>(96,97)</sup>. In a cross-sectional study, non-starchy vegetables consumption had been associated with lower liver fat deposition, and dark green or bright orange/yellow vegetables intake had been associated with lower visceral fat and improved insulin sensitivity<sup>(88)</sup>. Other positive effects attributed to fruits and vegetables consumption are related to antioxidants such as the polyphenols which are present in these foods<sup>(98)</sup>. Polyphenols display beneficial effects on metabolic homeostasis and exert anti-inflammatory and anti-fibrotic effects, inhibit *de novo* lipogenesis and stimulate  $\beta$ -oxidation in the NAFLD models<sup>(89)</sup>.

Although fruits and vegetables have so many benefits, their consumption were lower in NAFLD patients compared with healthy subjects or recommended amounts in reviewed studies<sup>(11,99,100)</sup>.

### MEDITERRANEAN DIET

The traditional Mediterranean diet (MD) is the most frequently studied diet in NAFLD scenario. Its plays a beneficial role in metabolic profile<sup>(101)</sup> and has been shown to reduce the risk of CV disease<sup>(102)</sup> and T2DM<sup>(103)</sup>, two highly relevant outcomes in NAFLD patients.

The main characteristics of the MD is a large intake of fruits, vegetables, whole grains, nuts, olive oil, fish and poultry and low consumption of dairy products, processed foods, saturated fats, red meats, foods high in sugar and moderate alcohol intake, usually red wine consumed with meals<sup>(104-106)</sup>. Several clinical and experimental studies have shown that the MD and the higher PUFA intake reduce hepatic triglyceride content, increase insulin sensitivity, and improve NASH<sup>(61,67,107,108)</sup> and has been consistently beneficial with respect to CV risk<sup>(109,110)</sup>. Estruch R et al., observed that consumption of extra virgin olive oil or nuts in the MD resulted in a substantial reduction in the risk of CV events among high-risk people (age 55+, smokers, hypertensive patients and type 2 diabetics)<sup>(102)</sup>.

The MD is also characterized by reduced carbohydrate intake (40% of calories), especially reduced sugars (sucrose and fructose) and refined carbohydrates, which may be partly responsible for its beneficial effect on NAFLD<sup>(111)</sup>. Reduction in fructose intake from artificially sweetened beverages improved MtS in obese individuals, regardless of dietary fruit consumption<sup>(112)</sup>. The recommendation of fruit consumption in the MD to be given safely due to the various healthy nutrients present fruit such as fiber and antioxidants.

Although the MD recommends drinking wine in moderation, it is unclear whether NAFLD patients should adopt this recommendation. Patients with cirrhosis should avoid alcohol, as any regular alcohol consumption puts them at higher risk of developing

HCC<sup>(113)</sup>. However, it remains uncertain about moderate alcohol consumption (up to two doses per day) in patients without cirrhosis. The specific protective effect for wine may be due to the phytosterols found in grapes<sup>(114)</sup>.

Adherence to the MD pattern leads to a significant decrease in liver fat in overweight and NAFLD patients<sup>(115)</sup> with or without T2DM<sup>(116,117)</sup> and have been included as therapeutic recommendations in the recent European guideline for the treatment of NAFLD<sup>(118)</sup>.

## COFFEE AND DARK CHOCOLATE

The coffee is one of the most consumed beverages in the world and can have beneficial effects on the liver. Coffee consumption has been associated with reduced liver enzymes including ALT, aspartate aminotransferase (AST) and gamma glutamyltransferase<sup>(119,120)</sup>. Coffee intake has also been associated with lower severity of liver disease and lower rates of liver disease progression<sup>(121)</sup>. In addition, drinking coffee was inversely related to alcoholic and nonalcoholic liver cirrhosis in case-control studies<sup>(122,123)</sup> and alcoholic cirrhosis in prospective study<sup>(124)</sup>. Epidemiological studies have suggested that coffee consumption is protective against chronic liver disease and reduces the risk of developing HCC<sup>(125-127)</sup>. Experimental data have shown potential beneficial effects on the liver following consumption of various coffee components, including caffeine<sup>(128)</sup>, coffee oils (kahweol and cafestol)<sup>(129)</sup> and aromatic extracts isolated from coffee beans<sup>(130)</sup>.

Caffeine is not only associated with a decreased of various liver diseases, but its consumption may also decrease mortality<sup>(131)</sup>. There is evidence that daily consumption of two to three cups of coffee has significant health benefits. Thus, coffee appears to have “hepatoprotective” health benefits<sup>(132)</sup>. Coffee is made up of more than 100 compounds, any of which may be responsible for their beneficial effects, so it is possible that the described hepatic benefits come from a synergistic effect of multiple compounds and not a particular compound<sup>(133)</sup>. It is important to mention that different types of coffee may show different effects on liver disease. Studies show that filtered coffee has a hepatoprotective role while unfiltered coffee showed a potentially deleterious effect<sup>(125,134)</sup>. Perhaps this difference is due to the presence of kahweol and cafestol which are released from ground coffee beans but removed by paper filters<sup>(135)</sup>. Anty et al. showed that espresso coffee had no beneficial effect on liver disease, particularly NAFLD, but the authors postulated that espresso coffee may not have been beneficial due to the addition of sucrose<sup>(133)</sup>.

Although so many studies show that coffee consumption has beneficial effects, a recent study suggests that coffee intake was not associated with lower chances of hepatic steatosis in NASH or alcoholic disease<sup>(136)</sup>. Due to divergences in scientific evidence on coffee consumption and prevention or treatment of NAFLD, further longitudinal and interventionist studies are needed for this evidence.

Like coffee, dark chocolate has antioxidant components, like to epicatequina, that can play a therapeutic role in NAFLD. Consumption of dark chocolate is associated with improved CV risk by reducing lipid peroxidation<sup>(137)</sup>. Loffredo L et al. showed that 40 g of dark chocolate supplementation per day reduced Nicotinamide adenine dinucleotide phosphate (NOX) oxidase in NASH patients<sup>(138)</sup>. In addition, the same authors recently published that the cocoa polyphenols improve endothelial function via Nox2

down-regulation in NASH patients<sup>(139)</sup>. NOX is considered the major cellular source of reactive oxygen species in humans<sup>(140)</sup> and its activation has been associated with liver damage<sup>(141)</sup>.

The supplementation of dark chocolate associated with cocoa and almonds in overweight and obese individuals resulted in favorable effects on lipid and lipoprotein profiles such as LDL, however, dark chocolate and cocoa alone had no effect on vascular health markers and oxidative stress<sup>(142)</sup>. A recent study showed a prebiotic effect of dark chocolate in overweight individuals. Supplementation of 10 g dark chocolate per day increased the abundance of symbiotic bacteria such as *lactobacillus*<sup>(143)</sup>. This modulation in the intestinal microbiota may be a potential therapeutic effect for NAFLD in the future.

## RECOMMENDATIONS FOR TREATMENT OF NAFLD

Lifestyle modification, including diet, exercise, and weight loss has been recommended to treat NAFLD patients. Excessive energy intake, particularly in the form of complex carbohydrates, increased fructose consumption, saturated fats and industrialized products, combined with a sedentary lifestyle have contributed to dysregulated metabolism and consequently leading to obesity and NAFLD. According to American Association for the Study of Liver Diseases and EASL guidelines a combination of a hypocaloric diet (daily reduction by 500–1.000 kcal) and moderate-intensity exercise is likely to provide the best likelihood to lose 7%–10% total weight and sustaining weight loss over time<sup>(118,144)</sup>.

The EASL guidelines recommended adherence to the Mediterranean diet with low-to-moderate fat and moderate-to-high carbohydrate intake and Low-carbohydrate ketogenic diets or high-protein. Besides this, it is suggested avoid fructose-containing beverages and foods. Additionally, recommended to moderate alcohol intake, strictly keep alcohol below the risk threshold (30 g/day, men; 20 g/day, women)<sup>(118)</sup>. Coffee shown the protective in NAFLD, as in liver disease of other etiologies, reducing histological severity and liver-related outcomes<sup>(126)</sup>. There are no liver-related limitations for coffee drinking<sup>(118)</sup>.

The protein intake is essential to improves CV risk factors and insulin sensitivity and decreases the risk of morbidity and mortality<sup>(74)</sup>. The EASL Clinical Practice Guidelines on nutrition in chronic liver disease recommended the optimal daily protein intake should not be lower than 1.2–1.5 g/kg actual body weight/day<sup>(83)</sup>.

Omega-3 fatty acids should not be used as a specific treatment of NAFLD or NASH, however, they might be considered to treat hypertriglyceridemia in NAFLD patients<sup>(144)</sup>.

Nowadays, pharmacological treatment of NAFLD is still limited and it is mostly based on the treatment of comorbidities. The drug therapy is based on understanding the pathogenesis of the disease, focusing on IR, oxidative stress, inflammatory process and on the mechanism involved directly and indirectly in the progression of fibrosis.

Antioxidant drugs have been investigated as promising treatments for NASH. Vitamin E administered at a daily dose of 800 IU/day have improved liver histology in nondiabetic adults with biopsy-proven NASH and therefore might be considered for this population<sup>(145)</sup>. However, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis<sup>(144)</sup>.

N-acetylcysteine (NAC), a glutathione precursor, leads to the reduction of oxidative stress. Some studies using NAC and

metformin have shown benefit in hepatic histology<sup>(146,147)</sup>. International guidelines recommend thiazolidinediones (pioglitazone) and glucagon-like peptide-1 analogues (liraglutide) showing significant results in enzymes and hepatic histology<sup>(118,144)</sup>.

For patients with severe obesity (BMI >35 kg/m<sup>2</sup>) and NASH, bariatric surgery is an option to be considered, as in most of these patients diet and physical activity are not effective and are not capable of achieving significant and sustained weight loss. The metabolic surgery improving obesity and diabetes, reduces liver fat and is likely to reduce NASH progression<sup>(148)</sup>. Prospective data have shown an improvement in all histological lesions of NASH, including fibrosis<sup>(149)</sup>.

More recently, it has been shown that unbalance of the intestinal microbiota (dysbiosis) and its bacterial metabolites may also contribute to the development of NAFLD. Evidence have shown that increased intestinal permeability leads to bacterial translocation and the endotoxins produced by these bacteria increases LPL activity. This augmentation of LPL activity promotes *de novo* fatty acid synthesis, TG production<sup>(150)</sup> and activation of inflammatory Toll-like receptors in hepatocytes<sup>(151)</sup>. Current studies have shown that the use of pre- and probiotics can modulate intestinal microbiota in several diseases<sup>(152)</sup>, including NAFLD<sup>(153,154)</sup>. In addition,

probiotics improved intestinal microbiota composition associated with a reduction in liver inflammation, diminished LPS concentrations, reduction of aminotransferases concentrations<sup>(155,156)</sup>, improvement of inflammatory factors<sup>(157,158)</sup> and also improvement of metabolic parameters of NAFLD, such as, visceral fat, total cholesterol and IR<sup>(159)</sup>.

It is important to note that, although there are several studies showing efficacy of pre- and probiotic supplementation in NAFLD patients, the guidelines do not recommend their supplementation as NAFLD/NASH treatment.

#### Authors' contribution

Oliveira CPMS was responsible for study design. Duarte SMB, Stefano JT, Vanni DS writing the manuscript. Stefano JT, Carrilho FJ and Oliveira CPMS helped on the revision of the manuscript.

#### Orcid

Sebastião Mauro Bezerra Duarte: 0000-0001-6988-7701.  
Jose Tadeu Stefano: 0000-0002-0218-1920.  
Denise Siqueira Vanni: 0000-0002-2624-5116.  
Flair José Carrilho: 0000-0002-7682-3105.  
Claudia P. Oliveira: 000-0002-2848-417X.

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**RESUMO** – A doença hepática gordurosa não alcoólica (DHGNA) afeta aproximadamente de 20% a 30% da população geral sendo prevalente entre os indivíduos obesos. Os fatores de risco associados à DHGNA são: doenças relacionadas à síndrome metabólica, fatores genéticos e meio ambiente. Nesta revisão, fornecemos uma compilação bibliográfica avaliando como as evidências relacionadas aos componentes da dieta, incluindo ingestão calórica, de gorduras, de proteínas, de fibras e de carboidratos, especialmente a frutose, poderiam ser um estímulo para o desenvolvimento e progressão da DHGNA. Foi demonstrado que a dieta é um fator importante para o desenvolvimento da DHGNA e sua associação se estende além do consumo total de calorias.

**DESCRITORES** – Hepatopatia gordurosa não alcoólica. Ingestão de energia. Gorduras na dieta. Carboidratos da dieta. Frutose.

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