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Nonalcoholic Fatty Liver Disease in Metabolic Syndrome, Epidemiology and Pathogenesis

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ABSTRACT

The prevalence of metabolic syndrome varies according to sex, age and demographic situations,being linked to sociocultural, environmental and genetic factors. Central obesity, type 2 diabetes, dyslipidemia and hypertension are widely known risk factors in patients with this syndrome, representing more than one clinical diagnosis, being a pre-morbid condition with a high rate of cardiovascular, renal and hepatic complications. The nonalcoholic fatty liver disease **(NAFLD)** has been considered the hepatic manifestation of the metabolic syndrome, which may progress to cirrhosis and hepatocellular carcinoma, being associated with greater cardiovascular and endocrine-metabolic risk. Thus, it becomes necessary to expand the knowledge of the pathophysiological mechanisms involved in its genesis and progression. Hepatic steatosiscan progress to more advanced forms of the disease from the greater absorption and synthesis of fatty acids, de novo lipogenesis, reduction of the hydrolysis of triglycerides and mitochondrial beta-oxidation of fatty acids, contributing to increased production of free radicals and reactive oxygen species, with the release of many inflammatory mediators. Many cytokines and inflammatory mediators are involved in the pathophysiology of nonalcoholic steatohepatitis **(NASH)**, the most advanced form of hepatic steatosis. However, the most widely studied and described cytokines are adiponectin, leptin, TNF-α, Il-6, which are essential for the understanding and development of new diagnostic modalities and therapeutic strategies.

Keywords: Obesity; Diabetes; Hyperinsulinemia; Oxidative Stress; Nonalcoholic Steatohepatitis; Nonalcoholic Fatty Liver Disease; Metabolic Syndrome

INTRODUCTION

In the spectrum of liver diseases, one has been highlighted as a result of the obesity epidemic, the sedentary lifestyle and the eating habits acquired by the Western culture, which lead to cardiovascular and endocrine-metabolic diseases. The disease in question is the nonalcoholic fatty liver disease **(NAFLD)**, also referred to as nonalcoholic fatty liver, or nonalcoholic fatty disease of the liver. Clinical, experimental and epidemiological studies have described it as the hepatic manifestation of the metabolic syndrome [1,2].

It is an entity of spectral morphological evolution, which exhibits two distinct forms of presentation: hepatic steatosis **(HS)** and nonalcoholic steatohepatitis **(NASH)**. The first state is characterized by lipid accumulation in hepatocytes. This heterotopic fat triggers varying degrees of necroinflammatory phenomena, which corresponds to NASH, a condition associated with the progressive disease, which is the most severe form of the disease [3,4]. With inflammation, cell death and fibrosis, the process can result in the end stage of liver disease, or be a precursor to hepatocellular carcinoma [5].

The four risk factors included in the NCEP/ATP III criteria for diagnosis of MS are present, alone or in combination, especially in patients with NASH [6,7]. Excessive hepatic fat is now recognized as an independent marker for increased cardiovascular risk [5].

In a study of 304 patients with HS, but without overt diabetes, liver biopsy was performed in 163 cases (54%). Of these, 120 patients (73.6%) were classified as having NASH and, in 88% of them, metabolic syndrome was present. Of the patients classified with pure HS, 53% had metabolic syndrome ($P < 0.0001$). The logistic regression analysis of the study confirmed that the presence of metabolic syndrome represented a high risk of NASH among individuals with HS (OR, 3.2; 95% CI, 1.2 to 8.9; $P = 0.026$) [8].

The prevalence of hepatic steatosis has been estimated at 2.8% to 88%, depending on the population and the research methods [9-13]. In the Western world, it is reported between 20%-40% in the adult population and between 10%-20% in the area of Asia, with a tendency to increase. Notwithstanding, it is still difficult to define the exact prevalence, given that NAFLD is often not diagnosed by presenting regular laboratory tests [4], having already been described in nonobese and nondiabetic patients [14].

RISK FACTORS FOR LIVER DISEASE IN METABOLIC SYNDROME

Obesity

Obesity is a global epidemic, with more than 1 billion overweight adults and at least 300 million obese people worldwide [1]. In the United States, it is considered a public health problem and its prevalence has increased significantly over the past 30 years [2,15].

Overweight is the main risk factor for the development of MS. The NHANES III study showed that, according to the ATP III criteria, the following subjects have metabolic syndrome:

- 4.6% of men with normal BMI;
- 22.4% of overweight men;
- 59.6% of obese men;
- 6.2% of women with normal BMI;
- 28.1% of overweight women [16].

The Insulin Resistance Atherosclerosis Study **(IRAS)** showed that the best predictor of MS would be a high waist circumference. In men with waist circumference > 102 cm, the incidence of MS could reach, in 5 years, 46% [17].

Demographic analyses of obese people have added impressive evidence of how ethnic variations can influence the extent and incidence of NAFLD, this condition being common among Hispanic populations, probably due to the high level of obesity in this ethnic group [18].

Despite the high prevalence of other risk factors such as type 2 diabetes, the prevalence of NASH in the African-American population is not as high, which could explain the significantly lower chance of developing serious liver disease compared to Caucasians [19]. In two series of patients with NASH and one with cryptogenic cirrhosis, African-Americans accounted for only 1% and 0.6% of these groups, respectively [20].

Solgaet al. (2005) presented a study in which the NASH was completely absent in obese African-Americans. It is speculated that genetic and environmental factors (e.g., eating habits) can be related to the reduction of the incidence of liver disease in this ethnic population. Little information is available about the prevalence of NASH in Western countries. A Brazilian multicenter epidemiological study involving 1,280 patients with NAFLD described the prevalence of obesity in 44.7% of the studied sample [21].

NASH, however, can be found in 40% to 100% of cases of obesity in adults and in 15% to 25% of children [22,23]. Considering obesity as a growing epidemic, the prevalence and impact of NAFLD make NASH, potentially, the most common cause of advanced liver disease in the coming decades [24].

A clinical series in Korean patients showed that the body mass index **(BMI)** was useful in distinguishing between NASH and simple steatosis. In this study, the BMI of 28.9 was suggested as a threshold for NASH. It was proposed that abdominal fat is directly associated with the evolutionary stage of the disease [25]. In another study published in 2013, 9,159 apparently healthy Korean adults were evaluated, being determined that increased waist circumference was significantly associated with prevalence of NAFLD, insulin resistance and elevated alanine aminotransferase (> 40 IU/L in men and > 35 IU/L in women) [26].

In addition, the BMI is also able to predict the cardiovascular risk and the insulin resistance risk in children [27]. The literature describes the association between obesity and epicardial fat, which, in turn, is consistently associated with coronary artery disease and MS, being found a greater amount of epicardial fat in people with MS [28].

Diabetes and Insulin Resistance

Diabetes mellitus is a complex disease caused by genetic and environmental factors. Many diabetic patients have insulin resistance and MS even before the diagnosis of diabetes, which has been proven by several studies demonstrating the strong association between MS and the risk of developing diabetes [14,29].

Insulin resistance and hyperinsulinemia are the laboratory findings most associated with the presence of NAFLD in a large series of patients, even in lean subjects with normal glucose tolerance [2,30,31].

The meta-analysis published by Ford (2005) demonstrated that the relative risk for diabetes was 2.99 (95% CI, 1.96 to 4.57) when the MS definition was performed according to the NCEP criteria [32]. The Framingham Offspring study used the modified NCEP criteria do define MS and determine the prevalence of diabetes in a cohort of 3323 middle-aged individuals, with follow-up of 8 years. The relative risk for diabetes in men was 6.92 (95% CI, 4.47 to 10.81), and in women, 6.90 (95% CI, 4.34 to 10.94). Moreover, it showed that the population attributable risk for diabetes associated with MS was 62% in men and 47% in women [33].

According to Jimbaet al. (2005), the prevalence of hepatic steatosis is increased in subjects with glucose intolerance and in those newly diagnosed with diabetes, in the proportion of 43% and 62%, respectively [34]. In a prospective study of 100 patients with type 2 diabetes, the incidence of hepatic steatosis was 49%, which confirms this strong independent risk factor for NAFLD [31].

In Brazil, the prevalence of metabolic syndrome and diabetes in patients with NAFLD was estimated at 41.3% and 22.7%, respectively [35].

Targheret al. (2010) reported increased prevalence of NAFLD and its association with cardiovascular disease in diabetic patients, regardless of the association with other factors [36].

The frequency of risk factors associated with NAFLD in patients with type 2 diabetes mellitus was studied, and almost half of patients with type 2 diabetes mellitus were associated with liver disease, especially those with increased BMI, transaminases, GGT, uric acid, TNF- α, insulin and HOMA-IR, compared to subjects without NAFLD [37].

The possible pathogenic link between diabetes and NASH involves advanced glycation end products. These products constitute a wide variety of substances formed by aminocarbonyl interactions ofnonenzymatic nature between reducing sugars or oxidized lipids andproteins, nucleic acids or aminophospholipids [38].

The formation of these adducts occurs at high rates of type 2 diabetes, compared to healthy controls or patients with simple steatosis [39]. The interaction of these products with the surface receptor of the stellate cell has been linked to the induction of oxidative stress and consequent increase of potential fibrogenic responses in cultures of stellate cells [40].

The relationship between these advanced glycation end products and cell surface receptors results in intermediate oxygen reactive species, which play an important role in the pathogenesis of the disease in diabetic patients [25].

Hypertension

According to the I Brazilian Guideline for Diagnosis and Treatment of Metabolic Syndrome, the arterial hypertension, particularly the systolic hypertension, also represents an independent predictor of NAFLD [41].

Cotrimet al. (2011) confirmed these data when reporting a 64% prevalence of hypertension in patients with NAFLD [35]. Moreira et al. (2014) conducted a cross-sectional study of 1369 individuals older than 18 years. In this study, the prevalence of MS was 60.4% (95% CI: 54.2% to 67.2%) among hypertensive patients, while only 9.5% (95% CI: 7.0% to 13.0%) of normotensive individuals were affected by the syndrome. The ratioof the prevalence of MS in hypertensive/ normotensive patients was 6.32 (95% CI: 4.57 to 8.75; p <0.0005) [42].

Hsiao et al. (2007) demonstrated that the presence of severe hepatic steatosis was significantly correlated with the prevalence and severity of hypertension, serum glucose levels and triglyceride levels [43]. Moreover, a study in nonobese and nondiabetic patients with primary hypertension demonstrated that the prevalence of hepatic steatosis can double compared to the control group [44].

Abnormalities in the left ventricular diastolic function have been described in patients with hepatic steatosis, as well as a more severe coronary artery disease, characterized by vulnerable plaques, although observed in small cohorts [45].

It is known that hepatic steatosis represents a strong independent risk factor for cardiovascular disease and may play a central role in the cardiovascular risk associated with MS [46].

Dyslipidemia

Hypertriglyceridemia and hyperlipemia are present in, respectively, 64% and 66.8% of patients with hepatic steatosis [35].

Iincreased levels of triglycerides and low-density lipoproteins **(LDL)**, combined with low levels of high-density lipoproteins **(HDL)**, is the central pathway for the development of multiple comorbidities associated with obesity, such as NAFLD and cardiovascular diseases. In turn, hepatic steatosis also represents an important role in increasing the risk of cardiovascular diseases, since, in these patients, the liver produces various atherogenic factors, such as cytokines and LDL [35,47].

Smoking

Smoking is a major cause of morbidity and mortality worldwide. It represents a risk factor for metabolic and cardiovascular diseases. This practice reduces insulin sensitivity or induces its resistance, in addition to increasing cardiovascular risk factors, such as high plasma triglyceride levels. It also decreases the high-density lipoprotein and causes hyperglycemia. Several studies show that smoking is associated with metabolic abnormalities and increases the risk of metabolic syndrome. Cenaet al. (2013) conducted a cross-sectional study to estimate the prevalence of metabolic syndrome in a group of light and heavy/moderate smokers who want to quit smoking,finding a MS prevalence of 52.1% (57.3% and 44.9% for men and women, respectively) [48].

PATHOGENESIS OF LIVER INJURY IN METABOLIC SYNDROME

Several factors are likely involved in the pathogenic mechanisms, creating together a network of interactions that participate in both the development and progression of NAFLD [49,50]. The pathophysiological mechanisms of NAFLD are still under investigation. Nonetheless, the accumulation of triglycerides in hepatocytes, as a result of insulin resistance, is considered the first step in the pathogenic model [50,51].

The oxidative stress, resulting from mitochondrial oxidation of fatty acids, and the expression of inflammatory cytokines have been identified as secondary causative factors that lead to liver injury, fibrosis and inflammation [3,49, 52-54]. The intrahepatic accumulation of fatty acids is closely associated with insulin resistance and increases the susceptibility of hepatocytes to attacks such as oxidative stress, mitochondrial dysfunction, overproduction and release of secondary pro-inflammatory cytokines, as well as the endotoxin-mediated activation of the innate immune response. The increased susceptibility to these factors may explain the progression of NAFLD to NASH [51, 53-55].

Insulin resistance plays a central role in the pathogenesis of NAFLD. It is defined as an inappropriate response to the physiological effects of the insulin circulating in specific target tissues, in the skeletal muscle, in the liver and in the adipose tissue. Inflammatory cytokines activate various kinases, such as serine kinase IKKβ, mTOR/S6 kinase and mitogen-activated protein kinase **(MAPK)**, as well as suppressor of cytokine signaling **(SOCS)** proteins, which interfere with the action and signaling pathway of insulin in adipocytes and hepatocytes [56]. Molecular alterations in insulin signaling ultimately result in the accumulation of hepatic triglycerides. In the skeletal muscle, the peripheral insulin resistance affects, mainly,a large proportion of the total glucose uptake [57].

In the adipose tissue, this resistance decreases the lipogenic action of insulin, with consequent release of nonesterifiedfatty acids. In other words, insulin resistance increases the lipolysis of triglycerides and inhibits the esterification of free fatty acids in the adipose tissue. The result

is an increase in the serum levels of free fatty acids, which are absorbed by the liver [58]. High concentrations of plasma glucose and fatty acids result in increased hepatic uptake of lipids. This increased supply of fatty acids in the liver impairs mitochondrial β-oxidation, by stress in the enzymatic system. As a result, these substances accumulate in the hepatocyte, determining the onset of hepatic steatosis [58].

Additionally, insulin resistance also inhibits the alternative metabolism of free fatty acids **(FFAs)**, by oxidation. The hepatic export of very-low-density lipoprotein **(VLDL)** may be inhibited with decreasing synthesis of apolipoprotein B (apoB) and lower association thereof with triglycerides, by the microsomal triglyceride transfer protein **(MTP)** [59].

The Role of the Adipose Tissue and Cytokines

Cytokines are soluble molecules which are involved in intracellular communication, and are produced by a wide variety of cells in the body, including hepatic cells. They consist of several subfamilies, including interferon, interleukins, tumor necrosis factors **(TNF)**, transforming growth factor **(TGF)**, colony-stimulating factors and chemokines [60]. Furthermore, they may mediate many fundamental biological processes, including body growth, adiposity, lactation, hematopoiesis, as well as inflammation and immunity. They are implicated in various disorders, such as arthritis, atherosclerosis, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, as well as NAFLD [61].

Under physiological conditions, the hepatic production of constitutive cytokines is absent or minimal in the liver. However, pathological stimuli, such as lipid accumulation, induce the hepatic cells to produce these inflammatory molecules. Cytokines may play an active role in the development and progression of NAFLD through stimulation of the hepatic inflammation, cell necrosis, apoptosis, and fibrosis induction (Table 1). Notwithstanding, they are also essential for hepatic regeneration [61].

The total adipose tissue of mammals is composed of two distinct functional types: white and brown. The first is the energy storage site,where occurs the release of hormones and cytokines that modulate the body's metabolism and the insulin resistance. Their accumulation is associated with obesity. On the other hand, the brown adipose tissue, rich in mitochondria, is important for energy expenditure in the form of thermogenesis, since it can modulate the susceptibility to body weight gain. Physiologically, it is more concentrated in children, but can also be found in adults [62,63].

It is believed that the visceral adipose tissue plays a crucial role in the pathogenesis of hepatic steatosis, once it is involved in the production of more adipocytokines such as Tumor Necrosis Factor-alpha **(TNF-α)**, resistin and adiponectin, involved in insulin-resistance inducing and varying degrees of inflammation. Several therapeutic approaches, such as weight loss and the use of insulin sensitizers, are used in order to diminish the release of adipose tissue-derived cytokines, thus reducing the supply of free fatty acids to the liver [64].

Leptin is an adipocyte-derived peptide hormone related to food intake and energy expenditure, controlling body weight and satiety, by the hypothalamic negative feedback mechanism [65-67]. Its serum levels are increased in obesity, as a result of what has been characterized as leptin resistance, a phenomenon that may already be present in obese children [68,69].

Adiponectin is the most abundant cytokine, exclusively synthesized by the adipose tissue [68,70]. It acts by stimulating the secretion of anti-inflammatory cytokines, such as interleukin-10 **(IL-10)**, for example, which blocks the activation of the nuclear factor kaapaβ **(NFkβ)** and inhibits the release of TNF-α and interleukin-6 [71]. In the liver, it acts through the MAPK, through the peroxisome proliferator-activated receptor-alpha **(PPAR-α)** and through theinhibition of the Toll-like receptor 4 **(TLR-4)** signaling. There is evidence that adiponectin reduces hepatic and systemic insulin resistance,attenuates necroinflammationand hepatic fibrosis and is considered an indicator of severity for NAFLD [72-74]. Drugs that increase adiponectin levels can be considered therapeutic targets for NAFLD. The identification of molecules involved in the signaling pathways of adiponectin and the potential role of the resistance of their receptors in NASH have been little investigated and can be promising in the treatment of the disease [70,74].

Resistin is a cytokine secreted by the adipose tissue and the macrophages, acting probably as an insulin antagonist, contributing to the development of glucose intolerance in obese individuals. Evidence suggests a pro-inflammatory action of this cytokine, stimulating TNF-α and interleukin-12 **(IL-12)** in macrophages, through NFκβ20. In humans, its levels are high and can serve to discriminate steatosis from steatohepatitis [75].

TNF-α is produced by B lymphocytes, T **(Natural Killer)** lymphocytes, macrophages and fibroblasts, and plays a central role in the evolution of NAFLD to NASH28. This denomination refers to its biological property of inducing hemorrhagic necrosis in certain tumors. When synthesized in the inactive form, it becomes toxic in the tissue, inducing necrosis and angiogenesis. At low concentrations, TNF-α stimulates cell growth. On the other hand, at high concentrations, it inhibits cell growth induced by other cytokines, whose levels are associated with obesity and insulin resistance in animal and human models [68]. This cytokine has lipogenic and fibrogeniceffect, mediated by paracrine mechanism, which involves activation of Kupffer cells with secretion of soluble mediators, stimulating the transformation of Ito cells into myofibroblasts, which, in turn, begin to synthesize components of the extracellular matrix, and can be used in the diagnosis of NAFLD/NASH [76].

Oxidative stress is one of the agents responsible for the production of pro-inflammatory cytokines, among which are highlighted: TNF-α, transforming growth factor-alpha, transforming growth factor-beta **(TGF-α** and **TGF-β)**, interleukin-6 **(IL-6)**, interleukin-8 **(IL-8)**, NFκB and adiponectin. These cytokines are produced by lymphocytes and Kupffer cells via mechanisms mediated by free radicals and may act by altering the permeability of the mitochondrial membrane and inhibiting the respiratory chain [77,78].

Genetic polymorphism has been reported in patients with NAFLD and NASH. Mice genetically deficient for the TNF-α receptor were resistant to the development of NASH [68]. Although the inhibition of TNF-α in animal models of NAFLD has encouraged therapeutic perspectives in humans, the role of this cytokine remains under investigation [76]. In some patients, TNF-α levels were shown to be higher in obese than in lean individuals, and were correlated with insulin resistance. Moreover, a positive correlation has been shown between the degree of hepatic fibrosis and the circulating levels of TNF-α in patients with NASH [79].

Also known as nitrogen monoxide and azote monoxide, nitric oxide **(NO)** is a soluble, highly lipophilic gas, synthesized by endothelial cells, macrophages and certain brain neurons. NO is a enzyme product formed from L-arginine under the catalytic action of the nitric oxide synthase **(NOS)** enzyme, which produces equimolar concentrations of L-citrulline and NO. It is an important intracellular and extracellular signaling molecule, which acts by inducing guanylatecyclase and by producing cyclic guanosine monophosphate **(cGMP)**, producing, among other effects, smooth muscle relaxation, which causes, such as biological actions, vasodilation and bronchodilation [80].

Nitric oxide is capable of potentiating the cytotoxicity caused by oxidative stress, through the reaction between the superoxide anion and the formation of nitrotyrosine, whose intrahepatic accumulation is associated with the severity of steatohepatitis, strongly suggesting that the oxidative injury is implicated in the pathogenesis of this disease. Studies in obese mice have shown that in severe steatosis, hepatic injury is mediated by TNF-α and Interferon-γ. This demonstrates that inflammatory cytokines are directly involved in the positive regulation mediated by induced nitric oxide synthase **(iNOS)**. The expression of receptors for iNOS, by immunohistochemical method, was strongly positive in the hepatocytes of mice and rats fed with hyperlipidemic diet [80].

In the hepatic vasculature, insulin resistance can be detected earlier than inflammation or any other symptom in NAFLD. The administration of a hyperlipidemic diet induces insulin resistance in the endothelium of hepatic sinusoids, being mediated, at least partly, by the upregulation of iNOS [81]. Current evidence has highlighted the role of nitric oxide as a therapeutic target for its participation in the control ofglycemic and lipemic metabolism, in addition to the already known actions on neuronal transmission, vascular relaxation, immunological modulation and cytotoxicity. The blockade of iNOS decreased adiposity and improved insulin resistance in an experimental obese rat model, using animals fed with lipid diet [82].

Among the NO synthase isoforms, inducible isoforms have been associated with responses to a variety of inflammatory stimuli, such as those produced by pro-inflammatory cytokines and bacterial endotoxins. The iNOS modulates the secretion of large amounts of NO, and can contribute to the development of obesity related to glucose intolerance [80,83]. A key role for iNOS in the pathogenesis of obesity related to insulin resistance has been substantiated by observations that the interruption of the iNOS target protects against muscle insulin resistance and improves the

systemic action of insulin in obese mice [84]. The blockade of iNOS, in mice, also protected against adverse effects associated with high fat content in insulin resistant states and in a genetically determined obesity model [85,86]. Moreover, this enzyme was induced in the skeletal muscle and the adipose tissue of type 2 diabetic patients, whose expression correlated with the occurrence of insulin resistance and obesity [87,88].

Fetuin is a glycoprotein produced by the liver and secreted in the plasma. It acts physiologically as an inhibitor of ectopic deposition of calcium. Fetuin-A has the ability to bind and inhibit the receptor and the signaling pathway of insulin in the skeletal muscle and in hepatocytes [89]. In humans, elevated serum levels of this glycoprotein have been associated with obesity, insulin resistance, diabetes and NAFLD. Fetuin-A and adiponectin act together to regulate insulin resistance, and their serum levels are inversely correlated. Studies in cultured adipocytes showed that this glycoprotein inhibits the adiponectin's messenger ribonucleic acid **(mRNA)** coding [90].

Visfatin, which is another adipocytokine, richly found in the visceral adipose tissue, presents immunomodulatory properties, promoting B cell maturation and activation of leukocytes, synthesis and adhesion of molecules, in addition to production of pro-inflammatory cytokines. It is an insulin-mimetic molecule that reduces glucose levels and regulates energy balance [91,92]. A recent study showed a significant reduction in visfatin levels in the adipose tissue of patients with NAFLD, highlighting the participation of this adipocytokine in the pathogenic scenario of the disease [92].

Apelin is an adipocytokine whose plasma concentration is increased in obesity, and is correlated with insulin resistance and hyperinsulinemia. In the cardiovascular system, it induces endothelial relaxation mediated by nitric oxide, lowering blood pressure, associated with a positive inotropic activity [93].

Table 1: Role of different cytokines in NAFLD.

Cytokine	Biological activity in experimental models	Biologicalactivity in humans
Leptin	Pro-inflammatory Stellate cell activation	Does notincreasein NAFLD, nocorrelationwithhistology
Adiponectin	Anti-inflammatory	Lower in NAFLDthan in controls; inverserelationshipwithfibrosis
Resistin	Pro-inflammatory	Increased in NASH, possibly associated with fibrosis
TNF-α	Pro-inflammatory	Increased in NASH, correlateswith fibrosis
IL-6	Uncertain	Ongoingstudies
Visfatin	Uncertain	Ongoingstudies

Source: Adaptedfrom Tsochatzis, et al., 2009.

Role of Intestinal Microbiota

The transition from NAFLD to NASH is governed, in part, by systemic factors, bacterial products and TLR ligands, and metabolic products reaching the liver through the portal vein may be involved in this process. NAFLD is associated with increased permeability of the intestinal epithelium to the bacteria and their components, and can be detected in the portal vein and in the systemic circulation [50,52].

In obesity, the tuned communication between the intestinal epithelium and the intestinal microbiota becomes unbalanced, as a result of imbalances in the intestinal microbial composition. The intestinal microbiota communicates with the epithelial cells via surface molecules **(TLR4, CD14, TLR5)** and also via intracellular molecules **(TLR9, NLRP3/6-ASC-caspase 1 inflammasome)**, through specific receptors/ligands [94].

Recent evidence suggests that the microbiota and/or its products not only affect the liver through the portal vein, but also the peripheral organs, such as the adipose tissue, leading to the so called metabolic infection, important factor of NAFLD/NASH [52].

CONCLUSION

Metabolic syndrome contributes to the pathogenesis of NAFLD by determining the accumulation of fat in the hepatic tissue, developed from increased supply of free fatty acids, decreased oxidation, increased hepatic lipogenesis and reduced hepatic export of triglycerides via VLDL, resulting from peripheral insulin resistance and hyperinsulinemia.

The pathogenesis of NAFLD is multifactorial, and the development of NASH represents a complex process not fully understood. It has been suggested that its development occurs in multiple parallel stages. The cytokines most widely studied and described in the pathogenesis of NASH are adiponectin, leptin, TNF-α, IL-6, visfatin. Knowledge of these inflammatory mediators is of fundamental importance for the development of new diagnostic modalities and therapeutic strategies. The pathogenesis of NAFLD is multivariate, however, recent studies have highlighted the role of intestinal microbiota in the pathogenesis of NASH. Genetic polymorphisms have been described as determining factors for the development of the disease.

References

1. Boppidi H, Daram SR. Nonalcoholic fatty liver disease: hepatic manifestation of obesity and the metabolic syndrome. Postgrad Med. 2008; 120: 01-07.
2. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes. 2001; 50: 1844-1850.
3. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology. 1998; 114: 842-845.
4. Abenavoli L, Milic N, Di Renzo L, Preveden T, Medic-Stojanoska M, et al. Metabolic aspects of adult patients with nonalcoholic fatty liver disease. World Journal of Gastroenterology. 2016; 22: 7006-7016.
5. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckerl RH, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005; 112: 2735-2752.

6. Meigs JB, Nathan DM, Wolfsdorf JI, Mulder JE. The metabolic syndrome (insulin resistance syndrome or syndrome X). UpToDate. Disponível em. 2015.

7. Sheth SG, Chopra S. Epidemiology, clinical features, and diagnosis of nonalcoholic fatty liver disease in adults. UptoDate. Disponível em. 2015.

8. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology. 2003; 37: 917-923.

9. Angulo P. GI epidemiology: nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2007; 25: 883-889.

10. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology. 2005; 42: 44-52.

11. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol. 2003; 98: 960-967.

12. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. J Clin Gastroenterol. 2006; 40: S5-10.

13. Jimba S, Nakagami T, Takahashi M, Wakamatsu T, Hirota Y, et al. Prevalence of nonalcoholic fatty liver disease an d its association with impaired glucose metabolism in Japanese adults. Diabet Med. 2005; 22: 1141-1145.

14. Kim HJ, Kim HJ, Lee KE, Kim DJ, Kim SK, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. Arch Intern Med. 2004; 164: 2169-2175.

15. Baskin ML, Ard J, Franklin F, Allison DB. Prevalence of obesity in the United States. Obes Rev. 2005; 6: 5-7.

16. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, et al. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med. 2003; 163: 427-436.

17. Palaniappan L, Carnethon MR, Wang Y, Hanley AJ, Fortmann SP, et al. Predictors of the incident metabolic syndrome in adults: the Insulin Resistance Atherosclerosis Study. Diabetes Care. 2004; 27: 788-793.

18. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004; 40: 1387-1395.

19. Giday SA, Ashiny Z, Naab T, Smoot D, Banks A. Frequency of nonalcoholic fatty liver disease and degree of hepatic steatosis in African-American patients. J Natl Med Assoc. 2006; 98: 1613-1615.

20. Caldwell SH, Harris DM, Patrie JT, Hespenheide EE. Is NASH underdiagnosed among African Americans? Am J Gastroenterol. 2002; 97: 1496-1500.

21. Solga SF, Clark JM, Alkhuraishi AR, Torbenson M, Tabesh A, et al. Race and comorbid factors predict nonalcoholic fatty liver disease histopathology in severely obese patients. Surg Obes Relat Dis. 2005; 1: 6-11.

22. Tarantino G, Saldalamacchia G, Conca P, Arena A. Non-alcoholic fatty liver disease: further expression of the metabolic syndrome. J Gastroenterol Hepatol. 2007; 22: 293-303.

23. Povel CM, Boer JM, Reiling E, Feskens EJ. Genetic variants and the metabolic syndrome: a systematic review. Obes Rev. 2011; 12: 952-967.

24. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011; 34: 274-285.

25. Rivera CA. Risk factors and mechanisms of non-alcoholic steatohepatitis. Pathophysiology. 2008; 15: 109-114.

26. Ju, Deok Yun. The Influence of Waist Circumference on Insulin Resistance and Nonalcoholic Fatty Liver Disease in Apparently Healthy Korean Adults. Clinical and molecular Hepatology. 2013; 19: 140-147.

27. Kim C, Kim B, Joo N, Park Y, Lim H, et al. Determination of the BMI threshold that predicts cardiovascular risk and insulin resistance in late childhood. Diabetes Res Clin Pract. 2010; 88: 307-313.

28. Bertaso AG, Bertol D, Duncan BB, Foppa M. Gordura epicárdica: definição, medidas e revisão sistemática dos principais desfechos. Arquivos Brasileiros de Cardiologia. 2013; 101: 18-28.

29. Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, et al. DECODE Study Group. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. Arch Intern Med. 2004; 164: 1066-1076.

30. Bloomgarden ZT. Definitions of the insulin resistance syndrome: the 1st World Congress on the Insulin Resistance Syndrome. Diabetes Care. 2004; 27: 824-830.

31. Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, et al. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. J Gastroenterol Hepatol. 2004; 19: 854-858.

32. Ford ES. Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: A summary of the evidence. Diabetes Care. 2005; 28: 1769-1778.

33. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation. 2005; 112: 3066-3072.

34. Jimba S, Nakagami T, Takahashi M, Wakamatsu T, Hirota Y, et al. Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. Diabet Med. 2005; 22: 1141-1145.

35. Cotrim HP, Parise ER, Oliveira CP, Leite N, Martinelli A, et al. Nonalcoholic fatty liver disease in Brazil. Clinical and histological profile. Ann Hepatol. 2011; 10: 33-37.

36. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, et al. Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease in patients with type 1 diabetes. J Hepatol. 2010; 53: 713-718.

37. Ferreira VS, Pernambuco RB, Lopes EP, Morais CN, Rodrigues MC, et al. Frequency and risk factors associated with non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus. Arq Bras Endocrinol Metabol. 2010; 54: 362-368.

38. Monnier VM. Intervention against the Maillard reaction in vivo. Arch Biochem Biophys. 2003; 419: 1-15.

39. Hyogo H, Yamagishi S, Iwamoto K, Arihiro K, Takeuchi M, et al. Elevated levels of serum advanced glycation end products in patients with non-alcoholic steatohepatitis. J Gastroenterol Hepatol. 2007; 22: 1112-1119.

40. Fehrenbach H, Weiskirchen R, Kasper M, Gressner AM. Up-regulated expression of the receptor for advanced glycation end products in cultured rat hepatic stellate cells during transdifferentiation to myofibroblasts. Hepatology. 2001; 34: 943-952.

41. Souza MR, Diniz Mde F, Medeiros-Filho JE, Araújo MS. Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. Arq Gastroenterol. 2012; 49: 89-96.

42. Moreira GC, Cipullo JP, Ciorlia LAS, Cesarino CB, Vilela-Martin JF. Prevalence of Metabolic Syndrome: Association with Risk Factors and Cardiovascular Complications in an Urban Population. PLoS ONE. 2014; 9: e105056.

43. Hsiao PJ, Kuo KK, Shin SJ, Yang YH, Lin WY, et al. Significant correlations between severe fatty liver and risk factors for metabolic syndrome. J Gastroenterol Hepatol. 2007; 22: 2118-2123.

44. Donati G, Stagni B, Piscaglia F, Venturoli N, Morselli-Labate AM, et al. Increased prevalence of fatty liver in arterial hypertensive patients with normal liver enzymes: role of insulin resistance. Gut. 2004; 53: 1020-1023.

45. Tarquini R, Lazzeri C, Boddi M, Marra F, Abbate R, et al. Non-alcoholic fatty liver disease: a new challenge for cardiologists. G Ital Cardiol (Rome). 2010; 11: 660-669.

46. Lu H , Liu H , Hu F , L Zou , Luo S , et al. Independent Association between Nonalcoholic Fatty Liver Disease and Cardiovascular Disease: A Systematic Review and Meta-Analysis. Int J Endocrinol. 2013; 2013:7.

47. FonTacer K, Rozman D. Nonalcoholic Fatty Liver Disease: Focus on Lipoprotein and Lipid Deregulation. Journal of Lipids. 2011: 14.

48. Cena H, Tesone A, Niniano R, Cerveri I, Roggi C, et al. Prevalence rate of Metabolic Syndrome in a group of light and heavy smokers. Diabetol Metab Syndr. 2013; 5: 28.

49. Day CP. Genetic and environmental susceptibility to non-alcoholic fatty liver disease. Dig Dis. 2010; 28: 255-260.

50. Schnabl B, Brenner DA. Interactions Between the Intestinal Microbiome and Liver Diseases. Gastroenterology. 2014.

51. Podrini C, Borghesan M, Greco A, Pazienza V, Mazzoccoli G, et al. Redox homeostasis and epigenetics in non-alcoholic fatty liver disease (NAFLD). Curr Pharm Des. 2013; 19: 2737-2746.

52. Takaki A, Kawai D, Yamamoto K. Multiple hits, including oxidative stress, aspathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). Int J Mol Sci. 2013; 14: 20704-20728.

53. Sumida Y, Niki E, Naito Y, Yoshikawa T. Involvement of free radicals and oxidative stress in NAFLD/NASH. Free Radic Res. 2013; 47: 869-880.

54. Begriche K, Massart J, Robin MA, Bonnet F, Fromenty B. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. Hepatology. 2013; 58: 1497-1507.

55. Csak T. Fatty acids and endotoxin activate inflammasome in hepatocytes which release danger signals to activate immune cells in steatohepatitis. Hepatology. 2011; 54: 133-144.

56. Tanti JF, Ceppo F, Jager J, Berthou F. Implication of inflammatory signaling pathways in obesity-induced insulin resistance. Front Endocrinol (Lausanne). 2013; 3: 181.

57. Leavens KF, Birnbaum MJ. Insulin signaling to hepatic lipid metabolism in health and disease. Crit Rev Biochem Mol Biol. 2011; 46: 200-215.

58. Tessari P, Coracina A, Cosma A, Tiengo A. Hepatic lipid metabolism and non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis. 2009; 19: 291-302.

59. Tilg H. The role of cytokines in non-alcoholic fatty liver disease. Dig Dis. 2010; 28: 179-185.

60. Marra F, Bertolani C. Adipokines in liver diseases. Hepatology. 2009; 50: 957-969.

61. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med. 2009; 360: 1509-1517.

62. Cypess AM, Kahn CR. The role and importance of brown adipose tissue in energy homeostasis. Curr Opin Pediatr. 2010; 22: 478-484.

63. Giorgio V, Graziano FPF, Nobili V. Pediatric non alcoholic fatty liver disease: old and new concepts on development, progression, metabolic insight and potential treatment targets BMC Pediatr. 2013; 13: 40.

64. Singh A, Wirtz M, Parker N, Hogan M, Strahler J, et al. Leptin mediated changes in hepatic mitochondrial metabolism, structure, and protein levels. Proc Natl Acad Sci U S A. 2009; 106: 13100-13105.

65. Minokoshi Y, Toda C, Okamoto S. Regulatory role of leptin in glucose and lipid metabolism in skeletal muscle. Indian J Endocrinol Metab. 2012; 16: S562-568.

66. Farooqi IS, O'Rahilly S. Leptin: a pivotal regulator of human energy homeostasis. Am J Clin Nutr. 2009; 13: 980-984.

67. Tsochatzis EA, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. Mediators of inflammation. 2009: 8.

68. Stringer DM, Sellers EA, Burr LL, Taylor CG. Altered plasma adipokines and markers of oxidative stress suggest increased risk of cardiovascular disease in First Nation youth with obesity or type 2 diabetes mellitus. Pediatr Diabetes. 2008; 13: 269-277.

69. Buechler C, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. World J Gastroenterol. 2011; 17: 2801-2811.

70. Moschen AR, Wieser V, Tilg H. Adiponectin: key player in the adipose tissue-liver crosstalk. Curr Med Chem. 2012; 19: 5467-5473.

71. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism. 2011; 60: 313-326.

72. Polyzos SA, Kountouras J, Zavos C, Tsiaousi E. The role of adiponectin in the pathogenesis and treatment of non-alcoholic fatty liver disease. Diabetes Obes Metab. 2010; 12: 365-383.

73. Finelli C, Tarantino G. What is the role of adiponectin in obesity related non-alcoholic fatty liver disease? World J Gastroenterol. 2013; 19: 802-812.

74. Senates E, Colak Y, Yesil A, Coskunpinar E, Sahin O, et al. Circulating resistin is elevated in patients with non-alcoholic fatty liver disease and is associated with steatosis, portal inflammation, insulin resistance and nonalcoholic steatohepatitis scores. Minerva Med. 2012; 103: 369-376.

75. Hijona E, Hijona L, Arenas JI, Bujanda L. Inflammatory mediators of hepatic steatosis. Mediators Inflamm. 2010; 2010: 837419.

76. Moschen AR, Kaser S, Tilg H. Non-alcoholic steatohepatitis: a microbiota-driven disease. Trends Endocrinol Metab. 2013; 24: 537-545.

77. Zahran WE, Salah El-Dien KA, Kamel PG, El-Sawaby AS. Efficacy of Tumor Necrosis Factor and Interleukin-10 Analysis in the Follow-up of Nonalcoholic Fatty Liver Disease Progression. Indian J Clin Biochem. 2013; 28: 141-146.

78. Shifflet A, Wu GY. Non-alcoholic steatohepatitis: an overview. J Formos Med Assoc. 2009; 108: 4-12.

79. Ha SK, Chae C. Inducible nitric oxide distribution in the fatty liver of a mouse with high fat diet-induced obesity. Exp Anim. 2010; 59: 595-604.

80. Pasarín M, Abraldes JG, Rodríguez-Vilarrupla A, La Mura V, García-Pagán JC, et al. Insulin resistance and liver microcirculation in a rat model of early NAFLD. J Hepatol. 2011; 55: 1095-1102.

81. Dallaire P, Bellmann K, Laplante M, Gélinas S, Centeno-Baez C, et al. Obese mice lacking inducible nitric oxide synthase are sensitized to the metabolic actions ofperoxisome proliferator-activated receptor-gamma agonism. Diabetes. 2008; 57: 1999-2011.

82. Carvalho-Filho MA, Ueno M, Carvalheira JB, Velloso LA, Saad MJ. Targeted disruption of iNOS prevents LPS-induced S-nitrosation of IRbeta/IRS-1 and Akt and insulin resistance in muscle of mice. Am J Physiol Endocrinol Metab. 2006; 291: 476-482.

83. Tsuchiya K, Sakai H, Suzuki N, Iwashima F, Yoshimoto T, et al. Chronic blockade of nitric oxide synthesis reduces adiposity and improves insulin resistance in high fat-inducedobese mice. Endocrinology. 2007; 148: 4548-4556.

84. Noronha BT, Li JM, Wheatcroft SB, Shah AM, Kearney MT. Inducible nitric oxide synthase has divergent effects on vascular and metabolic function in obesity. Diabetes. 2005; 54: 1082-1089.

85. Fujimoto M, Shimizu N, Kunii K, Martyn JA, Ueki K, et al. A role for iNOS in fasting hyperglycemia and impaired insulin signaling in the liver of obese diabetic mice. Diabetes. 2005; 54: 1340-1348.

86. Engeli S, Janke J, Gorzelniak K, Böhnke J, Ghose N, et al. Regulation of the nitric oxide system in human adipose tissue. J Lipid Res. 2004; 45: 1640-1648.

87. Torres SH, De Sanctis JB, de L Briceño M, Hernández N, Finol HJ. Inflammation and nitric oxide production in skeletal muscle of type 2 diabetic patients. J Endocrinol. 2004; 181: 419-427.

88. Memoli B, De Bartolo L, Favia P, Morelli S, Lopez LC, et al. Fetuin-A gene expression, synthesis and release in primary human hepatocytes cultured in a galactosylated membrane bioreactor. Biomaterials. 2007; 28: 4836-4844.

89. Ix JH, Sharma K. Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, andAMPK. J Am Soc Nephrol. 2010; 21: 406-412.

90. Kukla M, Mazur W, Buldak RJ, Zwirska-Korczala K. Potential role of leptin, adiponectin and three novel adipokines-visfatin, chemerin and vaspin-in chronichepatitis. Mol Med. 2011; 17: 1397-1410.

91. Gaddipati R, Sasikala M, Padaki N, Mukherjee RM, Sekaran A, et al. Visceral adipose tissue visfatin in nonalcoholic fatty liver disease. Ann Hepatol. 2010; 9: 266-270.

92. Auguet T, Terra X, Porras JA, Orellana-Gavaldà JM, Martinez S, et al. Plasma visfatin levels and gene expression in morbidly obese women with associated fatty liver disease. Clin Biochem. 2013; 46: 202-208.

93. Aktas B, Yilmaz Y, Eren F, Yonal O, Kurt R, et al. Serum levels of vaspin, obestatin, and apelin-36 in patients with nonalcoholic fatty liver disease. Metabolism. 2011; 60: 544-549.

94. Sawada K, Ohtake T, Hasebe T, Abe M, Tanaka H, et al. Augmented hepatic Toll-like receptors by fatty acids trigger the pro-inflammatory state of non-alcoholic fatty liver disease in mice. Hepatol Res. 2014; 44: 920-934.