

## REVIEW

# Nonalcoholic fatty liver disease and metabolic syndrome: a concise review

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### Abstract

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological entity increasingly recognized as a major health burden in developed countries. In the last decade, several studies have independently provided evidence for a strong association between NAFLD and each component of the metabolic syndrome, including central obesity, hyperglycemia, dyslipidemia, and hypertension. This article focuses on epidemiological, clinical, pathogenic and therapeutic aspects, which link these two syndromes.

**Keywords:** NAFLD, NASH, metabolic syndrome, insulin resistance, adiponectin.

### ☐ Nonalcoholic Fatty Liver Disease: past and present context

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological entity increasingly recognized as a major health burden in developed countries, with an estimated prevalence of 20% to 40% in general population and significantly higher in certain individuals, such as those with obesity or other features of the metabolic syndrome [1–3].

NAFLD encompasses a spectrum of liver lesions occurred in the absence of significant alcohol intake and characterized by histological abnormalities that range from simple steatosis (NAFL) to non-alcoholic steatohepatitis (NASH), advanced liver fibrosis or cirrhosis [4]. Although authors have different points of view on the level of alcohol consumption in NAFLD definition, the maximum level of alcohol intake agreed by the *National Institutes of Health (NIH) Clinical Research Network* on NAFLD/NASH is one standard drink a day for women (70 g ethanol/week), and two standard drinks a day for men (140 g ethanol/week) [5].

The NASH term was coined in 1980 by Ludwig J *et al.* to describe a clinicopathologic syndrome, "hitherto unnamed disease that mimics alcoholic hepatitis", but occurring in non-drinking patients with obesity-associated diseases, among which diabetes mellitus. Findings on the liver biopsy specimens in the Ludwig study included macrovesicular steatosis, lobular inflammation, focal necroses with mixed inflammatory infiltrates, Mallory bodies, and fibrosis [6].

Despite various conditions that may cause fatty

infiltration of the liver, such as drug toxicity (i.e. glucocorticoids, estrogens, tamoxifen, methotrexate, amiodarone, perhexilene, diltiazem), gastrointestinal surgery, total parental nutrition, starvation and inflammatory bowel disease [2], the NAFLD term is reserved now for the spectrum of metabolic fatty liver disorders with undefined histology [5]. The use of the term NASH request histopathological evaluation because the parenchymal injury cannot be detected by imaging studies or laboratory tests [7]. At present, NAFLD is considered to be the most common cause of elevated aminotransferase levels in the asymptomatic patients and also the cause of most cases of cryptogenic cirrhosis in developed countries [8–10].

In the last decade, several studies have demonstrated independently a strong association between NAFLD and each component of the metabolic syndrome, including central obesity, hypertriglyceridemia and mixed hyperlipidemia, type II diabetes mellitus and hypertension. Increased NAFLD prevalence (57.5–74%) in obese patients has been reported [4]. Additionally, truncal obesity confers a higher risk of developing NAFLD, even in patients with a normal body mass index (BMI) [11]. Dyslipidemia and insulin resistance both are strongly associated with the presence of NAFLD. Prevalence of hypertriglyceridemia in different NAFLD case series is quite variable, but all evidences linked it to NAFLD. Jimba S *et al.* detected NAFLD by ultrasound in 62% of patients newly diagnosed with type 2 diabetes mellitus [12]. Recent evidence suggests that NAFLD should be considered as the hepatic manifestation of metabolic syndrome [13].

### ☐ Evolution of the metabolic syndrome concept

In the early 1960s, Avogaro P and Crepaldi G described a frequent simultaneous presence of obesity, hyperlipidemia, diabetes, and hypertension as “plurimetabolic syndrome” [14]. In 1988, Reaven GM coined the term *Syndrome X* to designate the association between insulin resistance, glucose intolerance, dyslipidaemia and hypertension. He postulated that the common feature of the proposed syndrome is insulin resistance and its compensatory hyperinsulinemia, and all other changes were secondary to this abnormality [15].

In 1998, *World Health Organization* (WHO) named this entity “metabolic syndrome” and developed a first set of criteria for a clinical diagnosis, “working definition”, revised in 1999 [16]. These included clinical evidence of insulin resistance, such as impaired glucose tolerance (IGT), impaired fasting glucose (IFG),

or type 2 diabetes plus with at least two out of four other additional factors (elevated triglycerides, low HDL cholesterol, elevated blood pressure, obesity, microalbuminuria) [17]. Later a number of expert groups have attempted to develop a unifying definition for this clinico-biological entity: in 2001 the *National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATP III)* [18], in 2003, the *American College of Endocrinology/American Association of Clinical Endocrinologists (ACE)* [19], and in 2005 the *International Diabetes Federation (IDF)* [20]. In 2005, *The American Heart Association and National Heart, Lung, and Blood Institute (AHA/NHLBI)* updated the *NCEP/ATP III* criteria to correspond with the new *American Diabetes Association* standard of a normal fasting glucose level [21].

The *WHO*, *NCEP ATP III*, *AHA/NHLBI* and *IDF* criteria for metabolic syndrome are summarized in Table 1.

Table 1 – Current definitions of metabolic syndrome [16–18, 20, 21]

Risk factors	Categorical cut points			
	NCEP ATP III (≥3 criteria)	AHA/NHLBI (3 criteria)	IDF (obesity + ≥2 other criteria)	WHO (Insulin resistance* plus any 2 of the following risk factors)
<b>Elevated waist circumference</b>				<b>Obesity</b>
• <i>males</i>	>102 cm	≥102 cm	Ethnicity-specific <sup>†</sup> ≥94 cm (for European men)	<b>waist to hip ratio</b> <i>males</i> >0.90; <i>females</i> >0.85; <b>and/or</b> BMI >30 kg/m <sup>2</sup>
• <i>females</i>	>88 cm	≥88 cm	≥80 cm (for European women)	
<b>Elevated triglycerides</b>	≥150 mg/dL (≥1.7 mmol/L) or specific treatment	≥150 mg/dL (≥1.7 mmol/L) or specific treatment	≥150 mg/dL (≥1.7 mmol/L) or specific treatment	≥150 mg/dL (≥1.7 mmol/L)
<b>Reduced HDL-C</b>				
• <i>males</i>	<40 mg/dl or specific treatment	<40 mg/dl or specific treatment	< 40 mg/dL or specific treatment	<35 mg/dL (<0.9 mmol/L)
• <i>females</i>	<50 mg/dl or specific treatment	<50 mg/dl or specific treatment	< 50 mg/dL or specific treatment	<39 mg/dL (<1.0 mmol/L)
<b>Elevated blood pressure (BP)</b>	≥130 / ≥85 mmHg or drug treatment for hypertension	≥130 / ≥85 mmHg or drug treatment for hypertension	≥130 / ≥85 mmHg or treatment of previously diagnosed hypertension	≥140 / ≥90 mmHg or treatment for hypertension
<b>Elevated fasting glucose</b>	≥110 mg/dL (≥6.1 mmol/L)	≥100 mg/dL or drug treatment	≥100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes	
<b>Microalbuminuria</b>				urinary albumin >20 mg/mL albumin-creatinine ratio >30 mg/g

\*Insulin resistance, identified by type 2 diabetes mellitus or impaired fasting glucose (fasting plasma glucose level, 100–125 mg/dL) or impaired glucose tolerance (2-hour plasma glucose level after 75 g glucose load, 140–199 mg/dL).

<sup>†</sup>Ethnicity specific values for other groups.

### ☐ NAFLD and metabolic syndrome: pathophysiological relationships

NAFLD and metabolic syndrome have been linked by relationships between central obesity, steatosis, and insulin resistance. The pathophysiological mechanism underlying NAFLD and its progression from steatosis to cirrhosis are not well understood at present, but all evidence suggested that numerous factors are implicated: insulin resistance, liver mitochondrial dysfunction, oxidative stress, apoptosis,

cytokine/adipocytokine pathways, genetic and environmental factors.

The “two-hits” theory of the pathophysiological model proposed since 1998 is currently the most widely accepted [22]. Insulin resistance is considered to play a central role in the pathophysiological process, linking all others factors and starting the sequence of events by liver fat accumulation, furthermore considered as the first hit [23].

The accumulation of lipid in the form of triglycerides within the hepatocytes triggers the

sequences of second hit (oxidative stress, lipid peroxidation, liver mitochondrial dysfunction, proinflammatory cytokines and adipocytokines release), considered to be responsible for the progression from NAFL to NASH [24].

### Insulin resistance

Current evidence suggests that both peripheral and hepatic insulin resistance are implicated in hepatic fat accumulation by a combination of increased peripheral lipolysis and increased visceral fat stores. Peripheral insulin resistance increases triglyceride lipolysis *via* elevated hormone-sensitive lipase (HSL) activity, and inhibits esterification of free fatty acids (FFAs), resulting in an increased FFAs delivery to the liver [25].

Accumulation of FFAs and their metabolites within hepatocytes leads to the development of hepatic insulin resistance [26]. Inside hepatocytes FFAs are metabolized by oxidation, generating ATP, or by esterification producing triglycerides, which are either incorporated into VLDL particles for export or stored within the hepatocyte. Resulting hepatic insulin resistance and its hyperinsulinemia increases *de novo* synthesis of fatty acids in hepatocytes. A consequence of increased FFAs synthesis is an increased production of malonyl-CoA, which inhibits the protein responsible for fatty acid transport into the mitochondria, thus reducing  $\beta$ -oxidation and enhancing fatty acid and triglyceride accumulation. Decrease of very low-density lipoproteins (VLDL) synthesis and damaged triglyceride export *via* VLDL also contributes to increased triglyceride accumulation within hepatocytes and initiates further hepatic damage [2, 27].

### Oxidative stress

The resulting accumulation of lipid within hepatocytes triggers the second hit, oxidative stress, considered responsible for further progression of NAFLD subtypes. Oxidative stress due to excessive generation of prooxidant reactive oxygen species (ROS) and subsequent lipid peroxidation has been suggested to play an important role in NASH pathogenesis and fibrogenesis [28, 29].

Mitochondria, peroxisomes, and microsomes are the potential sites within hepatocytes considered involved in ROS generation by several pathways, including mitochondrial and peroxisomal fatty acids oxidation, and increased activity of cytochrome P450 (CYP), particularly CYP2E1 and members of the CYP4A subfamily [30–32].

The most important consequences of increased ROS include lipid peroxidation, release of proinflammatory cytokines and activation of hepatic stellate cells (HSC) leading to fibrogenesis [28]. Lipid peroxidation products, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are considered to have an important role in promoting an immune response, because of hepatocyte proteins bindings and neoantigens forming [33]. They are directly involved in hepatocyte death and necrosis, inflammation, and HSC activation leading to liver fibrosis [27].

### Adipose tissue as an endocrine organ: a key target for NAFLD

Since the first description of adipose tissue as an endocrine organ by Cook KS *et al.* [34] a large variety of adipose tissue-derived secretory products (i.e. adiponectin leptin, resistin), has been discovered and described. There is substantial evidence showing that adipose tissue acts as an endocrine organ, and its expressed and secreted products, such as adiponectin, leptin resistin, and other cytokines, among those tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6 and IL-1 $\beta$ , play an important role in the pathogenesis of NAFLD. These biologically active compounds are collectively termed as adipocytokines. With the exception of adiponectin, secreted exclusively by the adipocyte, all other adipokines can also be synthesized and secreted by so-called stroma-vascular fraction (SVF) of the human adipose tissue, which consists of preadipocytes, endothelial cells, and leukocytes (including macrophages), and by other various tissues. These adipocytokines have either pro-inflammatory effects (i.e. leptin, TNF- $\alpha$ , IL-6, and resistin), or anti-inflammatory (i.e. adiponectin) effects in NAFLD [35–37]. The composition and function of adipose tissue is altered in obese state, an over-production of proinflammatory cytokines in rodent obesity models has been described [38, 39]. Obesity is associated with macrophage accumulation in adipose tissue, which correlates with increased expression of proinflammatory cytokines. Abdominal fat macrophage accumulation in obese was considered as a marker of low-grade chronic inflammation [40].

Both the adipose tissue and the liver are involved in lipid metabolism and consist of different cell types, able to produce various proinflammatory cytokines.

**Adiponectin**, an adipocytokine secreted exclusively by adipocytes [41], has been found to have insulin-sensitizing, anlipogenic, anti-atherogenic, anti-inflammatory and anti-apoptotic properties in a variety of cell types [42–44]. Low serum levels of adiponectin have been associated with several metabolic syndrome components, including central obesity, hyperlipidemia, and type 2 diabetes states, which are characterized by insulin resistance [45]. Similarly, Hui JM *et al.* have found that hypo adiponectinemia is a feature of NASH, and may be involved independently from insulin resistance in the NAFL progression. Based on their observations, they envisaged that hypo adiponectinemia may be one of the pathogenic links between central obesity and NASH development [46]. A specific role for adiponectin in the liver has been suggested, more evidence supporting the hypothesis that liver is a major target of adiponectin. Two types of adiponectin receptors, AdipoR1 (abundantly expressed in skeletal muscle) and AdipoR2 (predominantly expressed in the liver) have been cloned recently [47]. Additionally, hepatic mRNA expression of AdipoR2 in NASH inversely correlated with the histological grade of fibrosis but not with serum and hepatic adiponectin levels. Adiponectin has been shown to have hepatic cytoprotective properties, improving both hepatic and

peripheral insulin sensitivity, and preventing steatosis, inflammation, necrosis and fibrosis [48–50]. As anti-steatotic agent, adiponectin decreases hepatic accumulation of triglycerides as a consequence of decreased serum FFAs levels by increasing oxidation of nonesterified fatty acids in skeletal muscle [51]. In addition, adiponectin itself improves hepatocyte sensitivity to insulin [52], stimulates hepatic FFAs uptake and suppresses hepatic glucose production [53]. Adiponectin exerts a potent anti-inflammatory effect in liver by antagonizing both production and activity of TNF- $\alpha$  [54, 55] and by inducing various anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonist [56]. In addition, adiponectin has a direct antifibrotic effect, preventing activation of HSC during fibrogenesis [57]. Responsible for the reduction in adiponectin secretion appears to be increased TNF- $\alpha$  and ROS production [58].

TNF- $\alpha$  is considered to favor hepatic steatosis by interfering with insulin signaling and may play a role in inflammation pathogenesis of the NASH. Additionally, among other factors, helps progression from NASH to cirrhosis [46, 59]. Although their structural resemblance [60], TNF- $\alpha$  and adiponectin have been found to have opposite effects, suppressing each other's production in adipose tissue. Additionally, adiponectin and TNF- $\alpha$  antagonize each other's function in skeletal muscle [61]. Originally identified as a macrophage product, TNF- $\alpha$  was the first adipose tissue-derived secretory products proposed in 1993 as a molecular link between obesity and insulin resistance [62, 63]. Both, rodent obesity models and humans observations suggest that, in the absence of active infections or extrahepatic inflammatory conditions, plasma TNF- $\alpha$  levels is positively correlated with body fat mass [41]. Two TNF- $\alpha$  receptors have been identified and designated, type 1 (TNF-RI, p55) and type 2 (TNF-RII, p75), and a strong positive correlation between insulin resistance, severe NASH and a TNF- $\alpha$  gene polymorphism was described [64–65].

Several recent studies have suggested that NAFLD progression plays an important role for pro-inflammatory signaling pathways, such as nuclear factor  $\kappa$ B (NF- $\kappa$ B). Local FFAs accumulation triggers signals that activate NF- $\kappa$ B within hepatocytes, inducing NF- $\kappa$ B-sensitive genes that determines the over-production of TNF- $\alpha$  and of other pro-inflammatory cytokines (i.e. IL-1, IL-6), which may cause insulin resistance both locally in the liver and systemically [66–67].

**Leptin**, a polypeptide produced predominantly by adipocytes, may be involved in insulin resistance and NAFLD development. Elevated plasma levels of leptin have been related to fatty liver and steatohepatitis in the obese and **non-obese** patients [68]. Recently, leptin has been described to have a critical role in the development of hepatic fibrosis [69]. Several studies have provided evidence that genetic and environmental factors may be involved in NAFLD pathogenesis [70–72].

The **endocannabinoid system (ECS)**, an endogenous signaling system recently characterized physiologically, involved in modulating energy homeostasis and

metabolism, can play an important role in both steatosis and liver fibrogenesis. ECS contributes to hepatic steatosis by adiponectin down-regulation [73], lipolysis and de novo fatty acid synthesis in hepatocytes [74], and can be involved in hepatic fibrogenesis by modulating HCS activity [75].

### ▣ Initial assessment and diagnosis

Diagnosis in the early stages of NAFLD is usually first suspected in an overweight or obese person persons, no history of significant alcohol intake and other possible causes of steatose previously described, with asymptomatic elevation of alanine-transaminase (ALT) and aspartate transaminase (AST) levels, during a routine blood testing (AST/ALT ratio usually less than 1). Other presentation modalities are an abnormal hepatic ultrasonography (or other imaging methods) performed for an unrelated condition or the detection of hepatomegaly during routine physical examination [2, 5, 76].

A thorough medical history is indispensable in order to exclude alcohol-induced fatty liver disease and other illnesses or causes known to cause hepatic steatosis. In addition, medical history and physical examination should focus on metabolic disorders associated with insulin resistance, such as central or overall obesity, type 2 diabetes mellitus and hypertension [2, 3, 13, 77].

The initial laboratory assessment of a patient with suspected NAFLD should be determined by the common tests used to evaluate liver function (ALT, AST, gamma-glutamyl transpeptidase and alkaline phosphatase levels, serum bilirubin, prothrombin time, serum protein electrophoresis, and platelet count). Laboratory testing for exclusion of viral (hepatitis B and hepatitis C), autoimmune liver diseases, and screening for metabolic syndrome comprise initial assessment [2, 4, 5]. Abdominal ultrasound examination is the most commonly used imaging technique for detecting steatosis (increased echogenicity, “bright liver”), but is unable to quantify liver steatosis or distinguish between NAFLD subtypes [78, 79]. In addition, non-invasive imaging method not currently available can distinguish between simple steatosis and NASH, or predict fibrosis and the severity in NAFLD [80].

Liver biopsy and histological assessment remains the “gold standard” for diagnosing NASH, as well as for determining the stage of hepatic fibrosis and disease severity. However, several disadvantages (invasive method, sampling error, considerable inter/intra-observation variability, potential risks to complications, costly, poor patient acceptance), as well as problems (not among minimal histological criteria agreed for NAFLD subtypes, indistinguishable histological aspect for alcohol-induced fatty liver) [81–84] limit the liver biopsy in NAFLD patients.

Recently, a histological scoring system for NAFLD was developed and proposed for use in clinical trials. The NAFLD activity score (NAS) represents the sum of scores for three pathologic features (steatosis, lobular inflammation and hepatocellular ballooning), and ranges

from 0–8. A score of 5 or more occurs in cases that were considered to be diagnosed with NASH, scores of 3–4 are borderline NASH and scores of 0–2 equates to not diagnostic of NASH [85].

One of the most difficult and controversial decisions in clinical practice is whether to perform a liver biopsy in a patient with NAFLD. Additionally, no consensus currently exists regarding indications of liver biopsy in NAFLD. In addition, another problem rises: if in the absence of effective therapy in NAFLD and if liver biopsy does not change disease management, which are the benefits. Even so, a liver biopsy is essential when diagnosis is uncertain. In clinical practice, liver biopsy indication will have to be individualized, after other diseases have been excluded [85, 86].

Noninvasive approaches, including serum markers, routine laboratory findings, and clinical features have been studied and proposed as an alternative to liver biopsy in NAFLD [87–89], but none of these have proven accurate in predicting and differentiating liver histology and fibrosis severity.

### Management strategies

The goal of NAFLD management is to diminish steatosis and prevent the development of end stages liver diseases. The current therapeutic options are focused on the management of associated metabolic conditions such as obesity, diabetes mellitus and hyperlipemia. The management strategies include weight loss through lifestyle changes, pharmacological treatment of obesity, insulin-sensitizing agents, antihyperlipidemic agents, and cytoprotective and antioxidant agents [2, 4, 5, 90].

Most of the proposed treatments were tested within small trials, only a few had a placebo control, most were conducted over a short period and many lacked histological end-point.

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological entity increasingly recognized as a major health burden. Its prevalence is expected to increase worldwide, with the rising prevalence of obesity and metabolic syndrome.

Epidemiological, clinical, pathogenic and therapeutic aspects, which link NAFLD and metabolic syndrome, made possible to consider NAFLD as a hepatic manifestation of the metabolic syndrome.

Although many treatment modalities have been proposed and used, there is no consensus on the effective therapy in NAFLD. Large, well-controlled trials are needed to evaluate the potential benefits of pharmacological therapy and to determine optimal management for NAFLD. Prevention of obesity should be the first line of treatment in NAFLD.

A multidisciplinary program including weight loss and life style changes, and the correction and management of all other associated metabolic conditions is essential in the prevention and treatment of NAFLD.

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