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**Non-alcoholic fatty liver disease and diabetes: From physiopathological interplay to diagnosis and treatment**

Leite NC *et al* NAFLD and diabetes: a review

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**Abstract**

Non-alcoholic fatty liver disease (NAFLD) is highly prevalent in patients with diabetes mellitus and increasing evidence suggests that patients with type 2 diabetes are at a particularly high risk for developing the progressive forms of NAFLD, non-alcoholic steatohepatitis and associated advanced liver fibrosis. Moreover, diabetes is an independent risk factor for NAFLD progression, and for hepatocellular carcinoma development and liver-related mortality in prospective studies. Notwithstanding, patients with NAFLD have an elevated prevalence of prediabetes. Recent studies have shown that NAFLD presence predicts the development of type 2 diabetes. Diabetes and NAFLD have mutual pathogenetic mechanisms and it is possible that genetic and environmental factors interact with metabolic derangements to accelerate NAFLD progression in diabetic patients. The diagnosis of the more advanced stages of NAFLD in diabetic patients shares the same challenges as in non-diabetic patients and it includes imaging and serological methods, although histopathological evaluation is still considered the gold standard diagnostic method. An effective established treatment is not yet available for patients with steatohepatitis and fibrosis and randomized clinical trials including only diabetic patients are lacking. We sought to outline the published data including epidemiology, pathogenesis, diagnosis and treatment of NAFLD in diabetic patients, in order to better understand the interplay between these two prevalent diseases and identify the gaps that still need to be fulfilled in the management of NAFLD in patients with diabetes mellitus.

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**Key words:** Non-alcoholic fatty liver disease; Diabetes mellitus; Pathogenesis; Diagnosis; Treatment

**Core tip:** This review addresses the important interplay between non-alcoholic fatty liver disease and diabetes mellitus, with particular emphasis on physiopathological mechanisms, diagnosis and treatment.

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**Introduction**

Diabetes mellitus (DM) is an actual public-health concern: 347 million people worldwide have diabetes and the WHO projects that diabetes will be the 7th leading cause of death in 2030. It is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. There are two most important etiopathogenetic categories: type 1 DM (T1DM), which accounts for 5%-10% of the cases of DM and results from autoimmune destruction of the beta-cells of the pancreas and absolute deficiency of insulin; and type 2 DM (T2DM), which encompasses patients who have insulin resistance with relative insulin deficiency and accounts for 90%-95% of diabetic patients. Both T1DM and T2DM patients are at high risk of developing chronic macrovascular and microvascular degenerative complications. Nevertheless, beyond these classic complications, DM is also associated with liver-related mortality and increasing risk of hepatocellular carcinoma[[1](#_ENREF_1),[2](#_ENREF_2)].

Non-alcoholic fatty liver disease (NAFLD) prevalence is increased in patients with DM[[3](#_ENREF_3),[4](#_ENREF_4)]. Increasing evidence suggests that patients with T2DM are at a particularly high risk for non-alcoholic steatohepatitis (NASH), with varying degrees of liver fibrosis[[5](#_ENREF_5),[6](#_ENREF_6)]. Likewise, preexisting diabetes is an independent risk factor for NAFLD progression and for liver-related mortality and hepatocellular carcinoma in prospective studies[[1](#_ENREF_1),[7-9](#_ENREF_7)]. On the other hand, patients with NAFLD have an elevated prevalence of prediabetes[[10](#_ENREF_10)] and recent data has shown that NAFLD presence predicts the development of T2DM[[10](#_ENREF_10),[11](#_ENREF_11)].

There are pathogenetic mechanisms linking NAFLD and DM. Besides insulin resistance and hyperinsulinemia, disordered lipid metabolism, increased oxidative stress and inflammation contribute to both entities. It is possible that genetic and environmental factors interact with metabolic derangements to accelerate NAFLD progression in diabetic patients.

Ultrasound is the method of choice for screening patients for NAFLD. In patients with diabetes and histologically-proven NASH, abnormal liver enzymes may be seen in less than 20% of patients[[5](#_ENREF_5),[6](#_ENREF_6),[12](#_ENREF_12),[13](#_ENREF_13)]. Liver biopsy is still the gold standard method to diagnose and stage NAFLD. However, it is a costly and invasive procedure with some limitations as sampling error and intra-and inter-observer variability among pathologists[[6](#_ENREF_6)]. It was previously suggested that liver biopsy should be considered in all patients with DM and hepatic steatosis on ultrasound[[6](#_ENREF_6)]. Newer radiological techniques, biomarkers and clinical algorithms are being currently studied and may provide, in the near future, valuable noninvasive alternatives to histological diagnosis. Treatment is based mainly on lifestyle changes and antioxidants and numerous drugs have been studied searching for histological improvement with variable outcomes. The following review aims to discuss all these aspects from physiopathological mechanisms to diagnosis and treatment of NAFLD in DM.

**Definition of NAFLD**

In 1980, Ludwig *et al*[14] described a small series of patients with liver histology characterized by fat accumulation, hepatic necroinflammation and, in most cases, fibrosis in the absence of a history of excessive alcohol consumption. Since this original description, the histologic criterion for diagnosing NAFLD has evolved, and several grading systems have been proposed to assess histologic severity. In 1999, Matteoni *et al*[[15](#_ENREF_15)] proposed pathological NAFLD subtypes based on long-term outcome studies, and Brunt *et al*[[16](#_ENREF_16)] proposed a specific classification for NASH based on criteria used in other forms of chronic liver disease, using grades of necroinflammatory lesions and stages of fibrosis. Subsequently, the U.S. Pathology Committee of the NASH Clinical Research Network[17] proposed and validated a histological scoring system of specific lesions that addressed the full spectrum of NAFLD. In addition, a separate scoring system known as the NAFLD activity score (NAS) has been developed for use in clinical trials. It measures steatosis semi-quantitatively, as well as lobular inflammation and hepatocellular ballooning, to enable systematic documentation of changes in activity of NASH[[17](#_ENREF_17)]. However, a numerical value alone is not accepted for definitive diagnosis of steatohepatitis. In general, accumulation of greater than 5% of fat, particularly in the form of triglycerides, is considered to be the minimal requirement for histologic diagnosis of steatosis. NASH, in turn, encompasses steatosis plus lobular inflammation and ballooning degeneration, with or without a varying degrees of fibrosis, which is originally centered on the hepatic venulae.

**Clinical Presentation**

NAFLD is generally asymptomatic at presentation and is frequently found among individuals with conditions such as obesity, T2DM, metabolic syndrome and its individual components[[18](#_ENREF_18)]. The most common signs and symptoms are fatigue, right upper quadrant pain and hepatomegaly, as well as acanthosis nigricans, which are more frequently observed in the pediatric population. In fact, most patients with NAFLD are diagnosed by incidental elevated liver enzymes or imaging studies suggesting hepatic steatosis[[18](#_ENREF_18)]. By definition, NAFLD is established in patients who consume little or no alcohol presenting a histological picture that resembles alcohol-induced liver disease. Most studies defined a threshold for excessive alcohol consumption as more than 20 g/d in women and more than 30 g/d in men[[15](#_ENREF_15),[19](#_ENREF_19)]. In contrast, intake levels of 20 g/d (140g weekly) for men, and 10 g/d (70g weekly) for women have been endorsed as the acceptable thresholds to define “non-alcoholic” in the guidelines proposed by the Asia-Pacific Working Party for NAFLD (APWP-NAFLD) and by the National Institutes of Health Clinical Research Network[[20](#_ENREF_20),[21](#_ENREF_21)]. Additionally, definitive diagnosis of NAFLD requires exclusion of other secondary causes of hepatic steatosis, such as medications like prednisolone, tamoxifen, amiodarone and methotrexate among others, exposure to toxins as vinyl chloride, total parenteral nutrition, cachexia, intestinal bypass surgery, viruses infections like genotype 3 hepatitis C virus and human immunodeficiency virus.

**Epidemiology**

NAFLD is the commonest cause of liver disease in western countries, present in over 30% of the general population[[22](#_ENREF_22),[23](#_ENREF_23)]. The prevalence in Asian populations ranges from 6% up to 25%[[24](#_ENREF_24)]. The prevalence of NAFLD among children is 3%–10%, rising up to 40%–70% among obese children[[25](#_ENREF_25)]. The prevalence of NASH is difficult to assess because it requires a histological diagnosis, which is impractical in all patients with NAFLD. In a recent study in a cohort of 400 military personnel and their relatives, ultrasound prevalence of NAFLD was 46%, and NASH was found in 30% of these individuals, resulting in a NASH prevalence of 12% for the entire cohort[[26](#_ENREF_26)].

NASH cirrhosis is now the third most common indication for liver transplantation in the USA, behind hepatitis C and alcoholic liver disease, and is the only liver-related transplant indication that continues to increase[[27](#_ENREF_27)]. Liver disease is the third leading cause of death in NAFLD patients[[8](#_ENREF_8),[28](#_ENREF_28)], with hepatocellular carcinoma being the most frequent cause of liver-related death[[29](#_ENREF_29)].

T2DM is not only a risk factor for NAFLD but it is also related to a higher prevalence of NASH and fibrosis[[1](#_ENREF_1),[5](#_ENREF_5),[6](#_ENREF_6)]. Data regarding T1DM and NAFLD are scarce. Although T1DM is characterized by insulin deficiency, obesity and metabolic syndrome may eventually occur in these patients and lead to NAFLD[30]. Targher *et al*[31]found a NAFLD prevalence of 44% in patients with T1DM by ultrasound imaging, without histological confirmation. Nevertheless, the prevalence of NAFLD in patients with T2DM has been reported to be as high as 74%[[3](#_ENREF_3),[4](#_ENREF_4),[32](#_ENREF_31),[33](#_ENREF_32)]. Most of the studies evaluating NAFLD in T2DM have relied exclusively on ultrasonography or on elevated liver enzymes for diagnosis. Studies describing the histopathological spectrum of NAFLD in type 2 diabetes mellitus are still scarce[[6](#_ENREF_6)].

**NAFLD and Diabetes Mellitus: the natural history is a two-way traffic**

NAFLD represents a wide spectrum of conditions ranging from fatty liver, which follows in general a benign and stable clinical course, to NASH that may progress to cirrhosis. Less than 1% to 4% of patients with simple steatosis progress to advanced fibrosis. By contrast, NASH can lead to cirrhosis in 15% to 25% of individuals, with further liver-related complications and death[[8](#_ENREF_8),[11](#_ENREF_11),[34](#_ENREF_33)]. Among those with NASH-related cirrhosis, about 25% will develop major complications of portal hypertension within 3 years[[35](#_ENREF_34)].

The role of diabetes in NASH and fibrosis was initially evaluated in patients undergoing bariatric surgery. The prevalence of T2DM ranged from 14% to 28% in these studies and its presence was a predictor of NASH and fibrosis in morbidly obese patients[[36](#_ENREF_35),[37](#_ENREF_36)].

Younossi *et al*[[1](#_ENREF_1)], in a retrospective cohort study with 132 patients with histological diagnosis of NAFLD, reported that patients with DM had greater rates of cirrhosis and mortality than those with NAFLD without DM. In a recent prospective study of 328 asymptomatic patients, 16.5% had an established diagnosis of T2DM. The prevalence of NAFLD and NASH in the entire cohort was 46% and 12%, respectively. However, in diabetic patients, NAFLD was observed in 74% and NASH in 22%[[26](#_ENREF_26)].

Few studies evaluated the prevalence of NAFLD and the correlated factors with histopathological stages of NAFLD in patients with T2DM. Table 1 outlines a summary of these studies. The sample sizes varied from 32 to 92 patients, and NASH was present in 63%–87% of the patients, while the prevalence of any fibrosis ranged from 22% to 60% in these studies[[5](#_ENREF_5),[6](#_ENREF_6),[13](#_ENREF_13)]. In the largest single-centre study, we found high prevalences of the more severe stages of NAFLD: 78% for NASH and 34%-60% for moderate-severe fibrosis[[6](#_ENREF_6)]. No diabetes-related variable, such as glycemic control, diabetes duration or the presence of micro- and macrovascular complications, was associated with the more severe stages of NAFLD[[6](#_ENREF_6)]. Alanine aminotransferase (ALT), high triglycerides and low HDL-cholesterol were independently associated with NASH. On the other hand, male gender, older age and elevated values of gammaglutamyl transferase (GGT) were associated with moderate-severe fibrosis[[6](#_ENREF_6)]. Prashanth *et al*[5] reported prevalences of NASH and of any stage of fibrosis of 62.6% and 37.3%, respectively. Serum ALT and alkaline phosphatase levels, although within normal limits, were significantly higher in patients with NASH[[5](#_ENREF_5)]. Of note, NASH prevalence increased proportionally to the number of metabolic syndrome components presented. Also, no diabetes-related variable was predictive of the severity of NAFLD. In a study conducted in Mexico, 60 patients with DM were evaluated and 22 of them (37%) had elevated liver enzymes and/or steatosis on radiological examination[[38](#_ENREF_37)]. These patients underwent liver biopsy and the prevalence of NASH was 64%. There was no association of liver enzymes, lipid profile, glycated hemoglobin or body mass index with the presence of NASH. In another small group of 32 T2DM patients submitted to liver biopsies, 49% had NAFLD on histopathology, of which 87.5% had NASH. There was no significant correlation between liver enzymes and NASH or fibrosis[[13](#_ENREF_13)]. Subsequently, these researchers further reported that in 36 patients with NASH, 30.5% had any stage of fibrosis. Patients with any stage of fibrosis had higher levels of aminotransferases and a higher aspartate aminotransferase (AST) to ALT ratio[[12](#_ENREF_12)].

Although in most of the studies diabetic patients with NASH had higher serum ALT than those without NASH, and ALT levels were independently associated with the presence of NASH on liver biopsy, abnormal ALT levels were uncommon and did not have enough predictive value to be indicated as a screening test for NASH detection[[6](#_ENREF_6)]. Moreover, in patients with T2DM serum liver enzymes could be less representative of the severity of intrahepatic fat accumulation. In a case-control study, patients with T2DM showed approximately 80% more intra-hepatic fat content by magnetic resonance spectroscopy than age, sex, and body weight-matched non-diabetic controls[[39](#_ENREF_38)]. Liver fat content was underestimated by serum ALT compared with equally obese non-diabetic subjects.

A recent cross-sectional study demonstrated that in addition to diabetes, a family history of diabetes also increased the risk of NASH and fibrosis in non-diabetic individuals[[40](#_ENREF_39)]. The association between family history of diabetes with NASH and fibrosis remained significant even after adjusting for prediabetes status, suggesting that a family history of diabetes may provide additional risk stratification in non-diabetic patients with NAFLD.

In spite of apparent absence of association between any diabetes-related characteristic and the presence of more severe stages of NAFLD in previous studies, evidence is mounting that NAFLD may be associated with the occurrence of microvascular and macrovascular degenerative complications in diabetic patients[[3](#_ENREF_3),[31](#_ENREF_30),[41](#_ENREF_40),[42](#_ENREF_41)]. In patients with T1DM and T2DM, NAFLD was associated with higher rates of microalbuminuria, reduced glomerular filtration rate and retinopathy[[43](#_ENREF_42),[44](#_ENREF_43)]. Prospective studies have demonstrated a higher incidence of chronic kidney disease in patients with T2DM and NAFLD, independent of several established risk factors[[42](#_ENREF_41),[43](#_ENREF_42)]. Both subclinical atherosclerosis markers, like increased carotid intima-media thickness and aortic stiffness, as well as clinical cardiovascular diseases, were also more frequent in patients with T1DM and T2DM with the diagnosis of NAFLD than in their counterparts without NAFLD[[3](#_ENREF_3),[31](#_ENREF_30),[45](#_ENREF_44)]. It was demonstrated that the association between macrovascular disease and NAFLD was independent of the classic cardiovascular risk factors and components of the metabolic syndrome[[3](#_ENREF_3),[31](#_ENREF_30)].

Another issue to be considered is whether diabetes also accelerates the progression of NAFLD. The presence of DM was independently associated with advanced liver fibrosis in cross-sectional studies[[46](#_ENREF_45),[47](#_ENREF_46)]. However, findings from prospective studies with serial liver biopsies are still scarce and controversial. In a cohort study with 129 patients with biopsy-proven NAFLD reevaluated after a mean follow-up of 13.7 years, the progression of liver fibrosis occurred in 41%[8]. More pronounced insulin resistance during follow-up was associated with liver fibrosis progression. Unfortunately, fasting plasma glucose was not measured at baseline, and thus the prevalence of diabetes at onset of the study could not be reported[[8](#_ENREF_8)]. In another cohort of 103 NAFLD patients who underwent a second liver biopsy at an average interval of 3.2 years, fibrosis staging progressed in 37%. Preexisting diabetes and early stages of fibrosis at first biopsy were predictors of fibrosis progression[[7](#_ENREF_7)]. Otherwise, in a systematic review including ten longitudinal studies comprising 221 patients who had NASH on their initial biopsy, received no intervention of proven benefit regarding histology and underwent a second liver biopsy at least one year apart, only age and any degree of inflammation in the initial biopsy were the risk factors related to progression to advanced fibrosis. Other traditional parameters such as obesity, diabetes and hypertension were not statistically significant predictors[[48](#_ENREF_47)]. Overall, inclusion of heterogeneous studies, disagreement on criteria for NASH diagnosis, liver biopsy sampling error and variability among pathologists are remarkable limitations of long-term histological studies.

It is not clearly defined yet if NAFLD worsens glycemic control in patients with T2DM. NAFLD patients have shown both an impaired ability of insulin to suppress endogenous glucose production related to hepatic insulin resistance, and a reduction in glucose disposal, a measure of whole-body insulin sensitivity[[49](#_ENREF_48)]. Indeed, intrahepatic triglyceride content may influence insulin requirements in diabetic patients *via* an effect on the sensitivity of endogenous glucose production to insulin[[50](#_ENREF_49)].

On the other hand, NAFLD is known to be associated with insulin resistance, hyperinsulinemia and prediabetes, which includes both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). In an Australian study of 70 patients with NAFLD determined by ultrasound, 24% had IGT and 10% had diabetes on standard oral glucose tolerance test (OGTT). Over half of the NAFLD patients with a normal fasting glucose had abnormal glucose tolerance, as detected by OGTT. In addition, irrespective of the status of glucose tolerance, 2-h hyperinsulinemia during the OGTT occurred in all subjects with NAFLD, and fasting insulin resistance was found in 73%; fasting insulin resistance was assessed by the homeostasis model assessment method (HOMA-IR) and was defined by a HOMA-IR equal or greater than 2[[51](#_ENREF_50)]. In the Framingham Heart study with 2589 individuals, fatty liver was associated with T2DM, IFG, hypertension, metabolic syndrome, HDL-cholesterol, triglycerides, and adiponectin levels, even after multivariate adjustment for other fat depots, such as visceral adipose tissue, waist circumference, and body mass index[[52](#_ENREF_51)]. Moreover, several prospective observational studies have shown an increased incidence of T2DM in patients with NAFLD diagnosed by ultrasonography or by liver biopsy. However, most of them were not adjusted for main covariates, such as family history of T2DM, physical activity and fasting glucose and insulin levels[[8](#_ENREF_8),[53](#_ENREF_52)].

In a recent prospective community-based study in an urban adult population from Sri Lanka, individuals with ultrasonographic NAFLD showed an increased risk of developing T2DM. After three years follow-up, T2DM incidence rates were 64.2 and 34 per 1000 person/years for those with and without NAFLD, respectively, and NAFLD was an independent predictor of T2DM development[[54](#_ENREF_53)]. In a longitudinal cohort study of 7849 Korean individuals followed-up for 5 years, those who had both elevated ALT and ultrasonographic liver steatosis had an increased risk of future diabetes development[[55](#_ENREF_54)]. Another Korean study demonstrated that NAFLD had an independent and additive effect on the development of T2DM in patients with IFG at baseline[[56](#_ENREF_55)]. Similarly, Musso *et al*[[11](#_ENREF_11)] reported an increased risk for incident diabetes in patients with evidence of ultrasonographic and histological NAFLD in a recent meta-analysis of three large community-based cohorts with a range of follow-up from 4 to 10 years.

Overall, it appears that DM and NAFLD are two conditions with intense interplay roles, one adversely affecting the natural history of the other, and vice-versa.

**Pathogenetic mechanisms**

The pathophysiological hallmark of NAFLD is the underlying insulin resistance (Figure 1). Insulin resistance at the level of the adipocyte seems to be the primary defect in NAFLD, leading to increased lipolysis. Any grade of insulin secretion deficit associated with DM, would further increase lipase activity in adipose tissue. This leads first to elevated circulating and portal free fatty acids (FFAs) and subsequently to their increased skeletal muscle and hepatic delivery and uptake, which decreases insulin action in these tissues[[57](#_ENREF_56),[58](#_ENREF_57)]. Insulin resistance in these tissues leads to increased gluconeogenesis and glycogenolysis in liver as well as reduction in peripheral glucose disposal resulting in hyperglycemia. The pancreatic beta islet cells adapt to hyperglycemia by rising insulin secretion, leading to hyperinsulinemia. Hyperinsulinemia and hyperglycemia also upregulate several key lipogenic transcription factors, including sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate response element binding protein (ChREBP), promoting hepatic lipid synthesis or *de novo* lipogenesis[[59](#_ENREF_58)]. In patients with NAFLD, studies have shown that vast majority of hepatic fat origins from FFAs (59%), but 26% comes from *de novo* lipogenesis and 15% originates from the diet[[60](#_ENREF_59)].

Hepatic steatosis results when the balance between delivery and synthesis of FFA exceeds the liver capacity to oxidize or export them. Lipids are exported in the form of very-low-density lipoprotein (VLDL), and the synthesis of apolipoproteinB-100, which is the apolipoprotein contained in VLDL particles, is reduced in patients with NASH[[61](#_ENREF_60)]. Accumulation of lipids can exert toxic effects on the liver by inefficient oxidation or activation of inflammatory pathways. Although hepatic triglycerides (triacylglycerol) are thought to be protective for NAFLD progression, certain lipid metabolites such as diacylglycerol and ceramides may themselves cause cell injury and death and contribute to NASH development[[62](#_ENREF_61)].

FFAs may be oxidized within mitochondria, peroxisomes and microsomal system. Increased FFA oxidation causes an oxidative stress that further uncouples mitochondrial oxidation/phosphorylation and generates more reactive oxygen species (ROS). Indeed, abnormal mitochondrial morphology and function is frequently present in the hepatocytes of patients with NASH. These abnormalities might render hepatocytes even more susceptible to oxidative damage[[63](#_ENREF_62)]. Oxidative stress generated from ROS promote lipid peroxidation and augment inflammation by up-regulating key factors and pathways of NF-kB and toll-like receptor (TLR) signaling[[64](#_ENREF_63)].

Systemic subclinical inflammation also appears to be involved in the pathogenesis of NAFLD and NASH. Patients with NAFLD have higher circulating markers of inflammation than healthy controls. It has been shown that diabetic patients with NAFLD have higher circulating markers of inflammation than diabetic patients without NAFLD[[65](#_ENREF_64)]. It has been also described that, as obesity increases, there is an increase in macrophage infiltration in adipose tissue. These activated macrophages secrete inflammatory cytokines such as TNF-alpha and IL-6 that may exacerbate insulin resistance by decreasing insulin signaling. In turn, serum levels of adiponectin, an anti-inflammatory insulin sensitizing and potentially hepatoprotective adipokine, are reduced in patients with NAFLD and are lower in NASH than in simple steatosis[[66](#_ENREF_65),[67](#_ENREF_66)]. Furthermore, adiponectin does fall in prediabetes and in T2DM and could be a link between adipocyte dysfunction and NASH in these patients[[68](#_ENREF_67)]. It is now recognized that NAFLD and particularly NASH progression results of a complex interplay between insulin resistance with hyperinsulinemia, increased oxidative stress, hepatic and systemic inflammation. Hyperinsulinemia combined with ongoing liver inflammation and hepatocyte apoptosis may induce profibrotic factors. Thus, profibrotic factors can contribute to fibrosis progression by activating hepatocyte stellate cells.

NAFLD has recently been linked to alterations of gut microbiota and its metabolic effects. Increased absorption of lipopolysaccharides (LPS) resulting from a “leaky” small intestinal mucosa may cause activation of the innate immune system, by direct stimulation of TLR-signaling, leading to inflammation and insulin resistance[[69](#_ENREF_68)]. Of note, patients with T2DM had mean plasma levels of LPS higher than controls due to a higher small intestinal bacterial overgrowth and increased leakiness of the intestinal mucosa[[70](#_ENREF_69)].

Genetic factors may also play a role in the development of NAFLD. Based on the complex mechanisms involved in the pathogenesis of NAFLD, there is a low likelihood of finding a single candidate gene responsible for NAFLD or a clear genetic link between T2DM and NAFLD. Through several genome-wide association studies, the missense rs738409 C/G single-nucleotide polymorphism implying an amino acid change from isoleucine (I) to methionine (M) at the position 148 (I148M) of the protein encoding by the *patatin-like phospholipase domain–containing 3* gene (*PNPLA3*) was strongly associated with increased hepatic fat content and NAFLD histological severity[[71](#_ENREF_70),[72](#_ENREF_71)]. Genetic variation at *PNPLA3* seems to confer a markedly increased risk of severe histological features of NAFLD, but there is no association of this genetic polymorphism with body mass index, triglyceride, HDL- and LDL-cholesterol levels, or diabetes[[73](#_ENREF_72)]. Other polymorphisms in the microsomal triglyceride transfer protein or in the gene of superoxide dismutase 2 could play a role in the interaction between NAFLD and diabetes[[74](#_ENREF_73),[75](#_ENREF_74)].

The main physiopathological mechanisms involved in NAFLD progression from simple steatosis to NASH and fibrosis are summarized in Figure 2.

**Diagnosis**

When NAFLD is suspected, the first step to define its diagnosis is to exclude other known etiologies of chronic liver diseases like drug-related steatosis[[76](#_ENREF_75),[77](#_ENREF_76)], viruses and alcohol. As previously described, a careful history of alcohol ingestion must be taken. Of note, diabetic patients with excessive alcohol intake may have both alcoholic- and NAFLD[[78](#_ENREF_77)].

Liver enzymes may be elevated, but normal aminotransferases do not exclude the diagnosis of NAFLD, even in diabetic individuals[[4](#_ENREF_4),[79](#_ENREF_78)]. Aminotransferase levels have been reevaluated and new thresholds have been suggested for normal levels considering patients with NAFLD. These levels are 19 U/L for men and 30 U/L for women and this improved the sensitivity for diagnosing NAFLD[[80](#_ENREF_79)], although the diagnosis of NASH still cannot be performed based solely on aminotransferases. Although unspecific, serum ferritin levels may be high and it is important to discard hemochromatosis in patients with a high transferrin saturation index[[81](#_ENREF_80)]. Low-titer autoantibodies, such as anti-nuclear and anti-smooth muscle, can be found as an epiphenomenon in NAFLD[[82](#_ENREF_81)], although a liver biopsy may be indicated to exclude autoimmune liver disease.

The diagnostic approach to patients with NAFLD is based mainly on imaging, serological and histopathological methods. Apart from DM, other clinical conditions are associated with NAFLD, such as essential hypertension, obesity, hypertriglyceridemia, polycystic ovary disease and metabolic syndrome[[83-85](#_ENREF_82)]. Thus, NAFLD should also be investigated in these clinical settings, and routinely in DM.

The spectrum of NAFLD is similar in diabetic and non-diabetic individuals, and it complies from simple steatosis to advanced fibrosis, cirrhosis and hepatocellular carcinoma[[86](#_ENREF_85)]. The only reliable method that identifies these different stages is liver biopsy. However, owing to its potential complications and limitations like cost, sampling error and procedure risks, many non-invasive methods have been proposed to diagnose NAFLD and to predict those patients with a higher risk of having NASH[[87](#_ENREF_86),[88](#_ENREF_87)].

Imaging methods have a variable accuracy to identify liver steatosis[[89](#_ENREF_88)]. Liver ultrasonography (US) is a safe, inexpensive and readily available method. It is the most used technique to diagnose NAFLD with a sensitivity of 60%–94% and a specificity of 66%–95% for detecting steatosis[[90](#_ENREF_89),[91](#_ENREF_90)]. Its main limitation is that it is operator-dependent and cannot detect mild steatosis (5%-30%)[[90-92](#_ENREF_89)]. Additional dopplerfluxometry helps identify indirect signs of advanced liver disease. Recently, Ballestri *et al*[93] developed the ultrasonographic fatty liver indicator (US-FLI), a new score ranging from 2 to 8 points, which is capable of ruling out NASH based on US parameters like the intensity of liver and kidney contrast, posterior attenuation of US beam, vessel blurring, difficult visualization of gallbladder wall, difficult visualization of the diaphragm and areas of focal sparing. It has a negative predictive value for NASH of 94% and can be easily assessed[[93](#_ENREF_92)].

Computed tomography (CT) allows quantitative and qualitative evaluation of liver steatosis with a higher accuracy. Based on the difference of the hepatic-splenic attenuation, unenhanced CT can detect liver steatosis grades as low as 5%[[94](#_ENREF_93)]. Magnetic resonance (MR) imaging with appropriate sequences also provides high sensitivity and specificity. MR spectroscopy is one of the most accurate methods for the evaluation of liver steatosis, has a strong correlation with histology and can detect very low levels of steatosis[[95](#_ENREF_94)]. Recently, MR elastography has showed a high predictive value for excluding advanced fibrosis and a good accuracy for detecting NASH with an area under ROC curve of 0.93. MR elastography discriminated NASH from steatosis with a sensitivity of 94% and specificity of 73% with a cutoff of 2.74 kilopascals units (kPa)[[96](#_ENREF_95)]. However, MR is too expensive to be used routinely, but might be useful in patients under study protocols and in those with a strong suspicious of NAFLD with normal liver echogenicity on ultrasound[[97](#_ENREF_96)].

A novel method to diagnose and quantify steatosis is the controlled attenuated parameter (CAP)[[98](#_ENREF_97)]. CAP is a software that can be used simultaneously with liver transient elastography available by Fibroscan[[99](#_ENREF_98),[100](#_ENREF_99)].It is a simple and easily performed method that can detect liver steatosis as low as 5%. Sasso *et al*[[99](#_ENREF_98)] defined the best cutoff value of 292 for severe steatosis (> 66%) detection, with a negative predictive value of 100%. Its main limitation is the difficulty to obtain reliable measurements in obese patients. When liver steatosis is estimated using CAP, liver stiffness is also evaluated by Fibroscan elastography. In this method, vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the liver. The velocity of wave propagation relates directly to liver stiffness or fibrosis: the stiffer the tissue, the faster the shear wave propagates. The velocity of the shear wave propagation is measured in kilopascals (kPa). Higher tissue stiffness corresponds to increasing severity of fibrosis. Wong *et al*[[101](#_ENREF_100)] defined a cutoff of 10.3 kPa in NAFLD patients to predict advanced fibrosis with a sensitivity of 92% and specificity of 88%. The negative predictive value of this cutoff for advanced fibrosis was 99%. The possibility of evaluating fibrosis and steatosis simultaneously makes the Fibroscan a valuable tool in the study of NAFLD.

Many serological methods have been evaluated in the diagnosis of NAFLD regarding their accuracy for detecting NASH[[102](#_ENREF_101)]. In this setting, the AST/ALT ratio[[103](#_ENREF_102)], FIB-4 index[87], the BARD score[[47](#_ENREF_46)], NAFLD Fibrosis Score[[46](#_ENREF_45)], and the Enhanced Liver Fibrosis (ELF) test shall be addressed. The AST/ALT ratio has been used to identify patients with advanced fibrosis and a value > 1 may predict advanced fibrosis in patients with NAFLD[[103](#_ENREF_102)]. The FIB-4 index is easily calculated using the following formula: [Age (years) × AST (U/L)] / [platelet X√ALT (U/L)] being useful for the diagnosis of liver fibrosis, but not capable of diagnosing NASH. It was described that a FIB-4 index ≥ 2.67 had an 80% positive predictive value and a FIB-4 index ≤ 1.30 had a 90% negative predictive value to fibrosis in patients with NAFLD, although other non-invasive tests like the ELF and fibrotest were more accurate than FIB-4 index to predict advanced fibrosis[[87](#_ENREF_86),[104](#_ENREF_103)]. The NAFLD fibrosis score and the BARD score include DM as a variable in their formula. The BARD score is composed of three variables: an AST/ALT ratio ≥ 0.8 sums 2 points; a BMI ≥ 28 sums 1 point; presence of diabetes sums 1 point. The possible score ranges from 0 to 4 points. According to the results by Harrison *et al*[47], score values of 0 or 1 would have a high negative predictive value (NPV) for severe fibrosis. The NAFLD score comprises demographic and easily obtained laboratory variables as age, BMI, hyperglycemia, platelet count, albumin and AST/ALT ratio. The formulae {−1.675 + [0.037 × age (years)] + (0.094 × BMI) + [1.13 × IFG/diabetes (yes = 1, no = 0)] + (0.99 × AST/ALT) - [0.013 × platelet (109/l)] - [0.66 x albumin (g/dl)]} can be assessed online at http://NAFLDscore.com. According to several studies it has a high accuracy to predict NASH and advanced fibrosis. Musso *et al*[11] showed in a meta-analysis that the NAFLD score had a sensitivity and specificity higher than 90% to predict NASH and advanced fibrosis and suggested that the combination of two non-invasive methods like the NAFLD score and Fibroscan elastography could be a useful tool in this setting[[11](#_ENREF_11)]. The ELF panel, obtained through the assessment of three matrix turnover proteins (hyaluronic acid, TIMP-1 and PIIINP) displayed a high accuracy in predicting NASH, although its use may be limited by cost and local availability[[105](#_ENREF_104)]. Cytokeratin-18 (CK-18) is a serological marker of apoptosis that can be used alone or in combination and was highly accurate for NASH detection[[106](#_ENREF_105)]. Its main limitation for clinical use is that a well-established cutoff is not yet defined.

So far, studies evaluating the different serum biomarkers are comprised of general patients with NAFLD and there is no specific test to patients with DM. Recently, we studied several serum biomarkers in 78 biopsy-proven NAFLD diabetic patients and showed an association between low levels of adiponectin and TGF-β1 with the severe NAFLD stages[[67](#_ENREF_66)]. Maybe the combination of two methods like serum biomarkers and imaging methods might be the best tool for predicting NASH and advanced fibrosis.

The histological diagnosis of NAFLD is defined as the presence of lipid deposit in more than 5% of the hepatocytes independent of the localization into the hepatic lobule. However, the most important issue is the definition of NASH, owing to its prognostic value. Brunt *et al*[[16](#_ENREF_16)] in 1999 classified NAFLD into three different stages: mild, moderate and severe. Likewise, Matteoni’s classification of NAFLD was based on the severity of hepatic lesion as follows: type 1, isolated steatosis; type 2, steatosis and lobular inflammation; type 3, steatosis and ballonization of hepatocytes; and type 4, which added the presence of hyaline bodies and fibrosis to the previous stages. Stages 3 and 4 were considered as NASH17]. Kleiner *et al*[[17](#_ENREF_17)] in 2005 proposed an update on Brunt’s classification and defined a score named NAS, based on the sum of three criteria: steatosis (graded 0 to 3), lobular inflammation (graded 0 to 3) and ballonization (graded 0 to 2). A NAS ≥ 5 points implies an advanced inflammatory activity. However, this score should not be applied to diagnose NASH because many patients have NASH with a NAS < 4 points. The NAS is a useful tool to evaluate treatment response and should be used in this situation. Thus, the hallmarks to the diagnosis of NASH are the histological findings observed in liver biopsy and not its intensity. Currently, NASH is defined by the combination of steatosis and necroinflammatory lesions, like ballonization, with or without fibrosis[[107](#_ENREF_107)].

**Treatment**

There are very few randomized, blinded and controlled clinical trials of drugs with sufficient duration and adequate histological outcomes in patients with NAFLD and DM. Hence, data on treatment of NAFLD in diabetic patients are scarce, and treatment of NAFLD in diabetic patients is conducted based on evidences from mixed populations of diabetic and non-diabetic individuals.

Weight loss following caloric restriction and physical exercise improves insulin sensitivity and cardiometabolic risk factors. However, both implementation and maintenance of these lifestyle interventions pose challenges for most of the individuals[[108](#_ENREF_108),[109](#_ENREF_109)]. A 5% weight loss through lifestyle modification improved liver biochemistry and reduced hepatic steatosis[[110](#_ENREF_110)], however at least a 10% weight reduction was required for a significant improvement in inflammation, ballooning, and NAS[[110](#_ENREF_110)]. Notably, sedentary patients with NAFLD and DM should undergo a cardiovascular risk assessment before initiating a fitness program, especially before a high intensive training. In a retrospective study with 813 individuals with biopsy-proven NAFLD from the Nonalcoholic Steatohepatitis Clinical Research Network, neither moderate-intensity exercise nor total exercise per week were associated with NASH or stage of fibrosis. In this study, meeting vigorous recommendations was associated with decreased adjusted odds of having NASH. This study suggested that maybe the intensity of the exercise could be more important than its duration[[111](#_ENREF_111)]. Notwithstanding, Hallsworth *et al*[[112](#_ENREF_112)] showed that independent of weight loss, moderate anaerobic exercise seemed to improve insulin sensitivity and hepatic steatosis. In a recent randomized controlled trial, Bacchi *et al*[113] compared the effects of aerobic (AER) or resistance (RES) training on hepatic fat content in 31 type 2 diabetic subjects with NAFLD. Hepatic fat content was markedly reduced in both AER and RES training groups. In addition, hepatic steatosis defined as hepatic fat content > 5.56% by an in-opposed-phase magnetic resonance imaging technique was not detected in about one-quarter of the patients in each intervention group.

Because NAFLD is present in the majority of patients who undergo bariatric surgery, there has been growing interest in evaluating the role of foregut surgery in NAFLD treatment. In a prospective study with 381 severe obese adults followed-up for 5 years after surgery, it was observed significant improvements in steatosis, ballooning, NAS and resolution of NASH, changes already present at the first year[[114](#_ENREF_113)]. After 5 years, levels of fibrosis increased, but 95.7% of patients maintained a grade 1 fibrosis. As none of the patients had advanced fibrosis at entry, the effect of bariatric surgery on liver fibrosis could not be evaluated[[114](#_ENREF_113)]. In a meta-analysis that evaluated the influence of bariatric surgery on liver histology in adults with NAFLD, Mummadi *et al*[115] found that steatosis, NASH, and fibrosis improved or completely resolved in a significant proportion of patients. At this moment, there is still no clear evidence indicating foregut bariatric surgery as an established option to specifically treat NASH, but it may provide benefit in NAFLD treatment in otherwise eligible obese individuals[[115](#_ENREF_114)]. Table 2 summarizes the principal studies concerning non-pharmacological interventions in patients with NAFLD.

High doses of omega-3 polyunsaturated fatty acids (PUFAs) are effective in treating hypertriglyceridemia that is often a feature of NAFLD and T2DM. The efficacy of omega-3 PUFAs supplementation in NAFLD has recently been examined in a systematic review of nine eligible studies, involving 355 patients with NAFLD[[116](#_ENREF_115)]. This systematic review with different doses of omega-3 PUFAs demonstrated significant reductions in hepatic fat content. However, at this point, the optimal dose and duration of this therapy is not yet established. A large randomized placebo-controlled trial of two doses of eicosapentanoic acid is under way in the United States.

Many drugs have been evaluated in NAFLD management. The main studies on pharmacological treatments of NAFLD are resumed on Table 3. Statin therapy is recommended in patients with overt cardiovascular disease and in almost all patients with T2DM. Additionally, these drugs can be used in dyslipidemic subjects with increased baseline liver enzymes and may even produce some histological benefit in NASH[[117](#_ENREF_116),118]. Ursodesoxycholic acid (UDCA) is a secondary bile acid with lipid lowering, anti-apoptotic and anti-inflammatory properties. There has been initial interest in the use of UDCA to treat NAFLD, although double-blind, randomized, placebo-controlled trials with doses ranging from 13 to 28 mg/kg per day and pre- and post-treatment liver biopsies have yielded disappointing results[119,120].

Given the importance of insulin resistance in the pathogenesis of NAFLD, insulin-sensitizing agents have been investigated in the treatment of this condition in patients with and without diabetes. Metformin reduces endogenous glucose production and improves whole-body insulin sensitivity. It is the first-line choice in oral therapy for patients with T2DM. Metformin has beneficial effects on serum aminotransferases and insulin resistance. However, in patients with NAFLD without T2DM, a number of small randomized placebo-controlled clinical trials with different doses (1500-2000 mg/d) and short durations (6–12 mo) have failed to demonstrate an improvement in liver steatosis, inflammation or fibrosis[121-123]. In spite of these poor results, there is evidence from case-control and population-based studies that the use of metformin was associated with risk reduction for the development of hepatocellular carcinoma in diabetic patients[124,125].

Rosiglitazone and pioglitazone are peroxisome proliferator-activated receptor γ (PPAR γ) agonists that redistribute fat from the muscle and liver to peripheral adipose tissue and, thereby, improve insulin resistance. Concerns have been raised regarding an association between increased cardiovascular risk with rosiglitazone and its use has been restricted. Three studies of pioglitazone with doses ranging from 30 to 45 mg found a significant improvement in liver histology when compared with placebo in patients with NASH[126-128], but improvement of fibrosis was demonstrated in only one study[128]. Moreover, among these studies only one examined a cohort of patients with T2DM or impaired glucose tolerance with NASH; in this study, pioglitazone significantly improved steatosis, hepatocellular ballooning, inflammation and necroinflammation, compared with placebo. Improvement in the NAS was seen in 73% of patients treated with pioglitazone compared to 24% of placebo-treated patients, and there was a trend toward improvement in fibrosis in patients receiving pioglitazone[[127](#_ENREF_126)]. The PIVENS study[129] is a recent clinical trial that randomized 247 non-diabetic patients with biopsy-proven NASH to pioglitazone 30 mg/d, vitamin E 800 IU/d, or placebo for 24 mo. The primary outcome was histological improvement in the features of NASH. Pioglitazone, as compared to placebo, was not associated with a significantly higher rate of improvement in the composite NAS score. However, both vitamin E and pioglitazone treatment improved the scores of steatosis, inflammation, ballooning, and serum aminotransferase levels[129]. It seems that liver histology benefits obtained with pioglitazone therapy may disappear with its discontinuation. Nonetheless, there is a debate surrounding the long-term risk-benefit ratio of pioglitazone therapy. The most frequent side-effects of pioglitazone are weight gain of 2-5 kg and bone loss with fractures[130]. Pioglitazone treatment can also precipitate congestive heart failure in patients with preexisting cardiac failure[131]. In addition, increased bladder cancer risk has been recently associated with pioglitazone use in diabetic patients[[132](#_ENREF_131)].

Glucagon-like peptide-1 (GLP-1) agonists and dipeptidyl peptidase-IV (DPP-IV) inhibitors are new pharmacological agents with multiple anti-hyperglycemic actions. The biological activities of GLP-1 agonists include glucose-dependent insulin secretion, suppression of postprandial glucagon to reduce hepatic glucose release and slowing of gastric emptying. There is also evidence that GLP-1 agonists have beneficial effects on the liver, including suppression of hepatic lipogenesis and stimulation of lipid oxidation[[133](#_ENREF_132),[134](#_ENREF_133)]. A recent meta-analysis of two GLP-1 agonists, liraglutide and exenatide, in populations with and without diabetes, including data on liver enzyme tests from 12 of the 25 trials included, found that ALT concentrations decreased after treatment with the liraglutide but not with exenatide[[135](#_ENREF_134)]. Clinical trials with these agents in patients with NAFLD with or without T2DM are ongoing and their results are awaited in the next years.

Increased oxidative stress occurs in NAFLD and T2DM. Among antioxidant compounds, vitamin E has the most significant evidence supporting its use. In the PIVENS study[129], vitamin E supplementation, 800 UI/d, resulted in significant improvement in pathological features of NASH. The improvement in NAS was observed in 42% of patients receiving vitamin E compared with 19% of patients receiving placebo. Nevertheless, caution must be applied regarding the long-term safety of vitamin E, especially in doses greater than 400 UI/d, which may be associated with increased risk of all-cause mortality[[13](#_ENREF_135)6]. Currently, there is no evidence regarding vitamin E effectiveness and safety in diabetic patients with NASH or in patients with NASH-related cirrhosis.

GFT505, a dual peroxisome proliferator-activated receptor (PPAR)-α/δ agonist, improved peripheral and hepatic insulin sensitivity in a randomized crossover study to subsequent 8-week treatment periods with GFT505 (80 mg/d) or placebo. GFT505 also reduced liver enzyme concentrations and could be a promising drug candidate for the treatment of T2DM and NAFLD. There was no indication of PPARγ activation and no safety concern with GFT505[[137](#_ENREF_136)].

Obeticholic acid (OCA), a farnesoid X agonist receptor, is a semi-synthetic human bile acid that regulates glucose and lipid metabolism. Data from a small pilot study demonstrate that OCA improves insulin sensitivity compared with placebo. Also, of importance, OCA appears to improve liver injury in patients with T2DM and NAFLD[[138](#_ENREF_137)]. Larger studies with longer duration of therapy and follow-up are needed to evaluate long-term efficacy of these emerging therapies.

**Conclusion**

Patients with DM and NAFLD are prone to the severest stages of liver diseases and to cardiovascular and liver-related outcomes. The major challenge is to identify these patients by accurate non-invasive methods. Many algorithms and new imaging methods are available but they still need to be validated in this specific population. The ideal treatment would be effective for both NASH and diabetes, but it is not yet available. Given the importance of cardiovascular and liver outcomes in diabetic patients, effective interventions are urgently required in order to prevent progression to these life-threatening and prevalent complications.

**References**

1 **Younossi ZM**, Gramlich T, Matteoni CA, Boparai N, McCullough AJ. Nonalcoholic fatty liver disease in patients with type 2 diabetes. *Clin Gastroenterol Hepatol* 2004; **2**: 262-265 [PMID: 15017611 DOI: 10.1053/S1542-3565(04)00014-X]

2 **Trombetta M**, Spiazzi G, Zoppini G, Muggeo M. Review article: type 2 diabetes and chronic liver disease in the Verona diabetes study. *Aliment Pharmacol Ther* 2005; **22** Suppl 2: 24-27 [PMID: 16225467 DOI: 10.1111/j.1365-2036.2005.02590.x]

3 **Targher G**, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; **30**: 1212-1218 [PMID: 17277038 DOI: 10.2337/dc06-2247]

4 **Leite NC**, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009; **29**: 113-119 [PMID: 18384521 DOI: 10.1111/j.1478-3231.2008.01718.x]

5 **Prashanth M**, Ganesh HK, Vima MV, John M, Bandgar T, Joshi SR, Shah SR, Rathi PM, Joshi AS, Thakkar H, Menon PS, Shah NS. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *J Assoc Physicians India* 2009; **57**: 205-210 [PMID: 19588648]

6 **Leite NC**, Villela-Nogueira CA, Pannain VL, Bottino AC, Rezende GF, Cardoso CR, Salles GF. Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. *Liver Int* 2011; **31**: 700-706 [PMID: 21457442 DOI: 10.1111/j.1478-3231.2011.02482.x]

7 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]

8 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

9 **Porepa L**, Ray JG, Sanchez-Romeu P, Booth GL. Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. *CMAJ* 2010; **182**: E526-E531 [PMID: 20566726 DOI: 10.1503/cmaj.092144]

10 **Willner IR**, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001; **96**: 2957-2961 [PMID: 11693332 DOI: 10.1111/j.1572-0241.2001.04667.x]

11 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

12 **Amarapurka DN**, Amarapurkar AD, Patel ND, Agal S, Baigal R, Gupte P, Pramanik S. Nonalcoholic steatohepatitis (NASH) with diabetes: predictors of liver fibrosis. *Ann Hepatol* 2006; **5**: 30-33 [PMID: 16531962]

13 **Gupte P**, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, Patel N, Madan A, Amarapurkar A. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol* 2004; **19**: 854-858 [PMID: 15242486 DOI: 10.1111/j.1440-1746.2004.03312.x]

14 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]

15 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419 [PMID: 10348825 DOI: 10.1016/S0016-5085(99)70506-8]

16 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]

17 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

18 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]

19 **Bellentani S**, Saccoccio G, Costa G, Tiribelli C, Manenti F, Sodde M, Saveria Crocè L, Sasso F, Pozzato G, Cristianini G, Brandi G. Drinking habits as cofactors of risk for alcohol induced liver damage. The Dionysos Study Group. *Gut* 1997; **41**: 845-850 [PMID: 9462221]

20 **Farrell GC**, Chitturi S, Lau GK, Sollano JD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. *J Gastroenterol Hepatol* 2007; **22**: 775-777 [PMID: 17565629 DOI: 10.1111/j.1440-1746.2007.05002.x]

21 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]

22 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]

23 **Loomba R**, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 686-690 [PMID: 24042449 DOI: 10.1038/nrgastro.2013.171]

24 **Fan JG.** An introduction of strategies for the management of nonalcoholic fatty liver disease (NAFLD) recommended by Asia Pacific Working Party on NAFLD. *Chin J Hepatol* 2007; **15**: 552-553 [PMID: 17669255]

25 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]

26 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

27 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

28 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]

29 **Hashimoto E**, Yatsuji S, Tobari M, Taniai M, Torii N, Tokushige K, Shiratori K. Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *J Gastroenterol* 2009; **44** Suppl 19: 89-95 [PMID: 19148800 DOI: 10.1007/s00535-008-2262-x]

30 **McGill M**, Molyneaux L, Twigg SM, Yue DK. The metabolic syndrome in type 1 diabetes: does it exist and does it matter? *J Diabetes Complications* 2008; **22**: 18-23 [PMID: 18191073 DOI: 10.1016/j.diacomp.2006.10.005.]

31 **Targher G**, Bertolini L, Padovani R, Rodella S, Zoppini G, Pichiri I, Sorgato C, Zenari L, Bonora E. 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 [PMID: 20619918 DOI: 10.1016/j.jhep.2010.04.030]

32 **Lv WS**, Sun RX, Gao YY, Wen JP, Pan RF, Li L, Wang J, Xian YX, Cao CX, Zheng M. Nonalcoholic fatty liver disease and microvascular complications in type 2 diabetes. *World J Gastroenterol* 2013; **19**: 3134-3142 [PMID: 23716995 DOI: 10.3748/wjg.v19.i20.3134]

33 **Williamson RM**, Price JF, Glancy S, Perry E, Nee LD, Hayes PC, Frier BM, Van Look LA, Johnston GI, Reynolds RM, Strachan MW. Prevalence of and risk factors for hepatic steatosis and nonalcoholic Fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 2011; **34**: 1139-1144 [PMID: 21478462 DOI: 10.2337/dc10-2229]

34 **Rafiq N**, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009; **7**: 234-238 [PMID: 19049831 DOI: 10.1016/j.cgh.2008.11.005]

35 **Ratziu V**, Bugianesi E, Dixon J, Fassio E, Ekstedt M, Charlotte F, Kechagias S, Poynard T, Olsson R. Histological progression of non-alcoholic fatty liver disease: a critical reassessment based on liver sampling variability. *Aliment Pharmacol Ther* 2007; **26**: 821-830 [PMID: 17767466 DOI: 10.1111/j.1365-2036.2007.03425.x]

36 **Boza C**, Riquelme A, Ibañez L, Duarte I, Norero E, Viviani P, Soza A, Fernandez JI, Raddatz A, Guzman S, Arrese M. Predictors of nonalcoholic steatohepatitis (NASH) in obese patients undergoing gastric bypass. *Obes Surg* 2005; **15**: 1148-1153 [PMID: 16197788 DOI: 10.1381/0960892055002347]

37 **Lima ML**, Mourão SC, Diniz MT, Leite VH. Hepatic histopathology of patients with morbid obesity submitted to gastric bypass. *Obes Surg* 2005; **15**: 661-669 [PMID: 15946458]

38 **Kemmer NM**, Xiao SY, Singh H, Murray R, Abdo B. High prevalence of NASH among Mexican American females with type II diabetes mellitus. *Gastroenterology* 2001; **120** Suppl 1: A117 [abstract]

39 **Kotronen A**, Juurinen L, Hakkarainen A, Westerbacka J, Cornér A, Bergholm R, Yki-Järvinen H. Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. *Diabetes Care* 2008; **31**: 165-169 [PMID: 17934148 DOI: 10.2337/dc07-1463]

40 **Loomba R**, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, Bass NM. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* 2012; **56**: 943-951 [PMID: 22505194 DOI: 10.1002/hep.25772]

41 **Targher G**, Chonchol M, Pichiri I, Zoppini G. Risk of cardiovascular disease and chronic kidney disease in diabetic patients with non-alcoholic fatty liver disease: just a coincidence? *J Endocrinol Invest* 2011; **34**: 544-551 [PMID: 21427524 DOI: 10.3275/7614]

42 **Targher G**, Bertolini L, Rodella S, Lippi G, Zoppini G, Chonchol M. Relationship between kidney function and liver histology in subjects with nonalcoholic steatohepatitis. *Clin J Am Soc Nephrol* 2010; **5**: 2166-2171 [PMID: 20724519 DOI: 10.2215/CJN.05050610]

43 **Targher G**, Chonchol M, Zoppini G, Abaterusso C, Bonora E. Risk of chronic kidney disease in patients with non-alcoholic fatty liver disease: is there a link? *J Hepatol* 2011; **54**: 1020-1029 [PMID: 21145850 DOI: 10.1016/j.jhep.2010.11.007]

44 **Targher G**, Bertolini L, Chonchol M, Rodella S, Zoppini G, Lippi G, Zenari L, Bonora E. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and retinopathy in type 1 diabetic patients. *Diabetologia* 2010; **53**: 1341-1348 [PMID: 20369224 DOI: 10.1007/s00125-010-1720-1]

45 **Lee YJ**, Shim JY, Moon BS, Shin YH, Jung DH, Lee JH, Lee HR. The relationship between arterial stiffness and nonalcoholic fatty liver disease. *Dig Dis Sci* 2012; **57**: 196-203 [PMID: 21750929 DOI: 10.1007/s10620-011-1819-3]

46 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]

47 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]

48 **Argo CK**, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J Hepatol* 2009; **51**: 371-379 [PMID: 19501928 DOI: 10.1016/j.jhep.2009.03.019]

49 **Bugianesi E**, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005; **48**: 634-642 [PMID: 15747110 DOI: 10.1007/s00125-005-1682-x]

50 **Ryysy L**, Häkkinen AM, Goto T, Vehkavaara S, Westerbacka J, Halavaara J, Yki-Järvinen H. Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. *Diabetes* 2000; **49**: 749-758 [PMID: 10905483]

51 **Manchanayake J**, Chitturi S, Nolan C, Farrell GC. Postprandial hyperinsulinemia is universal in non-diabetic patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2011; **26**: 510-516 [PMID: 21155882 DOI: 10.1111/j.1440-1746.2010.06528.x]

52 **Speliotes EK**, Massaro JM, Hoffmann U, Vasan RS, Meigs JB, Sahani DV, Hirschhorn JN, O'Donnell CJ, Fox CS. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. *Hepatology* 2010; **51**: 1979-1987 [PMID: 20336705 DOI: 10.1002/hep.23593]

53 **Targher G**, Byrne CD. Clinical Review: Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab* 2013; **98**: 483-495 [PMID: 23293330 DOI: 10.1210/jc.2012-3093]

54 **Kasturiratne A**, Weerasinghe S, Dassanayake AS, Rajindrajith S, de Silva AP, Kato N, Wickremasinghe AR, de Silva HJ. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. *J Gastroenterol Hepatol* 2013; **28**: 142-147 [PMID: 22989165 DOI: 10.1111/j.1440-1746.2012.07264.x]

55 **Choi JH**, Rhee EJ, Bae JC, Park SE, Park CY, Cho YK, Oh KW, Park SW, Lee WY. Increased risk of type 2 diabetes in subjects with both elevated liver enzymes and ultrasonographically diagnosed nonalcoholic fatty liver disease: a 4-year longitudinal study. *Arch Med Res* 2013; **44**: 115-120 [PMID: 23398788 DOI: 10.1016/j.arcmed.2013.01.007]

56 **Bae JC**, Rhee EJ, Lee WY, Park SE, Park CY, Oh KW, Park SW, Kim SW. Combined effect of nonalcoholic fatty liver disease and impaired fasting glucose on the development of type 2 diabetes: a 4-year retrospective longitudinal study. *Diabetes Care* 2011; **34**: 727-729 [PMID: 21278140 DOI: 10.2337/dc10-1991]

57 **Cusi K**. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology* 2012; **142**: 711-725.e6 [PMID: 22326434 DOI: 10.1053/j.gastro.2012.02.003]

58 **Lomonaco R**, Ortiz-Lopez C, Orsak B, Webb A, Hardies J, Darland C, Finch J, Gastaldelli A, Harrison S, Tio F, Cusi K. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 1389-1397 [PMID: 22183689 DOI: 10.1002/hep.25539]

59 **Browning JD**, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 2004; **114**: 147-152 [PMID: 15254578 DOI: 10.1172/JCI22422]

60 **Donnelly KL**, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1343-1351 [PMID: 15864352 DOI: 10.1172/JCI23621]

61 **Charlton M**, Sreekumar R, Rasmussen D, Lindor K, Nair KS. Apolipoprotein synthesis in nonalcoholic steatohepatitis. *Hepatology* 2002; **35**: 898-904 [PMID: 11915037 DOI: 10.1053/jhep.2002.32527]

62 **Neuschwander-Tetri BA**. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology* 2010; **52**: 774-788 [PMID: 20683968 DOI: 10.1002/hep.23719]

63 **Sanyal AJ**, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256]

64 **Brenner DA**, Seki E, Taura K, Kisseleva T, Deminicis S, Iwaisako K, Inokuchi S, Schnabl B, Oesterreicher CH, Paik YH, Miura K, Kodama Y. Non-alcoholic steatohepatitis-induced fibrosis: Toll-like receptors, reactive oxygen species and Jun N-terminal kinase. *Hepatol Res* 2011; **41**: 683-686 [PMID: 21711427 DOI: 10.1111/j.1872-034X.2011.00814.x]

65 **Kelley DE**, McKolanis TM, Hegazi RA, Kuller LH, Kalhan SC. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol Endocrinol Metab* 2003; **285**: E906-E916 [PMID: 12959938 DOI: 10.1152/ajpendo.00117.2003]

66 **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 [PMID: 21040935 DOI: 10.1016/j.metabol.2010.09.003]

67 **Leite NC**, Salles GF, Cardoso CR, Villela-Nogueira CA. Serum biomarkers in type 2 diabetic patients with non-alcoholic steatohepatitis and advanced fibrosis. *Hepatol Res* 2013; **43**: 508-515 [PMID: 23067270 DOI: 10.1111/j.1872-034X.2012.01106.x]

68 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54 [PMID: 15239085 DOI: 10.1002/hep.20280]

69 **Musso G**, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Prog Lipid Res* 2009; **48**: 1-26 [PMID: 18824034 DOI: 10.1016/j.plipres.2008.08.001]

70 **Cuoco L**, Montalto M, Jorizzo RA, Santarelli L, Arancio F, Cammarota G, Gasbarrini G. Eradication of small intestinal bacterial overgrowth and oro-cecal transit in diabetics. *Hepatogastroenterology* 2002; **49**: 1582-1586 [PMID: 12397741]

71 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

72 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]

73 **Speliotes EK**, Butler JL, Palmer CD, Voight BF, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010; **52**: 904-912 [PMID: 20648472 DOI: 10.1002/hep.23768]

74 **Al-Serri A**, Anstee QM, Valenti L, Nobili V, Leathart JB, Dongiovanni P, Patch J, Fracanzani A, Fargion S, Day CP, Daly AK. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. *J Hepatol* 2012; **56**: 448-454 [PMID: 21756849 DOI: 10.1016/j.jhep.2011.05.029]

75 **Rubin D**, Helwig U, Pfeuffer M, Schreiber S, Boeing H, Fisher E, Pfeiffer A, Freitag-Wolf S, Foelsch UR, Doering F, Schrezenmeir J. A common functional exon polymorphism in the microsomal triglyceride transfer protein gene is associated with type 2 diabetes, impaired glucose metabolism and insulin levels. *J Hum Genet* 2006; **51**: 567-574 [PMID: 16721486 DOI: 10.1007/s10038-006-0400-y]

76 **Osman KA**, Osman MM, Ahmed MH. Tamoxifen-induced non-alcoholic steatohepatitis: where are we now and where are we going? *Expert Opin Drug Saf* 2007; **6**: 1-4 [PMID: 17181445 DOI: 10.1517/14740338.6.1.1]

77 **Farrell GC**. Drugs and steatohepatitis. *Semin Liver Dis* 2002; **22**: 185-194 [PMID: 12016549 DOI: 10.1055/s-2002-30106]

78 **Scaglioni F**, Ciccia S, Marino M, Bedogni G, Bellentani S. ASH and NASH. *Dig Dis* 2011; **29**: 202-210 [PMID: 21734385 DOI: 10.1159/000323886]

79 **Mofrad P**, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003; **37**: 1286-1292 [PMID: 12774006 DOI: 10.1053/jhep.2003.50229]

80 **Kunde SS**, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. *Hepatology* 2005; **42**: 650-656 [PMID: 16037946 DOI: 10.1002/hep.20818]

81 **Chandok N**, Minuk G, Wengiel M, Uhanova J. Serum ferritin levels do not predict the stage of underlying non-alcoholic fatty liver disease. *J Gastrointestin Liver Dis* 2012; **21**: 53-58 [PMID: 22457860]

82 **Vuppalanchi R**, Gould RJ, Wilson LA, Unalp-Arida A, Cummings OW, Chalasani N, Kowdley KV. Clinical significance of serum autoantibodies in patients with NAFLD: results from the nonalcoholic steatohepatitis clinical research network. *Hepatol Int* 2011; Epub ahead of print [PMID: 21557024 DOI: 10.1007/s12072-011-9277-8]

83 **Neuschwander-Tetri BA**. Fatty liver and the metabolic syndrome. *Curr Opin Gastroenterol* 2007; **23**: 193-198 [PMID: 17268250 DOI: 10.1097/MOG.0b013e32801421a9]

84 **Targher G**. Non-alcoholic fatty liver disease, the metabolic syndrome and the risk of cardiovascular disease: the plot thickens. *Diabet Med* 2007; **24**: 1-6 [PMID: 17227317 DOI: 10.1111/j.1464-5491.2007.02025.x]

85 **Targher G**, Byrne C. Diagnosis and Management of Nonalcoholic Fatty Liver Disease and Its Hemostatic/Thrombotic and Vascular Complications. *Semin Thromb Hem* 2013; **39**: 214-228 [PMID: 23397556 DOI: 10.1055/s-0033-1334866]

86 **Younossi Z**, Matteoni C, Gramlich T, Boparai N, Price L, McCullough A. Diabetes and non-alcoholic fatty liver disease: A worrisome combination. *Gastroenterology* 1999; **116**: A1292 [abstract]

87 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]

88 **Wieckowska A**, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. *Sem Liver Dis* 2008; **28**: 386-395 [PMID: 18956295 DOI: 10.1055/s-0028-1091983]

89 **Siegelman ES**, Rosen MA. Imaging of hepatic steatosis. *Semin Liver Dis* 2001; **21**: 71-80 [PMID: 11296698]

90 **Saadeh S**, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 745-750 [PMID: 12198701]

91 **Debongnie JC**, Pauls C, Fievez M, Wibin E. Prospective evaluation of the diagnostic accuracy of liver ultrasonography. *Gut* 1981; **22**: 130-135 [PMID: 7215943]

92 **Foster KJ**, Dewbury KC, Griffith AH, Wright R. The accuracy of ultrasound in the detection of fatty infiltration of the liver. *Br J Radiol* 1980; **53**: 440-442 [PMID: 7388276]

93 **Ballestri S**, Lonardo A, Romagnoli D, Carulli L, Losi L, Day CP, Loria P. Ultrasonographic fatty liver indicator, a novel score which rules out NASH and is correlated with metabolic parameters in NAFLD. *Liver Int* 2012; **32**: 1242-1252 [PMID: 22520641 DOI: 10.1111/j.1478-3231.2012.02804.x]

94 **Limanond P**, Raman SS, Lassman C, Sayre J, Ghobrial RM, Busuttil RW, Saab S, Lu DS. Macrovesicular hepatic steatosis in living related liver donors: correlation between CT and histologic findings. *Radiology* 2004; **230**: 276-280 [PMID: 14695401 DOI: 10.1148/radiol.2301021176]

95 **van Werven JR**, Marsman HA, Nederveen AJ, Smits NJ, ten Kate FJ, van Gulik TM, Stoker J. Assessment of hepatic steatosis in patients undergoing liver resection: comparison of US, CT, T1-weighted dual-echo MR imaging, and point-resolved 1H MR spectroscopy. *Radiology* 2010; **256**: 159-168 [PMID: 20574093 DOI: 10.1148/radiol.10091790]

96 **Chen J**, Talwalkar JA, Yin M, Glaser KJ, Sanderson SO, Ehman RL. Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. *Radiology* 2011; **259**: 749-756 [PMID: 21460032 DOI: 10.1148/radiol.11101942]

97 **Schwenzer NF**, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *J Hepatol* 2009; **51**: 433-445 [PMID: 19604596 DOI: 10.1016/j.jhep.2009.05.023]

98 **Castera L**, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 666-675 [PMID: 24061203 DOI: 10.1038/nrgastro.2013.175]

99 **Sasso M**, Beaugrand M, de Ledinghen V, Douvin C, Marcellin P, Poupon R, Sandrin L, Miette V. Controlled attenuation parameter (CAP): a novel VCTE™ guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound Med Biol* 2010; **36**: 1825-1835 [PMID: 20870345 DOI: 10.1016/j.ultrasmedbio.2010.07.005]

100 **de Lédinghen V**, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. *Liver Int* 2012; **32**: 911-918 [PMID: 22672642 DOI: 10.1111/j.1478-3231.2012.02820.x]

101 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745 DOI: 10.1002/hep.23312]

102 **McPherson S**, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 1265-1269 [PMID: 20801772 DOI: 10.1136/gut.2010.216077]

103 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362 [PMID: 10573511]

104 **Ratziu V**, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, Tahiri M, Munteanu M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V, Poynard T. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 6 [PMID: 16503961]

105 **Guha IN**, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; **47**: 455-460 [PMID: 18038452 DOI: 10.1002/hep.21984]

106 **Chen J**, Zhu Y, Zheng Q, Jiang J. Serum cytokeratin-18 in the diagnosis of non-alcoholic steatohepatitis: A meta-analysis. *Hepatol Res* 2013; Epub ahead of print [PMID: 23834322 DOI: 10.1111/hepr.12197]

107 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

108 **Bellentani S**, Dalle Grave R, Suppini A, Marchesini G. Behavior therapy for nonalcoholic fatty liver disease: The need for a multidisciplinary approach. *Hepatology* 2008; **47**: 746-754 [PMID: 18098321 DOI: 10.1002/hep.22009]

109 **Nobili V**, Manco M, Devito R, Di Ciommo V, Comparcola D, Sartorelli MR, Piemonte F, Marcellini M, Angulo P. Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: a randomized, controlled trial. *Hepatology* 2008; **48**: 119-128 [PMID: 18537181 DOI: 10.1002/hep.22336]

110 **Musso G**, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia* 2012; **55**: 885-904 [PMID: 22278337 DOI: 10.1007/s00125-011-2446-4]

111 **Kistler KD**, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol* 2011; **106**: 460-468; quiz 469 [PMID: 21206486 DOI: 10.1038/ajg.2010.488]

112 **Hallsworth K**, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut* 2011; **60**: 1278-1283 [PMID: 21708823 DOI: 10.1136/gut.2011.242073]

113 **Bacchi E**, Negri C, Targher G, Faccioli N, Lanza M, Zoppini G, Zanolin E, Schena F, Bonora E, Moghetti P. Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 randomized trial). *Hepatology* 2013; **58**: 1287-1295 [PMID: 23504926 DOI: 10.1002/hep.26393]

114 **Mathurin P**, Hollebecque A, Arnalsteen L, Buob D, Leteurtre E, Caiazzo R, Pigeyre M, Verkindt H, Dharancy S, Louvet A, Romon M, Pattou F. Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. *Gastroenterology* 2009; **137**: 532-540 [PMID: 19409898 DOI: 10.1053/j.gastro.2009.04.052]

115 **Mummadi RR**, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 1396-1402 [PMID: 18986848 DOI: 10.1016/j.cgh.2008.08.012]

116 **Parker HM**, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012; **56**: 944-951 [PMID: 22023985 DOI: 10.1016/j.jhep.2011.08.018]

117 **Foster T**, Budoff MJ, Saab S, Ahmadi N, Gordon C, Guerci AD. Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis Heart Study randomized clinical trial. *Am J Gastroenterol* 2011; **106**: 71-77 [PMID: 20842109 DOI: 10.1038/ajg.2010.299]

118 **Athyros VG**, Tziomalos K, Daskalopoulos GN, Karagiannis A, Mikhailidis DP. Statin-based treatment for cardiovascular risk and non-alcoholic fatty liver disease. Killing two birds with one stone? *Ann Med* 2011; **43**: 167-171 [PMID: 21476786 DOI: 10.3109/07853890.2011.561363]

119 **Lindor KD**, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, Lymp JF, Burgart L, Colin P. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology* 2004; **39**: 770-778 [PMID: 14999696 DOI: 10.1002/hep.20092]

120 **Leuschner UF**, Lindenthal B, Herrmann G, Arnold JC, Rössle M, Cordes HJ, Zeuzem S, Hein J, Berg T. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. *Hepatology* 2010; **52**: 472-479 [PMID: 20683947 DOI: 10.1002/hep.23727]

121 **Uygun A**, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A, Yesilova Z, Gulsen M, Dagalp K. Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2004; **19**: 537-544 [PMID: 14987322]

122 **Haukeland JW**, Konopski Z, Eggesbø HB, von Volkmann HL, Raschpichler G, Bjøro K, Haaland T, Løberg EM, Birkeland K. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand J Gastroenterol* 2009; **44**: 853-860 [PMID: 19811343 DOI: 10.1080/00365520902845268]

123 **Shields WW**, Thompson KE, Grice GA, Harrison SA, Coyle WJ. The Effect of Metformin and Standard Therapy versus Standard Therapy alone in Nondiabetic Patients with Insulin Resistance and Nonalcoholic Steatohepatitis (NASH): A Pilot Trial. *Therap Adv Gastroenterol* 2009; **2**: 157-163 [PMID: 21180541 DOI: 10.1177/1756283X09105462]

124 **Hassan MM**, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK, Javle M, Moghazy DM, Lozano RD, Abbruzzese JL, Vauthey JN. Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. *Cancer* 2010; **116**: 1938-1946 [PMID: 20166205 DOI: 10.1002/cncr.24982]

125 **Lai SW**, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. *Am J Gastroenterol* 2012; **107**: 46-52 [PMID: 22085817 DOI: 10.1038/ajg.2011.384]

126 **Lutchman G**, Modi A, Kleiner DE, Promrat K, Heller T, Ghany M, Borg B, Loomba R, Liang TJ, Premkumar A, Hoofnagle JH. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. *Hepatology* 2007; **46**: 424-429 [PMID: 17559148 DOI: 10.1002/hep.21661]

127 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

128 **Aithal GP**, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, Austin AS, Freeman JG, Morgan L, Webber J. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* 2008; **135**: 1176-1184 [PMID: 18718471 DOI: 10.1053/j.gastro.2008.06.047]

129 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

130 **Kelly IE**, Han TS, Walsh K, Lean ME. Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. *Diabetes Care* 1999; **22**: 288-293 [PMID: 10333947]

131 **Hernandez AV**, Usmani A, Rajamanickam A, Moheet A. Thiazolidinediones and risk of heart failure in patients with or at high risk of type 2 diabetes mellitus: a meta-analysis and meta-regression analysis of placebo-controlled randomized clinical trials. *Am J Cardiovasc Drugs* 2011; **11**: 115-128 [PMID: 21294599 DOI: 10.2165/11587580-000000000-00000]

132 **Lewis JD**, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP, Vaughn DJ, Nessel L, Selby J, Strom BL. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care* 2011; **34**: 916-922 [PMID: 21447663 DOI: 10.2337/dc10-1068]

133 **Ben-Shlomo S**, Zvibel I, Shnell M, Shlomai A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishman S. Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. *J Hepatol* 2011; **54**: 1214-1223 [PMID: 21145820 DOI: 10.1016/j.jhep.2010.09.032]

134 **Svegliati-Baroni G**, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, Faraci G, Pacetti D, Vivarelli M, Nicolini D, Garelli P, Casini A, Manco M, Mingrone G, Risaliti A, Frega GN, Benedetti A, Gastaldelli A. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver Int* 2011; **31**: 1285-1297 [PMID: 21745271 DOI: 10.1111/j.1478-3231.2011.02462.x]

135 **Vilsbøll T**, Christensen M, Junker AE, Knop FK, Gluud LL. Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. *BMJ* 2012; **344**: d7771 [PMID: 22236411 DOI: 10.1136/bmj.d7771]

136 **Miller ER**, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* 2005; **142**: 37-46 [PMID: 15537682 DOI: 10.7326/0003-4819-142-1-200501040-00110]

137 **Cariou B**, Hanf R, Lambert-Porcheron S, Zaïr Y, Sauvinet V, Noël B, Flet L, Vidal H, Staels B, Laville M. Dual peroxisome proliferator-activated receptor α/δ agonist GFT505 improves hepatic and peripheral insulin sensitivity in abdominally obese subjects. *Diabetes Care* 2013; **36**: 2923-2930 [PMID: 23715754 DOI: 10.2337/dc12-2012]

138 **Mudaliar S**, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, Adorini L, Sciacca CI, Clopton P, Castelloe E, Dillon P, Pruzanski M, Shapiro D. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 574-82.e1 [PMID: 23727264 DOI: 10.1053/j.gastro.2013.05.042]

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**Table 1 Studies with histopathological evaluation of diabetic patients with non-alcoholic fatty liver disease**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Sample size** | **Metabolic Syndrome and its components** | **Diabetes****duration (yr)** | **Diabetes-related complications** | **Elevated enzyme levels** **AST/ALT/GGT** | **Risk factors for NASH** | **Fibrosis (%)** |
| Amarapurka*et al*[12] | 36  |  |  |  |  |  | 30.5 |
| Gupte*et al*[13] | 32 |  |  |  | ALT and/or AST31% | No risk factors | 22 |
| Kemmer*et al*[38] | 22 (females) |  |  |  |  | No risk factors |  |
| Leite *et al*[6] | 92 | hypertension88%dyslipidaemia86% | 7.8  | Microvascular 46%Macrovaascular 26% | 14%/16%/13% | hypertriglyceridemia high ALT low HDL-chol | 34-60 |
| Prashanth*et al*[5] | 83 |  metabolic syndrome77% | 8.2 |  | ALT 7% |  MS componentsHigh ALT High AP | 37 |

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gammaglutamyltransferase; AP: Alkaline phosphatase; NASH: Non-alcoholic steatohepatitis; HDL-chol: HDL-cholesterol.

**Table 2 Current data on non-pharmacological treatments of** **non-alcoholic fatty liver disease**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Sample size** | **Type 2 diabetes** | **Type of intervention** | **Study design/****duration** | **Liver enzymes** | **Imaging** | **Histology** |
| Kistler *et al*[111] | 813 adults | 25% | Inactive or moderate or vigorous exercise | Retrospective analysis of biopsy-proven NAFLD | Vigorous recommendations was associated with ↓ GGT levels compared with being inactive |  | Vigorous exercise was associated with a ↓ adjusted odds of NASH |
| Hallsworth *et al* [112] | 19 adults |  | Resistance exercise  | Randomly assigned to either exercise or standard care. 8 weeks. | No significant changes in ALT levels | resistance exercise: 13% relative ↓ in liver lipid by 1H-MRS |  |
| Bacchi *et al* [113] | 31 adults | 100% | Aerobic (AER) or resistance (RES) training | Randomized controlled study. 4 mo |  | hepatic fat content was ↓ in both by in-opposed-phase MR imaging |  |
| Mathurin *et al* [114] | 381 adults | 25% | Bariatric surgery | Prospective study. follow- up of 5 years. | Significant ↓ in ALT and GGT levels 1 and 5 years after bariatric surgery |  | Significant ↓ in NASHfibrosis ↑, 96% with F1 |
| Mummadi *et al* [115] | 766 paired liver biopsies |  | Bariatric surgery | Systematic review and meta-analysis (15 studies) |  |  | ↓ or resolution 81.3% in NASH and 65.5% in fibrosis  |

↓: Decrease; ↑: Increase. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gammaglutamyl transferase; AP: Alkaline phosphatase; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; 1H-MRS: Proton magnetic resonance spectroscopy; F1: Stage 1 of fibrosis.

**Table 3 Current data on pharmacological treatments of** **non-alcoholic fatty liver disease**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Sample** **size** | **Type 2 diabetes** | **Type of intervention/ drug** | **Study design/****duration** | **Liver enzymes** | **Imaging** | **Histology** |
|  Parker *et al*[116] | 355  |  | Omega-3 PUFA: 0.8-13.7 g/d | Systematic review and meta-analysis (9 studies) median duration oftreatment: 6 mo | Significant efficacy of PUFAon ALT and AST levels | Significant efficacy of PUFAon liver fat (US, 1H-MRS) |  |
| Lindor *et al*[119] | 166  |  | UDCA: 13- 15 mg/kg per day | Randomized placebo-controlled study.24 mo  | No significant changes in ALT, AST and GGT levels with UDCA |  | No significant changes in NASH or fibrosis with UDCA |
| Leuschner *et al*[120] | 185  |  | UDCA: 23-28 mg/kg per day | Randomized placebo-controlled study. 18 mo  | No significant changes in ALT and AST, ↓ GGT levels with UDCA |  | No significant changes in NASH or fibrosis with UDCA |
| Uygun *et al*[121] | 36  | 0% | Metformin: 1.7 g/d | Randomized placebo-controlled study. 6 mo  | Significant ↓ in ALT and AST levels with metformin | significant efficacy of metformin on liver fat (US) | No significant ↓ ininflammatory activity or fibrosis with metformin |
| Haukeland *et al*[122] | 48 | 27% | Metformin: 2.5-3 g/d | Randomized placebo-controlled study. 6 mo  | No significant changes in ALT, AST levels with metformin | No significant ↓ on liver fat (CT) with metformin | No significant changes in NASH with metformin |
| Shields *et al*[124] | 19  | 0% | Metformin: 500 mg-1 g/d | Randomized placebo-controlled trial.12 mo | No significant changes in ALT and AST levels with metformin |  | No significant changes in NASH or fibrosis with metformin |
| Lutchman *et al* [127] | 18  | 0% | Pioglitazone: 30 mg/d  | Prospective open study. 12 mo. | ALT levels normalized in 72% | hepatic fat content was ↓ byMR imaging  | significant ↓ innecroinflammation and fibrosis with pioglitazone |
| Belfort *et al* [128] | 55 | 48% | Pioglitazone: 45 mg/d | Randomized placebo-controlled study. 6 mo | Significant efficacy of pioglitazoneon ALT and AST levels | Significant efficacy of pioglitazoneon liver fat (1H-MRS) | Significant ↓ innecroinflammation but not in fibrosis with pioglitazone |
| Aithal *et al* [129] | 74 | 0% | Pioglitazone: 30 mg/d | Randomized placebo-controlled trial.12 mo | Significant ↓ in ALT and GGT levels |  | Significant ↓ ininflammatory activity and fibrosis with pioglitazone |
| Sanyal *et al* [130] | 247 | 0% | Vitamin E: 800 UI/dPioglitazone: 30 mg/d | Randomized placebo-controlled trial.24 mo | Significant ↓ in ALT, AST and GGT levels with both treatments |  | Significant ↓ of NASH with vitamin E. No changes in fibrosis with either treatment |

↓: Decrease; ↑: Increase. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gammaglutamyl transferase; AP: Alkaline phosphatase; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; PUFA: Polyunsaturated fatty acids; UDCA: Ursodeoxycholic acid; US: Ultrasonography; MR: Magnetic resonance; 1H-MRS: Proton magnetic resonance spectroscopy; CT: Computed tomography.

**Figure 1 pivotal role of insulin resistance in non-alcoholic fatty liver disease pathophysiology.** ↓: Decrease; ↑: Increase. FFA: free fatty acids; DM: diabetes mellitus; NAFLD: Non-alcoholic fatty liver disease.

**Figure 2 Main physiopathological mechanisms influencing non-alcoholic fatty liver disease**. **Progression to non-alcoholic steatohepatitis and fibrosis.** ↓: Decrease; ↑: Increase.NASH: non-alcoholic steatohepatitis; FFA:free fatty acids.