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Review Article

Focus on Therapeutic Strategies of Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the Western world (it affects 30% of the general adult population). The NAFLD encompasses a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), defined by steatosis, hepatocellular damage, and lobular inflammation in individuals without significant alcohol consumption and negative viral, congenital, and autoimmune liver disease markers. Currently, NAFLD is considered an emerging epidemic in light of the dramatic increase in obesity rates. With the progressive nature of NASH and its rising prevalence there is a significant need for a specific and targeted treatments since to date there has not been any validated therapies for NAFLD other than weight loss, which is well known to have a poor long-term success rate. In recent years, visceral adipose tissue has taken an important role in NAFLD pathogenesis, and current therapeutic approaches aim at reducing visceral obesity and free fatty acid overflow to the liver. This paper is focused on the treatments used for NAFLD and the potential new therapy.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the Western world (it affects 30% of the general adult population) [1].

The NAFLD is an umbrella term for a group of diseases defined by a hepatic fat infiltration >5% hepatocyte, in the absence of excessive alcohol intake, defined by two standard drinks (20 g ethanol) daily for men and one standard drink (10 g ethanol) daily for women.

The NAFLD encompasses a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), defined by steatosis, hepatocellular damage, and lobular inflammation [2] in individuals without significant alcohol consumption and negative viral, congenital, and autoimmune liver disease markers.

While steatosis does not carry the risk of progressive liver disease, patients with NASH are at risk of developing cirrhosis (20–30% of patients) [3].

NASH may progress to decompensated liver disease and result in liver failure.

Furthermore, NASH confers an increased risk of cardiovascular disease (CVD) and diabetes [4] both directly and through its association with other cardiometabolic abnormalities, including obesity and metabolic syndrome [5].

Currently NAFLD is considered an emerging epidemic in light of the dramatic increase in obesity rates. With the progressive nature of NASH and its rising prevalence, there is a significant need for a specific and targeted treatments since to date there has not been any validated therapies for NAFLD other than weight loss, which is well known to have a poor long-term success rate.

This paper is focused on the treatments used for NAFLD and the potential new therapy. Computerized advanced search for primary evidence was performed in PubMed (Public/Publisher MEDLINE) by using a combination of terminology and methodology search filters [6].

1.1. Pathogenesis: The Two-Hit Hypothesis. Currently the pathogenesis of NAFLD is unclear. NAFLD seems to be a multifactorial disease, combining both genetic and

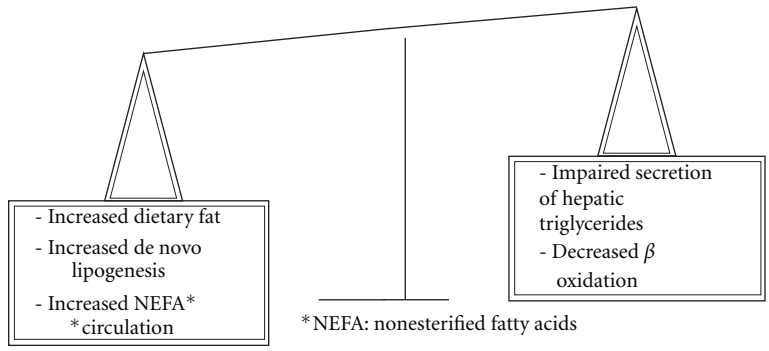


Figure 1: Pathways contributing to steatosis. An imbalance between fatty acid uptake, *de novo* synthesis and elimination of free fatty acids through oxidation and secretion into the blood with very low density lipoprotein triglycerides (VLDL), contributes to the development of steatosis.

environmental factors. Several theories have been proposed and the "two-hit hypothesis" is the most accredited theory.

Increased *de novo* lipogenesis [7], impaired secretion of hepatic triglyceride [8], decreased β-oxidation [9], and increased circulation of nonsterified fatty acids (NEFAs) released from adipose tissue [10] contribute to the steatosis when there is an imbalance between fatty acid uptake, *de novo* synthesis, and elimination of free fatty acids through oxidation and resecretion into the blood within very low density lipoprotein triglycerides (VLDL) (Figure 1).

Steatosis represent the "first hit." This increases the vulnerability of the liver to oxidative stress and inflammatory insults (the "second hit") as hepatic lipid peroxidation [11], mitochondrial dysfunction [12], and inflammatory cells activation [13], which cause hepatocyte injury and the possible progression to NASH and cirrhosis.

The variable progression of NAFLD may be linked, in some patients, to genetic or environmental susceptibility that leads to hepatic fibrosis and ultimately cirrhosis [14].

According to new research on obese mice, the theory on the development of the NAFLD has been challenged. The same event can be the cause of fat infiltration, necroinflammation, and fibrosis; in this context the hepatic triglycerides (TG) accumulation may protect the hepatocyte from toxic free fatty acids (FFAs) improving hepatic steatosis but exacerbating liver injury and fibrosis [15].

Furthermore, adipokines and cytokines produced by adipose tissue play an important role in the pathogenesis of NAFLD. Some adipokines such as adiponectin and leptin may positively influence NAFLD while others, such as TNF-α and resistin, may negatively influence it [16]. Also insulin resistance (IR) seems to play a major role in the development of NAFLD in the accumulation of fat in the liver to progression in NASH [16]. Dysregulation of adipokines and cytokines is involved in the development of IR, fatty liver, and its progression to NASH [17].

2. Diagnosis

Diagnosis of NAFLD is difficult because a completely reliable test to distinguish alcoholic and nonalcoholic fatty liver disease has not yet been found. Furthermore for the nature of NAFLD is mandatory to identify patients with progressive liver disease who are at risk of end-stage liver disease [18].

The diagnostic gold standard is *liver biopsy* (LB) but it is invasive, risky (complications like haemorrhage), costly and suffer for sampling [19].

The NASH Clinical Research Network designed and validated an histological score system (NAFLD activity score-NAS) to define the spectrum of NAFLD [20]; however, this score is not completely clear and it may incur in errors. So we need to find an alternative, not invasive exam for the diagnosis of this disorder and some exams to define which really need a LB. Biochemical and radiological methods are under development.

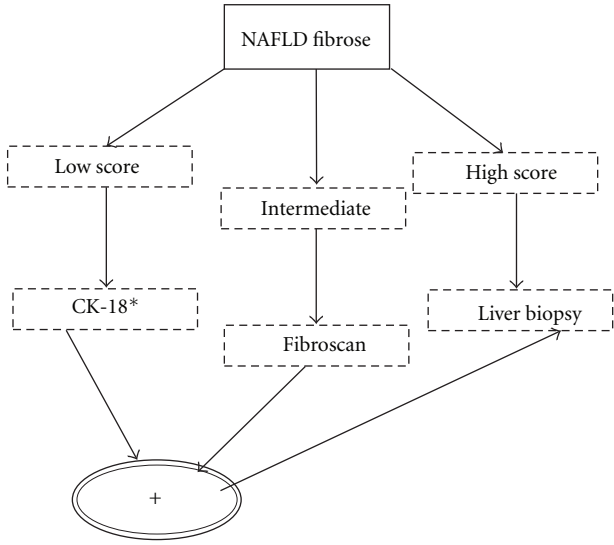
Transient elastography (Fibroscan), which measures liver stiffness (index of liver disease staging), accurately predict hepatic fibrosis, in a variety of clinical conditions like NASH, alcoholic hepatitis, viral hepatitis, and autoimmune liver disease [21–26], but Fibroscan has some limitations like the rate of unsuccessful examination in patients with metabolic syndrome because it has limited accuracy in the presence of obesity and steatosis [27, 28].

A method to evaluate advanced fibrosis is NAFLD fibrosis score. It is easy to calculate from routine parameters (age, hyperglycaemia, BMI, platelet count, albumin, and AST/ALT ratio) and has been independently validated in populations of various ethnicities, BMI, and diabetic status [29].

The NAFLD fibrosis score facilitates the identification of NAFLD patients with more advanced disease who require ongoing followup, and considerably reduces the requirement for liver biopsy in the minority of patients with an indeterminate score (25%) [30].

Nevertheless, the test is more useful to predict the absence of advanced fibrosis than its presence because its sensitivity is low and the specificity and the negative predictive value are high [31]. Several biomarkers are being investigated to differentiate between NASH and simple steatosis. *Adipocyte fatty acid binding protein* (AFABP) has a role in interaction between adipocytes and macrophages which leads to inflammation and insulin resistance [32].

Values of *blood cytokeratin 18 fragment* (CK-18) are linked with the degree of hepatocellular apoptosis, a character of NASH [33].



*CK-18: blood cytokeratin 18 fragment

Figure 2: Possible algorithm for the diagnosis of NAFLD. *Limits*: fibroscan is possible in BMI <30 kg/m^2. Adapted by Musso et al. [29].

Fibroblast growth factor 21 (FGF21) serum levels are high in NAFLD patients and its expression in the liver increases with the steatosis grade [34].

These results make biomarkers promising which could be used as noninvasive tests for NASH but they have not been adequately evaluated in independent cohorts, necessitating further research in this field.

A recent work showed that in patients with NAFLD all three biomarkers were associated with lobular inflammation, and CK-18 is the most accurate biomarker for NAFLD and NASH. Furthermore, a two-step approach using CK-18 and FGF21 improves the accuracy in diagnosing NASH [35].

Recent studies have underlined the role of non-HDL-cholesterol (non-HDL-C) as a superior predictor of cardiovascular incident than LDL-cholesterol [36].

Furthermore, recent study has shown that non-HDL-C levels increased more in patients with NASH than in those with steatosis, defining this value as a possible biomarker to estimate a patient's risk of NASH and need for a LB [37].

More studies are needed to confirm this finding (Figure 2).

3. Therapy

In recent years visceral adipose tissue has taken an important role in NAFLD pathogenesis, and current therapeutic approaches aim at reducing visceral obesity and free fatty acid overflow to the liver (Table 1).

3.1. Lifestyle Interventions. Lifestyle interventions (diet and exercise) are the standard treatment of NAFLD. The purpose of lifestyle modification is weight loss; moreover, it has an important role in the decrease of multiple cardiometabolic risk factors.

A weight loss ≥5% improved steatosis and cardiometabolic risk factors, while a weight loss ≥7% improved necroinflammation and NAS [38, 39].

The effects of weight loss are inconstant for the limited compliance of patients to the diet and the exercise [40].

The correct composition of the diet for NAFLD is unknown but the importance of the diet in this disease is underlined by the improvement of insulin sensitivity, the reduction of hepatic FFAs supply, and adipose tissue inflammation [41].

Different studies show that caloric restriction is the most important goal for improving hepatic steatosis, but a different nutrient composition may carry additional benefits according to individual patients features. Recent studies are suggested that cholesterol, trans fat, and excessive fructose had a role in the pathogenesis of NAFLD [42].

Regular exercise has well-known benefits on metabolic abnormalities. Recent studies suggest aerobic exercise may reduce hepatic steatosis through hepatic adenosine monophosphate-activated protein kinase (AMPK) activation. This evidence could define the exercise's benefits on NAFLD not only for the weight loss [43].

3.2. Lipid-Lowering Drugs: Fibrates, Statins, ω-3 Polyunsaterated Fatty Acids (PUFA). In the literature, there are some trials about the potential therapeutic role of lipid-lowering drugs in NAFLD. It seems that *fibrates* improve serum transaminases and may have a positive impact on IR and NAFLD [44]; *statins* are safe [45], improve serum levels of transaminases, and show beneficial effects on necroinflammation [46, 47]. Finally, *PUFA* improves biochemical and ultrasonographic steatosis [48].

Further studies are needed to elucidate this issue.

3.3. Insulin Sensitizers: Metformin and Thiazolidinediones (TZDs). Some works analysed the efficacy of *metformin* in NAFLD [49, 50]. Metformin reduced hepatic expression of TNF-α, a mediator of hepatic insulin resistance and necroinflammation, increased FFAs oxidation, and suppressed lipogenesis through AMPK activation [51]. Metformin had positive metabolic effects, improved weight loss and levels of liver enzymes [52].

The association between metformin and lifestyle intervention has given promising results, on the contrary there were controversial results on the improvement of histological steatosis, necroinflammation, and fibrosis [49, 53], necessitating further studies.

Few works have been studied on the role of *TZDs* pioglitazone and rosiglitazone in the treatment of NAFLD. They improved steatosis and necroinflammation, but their role on fibrosis had discordant results and is still not clear; however, TZDs significantly reduced the risk of fibrosis progression [54–56].

For the paucity of drugs used in the management of NAFLD, in recent years authors are investigating new therapeutic strategies.

Table 1: Synthesis of possible targets of therapeutic strategies in use and in study for NAFLD. Focus on biochemical, ultrasonographic, and histological features of NAFLD: altered levels of transaminases, steatosis, necroinflammation, and fibrosis.

	Biochemical features	Steatosis	Inflammation	Fibrosis
Diet	+	+ (>5% weight loss)	+ (>7% weight loss)	
Exercise		+		
Fibrates	+			
Statins	+		+	
PUFA	+	+		
Metformin	+	±	±	±
TZDs		+	+	±
Rimonabant		+		+
Orlistat*				
ARBs*				
Antioxidant agents	+			
UDCA	+			
Pentoxifylline	+	+	+	
Probiotics*				
GTE		+	+	
Incretin anlogs/antagonists			+	
Thyromimetics		+		
PXR		+		+
FXR			+	+
Bariatric surgery		+	+	+

* Target not clear.
PUFA: ω-3 polyunsaterated fatty acids; TZDs: thiazolidinediones; ARBs: angiotensin II type 1 receptor blockers; UDCA: ursodeoxycholic acid; GTE: green tea extract; PXR: pregnane X receptor; FXR: farnesoid X receptor.

3.4. Endocannabinoid Receptor Antagonists. The endocannabinoid pathway plays a significant role in the regulation of appetite and body weight, hepatic lipid metabolism and fibrosis. The endocannabinoid receptors-1 (CB-1) are overexpressed in NASH, suggesting a direct involvement of endocannabinoid system in the regulation of hepatocyte metabolism [57].

Rimonabant, a CB-1 blocker, in obese rats with NASH led to the improvement in lipid profile and the reduction in hepatic steatosis and fibrogenesis [58]. Depression and anxiety were more common with rimonabant. Concern about psychiatric adverse effects led to withdrawal of rimonabant, [59] but the development of peripherally acting CB1 antagonists is an area of intense research.

3.5. Orlistat. Orlistatis an inhibitor of gastric and pancreatic lipase. It inhibits the absorption of dietary triglycerides and has a role in reducing weight and IR in obesity. It could be used in patients with NAFLD for weight loss improvement.

The group of *Zelber-Sagi* have not noticed a difference of weight reduction between the orlistat and placebo groups. Therefore, other mechanisms of its beneficial effects in patients with NAFLD should be considered as the improvement of insulin sensitivity and the reduction of free fatty acids levels [60].

3.6. Angiotensin II Type 1 Receptor Blockers (ARBs). The association between the angiotensin II type 1 receptor polymorphisms and the presence and severity of NAFLD [61] could be the base of evaluation of ARBs for reducing hepatic lipid accumulation in these patients [62].

3.7. Antioxidant Agents. The oxidative stress seems to have a role in the second hit of NAFLD. Several works have studied the effects of vitamins C and E in patients with NAFLD with the result of biochemical improvement (decrease of transaminases levels) and discordant results about histological improvement [63, 64].

These data are so limited to give any significance in the therapeutic strategies of NAFLD.

3.8. Ursodeoxycholic Acid (UDCA). UDCA also has a role of antioxidant, improving liver enzymes but there are not enough data to indicate its use in NAFLD [65].

3.9. TNF-α Inhibitor. Anti-TNF therapy is based on the role of TNF-α in necroinflammation and insulin resistance. *Pentoxifylline* (a TNF-α inhibitor) in several trials improved liver enzymes and cytokine-mediated systemic inflammation [66] and reduced histological steatosis and necroinflammationin in patients with NASH [63] as demonstrated in the work of Zein at al. [67].

Further studies are required to confirm these results.

3.10. Probiotics. Gut microbiota is linked to NAFLD by its proinflammatory metabolites through endotoxin-mediated

toll-like receptor-4 axis activation [68] and may trigger hepatic *de novo* lipogenesis enzymes [69]; moreover, small intestinal overgrowth is plausibly linked to NAFLD pathogenesis [70]. Prebiotics are defined as "a nondigestible food ingredient that beneficially affects the host, by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon." Prebiotic fibres alter the gut microbiota in a manner that is advantageous to the host [71].

Animal models have shown that prebiotics reduces plasma lipid and hepatic triglyceride concentration and have a cholesterol-lowering effect, also prebiotic-rich diet may ameliorate NAFLD by attenuating *de novo* fatty acid synthesis [72, 73].

To date, continued ingestion of prebiotics is necessary to maintain these changes and the minimum dose and the duration of the therapy has not been defined, necessitating further studies [74].

In the literature, there are few studies on the effects of prebiotic on NAFLD patients, which showed a decrease in cholesterol and triglycerides levels, but the reduction is lower than in animal models, probably for the relatively lower dose administered [75] and for the lower rate of the hepatic *de novo* lipogenesis in humans [76].

Further studies in humans are required to understand better the potential to translate the positive effects of prebiotic fibres noted in animal models to human clinical application.

3.11. Green Tea Extract (GTE). Catechins are the major polyphenols present in green tea and have antioxidant, antinflammatory, and enzyme inhibition activities [77]. GTE protects against hepatic steatosis and injury in the *ob/ob* mice model of NAFLD [78] through several activities which include decreasing absorption of dietary lipid and lipogenic substrates [79], suppressing adipose lipolysis and decreasing hepatic and adipose lipogenesis [80]. GTE also increases energy expenditure by upregulating β-oxidation and thermogenesis responses [81] and improving insulin sensitivity [82].

GTE also has direct and indirect antioxidant and anti-inflammatory properties that could prevent the progression from steatosis to NASH by directly scavenging reactive oxygen and nitrogen species, by upregulating the transcription of genes related to the cellular antioxidant defense, and by directly suppressing inflammatory responses [83].

To date, no RTS in humans with NAFLD have examined the potentially beneficial effects of green tea. Such studies are necessary to provide direct evidence that green tea could have a role in the reduction of development and/or progression of NAFLD in humans.

3.12. Incretin Analogs/Antagonists. Incretin analogs and antagonists are a new class of drugs for the treatment of diabetes. They are dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) analogs and glucose-dependent insulinotropic polypeptide (GIP) agonists. Their action stimulates pancreatic β-cell insulin secretion and growth.

The focus for the use of these medicaments in NAFLD is the demonstration of reduced incretin action in NASH patients [84].

In animal models GLP-1 analogs decrease hepatic lipogenesis and oxidative stress [85].

Studies on GIP action suggest that GIP may be an important mediator of the adypocite response to nutritional excess and may have a role in the metabolic risk of NAFLD [86].

3.13. Thyromimetics. Recent research on antiobesity and low density lipoprotein (LDL) cholesterol lowering effects of thyroid hormones have developed a class of drugs with these beneficial effects, such as thyromimetics [87].

Animal models showed steatosis improvement and reduction in hepatic lipoperoxidaton [88].

More research is needed in human trials.

3.14. Nuclear Transcription Factors: Pregnane X Receptor and Farnesoid X Receptor. Studies on mice have shown that pregnane X receptor (PXR) is implicated in hepatic steatosis and in lipid and glucose metabolism [89].

PXR has also been proposed as a potential target for antifibrotic therapy [90] but other studies are needed to confirm these features and to characterize synthetic PXR agonists.

Farnesoid X receptor (FXR) is a regulator of lipid and glucose homeostasis. In the liver, FXR influences lipid homeostasis and antagonizes inflammatory and fibrogenetic process [91].

Several synthetic FXR agonists are studied for the therapy of metabolic and hepatic disorders [92].

3.15. Bariatric Surgery. A key point of NAFLD is exogenous fat accumulation which is reduced by surgery. Different surgical procedures, as laparoscopic adjustable banding, biliopancreatic diversion, vertically banded gastroplasty, and Roux-en y gastric bypass have given good results improving steatosis, hepatocellular injury, and fibrosis in NAFLD patients without signs of other liver injury [93], but there are no trials about the long-term effects of these procedures.

For patients with NASH not responding to lifestyle intervention, pharmacological treatment should be considered.

4. Conclusion

NAFLD in these years has obtained a prominent role in the spectrum of liver diseases for the increased frequency and recognition. Also, in the absence of a target therapy, it may appear as the leading cause of cirrhosis and liver transplantation by 2020. To date the gold standard for the therapy of NAFLD is lifestyle intervention. A gradual weight loss is desirable, because a faster weight loss has exacerbated liver injury [94].

For patients not compliant to lifestyle intervention, there is not a validated regimen, the result of recent studies

suggest that a combination therapy occur targeting different mechanisms involved in the pathogenesis of NAFLD [64, 95].

In the future another feature to study may be the association between lifestyle intervention and drugs, to assess if they could be a synergistic effect on liver histology [96].

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