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A COMPREHENSIVE REVIEW ON EVALUATION OF CLINICAL, BIOCHEMICAL AND IMAGING OUTCOMES OF SAROGLITAZAR AND VITAMIN E IN NON ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of diseases, involving excessive lipid deposition in the liver and is often accompanied by obesity, diabetes, dyslipidaemia, abnormal blood pressure, and other metabolic disorders. NAFLD is also called as metabolic dysfunction-associated steatotic liver disease [MASLD]. The estimated global incidence of NAFLD is 47 cases per 1000 population and is higher among males than females. In India, the overall prevalence of NAFLD in the general population is close to 40 percent. It is diagnosed by Ultrasound and LFT. Current treatment strategies mainly involve lifestyle modifications, such as weight loss and dietary modifications. Agents tested with some success in non-diabetic patients with NASH include pioglitazone, liraglutide, vitamin E and to a lesser degree, pentoxifylline. In patients with T2DM and NASH only pioglitazone has shown to significantly improve liver histology, with only a handful of patients with diabetes having been studied with other modalities. Glucagon-like peptide-1 analogue, FXR agonist, multi- strain reduces the fatty liver index, and aminotransferase levels in NAFLD patients. Sodium- dependent glucose cotransporter inhibitors, and peroxisome proliferator-activated receptor agonists have shown benefits in improving metabolic parameters and reducing hepatic lipid accumulation and inflammation. Saroglitazar is a drug that acts as a dual Peroxisome Proliferator-Activated Receptor (PPAR) alpha/gamma agonist and reduces liver fat and inflammation and has been shown to be efficacious in NAFLD by reduction of transaminase levels, improvement in overall metabolic health, reduction of liver fat content, and improvement of liver stiffness and histology. On the other hand, Vitamin E, a fat-soluble vitamin, which reduces liver inflammation. Antioxidants, anti-inflammatory and anti – apoptotic properties of vitamin E accompanied by ease-of-use and exceptional tolerability have made vitamin E a pragmatic therapeutic choice in NAFLD.

KEYWORDS: NAFLD, Metabolic disorders, MASLD, Saroglitazar, Vitamin E.

INTRODUCTION

- Non-alcoholic fatty liver disease (NAFLD) is emerging as the leading chronic liver disease worldwide.[8] NAFLD is viewed as the hepatic manifestation of metabolic syndrome and is commonly associated with metabolic risk factors, including obesity, dyslipidaemia, hypertension, and diabetes.[9,10] The rising rates of obesity and type 2 diabetes worldwide are paralleled by a rise in the global prevalence of NAFLD.[11] Non- alcoholic fatty liver disease (NAFLD) is a broad term used to cover a spectrum of conditions that are characterized by evidence of hepatic steatosis on imaging or histology (macro-vesicular steatosis), and absence of secondary causes of hepatic steatosis such as significant alcohol consumption, chronic use of medications that can cause hepatic steatosis or hereditary disorders.[12]
- The definition of significant alcohol consumption has not been consistent. For non- alcoholic

steatohepatitis (NASH) clinical trials, it has been defined as ongoing or recent consumption of more than 14 standard drinks on average per week in women and more than 21 standard drinks on average per week in men. Non-alcoholic fatty liver disease is most often diagnosed incidentally on imaging or when it presents with complications. The prevalence of NAFLD in Western countries is around 20 to 30%.[13] NAFLD is considered to be the liver manifestation of metabolic syndrome. 50 to 70% of people with diabetes are found to have NAFLD.[14]

- NAFLD has several phases of progression, which include simple steatosis, steatohepatitis, fibrosis, cirrhosis, and ultimately could even progress to hepatocellular carcinoma. The disease has a benign course; it is a silent liver disease when the only histological finding is steatosis.
- The presence of hepatic injury with inflammation with or without fibrosis constitutes non-alcoholic steatohepatitis (NASH).[15,16]

NAFLD is broadly classified into two sub-types[19]

- Non alcoholic fatty liver (NAFL), the non-progressive form of NAFLD
- NASH, the progressive form of NAFLD.

ETIOLOGY

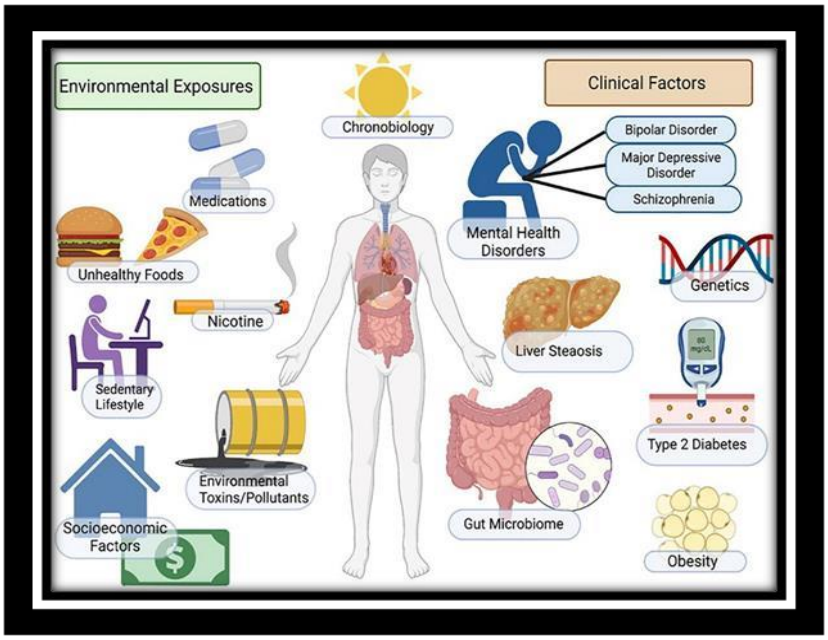

Figure 2: Factors regulating metabolism and NAFLD.

- Obesity, diabetes, dyslipidaemias, insulin resistance, and metabolic syndrome are known to be associated with the development of non-alcoholic fatty liver disease (NAFLD).[22] A temporal association has also been shown between inorganic arsenic exposure and the development of NAFLD reflected by elevated alanine transferase (ALT).[23]
- Due to its close association with metabolic syndrome, NAFLD correlates with cardiovascular risk factors, which also contributes to mortality in these patients in addition to end-stage liver cirrhosis.

Epidemiology

- Non-alcoholic fatty liver disease (NAFLD) incidence is rapidly increasing, especially in Western countries. Rising obesity levels, increasing incidence of childhood obesity, sedentary lifestyles, consumption of unhealthy quick eats, and a longer lifespan are some of the likely contributors.
- The incidence and prevalence of NAFLD are underestimated as ultrasonography is commonly used to screen for fatty liver disease. The prevalence of NAFLD is 80% to 90% in obese adults, 30% to 50% in patients with diabetes mellitus, 90% or more in patients with hyperlipidaemia, 3 to 10% in children, and as high as 40% to 70% among children with obesity.[24]
- NAFLD is estimated to afflict one billion individuals globally and may be present in approximately 25% of the world population.[25] There is considerable variability in the prevalence of NAFLD across the various geographic regions in the world. The Middle East and South America have the highest and Africa the lowest prevalence of NAFLD.
- In the United States, up to 80 million individuals may have NAFLD.[26] Globally, the rates of NAFLD in non-obese individuals average ~10%–30% in Western and Eastern countries.[27]
- There are also gender and racial/ethnic differences in the prevalence of non-obese NAFLD; the prevalence of NAFLD is much higher in non-obese South Asian men than in non-obese South Asian women and in men and women of other ethnic groups,[28] and the prevalence of NAFLD is higher in Hispanic and white than in Black individuals.[29]
- Non-obese subjects with NAFLD also have a

markedly higher cardiovascular risk.[30] Over the last 3 decades, the prevalence of NAFLD in the United States has risen from 20% in 1988–1994 to 32% in 2012–2016.

- Although the prevalence of viral hepatitis has been declining in the United States, there has been a tremendous increase in NAFLD.
- These rising trends in the prevalence and incidence of NAFLD are also noted in children and adolescents in the United States. Similar rises are seen elsewhere in the world, including Europe, China, India, and other regions. Therefore, NAFLD has become a global health problem that imposes a significant socioeconomic burden.

PATHOPHYSIOLOGY

- Both environmental and genetic factors contribute to the development of non-alcoholic fatty liver disease (NAFLD) and its progression. First-degree relatives of patients with NAFLD are at higher risk than the general population. Histone amino-terminal ends maintain the chromatin structure and gene expression that is cAMP- responsive element-binding protein H (CREBH) or sirtuin (SIRT1). Genetic studies have shown that activation of SIRT1 is thought to play a role in the development of NAFLD. The trigger of the progression of NAFLD to cancer is via abnormal DNA methylation.[31]

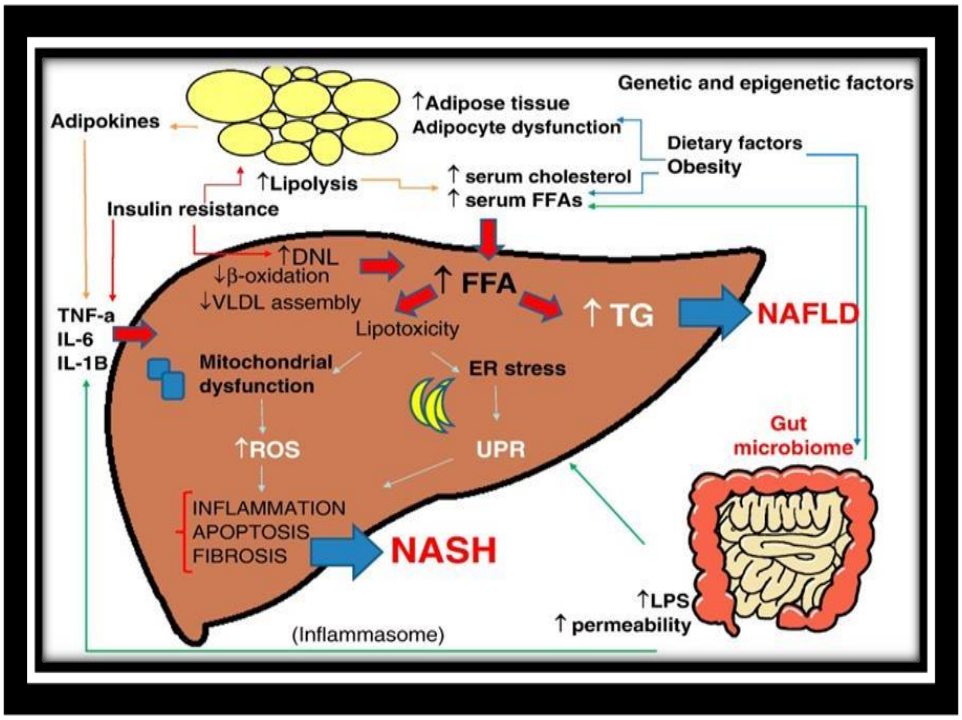

Figure 3: The cross talk between liver and peripheral organs in the pathogenesis of NAFLD.

- Day and James proposed a two-hit model of pathogenesis in 1998. The first hit is caused by insulin resistance, which leads to the accumulation of fat droplets that are triglycerides in the cytoplasm of hepatocytes, leading to the development of steatosis.
- Insulin resistance causes excess delivery of free fatty acid and triglycerides to the liver and decreased excretion leading to accumulation. Also, excess carbohydrates are a stimulus for de novo fatty acid synthesis in the liver.
- The second hit causing hepatocellular injury and the development of NASH is multifactorial. Excessive fatty acids in the liver make the liver more vulnerable to injury. Peroxisomal fatty acid oxidation, reactive oxygen species (ROS) production from the mitochondrial respiratory chain, cytochrome P450 metabolism of fatty acids, and hepatic metabolism of gut-derived alcohol are hypothesized to cause the injury.

- Obesity also contributes to the second hit as adipose tissue releases inflammatory mediators such as leptin, tumor necrosis factor (TNF)- alpha, and interleukin (IL)-6, causing hepatocyte damage. The hepatocytes undergo ballooning, cytoskeletal aggregation, apoptosis, and necrosis.[32]
- Insulin resistance is also a part of the second hit. The sinusoidal collagen deposition caused by the activation of hepatic stellate cells and the portal fibrosis caused by the ductular proliferation leads to the development and progression of NASH. These changes have correlated with insulin resistance, which is now believed to cause the progression of steatosis to NASH and progressive fibrosis.[33]

HISTOPATHOLOGY

- Non-alcoholic fatty liver disease (NAFLD) is more than 5% of hepatocytes with fat droplets on liver biopsy.
- Functionally, the liver is subdivided into three

zones; the classification is made based on the oxygen supply. Zone 1 has the highest oxygenation (oxygenated blood from hepatic arteries) and encircles the portal tracts, and zone 3 encircles the central veins where the oxygenation is poor.

- The American Association for the Study of Liver Diseases (AASLD) defined the histopathological abnormalities required in the diagnosis of NASH, which include steatosis (macro more than micro), lobular inflammation, and hepatocellular ballooning is seen most apparently in the zone 3 steatotic liver cells.

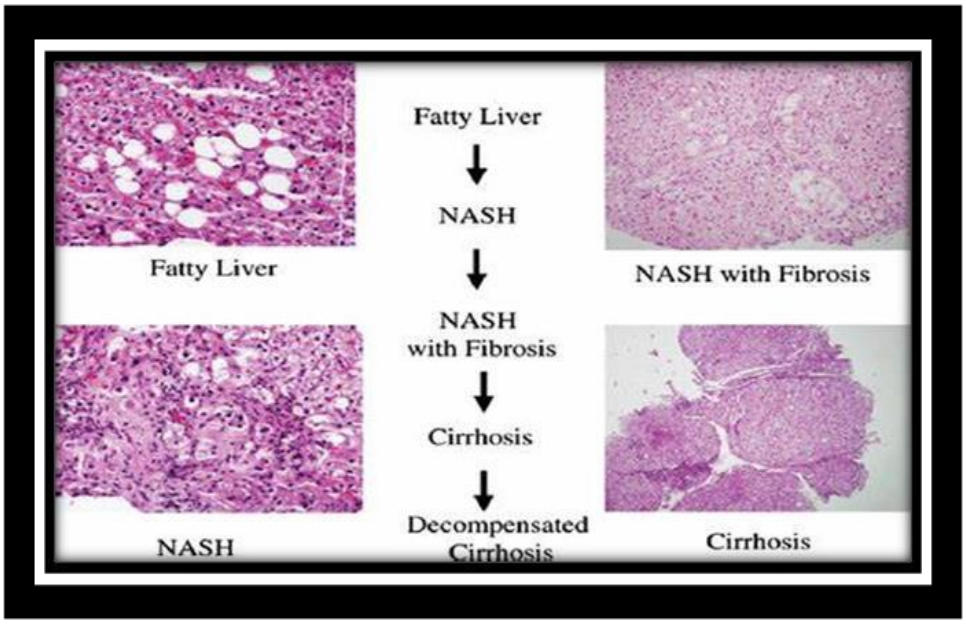

Figure 4: Progression of NAFLD to cryptogenic cirrhosis; steatosis.

- Fibrosis, although not necessary for the diagnosis, is usually present. Some other findings seen are Mallory-Denk bodies (MDB, eosinophilic intracytoplasmic inclusions), megamitochondria, glycogenated nuclei, and iron deposition.[34]
- Fibrosis starts in the acinar zone 3 and has the appearance of chicken wire from the deposition of collagen and other extracellular matrices along the sinusoids. NASH- related cirrhosis is macronodular or mixed. When cirrhosis develops, the other histological features may not be evident.[33]

SIGNS AND SYMPTOMS

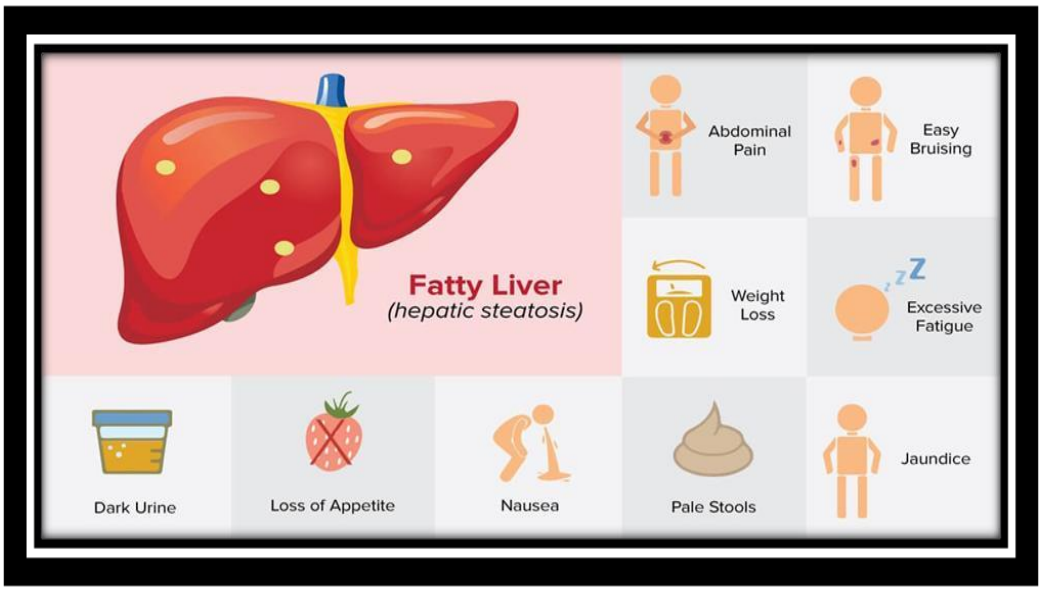

Figure 5: Fatty liver symptoms.

- Patients with non-alcoholic fatty liver disease (NAFLD) could present with many non- specific symptoms way before the diagnosis is made, although most patients are asymptomatic. Fatigue is

one of the most common presenting symptoms. Sharp or dull aching upper abdominal pain, thirst, bloating, and sleep disturbances.[35] Patients who develop NASH-associated cirrhosis, end-stage liver disease, or hepatocellular carcinoma (HCC) present with symptoms like:

- Nausea
- Vomiting
- Jaundice
- Pruritis
- Ascites
- Memory impairment
- Easy bleeding
- Loss of appetite

The most common clinical sign is mild to moderate hepatomegaly. Advanced stages of the spectrum can demonstrate signs of end-stage liver disease, such as:

- Jaundice
- Spider angiomas
- Gynecomastia
- Ascites

DIAGNOSIS

- Mildly elevated serum aminotransferases are the primary abnormality in non-alcoholic fatty liver disease (NAFLD), although the liver enzymes are normal in the majority of patients. The ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) is less than 1.
- Gamma-glutamyl transferase (GGT), when elevated in non-alcoholic fatty liver disease (NAFLD), can be a marker of increased mortality, with the progression of the disease, hypoalbuminemia, hyperbilirubinemia, thrombocytopenia, and prolonged prothrombin time present due to hepatic synthetic dysfunction.
- A wide spectrum of non-invasive diagnostic methods have been developed and clinically tested over the last years, the most important- and tested ones being vibration-controlled transient elastography (VCTE) and non-invasive fibrosis tests (i.e. NAFLD Fibrosis Score or FIB-4 Score).[36,37,38]
- Nevertheless, once biopsy specimens are obtained, pathologists should report the grades of hepatic steatosis [reported as a percentage of lipid-containing hepatocytes mild (Grade 1: 5–33%), moderate (Grade 2: 34–66%), severe steatosis (Grade 3: >66%)],[39] hepatocyte ballooning [absent (0), rare (1), or prominent(2)] and necro-inflammatory activity [absent (0), mild (1), moderate (2), or severe (3)].

Diagnostic tools	Technique/principle	Features
Serological tests	Aspartate aminotransferase (AST)	Since some NAFLD patients have normal AST and ALT values, elevated levels are not indicative of NAFLD
	Alanine aminotransferase (ALT)	
	AST/ALT	More than one indicates fibrosis
Imaging techniques	Ultrasonography	Does not distinguish between fatty liver and NASH; sensitive when steatosis is greater than 30% of the liver cells
	Magnetic resonance imaging (MRI), computerized tomographic (CT) scanning	More sensitive than ultrasonography; inadequate capacity to differentiate between NASH and fatty liver; costly
	Transient elastography	Able to diagnose fibrosis, but costly
Liver biopsy	Histological assessment of liver tissues: hepatic lesions like fatty liver, inflammation, and enlargement are graded, and fibrosis is staged.	Gold standard but invasive and may be implicated with complications and sampling variableness; able to detect inflammation and steatosis

- Finally, the NAFLD activity score (NAS)[40] should be reported as the sum of the three characteristics (steatosis, ballooning, inflammation) and ranges between 0 and 8 points; however, NAFLD is defined by the presence of steatosis so usually, a minimum of 1 point (for steatosis) should be reported to establish a NAFLD diagnosis. Ultrasound of the abdomen is routinely used to evaluate fatty liver, but a liver biopsy is considered the gold standard for the diagnosis of NAFLD.A non-invasive clinical scoring system called NAFLD in metabolic syndrome (MS) score was developed to predict the development of NAFLD in patients with metabolic syndrome. The clinical predictors included are BMI greater than or equal to 25, AST/ALT greater than or equal to 1, type 2 diabetes mellitus, and obesity.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of non-alcoholic fatty liver disease (conditions that can also cause hepatic steatosis) include:

- Alcoholic liver disease
- Hepatitis C, particularly genotype 3
- Wilson disease
- Medications such as amiodarone, methotrexate, tamoxifen, glucocorticoids, valproate, anti-retroviral agents for HIV
- Reye syndrome
- Mitochondrial hepatopathies
- Kwashiorkor/anorexia nervosa
- Mitochondrial disorders

Staging

The grading and stages of non-alcoholic fatty liver disease (NAFLD) are described below.[34,39,41]

Grades

- Grade 1 (mild): Steatosis up to 66%, occasional ballooning in zone 3, scattered polymorphs with or without lymphocytes, mild or no portal inflammation
- Grade 2 (moderate): Any degree of steatosis, obvious ballooning predominantly in zone 3, intralobular inflammation with polymorphs and chronic inflammation, and mild to moderate portal inflammation
- Grade 3 (severe): Panacinar steatosis, ballooning, and obvious disarray predominantly in zone 3, intralobular inflammation with scattered polymorphs with or without mild chronic and mild to moderate portal inflammation

Stages

- Stage 0: No fibrosis
- Stage 1: Zone 3 perisinusoidal fibrosis only
- Stage 2: Zone 3 perisinusoidal and periportal fibrosis
- Stage 3: Bridging fibrosis
- Stage 4: Cirrhosis

Table 1: Histological scoring system for liver fibrosis.

Appearance	Ishak stage: categorical description	Ishak	Metavir
	No fibrosis (normal)	0	F0
	Fibrosis expansion of some portal areas ± short fibrous septa	1	F1
	Fibrosis expansion of portal areas ± short fibrous septa	2	F2
	Fibrosis expansion of most portal areas with occasional portal to portal (P-P) bridging	3	
	Fibrosis expansion of portal areas with marked portal to portal (P-P) bridging as well as portal to central (P-C)	4	F3
	Marked bridging (P-P and / or P-C) with occasional nodules (incomplete cirrhosis)	5	
	Cirrhosis, probable or definite	6	F4

Prognosis

- Patients with non-alcoholic fatty liver disease (NAFLD) exhibit increased mortality rates when compared to the general population.
- These patients have a high risk of mortality from cardiovascular causes as these patients have metabolic derangements.
- Cardiovascular causes of mortality are higher in these patients than liver causes.[42] NAFLD is a slowly progressive disease; simple steatosis is reversible and non- progressive, whereas NASH can progress to cirrhosis.
- Over a 13 year follow-up, the progression of cirrhosis presented in 41% in a study by Ekstedt et al.[43]
- A meta-analysis done by White et al. showed that in cohorts of NAFLD or NASH with few or no cases of cirrhosis, the risk of developing HCC was minimal at 0 to 3% over 20 years, and in cohorts with NASH with cirrhosis, the risk was high at 2.4 % over seven years.[44]

Complications

The most important complications of non-alcoholic fatty liver disease (NAFLD) in the descending order are:

- Cardiovascular disease
- Hepatocellular carcinoma
- End-stage liver disease

The severity of these complications is proportional to the severity of the histological stage and grade of liver disease.

- The positive likelihood ratio of developing NAFLD is 2.32 (low when the score is less than 3), and the risk is 7.77 (high when five or more). Some of the other scoring systems are NAFLD fibrosis score (NFS), FIB-4 (fibrosis-4) index, original ELF (enhanced liver fibrosis) test, AST-to-platelet ratio index (APRI), AAR, Fibro-meter, NAFLD-MS score.

TREATMENT

- Lifestyle changes are recommended for all patients with non-alcoholic fatty liver disease (NAFLD), even without NASH, as these patients have metabolic derangement and are at risk for the development of cardiovascular diseases.[45]
- The recommendation is a weight loss of 3 to 5% in simple steatosis and a weight loss of 7% to 10% in NASH. Adequate control of risk factors like hyperlipidaemia with statins and hypertension and adequate glycaemic control are required.

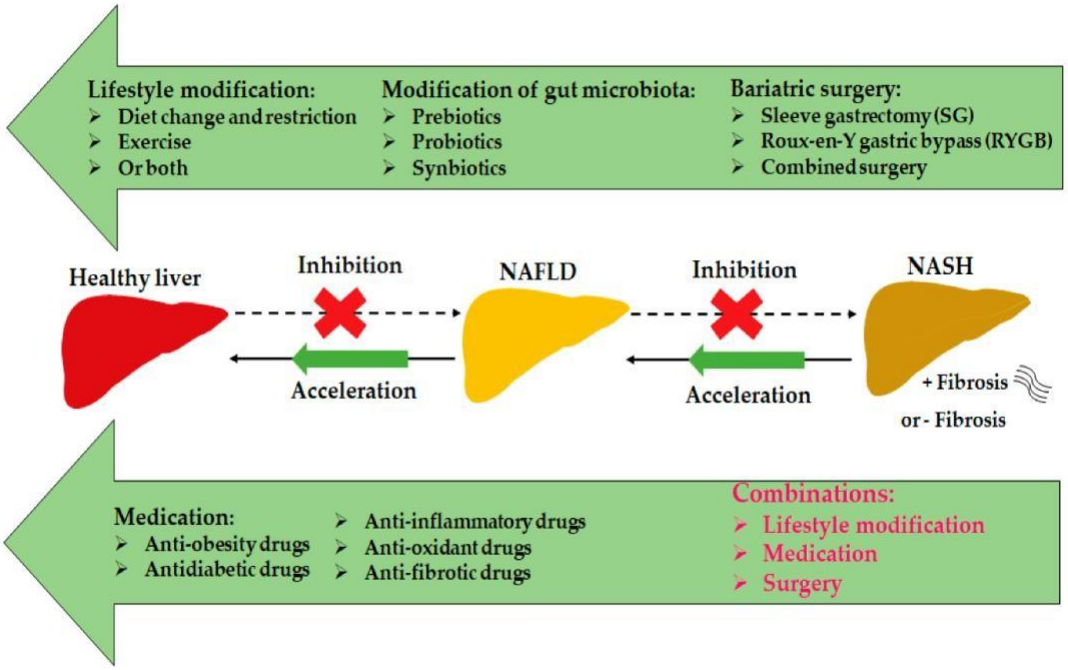

Figure 6: Treatment options for NAFLD.

- Patients with NASH are to be followed by hepatologists or gastroenterologists. NASH with cirrhosis requires hepatocellular carcinoma surveillance with an ultrasound every six months. Several clinical trials are being conducted using anti-fibrotic, anti-apoptotic, and immune therapies for the treatment of NAFLD.[42]
- Five medications are currently approved by the US Food and Drug Administration (FDA) for chronic weight management: orlistat (lipase inhibitor), phentermine/topiramate extended release (sympathomimetic plus anticonvulsant), naltrexone extended release/bupropion extended release (opioid antagonist plus aminoketone antidepressant), and liraglutide and semaglutide (glucagon-like peptide 1 receptor agonists [GLP-1 RAs])[46]

- In addition to significant weight loss, semaglutide has been shown to improve NASH, although not fibrosis, and it is also associated with significant cardioprotective and nephroprotective effect.

Vitamin E

- The anti-oxidative effect of Vitamin E is thought to contribute to its promising results in randomized trials showing a significant improvement in NASH. In 2010, the so far largest randomized trial on Vitamin E was published (PIVENS-Trial).[47]

- It included 247 adults with biopsy-proven NASH but without diabetes and compared Vitamin E (800 IU once daily) versus Pioglitazone (30 mg once daily) versus Placebo with the primary study endpoint defined as an improvement in histologic findings (improvement by 1 or more points in a hepatocellular ballooning score; no increase in fibrosis score; and either decrease of NAS to ≤3 points or of at least ≤2 points, with at least a 1-point decrease in either lobular inflammation or steatosis).[47]

- Vitamin E treatment resulted in a significantly higher rate of NASH improvement (43% vs. 19%, p = 0.001) as compared with placebo. However, the grade of fibrosis did not improve.[47] Most importantly, adverse events in the Vitamin E group were not significantly different compared to Pioglitazone or placebo

- A study evaluating the effect of Vitamin E on clinical outcomes in 236 NASH patients with bridging fibrosis or cirrhosis found that indeed 800 IE/day decreased the risk of death or transplantation and hepatic decompensation —both in diabetic and in non- diabetic patients[48] and therefore adds important data into the daily clinical use of Vitamin E. Nonetheless, the latter study was no randomized controlled trial and therefore results should be interpreted cautiously.

- While the PIVENS trials only included non-diabetic NASH patients, it has been shown that Vitamin E treatment alone (800 IE/day) was ineffective in reaching the primary endpoint (two-point reduction in NAS from two different parameters, without worsening of fibrosis) in a randomized trial including 105 patients with type 2 diabetes and biopsy- proven[49] NASH. Again no improvement in fibrosis was seen. Possible side effects of Vitamin E include an increased bleeding risk, prostate cancer, heart failure and hemorrhagic stroke and those should be discussed with the patient, even though they are rarely seen.[50,51]

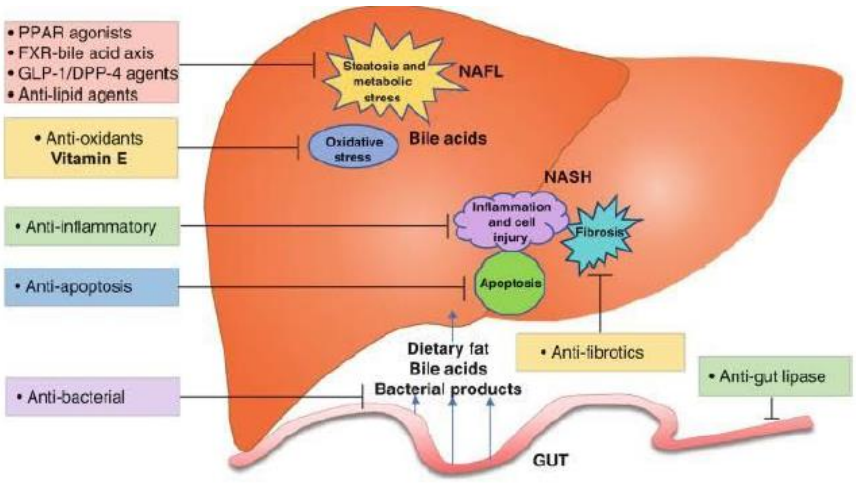

Figure 7: Function of Vitamin E in regulating NAFLD.

- As of 2022 the current (2016) EASL guidelines cautiously recommend ("could be used") Vitamin E treatment for selected patients with NASH and at least significant fibrosis (≥F2) while the current practice guidance endorsed by the AASLD states that Vitamin E (800 IU/day) "may be considered" for treating non-diabetic patients with NASH.[51] Most importantly, Vitamin E is currently not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis and cryptogenic cirrhosis.

Pioglitazone

- Pioglitazone even though the PPAR-y ligand Pioglitazone (30 mg/day) did not reach the pre-defined primary study endpoint in the PIVENS trial, which was set at a significance level of p = 0.025 due to two primary comparisons, 34% in the Pioglitazone group versus 19% in the placebo group (p = 0.04) showed an improvement in liver histology as defined in the primary outcome.[47]

- Most importantly, 47% with Pioglitazone versus 21% with Placebo showed a resolution of definite

NASH (p = 0.001). Similar to the Vitamin E treatment arm, fibrosis was not affected by Pioglitazone treatment.

- Adverse events per se were not increased in the Pioglitazone treatment arm; importantly, however, a significant mean weight gain of +4.7 kg at week 96 was seen, which however could be part of the therapeutic action (lipid partitioning with the expansion of subcutaneous adipose tissue).[52]
- While all diabetic patients were excluded from the PIVENS Trial, a randomized controlled trial including 101 patients with either pre- or type 2 diabetes found that 51% in the Pioglitazone group (45 mg/day) had resolution of NASH and 58% achieved the primary outcome of the study (reduction of ≥2 NAS points in two histologic categories without worsening of fibrosis), both significantly.[53]

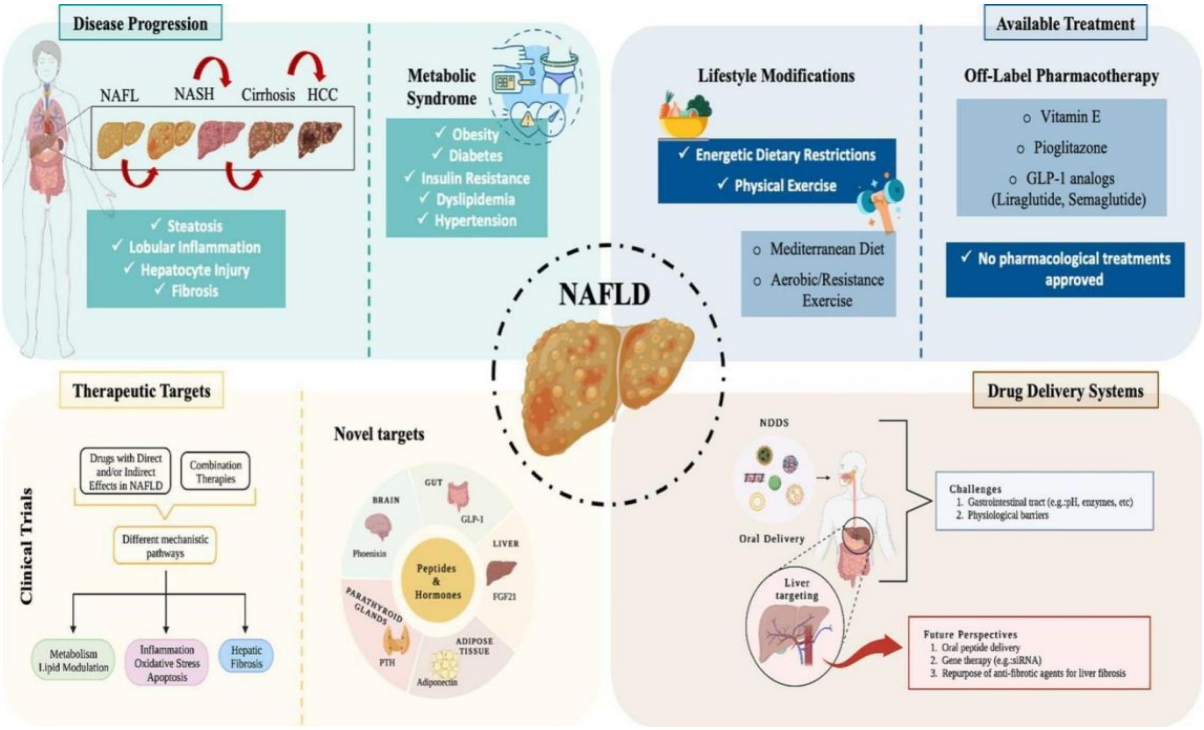

Figure 8: NAFLD current therapies and drug delivery perspectives.

- Interestingly, in their study, Pioglitazone treatment was also associated with a significant improvement in fibrosis score. However, weight gain was also significantly higher in the treatment group.[53]
- Nevertheless, it seems that a significant reduction in fibrosis score under Pioglitazone treatment is only seen in type 2 diabetic patients since Bril et al. showed a significant reduction of fibrosis with 45 mg/day. Pioglitazone treatment was only seen in type 2 diabetic patients, not in those with prediabetes.[54] While the dosage in this study was higher than in the PIVENS Trial (45 mg/day vs. 30 mg/day) duration of therapy was shorter and one could argue that similar results could have been seen in non-diabetic patients if the study drug dose was higher.Positive side effects of Pioglitazone treatment being improvement of insulin sensitivity and diabetic control should be weighed against its negative side effects including weight gain, fluid retention, bone loss and a possible increase in bladder cancer.[50,51]
- However, as long as weight gain is not due to fluid retention it may be due to induction of a healthy obese phenotype and therefore could be clinically acceptable. Most importantly, Pioglitazone is contraindicated in patients with NYHA class III or IV heart failure.[52]
- Finally, the current EASL guidelines state that Pioglitazone "could be used" for the treatment of patients with NASH and significant fibrosis, while the AASLD suggests that it "may be used" for treating biopsy-proven NASH patients with and without a type 2 diabetes.[51]

Other pharmacological treatment options

- Apart from Vitamin E and Pioglitazone, several trials testing mechanistically different types of medication in NAFLD have been published throughout the last few years and have shown promising results.
- However, none have yet made their way into national- or international guidelines. Nevertheless, we will outline the most important clinical findings in the following chapters, stratified by pharmacological mechanisms of action, while a

detailed review of emerging therapeutic targets for NAFLD can be found elsewhere.[55]

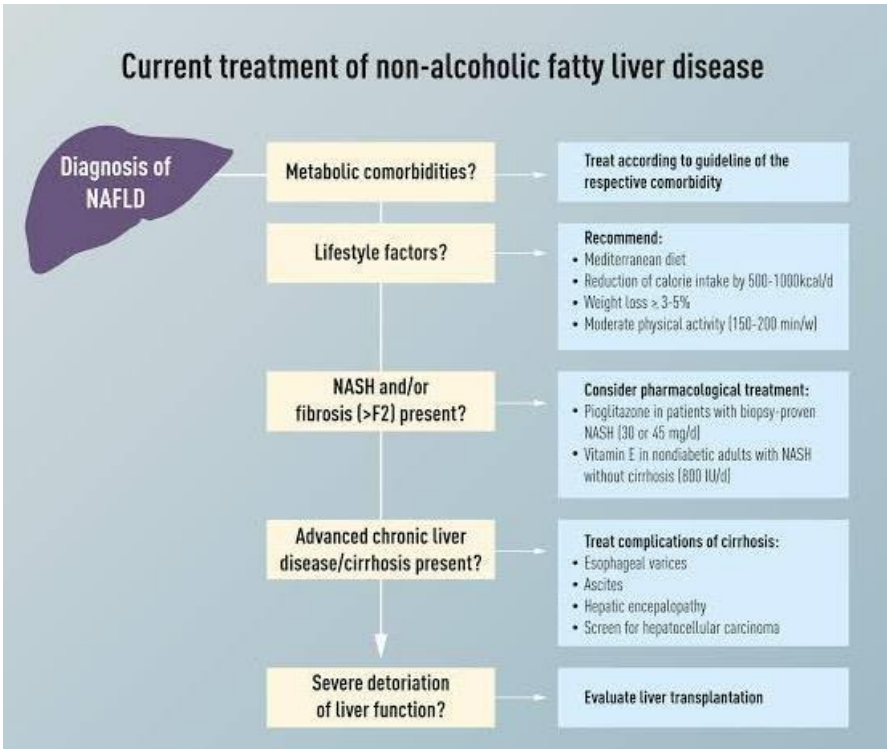

Figure 9: Algorithm for management of NAFLD.

GLP-1 agonists

- The glucagon-like peptide-1 receptor agonist semaglutide has shown a significantly higher percentage of patients with NASH resolution (and no worsening of fibrosis) compared to placebo in a 72-week, double-blind phase 2 trial involving 320 patients with biopsy-confirmed NASH and fibrosis stage 1–340. Improvement in fibrosis stage was seen in 43% of NASH patients and 33% of placebo patients, but this difference was not statistically significant.[56] Importantly, around 38% of patients in the study had no (!) diabetes mellitus, however all had at least a BMI >25. The Semaglutide dosage used (0.1, 0.2 or 0.4 mg once-daily) was significantly higher than in its main indication (treatment of diabetes mellitus type II).
- A previous study investigating the efficacy of the GLP-1 agonist liraglutide in 52 overweight patients with clinical evidence of NASH showed a significantly higher rate of NASH resolution in the liraglutide group compared to placebo.[57] Most importantly, 9% in the liraglutide group versus 36% in the placebo ($p = 0.04$) group showed a progression of fibrosis.[57]
- A recent meta-analysis consisting of 11 RCTs that investigated GLP-1 agonists in NAFLD patients concluded that their overall clinical effect lies mainly in NASH resolution rather than fibrosis improvement.[58] Thus current guidelines do not recommend GLP-1 agonists for patients with NAFLD outside their labeled indications (treatment of diabetes mellitus and/or obesity). Recent data suggested possible positive effects of dual GLP-1/Glucagone or GLP-1/GIP Receptor ligands[59,60] and those might be promising future targets, although further studies are needed to prove their clinical efficacy.

DPP-IV inhibitors and SGLT2 inhibitors

- Studies investigating the effect of DPP-IV inhibitors have all shown disappointing results and therefore DPP-IV inhibitor treatment is not recommended for NAFLD patients outside their labeled indications.[51]
- However, studies investigating sodium-glucose cotransporter protein 2 (SGLT2) inhibitors have consistently shown a reduction in liver transaminases and improvement of imaging-based biomarkers[61] and, therefore, might be a treatment option not only in diabetic NAFLD patients but also in those without diabetes, although large randomized trials are still needed to confirm this assumption.

FXR ligands

- In the FLINT trial[62], the effect of the steroidal farnesoid X nuclear receptor (FXR) ligand obeticholic acid (25 mg/daily) was tested in a 72-week randomized trial involving 283 patients with non-cirrhotic biopsy-proven NASH.
- Significantly more patients in the obeticholic acid arm (45%) versus placebo (21%) showed improved liver histology[62] (defined as decrease in NAS ≥2 points without worsening of fibrosis). Nevertheless, while the primary endpoint was reached, no statistically significant effect on the resolution of

NASH was seen, which could limit direct clinical usefulness.

- Importantly however obeticholic acid improved fibrosis in 35% of patients versus only 19% in the placebo arm (p = 0.004).[62] Pruritus was the main side effect of obeticholic acid (33% vs. 6% placebo). In 2019 interim data from the REGENERATE trial,[63] including 1968 patients with biopsy-proven NASH and fibrosis stages F2-3 or F2 with at least one accompanying comorbidity, with 931 patients included in the interim analysis was published.[63]
- Primary endpoints for the 18-month interim analysis were fibrosis improvement (≥1 stage) with no worsening of NASH or NASH resolution without worsening of fibrosis.[63] Improvement in fibrosis was seen in 12% of the placebo group, 18% with obeticholic acid 10 mg (p = 0.045) and 23% in the obeticholic acid 25 mg (p = 0.0002) group.
- The proportion of NASH resolution was not significant between the groups.[63] Similar to the previous study, pruritus was the most common adverse event.
- Both studies, FLINT and REGENERATE, however also showed an unfavorable effect on patients' lipid profile, that is, decrease in HDL and increase in LDL and this should be cautiously monitored in NAFLD patients under FXR ligand therapy.
- Results were also published regarding monotherapy with non-steroidal FXR agonists such as cilofexor[64,65] and tropifexor[66] where the primary endpoint was not met in both studies. The ATLAS trial however tested a combination therapy of a non-steroidal FXR agonist (cilofexor) with a lipogenesis inhibitor (firsocostat) and found a significant improvement of NAS subcomponents (steatosis, lobular inflammation and ballooning); however, there were no effects on fibrosis.[65]
- In summary, FXR ligands have shown first promising results in the RCTs investigating their clinical efficacy. Nevertheless, open questions regarding optimal dosing to minimize the potentially deleterious side effects of dyslipidemia and pruritus and the pathophysiological mechanisms behind those side effects are still unanswered and warrant further research.[67]

FGF19 mimetics

- Recently published data investigating the effects of Aldafermin, an analogon of the FXR- regulated Fibroblast-Growth-Factor 19 (FGF19), in patients with NASH and fibrosis stage 2 or 3 did not show improvement of fibrosis or resolution of NASH after 6 months of therapy, while improved hepatic fat content measured via MRI-PDFF was seen.[68] However, due to the rather short time of therapy (6 months) results of ongoing long-term studies (ALPINE) are eagerly awaited.

FGF-21 mimetics

- Pegbelfermin showed a reduction in hepatic fat (measured via MRI-PDFF) and liver transaminases over a 16-week treatment period as well as an improved lipid profile;[69] however, no histological readouts were available which hampers applicability of the results and warrants further studies on this compound.
- A Phase IIa study showed promising results (48% fibrosis improvement ≥1 stage; 28% both NASH resolution and fibrosis improvement) for the FGF-21 mimetic efruxifermin[70] that calls for Phase IIb trials.

PPAR agonists

- Apart from the PPARγ agonist Pioglitazone which has found its way into international guidelines, several studies have reported data on the effects of PPAR-δ, -α/δ, - α/γ and most recently Pan-PPAR agonists.
- Peroxisome proliferator activated receptors (PPARs) are nuclear receptors playing key roles in the regulation of metabolic homoeostasis, inflammation, cellular growth and differentiation.
- There are mainly three isoforms: alpha (α) present in liver, beta (β)/delta (δ) in skeletal muscle, and gamma (γ) in adipose tissue. Drugs acting on both PPARα and γ (glitazars) address two important issues of NAFLD—dyslipidemia and IR, and thus are the area of interest.
- Several glitazars (Tesaglitazar, Muraglitazar, Aleglitazar) have been tried in the treatment of DD but their development was terminated because of the adverse events due to their significant γ action. Saroglitazar, a novel dual PPAR α/γ agonist, with predominant PPARα effect and moderate PPARγ effect, lacks these side effects. Saroglitazar received approval from Drugs Controller General of India for treatment of patients with DD in 2013.

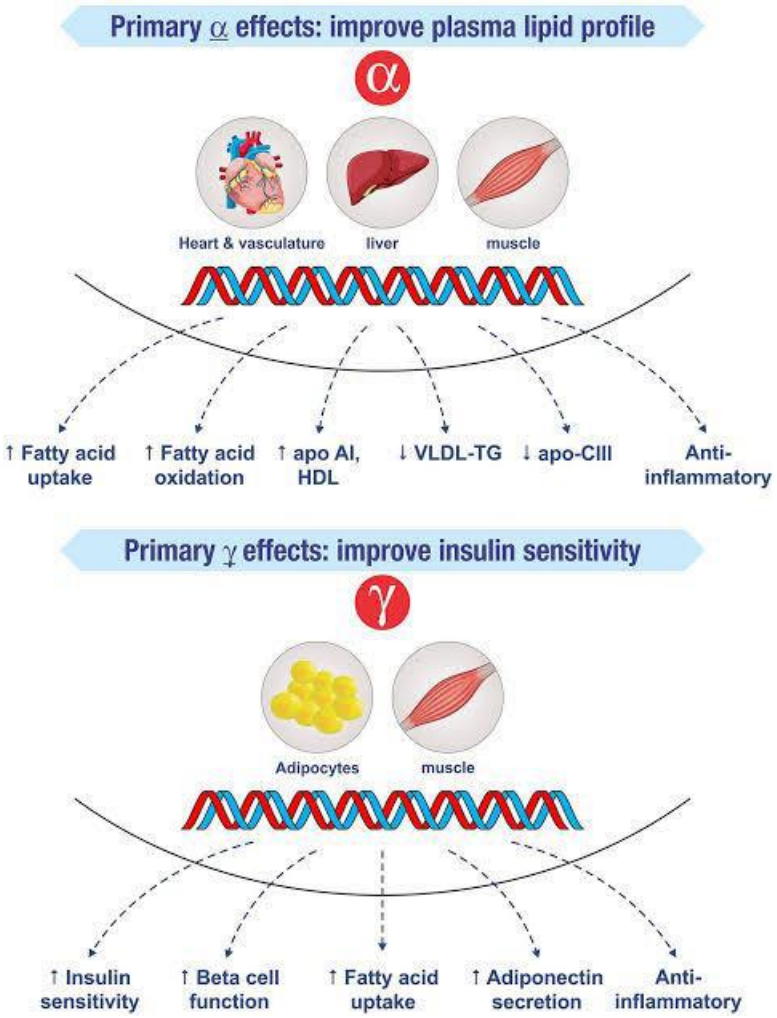

Figure 10: Mechanism of action of Saroglitazar.

- The PPARδ agonist seladelpar has shown an improvement in liver enzymes however without changes in hepatic fat (measured via MRI-PDFF), no full manuscript has yet been published.[71]
- Two Phase II trials have investigated the effects of saroglitazar, a PPARα/γ agonist, and found improvement of ALT and hepatic fat (measured via MRI-PDFF)[72] but no improvement of NAS (primary endpoint: delta change of NAS from baseline to Week 24 biopsy).[73]
- Finally and most recently the Pan-PPAR agonist Lanifibranor reached the primary endpoint of a decrease in SAF-A score of at least two points in a large Phase 2b Trial[73]

—a dose-dependenteffect was seen with more patients achieving the primary endpoint with 1200 mg versus 800 mg.

- Most importantly, resolution of NASH without worsening of fibrosis (49% with 1200 mg Lanifibranor, vs. 39% with 800 mg vs. 22% Placebo), improvement in fibrosis of at least one stage without worsening of NASH (48% vs. 34% vs. 22%) and resolution of NASH plus improvement in fibrosis stage of at least 1 (35% vs. 25% vs. 9%) all favored the study drug as compared to placebo.
- Diarrhea, nausea, peripheral edema, anemia and weight gain were all seen more frequently in patients receiving Lanifibranor.[73]

THR-beta agonists

- The Thyroid Hormone Receptor Beta (THR-B) Agonist Resmetirom (MGL-3196) reduced hepatic fat content (assessed via MRI-PDFF) after 12 and 36 weeks of treatment with positive effects on lipid profiles.[74] Here, a large Phase III trial (MAESTRO) is ongoing to evaluate the effects of Resmetirom on hard clinical endpoints defined as the resolution of NASH without worsening of fibrosis and prevention of progression to cirrhosis. The results are eagerly awaited. Another agent, VK2809, also showed an improvement in MRI-PDFF measured liver fat content after 12 weeks of treatment in a Phase IIa trial.[75] A complete Phase II trial (VOYAGE) is currently ongoing.

Anti-inflammatory/anti-fibrotic therapies

- Disappointing data from studies investigating anti-inflammatory/anti-fibrotic effects have been published within the last years, the largest negative studies were with Selonsertib, a selective ASK-1

inhibitor, in the STELLAR Trials,[76] Cenicriviroc, a C-C chemokine receptor type 2 and 5 dual antagonists, in the CENTAUR Study[77] and Simtuzumab, a monoclonal Lysyl oxidase-like 2 antibody.[78]

Combination therapies

- Since several studies have shown "not as good as expected" results regarding the effects of a single drug on either resolution of NASH and/or improvement of fibrosis, a very elegantly written review by Dufour JF et al.[79] has recently outlined possible promising combination therapies that could show significant results in both clinically relevant endpoints (NASH resolution, fibrosis improvement). Nevertheless, the primary endpoint of ≥1 stage improvement of fibrosis without worsening of NASH was not reached in any of the combination therapies tested in the ATLAS trial (cilofexor/firsocostat; cilofexor/selonsertib; firsocostat/selonsertib vs. placebo).[65]
- Future studies investigating combination therapies are therefore eagerly awaited.

Bariatric surgery

- In morbidly obese patients with NAFLD/NASH, bariatric surgery may lead to improvement of NASH and/or even fibrosis.[80] This might be due to the high remission rates of type II diabetes after bariatric surgery where studies have shown that around 72–75% showed diabetes resolution up to 2 years after surgery.[80,81,82]
- Glycemic control seems to be significantly improved by bariatric surgery. Additionally, the positive effects on lipid metabolism and inflammatory activity are thought to contribute to positive effects on severity of NAFLD.[80] Nevertheless, and importantly, NASH per se is currently not (yet) an established indication for bariatric surgery.
- Several studies have investigated the effects of bariatric surgery on histologic results comparing pre- and post-surgery liver biopsies and those have been elegantly summarized in a recent review. Importantly almost all showed an improvement in all components that determine NAFLD severity: steatosis, inflammation and fibrosis. However, it needs to be emphasized that in some patients worsening of NAFLD was seen.
- Studies have shown that while NASH resolution was achieved in the majority of patients, a considerable number were still found with histologically advanced fibrosis despite NASH resolution.[83]
- While the end-stage liver disease is a well-known contraindication for bariatric surgery no study has yet shown reduced liver-related mortality.
- A small case-control study has even investigated the effects of laparoscopic sleeve gastrectomy in 13 patients with cirrhosis that were matched to 26 non-cirrhotic patients: no postoperative mortality was seen in either group and complication rates did not differ between cirrhotic versus non-cirrhotic patients.[84]
- Statins can usually safely be used in patients with NAFLD and dyslipidemia and may also even counteract NASH. Although, probably due to concerns about safety and statin use in chronic liver disease patients, real-life data from the United States has shown that only 56% of NAFLD patients with at least one indication for statin therapy were actually prescribed statins.[85]
- Most importantly in patients under secondary prophylaxis of variceal bleeding, the addition of statin therapy to standard of care has shown a survival benefit in patients with Child-Pugh class A or B cirrhosis; however, only five patients with NAFLD have been included in this study. In regard to adverse events, no statistical difference was seen between the simvastatin and placebo arm, however, rhabdomyolysis occurred in two (2.8%) patients.
- A meta-analysis and retrospective cohort study has also shown a survival benefit of statin therapy in patients with ACLD. A recent study has however reported increased adverse events rates in patients with decompensated cirrhosis under 40 mg/day of simvastatin (combined with rifaximin) therapy, compared to 20 mg/day.
- Current recommendations state that statin therapy may be used in patients with NASH cirrhosis; however, it should be avoided in decompensated cirrhosis.
- Even though metformin does not play a role in the treatment of NASH, outside its classical indication in the treatment of diabetes, promising data have been published regarding positive clinical effects of metformin on prognosis (mortality, hepatic decompensation) and even HCC development.
- Nevertheless, Metformin use in NAFLD ACLD is not recommended outside its clinical indication, although if indicated its pleiotropic effects on clinical outcomes could be beneficial for the individual patient.
- Current pharmacological treatments include Vitamin E or Pioglitazone, while large randomized trials have shown promising results for GLP-1 agonists, FXR and PPAR ligands. Once patients develop advanced chronic liver disease (i.e. cirrhosis) management should focus on liver-related complications such as esophageal varices and associated bleeding and prevention of hepatic decompensation such as ascites or hepatic encephalopathy.
- Most importantly screening for hepatocellular carcinoma should be performed in all cirrhotic patients, while it may be performed in selected patients with biopsy-proven advanced fibrosis (F3) or where non-invasive fibrosis markers are suggestive of advanced fibrosis.
- Finally, in patients with end-stage liver disease due to NAFLD, liver transplantation should be considered and the patient referred to a tertiary care

liver transplant center.

Alternative medicine

No alternative medicine treatments are proved to cure non alcohic fatty liver disease. But researchers are studying whether some supplements or natural compounds could be helpful, such as:[45]

- **Caffeinated coffee:** Some studies suggest that coffee may benefit the liver by reducing the risk of liver diseases like NAFLD and lowering the chance of scarring. It's not yet clear how coffee may prevent liver damage. But certain compounds in coffee are thought to lower inflammation and slow scar tissue growth.

Lifestyle and home remedies[45]

- **Lose weight.** If you're overweight or obese, reduce the number of calories you eat each day and increase your physical activity to lose weight slowly. Eating fewer calories is key to losing weight and managing this disease.

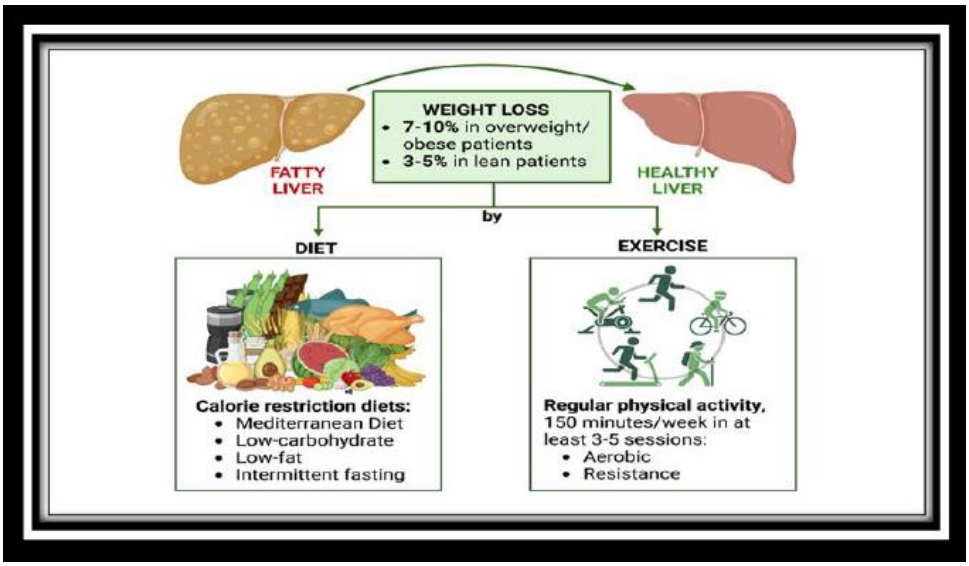

Figure 11: Life style treatment of NAFLD.

Choose a healthy diet. Eat a healthy diet that's rich in fruits, vegetables and whole grains. Your health care team may suggest avoiding or limiting certain foods and drinks, such as white bread, red and processed meats, juices, and sweetened drinks. Keep track of all calories you take in.

Exercise and be more active. Aim for at least 150 minutes of exercise a week. If you're trying to lose weight, you might find that more exercise is helpful. But if you don't already exercise regularly, get your health care team's OK first and start slowly.

Manage your diabetes. Follow your health care team's advice to manage your diabetes. Take your medicines as told by your care team and watch your blood sugar closely.

Lower your cholesterol and blood pressure. Improve your cholesterol levels and blood pressure if they are high. A healthy diet, exercise and medicines can help keep your cholesterol, triglycerides and blood pressure at healthy levels.

Protect your liver. Avoid things that could harm your liver health. For example, don't drink alcohol. Follow the instructions on all medicines and nonprescription drugs.

CONCLUSION

Saroglitazar outperformed Vitamin E in improving lipid profiles, glycemic control, and liver fibrosis in NAFLD patients, making it the more effective treatment option. Vitamin E showed benefits primarily in reducing liver enzymes but was less impactful overall. Saroglitazar is suggested as the prefered choice, especially for patients with metabolic complications. The findings suggest that Saroglitazar could be considered a preferred therapeutic agent in the management of NAFLD, particularly for patients with concomitant dyslipidemia or type 2 diabetes. However, further large-scale studies and long-term follow-up are needed to confirm these findings and to explore the potential benefits of combination therapy or sequential treatment approaches.

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