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REVIEW

New therapeutic strategies in nonalcoholic fatty liver disease: a focus on promising drugs for nonalcoholic steatohepatitis

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Abstract
The prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing worldwide. Globally, it is currently the most common liver disease and is estimated to affect up to 25% of the population. In the first stage, NAFLD is characterized by simple hepatic steatosis (NAFL, nonalcoholic fatty liver) that might progress to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis or hepatocellular carcinoma. In this review, we discuss the global burden of NAFLD, together with future perspectives on how this epidemic could be restrained. There is also an urgent need for the development of new medical strategies for NAFLD patients. We aim to present the beneficial effects of life-style modifications that should be advised to both non-obese and obese NAFLD patients. Since there are currently no medications directly used for the treatment of more advanced NAFLD stages, the central part of this review summarizes ongoing and recently completed clinical trials testing promising drugs for NASH resolution. The marketing of new therapeutic agents would greatly increase the odds of reducing the global burden of NAFLD.

Keywords Nonalcoholic fatty liver disease · Obesity · Therapy · New drugs

NAFLD development and progression

Nonalcoholic fatty liver disease (NAFLD) is an umbrella term for a range of liver conditions affecting people who drink little to no alcohol. As the name implies, the main characteristic of NAFLD is excessive accumulation of fat in hepatocytes. NAFLD can range from relatively benign non-alcoholic fatty liver (NAFL) to the aggressive form called nonalcoholic steatohepatitis (NASH), characterized by both fatty liver and liver inflammation. Since NAFL and NASH are chronic diseases, without proper treatment, they may lead to life-threatening complications such as fibrosis, cirrhosis, liver cancer or liver failure (Fig. 1).

Nonalcoholic fatty liver, the first stage of NAFLD, is defined as the accumulation of excessive fat in the liver in the absence of excessive alcohol consumption and the lack of any secondary cause. It is diagnosed in patients with visible lipid accumulation in at least 5% of hepatocytes; however, diagnosis is hampered by lack of characteristic symptoms [1, 2]. NAFL develops due to impaired hepatocyte metabolism, in particular because of excessive fatty acid (FA) uptake [3]. Other possible etiologies include decreased fatty acid oxidation, exaggerated de novo lipogenesis or reduced VLDL synthesis and secretion by hepatocytes [3, 4]. All of the mentioned above changes result in abnormal fatty acid metabolism that ultimately lead to lipid accumulation in the liver and might also be related to other complications, such as insulin resistance for example. Although most patients suffer from a mild course of illness, approximately 25% of cases progress and subsequently lead to the development of steatohepatitis, hepatic fibrosis, liver cirrhosis and hepatoma [5]. As shown in Fig. 1, remission of NAFL can be relatively easily achieved; however, it can also progress to more severe forms of NAFLD such as non-alcoholic steatohepatitis (NASH). NASH is characterized by excessive accumulation of fat combined with the development of inflammation. Constant worsening of this disease may ultimately lead to irreversible liver damage—including fibrosis, cirrhosis and hepatocellular carcinoma [6].

NAFLD can affect both lean and obese individuals; however, the association between NAFLD and metabolic syndrome is well documented and widely recognized. It is

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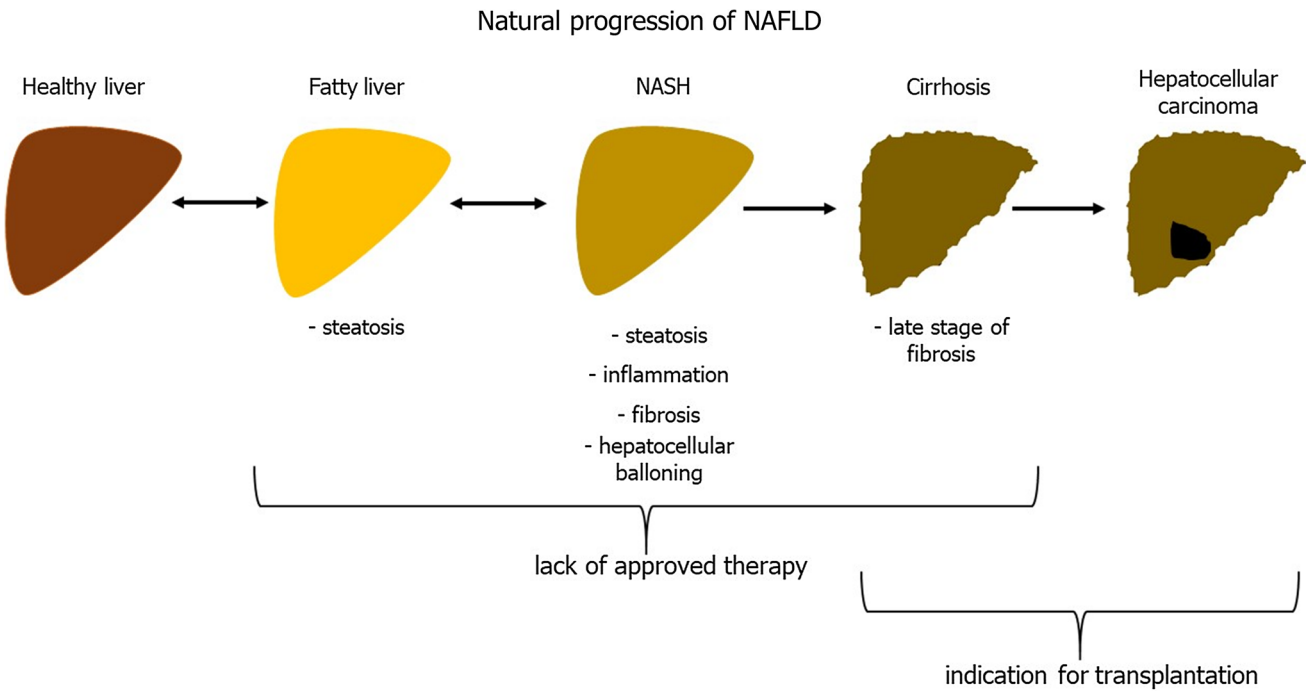

Fig. 1 Schematic presentation of NAFLD progression

known that obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia are the most common metabolic risk factors associated with both the development and progression of NAFLD [6]. According to the World Health Organization, in 2016, more than 1.9 billion adults were overweight (BMI ≥ 25–29.9 kg/m^2), and of these, over 650 million were obese (BMI ≥ 30 kg/m^2) [7]. Since the worldwide prevalence of obesity nearly tripled between 1975 and 2016, not surprisingly, the global epidemic of NAFLD is also spreading. Using BMI, one of the most classical epidemiological indexes to assess obesity, Loomis and coworkers reported that the risk of NAFLD/NASH increased linearly with BMI [8]. In comparison to control subjects with normal BMI, the risk of NAFLD/NASH was from 4.1- to 14-fold higher in patients with a higher BMI. Additionally, it was approximately 50% higher in men and doubled in those with diabetes [8]. In line with these data, a recent study with 3202 individuals reported that higher BMI (overweight/obesity) is an independent, dose-dependent risk factor for fatty liver disease [9].

There is also a strong link between obesity and T2DM. According to data collected between 1999 and 2006, the prevalence of overweight and obesity among American adults with diabetes was 80.3% and 49.1%, respectively [10]. The same trend was also observed in Europe, where people with BMI ≥ 25 were responsible for approximately 80% of T2DM cases [11]. Recently, a very important comparison between NAFLD and T2DM was made by Alkhouri and Scott [12]. The authors redefined the NAFLD spectrum by highlighting its similarities to T2DM: NAFLD is very common, the majority of patients suffer from the less advanced form, and it remains asymptomatic most of the time and can slowly progress from a relatively mild disease (hepatic steatosis) to a life-threatening disease (liver failure, hepatocellular carcinoma). The authors compared NAFL with prediabetes and stressed that management of both diseases should rely on lifestyle modifications. Next, upon progression of disease to NASH or T2DM, patients should be additionally treated with pharmacological agents to maximize the chances of disease remission. Finally, the development of NASH cirrhosis/HCC or T2DM with complications (i.e., neuropathy, nephropathy, retinopathy) requires the most aggressive treatment [12].

Molecular events implicated in the development of NAFLD

Obesity and metabolic dysfunctions such as insulin resistance or dyslipidemia are the best-known mechanisms leading to excessive accumulation of triglycerides in hepatocytes. It has been shown that obese patients are characterized by enhanced lipolysis of triglycerides and fatty acid release from adipose tissue [3]. This excessive breakdown of triglycerides causes accumulation of fatty acids in the form of diacylglycerol not only in the liver but also in other tissues [13]. In the case of the liver, hepatic uptake of circulating fatty acids is mediated by fatty acid transporters: FATP (fatty

acid transport proteins), CD36 (cluster of differentiation 36) and caveolins that are located in the hepatocyte plasma membrane [14]. The levels of these proteins are increased in the livers of NAFLD patients, which together with hyperlipidemia leads to enhanced FA uptake by hepatocytes [3]. In line with these data, knockdown of FATP2, FATP5 or CD36 in mice ameliorated hepatic steatosis induced by high-fat diet [15–17]. Once in the cytosol, FAs are stored in the form of triacylglycerols to be exported from hepatocytes or metabolized via oxidation. Importantly, all of these processes are disturbed in NAFLD patients leading to excessive TAG accumulation in hepatocytes. Catabolism of FAs is controlled on many stages, but the PPARα transcription factor is the master regulator of β-oxidation (occurring in mitochondria and in peroxisomes) and ω-oxidation (performed in cytochromes). The first link between FA oxidation and PPARα was established after demonstrating that an Acyl-CoA oxidase gene (encoding the rate-limiting enzyme in peroxisomal β-oxidation) is a direct PPARα target gene [18]. PPARα induces not only peroxisomal oxidation of long-chain FAs but also the transcription of a wide panel of genes related to FA oxidation in the mitochondria and cytochromes [19]. Interestingly, hepatic PPARα levels were reduced in patients with NASH in comparison to subjects with steatosis and healthy controls. Thus, the amount of PPARα might be an important transition marker during NAFL progression towards NASH [20].

One can expect that augmented hepatic lipid accumulation in NAFLD would stimulate FA oxidation. In fact, data from human studies are conflicting, showing enhanced, unchanged or decreased FA catabolism in steatosis or NASH [3]. However, the common feature from human NAFLD specimens and animal models is hepatic oxidative stress linked to mitochondrial dysfunction and FA oxidation. Liver biopsies collected from NASH patients were characterized by increased ROS levels and reduced expression of antioxidant genes. Lipid oxidation and oxidative damage of mitochondrial DNA further diminishes mitochondrial function compromising cellular respiration and metabolism [21, 22].

Export of FAs from the liver is another important process regulating hepatic lipid content. In a simplified model, liver steatosis begins when accumulation of lipids in hepatocytes does not match oxidation and secretion. On one hand, FAs are delivered to the liver from adipose tissue and from the small intestine; on the other hand, they are secreted from hepatocytes as water-soluble VLDL particles [3]. Formation of VLDL in the endoplasmic reticulum (ER) strictly depends on apolipoprotein B100 (apoB100) synthesis and the activity of microsomal triglyceride transfer protein (MTTP) Thus, both proteins are considered to be key components regulating VLDL secretion from hepatocytes. In the first step, loading of ApoB100 with lipids is catalyzed by MTTP, and then the nascent VLDL particle is transferred to the Golgi apparatus to ultimately be secreted from hepatocytes [23]. As demonstrated by Fujita and coworkers, dysfunctional VLDL synthesis and release is a NASH-specific dysfunction [22]. Although the serum level of VLDL-TG was higher in NAFL subjects in comparison to controls, it was reduced in the NASH group. In line with these data, liver biopsies collected from NASH patients were also characterized by reduced expression of MTTP, ApoB100 and PPARα in comparison to NAFL specimens [22]. Enhanced lipid secretion from hepatocytes in steatosis might compensate to some extent the intrahepatic lipid accumulation. However, lipid export from hepatocytes seems to be biphasic during NAFLD progression. After initial increase, secretion reaches a plateau and even decreases. The VLDL-TG secretion rate increased linearly with increasing intrahepatic lipid content, but reached a plateau when fat content exceeded 10% [24].

Similar to other metabolic diseases, the molecular pathways regulating the development and progression of NAFLD are very complex. Although we already have an accurate understanding of lipid metabolism, we still need a deeper knowledge of factors controlling the transition from fatty liver to NASH. However, one should not forget that the current understanding of NAFLD etiology allows us to relatively easily prevent this disease.

Global burden of NAFLD disease

Lifestyle modifications during the last decades have resulted in the growing incidence of noncommunicable disease all over the world. The global expansion of noncommunicable diseases, commonly known as chronic or lifestyle-related diseases, has dramatically changed health priorities not only in 'western' countries but also in developing ones. The new epidemic of NAFLD is related to the burden of liver diseases paralleling the worldwide increase of obesity and T2DM. The global prevalence of NAFLD is currently estimated to be 24%; however, one should not forget that the diagnostic tools that are currently used are inaccurate [25]. Noninvasive ultrasound examination is poorly sensitive for milder forms of hepatic steatosis; whereas, studies based on elevated liver enzymes systematically underestimated the true prevalence. Levels of ALT/AST are variably elevated in NAFLD and may be normal in 50–80% of cases [26]. Even, the 'gold' standard in NAFLD diagnosis—a liver biopsy—might give some variability because a relatively small liver fragment is used for histopathological examination [2]. Nevertheless, a meta-analysis published by Younossi and coworkers in 2016 reported the prevalence of NAFLD in different geographical regions. According to the study, the highest rates of NAFLD were reported in South America (31%) and the Middle East (32%), followed by Asia (27%), the United States (24%), Europe (23%) and Africa (14%) [25]. Globally, more than a

billion people worldwide are affected [27]. More recently, Estes and coworkers published an alarming study that modeled NAFLD prevalence and incidence from 2016 to 2030 [28]. Future NAFLD disease burden was analyzed in eight countries (China, France, Germany, Italy, Japan, Spain, United Kingdom and United States) accounting for a quarter of the world's population. According to the authors, over these 15 years, the total NAFLD population would increase by 18.3% to 100.9 million cases, with a prevalence of 28.4% (Fig. 2). The highest prevalence in 2030 was estimated for Italy (29.5%), United States (28.4%) and Spain (27.6%). Alongside the significant rise of total NAFLD patients, the authors also projected a growing number of different disease stages: NAFL, NASH or cirrhosis. Additionally, in all analyzed countries, the prevalence of HCC cases related to NAFLD were estimated to increase, ranging from 3240 cases in Japan (47% increase in 2030) to 24,860 cases in United States (130% increase in 2030) [28].

Finally, a meta-analysis performed in 2014 among United States patients undergoing liver transplantation showed that NASH is the third most common indication for this type of surgery [29]. Today, liver transplantation is the best therapeutic option for patients suffering from acute or chronic liver failure and/or hepatocellular carcinoma. Although liver transplantation is often a life-saving surgery for patients, the disproportion between recipients and donors is still an ongoing problem. A recently published, population-based ELITA study analyzed data from the European Liver Transplant Registry [30]. In that study, 60,527 patients who received liver transplantation between January 2007 and June 2017 were classified into five groups based on the etiology of liver disease: 1) hepatitis C virus (HCV); 2) hepatitis B virus (HBV); 3) alcoholic liver disease (ALD); 4) NASH and 5) all other indications. In line with previous reports, alcoholic liver disease has emerged as the most common indication for liver transplantation in Europe and in the USA [30, 31]. Furthermore, the authors demonstrated that the introduction of direct-acting antiviral drugs in 2014 led to a dramatic decline in the number of liver transplants performed in patients with decompensated cirrhosis due to HCV infection (−60%), and in those with hepatocellular carcinoma associated with HCV (−41%). In contrast, the number of patients enrolled in liver transplantation due to NASH has constantly increased over a 10-year period. Since the absolute number of liver transplantations caused by ALD, HCV and HBV in Europe is decreasing from 2014, there is a high chance that in the near future, NASH will become one of the main indications for this surgery [30]. Similar trends have been noted in the USA [32].

It is worth mentioning that patients with early-stage NAFLD (fatty liver with no signs of inflammation) may easily revert this disease phenotype. In such individuals, a great improvement in NAFLD severity is observed after life-style modification and weight loss. However, in more advanced NAFLD stages, the prescription of drugs to reduce insulin resistance and hyperlipidemia is highly recommended [33]. Importantly, so far there is no drug approved for direct therapy of NAFL or NASH [34].

Dietary changes and physical activity in NASH

NASH is becoming one of the most frequent causes of cirrhosis and liver transplantation for nonalcoholic steatohepatitis and other fatty liver diseases [35, 36]. Since there is no approved drug for NASH therapy, it is crucial to look for therapeutic methods that can lead to prevention or reversal of NASH progression. It is known that high-fat, high-sugar, hypercaloric diets increase the risk of hepatic steatosis [37]. On the other hand, weight loss achieved by caloric restriction reduces hepatic inflammation and fibrosis, and diminishes

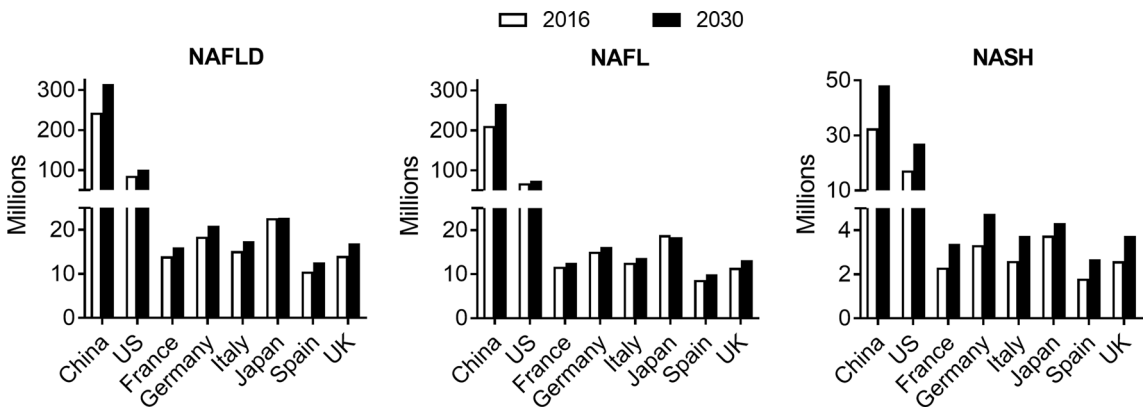

Fig. 2 Distribution of NAFLD, NAFL and NASH populations in 2016 and 2030 Data were collected and modified from Estes et al. [28]. US—United States, UK—United Kingdom

nonalcoholic steatohepatitis [38]. Studies revealed that even a loss of 7–10% of weight results in improvements in NAS score, and a loss of ≥ 10% of weight results in 90% of NASH resolution, 45% of fibrosis regression and a 100% steatosis resolution [38]. A calorie-restricted diet is the most important factor in nutritional interventions in NASH [39]. Weight reduction (at least 7%) achieved by several types of diet is indicated for remission of NASH [40]. The Mediterranean diet was recommended for NAFLD patients by the recent EASL–EASD–EASO Clinical Practice Guidelines [41]. This dietary pattern is characterized by a high intake of olive oil, which is rich in monounsaturated fat, nuts, fruits and legumes, vegetables, and fish and a low intake of red meat, processed meats, and sweets. The Mediterranean diet minimizes consumption of processed, high-sugar food and high-fructose food. Fructose has been shown to increase hepatic TNF production, lipid peroxidation and might promote hepatic steatosis and NAFLD [40]. It has been shown that diets enriched with omega-3 polyunsaturated fatty acids (PUFA) ameliorate steatohepatitis, together with a reduction in intrahepatic triglyceride content [42, 43]. Additionally, NAFL and NASH patients tend to consume lower amount of omega-3 PUFA versus a control group of patients [44].

Another lifestyle modification important for the treatment of NAFLD that is closely related to weight loss is physical activity and exercise. It was shown that exercise significantly reduces steatosis [45] and lowers the risk of NAFLD patients progressing to NASH [46]. A systematic review by Hashida and coworkers compared aerobic versus resistance exercise programs for NAFLD patients. The authors demonstrated that both exercise programs reduced hepatic steatosis in NAFLD with a similar frequency, duration, and period of exercise (40–45 min/session 3 times/week for 12 weeks). One should not forget that resistance exercise versus aerobic is characterized by lower intensity and energy consumption. Thus, this kind of physical activity may be more feasible for NAFLD patients with poor cardiorespiratory fitness that are accustomed to a sedentary lifestyle [47]. Since 10–20% of people suffering from NAFLD have a normal BMI, Wong and coworkers investigated if weight reduction is also beneficial for this group of patients. The authors enrolled 78 volunteers (BMI < 25) who were randomized to a 12-month lifestyle intervention program involving regular exercise, or to standard care. The primary outcome was remission of NAFLD at month 12. Patients were then prospectively followed for 6 more years. Importantly, remission of NAFLD was achieved in 67% of non-obese patients after lifestyle intervention. Furthermore, for half of all NAFLD patients enrolled in the study, a weight reduction of only 3–5% was effective in treating the disease [48].

Current strategies for NAFLD treatment

Successful treatment of patients suffering from NAFLD is challenging due to its complex etiology, difficult diagnosis, the wide spectrum of NAFLD stages and the presence of concurrent diseases. Thus, an individually tailored approach is required to improve outcomes not only for NAFL patients but also for those diagnosed with more advanced NASH stages. Because epidemiological studies demonstrated a tight link between NAFLD and an unhealthy lifestyle, its modification is a mandatory starting point for all patients [12, 49] (Table 1). According to EASL–EASD–EASO Clinical Practice Guidelines [41], comprehensive lifestyle modifications should combine: (1) energy restriction (500–1000 kcal/

Table 1 Treatment of NAFLD. clinical practice guidelines recommended by joined EASL–EASD–EASO associations (41)

Disease		Current therapy	Future perspectives
NAFL	Simple steatosis Steatosis and mild inflammation	Lifestyle modifications Energy restriction	Cenicriviroc Elafibranor
NASH	Early—no or little fibrosis (F0–F1)	Macronutrient composition Exclusion of fructose intake Strict daily limit for alcohol consumption Physical activity Bariatric surgery	Obeticholic acid Liraglutide Semaglutide
	Advanced—significant and bridging fibrosis (F2–F3)	As above, plus pharmacotherapy Pioglitazon Vitamin E Statins	
Cirrhosis	Late stage fibrosis (F4)	Liver transplantation	
HCC	Liver tumor	Resection Liver transplantation Radiotherapy Chemotherapy (e.g., sorafenib)	JX-594 Hepcortespenlisimut-L

Please see text for detailed description, F0–F4—fibrosis stage, HCC—hepatocellular carcinoma

day); (2) macronutrient composition (low-to-moderate fat and moderate-to-high carbohydrate intake); (3) exclusion of fructose intake both in beverages and foods; (4) strict daily limit for alcohol consumption (below 30 g for man and 20 g for women); and (5) physical activity (at least 150–200 min/week of moderate intensity in 3–5 sessions) (Table 1). All of the abovementioned lifestyle modifications have beneficial effects on weight reduction and metabolic control. In fact, this 'therapy' is a very effective first line of treatment recommended for NAFL and early NASH (no or mild fibrosis: F0–F1) patients. Importantly, pharmacotherapy in addition to lifestyle modifications is recommended for progressive NASH (≥ F2 stage) patients. Additionally, patients with early-stage NASH, but with a high risk of fibrosis progression should also be enrolled for treatment with prescribed drugs.

Unfortunately, despite intensive studies, there is not a single drug for NASH approved by the Food and Drug Administration. Thus, no specific therapy can be recommended and all currently prescribed drugs are used off-label. Nevertheless, there are some medicines that are already used worldwide [50]. Among the insulin sensitizers currently available on the market, only pioglitazone was demonstrated to have some positive effects in NASH patients (improved histology and biochemistry) [51–53]. Although no clear statement about pioglitazone can be made (still off-label use outside T2DM), this medication could be used for NASH according to the clinical guidelines published by the joint EASL–EASD–EASO Associations [41]. However, as discussed below, its usefulness for NASH is still under investigation. Vitamin E is another drug currently proposed by the EASL–EASD–EASO for NASH; however, its beneficial effects and long-term safety issues require further studies [53, 54]. In addition, statins might be used to improve patients' lipid profile and prevent cardiovascular risk; however, they have not been adequately tested in NASH [41].

Unfortunately, for many NASH patients, pharmacotherapy and lifestyle modifications are not sufficient to reduce liver fibrosis and inflammation. Current problems with resolution of the histological lesions indicate an urgent need for the development of new pharmacotherapies to manage this disease [38] (Fig. 3).

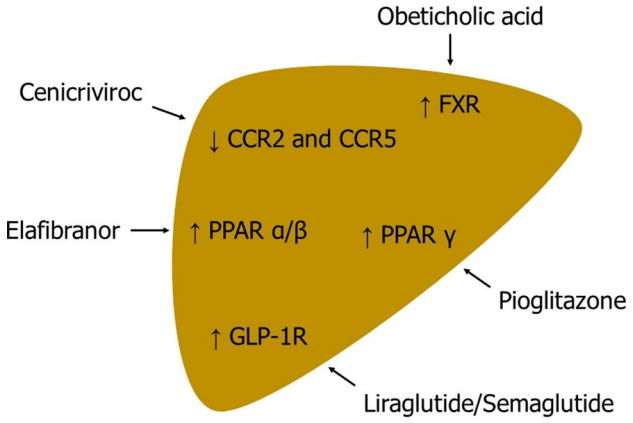

Fig. 3 Therapeutic action of promising drugs, that are currently tested in the clinical trials for NASH resolution

Promising drugs for NASH

Cenicriviroc

Cenicriviroc (CVC) is an oral, dual antagonist of C–C chemokine receptor types 2 and 5. Blockade of CCR2, a chemokine receptor predominantly expressed on monocytes and macrophages, results in reduced recruitment, migration and infiltration of these cells to the injured parts of the liver [55, 56]. Parallel CCR5 inhibition impairs the migration, activation and proliferation of activated hepatic stellate cells [56, 57]. In one study, 288 NASH patients took part in the CENTAUR phase 2b clinical trial (ClinicalTrails.gov Identifier: NCT02217475) to test the efficacy and safety of CVC in adults with NASH (Table 2). For this 2-year study, patients were divided into 3 groups: A—application of 150 mg daily CVC for 2 years, B—application of placebo for 1 year followed by application of CVC for another year, C—application of placebo for 2 years. Currently, only the results from the 1st year of the study are available; so, this review will focus on them. In the 1st year of the CENTAUR double-blinded study, 144 patients received once-daily 150 mg CVC, and the second group of 144 subjects was enrolled into placebo treatment. The primary endpoint of the study was a 2-point improvement in the NAFLD Activity Score (NAS) without worsening of fibrosis was not achieved (16% CVC vs. 19% placebo; $p = 0.52$). Nonetheless, more CVC patients had improvement in fibrosis by ≥ 1 stage without worsening of steatohepatitis (20% CVC vs. 10% placebo, $p = 0.02$). CVC also impacted inflammation, as reflected by marked reductions in circulating biomarkers—CRP, IL-6, IL-1β and soluble CD14. Due to its antifibrotic properties in subjects with NASH after 1 year of the CENTAUR clinical trial, CVC is currently being tested in the AURORA phase 3 clinical study for efficacy and safety for the treatment of liver fibrosis in adults with NASH (ClinicalTrails.gov Identifier: NCT03028740) [58].

Elafibranor

Another promising drug currently tested for resolution of NASH is elafibranor—a dual PPARα/δ agonist. PPARα is an important player in the context of steatohepatits: it modulates fatty acid transport and β-oxidation in the liver. Moreover, PPARα activation by fibrates improves plasma

lipids by decreasing triglycerides and increasing HDL levels [59]. It was shown that in advanced NASH, the PPARα level is reduced, but it recovers after improvement in disease [20]. PPARδ also regulates metabolism in the liver—its activation enhances fatty acid transport and oxidation. Furthermore, use of PPARδ agonists results in elevation of plasma HDL levels and proper glucose homeostasis [60]. In phase 2b of the GOLDEN-505 clinical trial, the efficacy of NASH treatment with elafibranor was evaluated (ClinicalTrails.gov Identifier: NCT01694849) (Table 2). Here, 276 subjects were randomly divided into three groups: A—93 patients received 80-mg elafibranor per day, B—91 of patients received 120-mg elafibranor daily, and C—92 patients were in the placebo group. The GOLDEN-505 trial aimed for NASH reversal without worsening of fibrosis (absence of at least 1 NASH feature: steatosis, hepatocyte ballooning or inflammation). This primary outcome was later modified to "disappearance of ballooning with resolved lobular inflammation or the persistence of mild lobular inflammation only", to highlight the importance of hepatocyte ballooning as a main feature of steatohepatitis. In this study, 19% of patients met this primary outcome in comparison to 12% from placebo group. Additionally, in the case of subjects with NAS ≥ 4, a significant effect of 120 mg, but not 80-mg elafibranor was observed when compared to the placebo group. In these patients, improvement in steatosis, hepatocyte ballooning and lobular inflammation was observed. NAS was ameliorated by ≥ 2 points in twice as many patients as the control group (48% elafibranor vs. 21% placebo; $p = 0.013$). Furthermore, the liver fibrosis stages were reduced in patients with NASH resolution after elafibranor treatment. Both doses of the tested drug improved serum levels of liver enzymes (ALT, GGTP, ALP) and lipid profile (triglycerides, LDL, HDL). In addition, a reduction in serum inflammatory markers (CRP, fibrinogen, haptoglobin) was obtained. Additionally, in diabetic patients (40% of participants), the level of fasting serum glucose, HbA1c and markers of insulin resistance were improved. Importantly, all of the above-listed beneficial effects of elafibranor fulfilled the requirements of the study secondary endpoints [61]. After successful phase 2b trials, elafibranor is currently being investigated in phase 3 of the RESOLVE-IT study (ClinicalTrails.gov Identifier: NCT02704403) to evaluate efficacy and safety in NASH patients.

Obeticholic acid

Obeticholic acid (OCA) is a 6a-ethyl derivative of one of the human bile acids: chenodeoxycholic acid, which is a natural Farnesoid X receptor (FXR) agonist. Due to synthetic modification, OCA stimulates FXR activity 100-fold more intensely than chenodeoxycholic acid [62]. Thanks to this feature, OCA exerts anticholestatic and hepatoprotective properties by regulating the metabolism of cholesterol and bile acids [63]. Additionally, it possesses anti-inflammatory and antifibrogenic activity [64]. In phase 2a of a clinical trial on OCA in diabetic and NAFLD patients (ClinicalTrails.gov Identifier: NCT00501592), weight loss as well as increased insulin sensitivity and a reduction in markers of liver inflammation and fibrosis were observed (Table 2). Next, 283 patients with NASH were included in the phase 2b—FLINT trial (ClinicalTrails.gov Identifier: NCT01265498) in which they were administered 25 mg OCA daily for 72 weeks. Importantly, the primary endpoint of the study—decreased NAS score by at least 2 points without worsening of fibrosis—was achieved (45% OCA vs. 21% placebo; $p = 0.0002$). Moreover, patients treated with OCA were characterized by reduced fibrosis (35% OCA vs. 19% placebo; $p = 0.004$). A significant reduction in steatosis, lobular inflammation and hepatocyte ballooning was accomplished as well. Decreased body weight and reduced ALT levels occurred in the group of patients treated with OCA [65]. Currently, OCA is being investigated in the phase 3 of the REGENERATE study (ClinicalTrails.gov Identifier: NCT02548351) to evaluate its impact on NASH with fibrosis and in phase 3 of the REVERSE trial (ClinicalTrails.gov Identifier: NCT03439254) to evaluate its efficacy and safety in subjects with compensated cirrhosis due to NASH.

Pioglitazone and Vitamin E

The purpose of the phase 3 PIVENS study (ClinicalTrails.gov Identifier: NCT00063622) was to assess if therapy with vitamin E or pioglitazone will lead to an improvement in the NAS score of nondiabetic patients with NASH. Pioglitazone is a drug from the thiazolidinedione class, which is commonly used for T2DM treatment. By activating the PPARγ nuclear receptor, pioglitazone regulates the expression of genes involved in glucose and lipid metabolism. It has a beneficial impact on lowering insulin resistance in the liver, muscles and adipose tissue, and decreases gluconeogenesis in the liver [66]. The second tested agent, vitamin E, is an antioxidant with anti-inflammatory and anti-apoptotic activity [67]. In patients with NASH, the level of α-tocopherol, which is a constituent of vitamin E, is lower versus healthy controls [68]. Thus, it was suggested that supplementation with vitamin E could be beneficial for the treatment of NASH (Table 2). In this study, 247 participants were divided into three groups: A—30 mg daily pioglitazone and placebo (instead of vitamin E), B—vitamin E (800 IU daily) and placebo (instead of pioglitazone) and C—placebo only. The primary outcome was improvement of histological features—a decrease by ≥ 1 point in hepatocellular ballooning score, lack of an increase in fibrosis score, and a general reduction in the NAS score to ≤ 3 or by at least 2 points. The vitamin E treatment reached this primary endpoint—it revealed a greater

improvement rate in NASH than placebo (43% vitamin E vs. 19% placebo; $p=0.001$). Treatment with pioglitazone was also beneficial for resolution of NASH (34% pioglitazone vs. 19% placebo; $p=0.04$), however, it did not reach the expected $p=0.025$ value. For secondary outcomes, in both groups, there was a significant reduction in steatosis, lobular inflammation and NAS score, but not in fibrosis and portal inflammation. Scores for hepatocyte ballooning were significantly improved only after vitamin E treatment ($p=0.01$), but steatohepatitis was resolved with statistical significance in the group of patients who received pioglitazone ($p=0.001$). A significant reduction in ALT, AST, ALP and GGTP levels was observed in the serum of patients from both groups [53]. As a result of this promising study, pioglitazone was further evaluated in phase 4 of a clinical trial to assess its effect on NASH in prediabetic and diabetic patients (ClinicalTrails.gov Identifier: NCT00994682). Here, 101 patients were qualified for the study, and received 30 mg daily pioglitazone (if well tolerated, the dosage was increased to 45 mg per day after 2 months of the trial) or placebo for 18 months. The primary outcome, which was reduction of NAS by more than 2 points without worsening of fibrosis, was achieved both in prediabetic and diabetic patients. Resolution of NASH, which was a secondary endpoint, was obtained only in patients with diabetes (60% pioglitazone vs. 16% control; $p=0.002$). In both the prediabetic and diabetic groups of patients treated with pioglitazone, a significant reduction of steatosis, NAS score, insulin resistance, and serum triglyceride level was observed. However, only in diabetic patients was a significant reduction of fibrosis ($p=0.035$), inflammation ($p=0.013$) and ballooning ($p=0.006$) accomplished. The general conclusion of this study was that similar results regarding NASH treatment were achieved by both prediabetic and diabetic patients [69].

GLP-1 analogs

Glucagon-like peptide 1 (GLP-1) is a gut-derived incretin hormone which possesses glucose-lowering features: it is able to induce insulin secretion and reduce production of glucagon. It also suppresses appetite and retards gastric emptying. Endogenous GLP-1 is degraded within a few minutes; so for therapeutic purposes, a GLP-1 analog—liraglutide—with a half-life of 13 h was developed [70]. Liraglutide use leads to weight loss, and improvement in metabolic regulation and beta cell function [71, 72]. It is used to maintain glycemic control in patients with type 2 diabetes. Liraglutide was also shown to improve lipid transport, beta-oxidation and de novo lipogenesis in in vitro-treated hepatocytes [73–75]. In phase 2 of the LEAN study (Liraglutide Efficacy and Action in NASH), 26 patients received 1.8 mg liraglutide daily by subcutaneous injection, and the other 26 subjects were injected with 1.8 mg placebo (ClinicalTrails.gov Identifier: NCT01237119) (Table 2). The primary outcome was improvement in liver histology (resolution of steatohepatitis without worsening of fibrosis) and was met in 39% of liraglutide patients versus 9% of placebo patients ($p=0.019$). Among the secondary endpoints, the amelioration of NAS score and its components (steatosis, ballooning and inflammation), stage of fibrosis and serum biomarker levels (liver enzymes, lipid profile) were listed. Patients treated with liraglutide showed a significant reversal of steatosis (83% liraglutide vs. 45% placebo; $p=0.009$) and hepatocyte ballooning (61% liraglutide vs. 32% placebo; $p=0.05$). Additionally, a smaller proportion of liraglutide-treated patients showed progression of fibrosis (9% liraglutide vs. 36% placebo; $p=0.04$). Liraglutide treatment significantly reduced body weight, BMI and depleted the concentration of serum liver injury biomarkers. Nonetheless, no differences in lobular inflammation and NAS score were observed between these two groups of patients [70]. The authors of this study did not continue to evaluate liraglutide in clinical trials, but they initiated a new phase 2b to investigate the efficacy and safety of another GLP-1 inhibitor—semaglutide (ClinicalTrails.gov Identifier: NCT02970942). However, liraglutide is now tested by another group in the phase 3 CGH-LiNASH study to compare the effects of liraglutide and bariatric surgery on weight loss, liver function, body composition, insulin resistance, endothelial function and biomarkers of NASH in obese Asian adults (ClinicalTrails.gov Identifier: NCT02654665) (Table 2).

Conclusions

From the global perspective, a growing number of patients suffering from metabolic diseases require urgent action. All decision makers—not only physicians, scientists or politicians—should join together in a community effort to promote healthy food and physical activity. In our opinion, there are still many possibilities to stop this negative trend. For example, more attention should be paid to education programs for young people, parents and teachers. Unfortunately, as described above, the global epidemic of NAFLD is predicted to spread even further. Furthermore, there are a constantly growing number of people on a waiting list for liver transplantation due to the liver cirrhosis or liver cancer, urging the development of new therapeutic strategies.

Table 2 Summary of clinical trials of several promising drugs tested for NASH resolution

Tested agent	Mechanism of action	Title of trial	Trial ID	Phase	Duration	Results	
						Resolution of NASH	Decreased fibrosis
Cenicriviroc	Blockade of CCR2 and CCR5	Efficacy and safety study of Cenicriviroc for the treatment of NASH in adult subjects with liver fibrosis (CENTAUR)	NCT02217475	2b	2014–2017	No	Yes
		AURORA: Phase 3 study for the efficacy and safety of CVC for the treatment of liver fibrosis in adults with NASH	NCT03028740	3	Ongoing since 2017	–	–
Elafibranor	Dual PPAR α/δ activation	Phase IIb study to evaluate the efficacy and safety of GFT505 versus placebo in patients with NASH	NCT01694849	2b	2012–2015	Yes	Yes
		Phase 3 study to evaluate the efficacy and safety of elafibranor versus placebo in patients with NASH (RESOLVE-IT)	NCT02704403	3	Ongoing since 2016	–	–
Obeticholic acid	Farnesoid X receptor activation	Study of INT-747 in patients with diabetes and presumed NAFLD	NCT00501592	2a	2007–2009	No	No
		The Farnesoid X receptor (FXR) ligand obeticholic acid in NASH treatment trial (FLINT)	NCT01265498	2b	2011–2014	No	Yes
		Randomized global phase 3 study to evaluate the impact on NASH with fibrosis of obeticholic acid treatment (REGENERATE)	NCT02548351	3	Ongoing since 2015	–	–
		Study evaluating the efficacy and safety of obeticholic acid in subjects with compensated cirrhosis due to NASH (REVERSE)	NCT03439254	3	Ongoing since 2017	–	–
Vitamin E	Antioxidant, anti-inflammatory and anti-apoptotic activity	Pioglitazone versus vitamin E versus placebo for treatment of non-diabetic patients with NASH (PIVENS)	NCT00063622	3	2005–2009	Yes	No
Pioglitazone	PPARγ activation	Pioglitazone versus vitamin E versus placebo for treatment of non-diabetic patients with NASH (PIVENS)	NCT00063622	3	2005–2009	Yes	No
		University of Texas H.S.C. San Antonio Pioglitazone in NASH trial	NCT00994682	4	2008–2014	Yes	Yes

Table 2 (continued)

Tested agent	Mechanism of action	Title of trial	Trial ID	Phase	Duration	Results	
						Resolution of NASH	Decreased fibrosis
GLP-1 analogs	GLP-1R activation	Liraglutide efficacy and action in NASH (LEAN)	NCT01237119	2	2010–2014	Yes	Yes
		Comparing effects of liraglutide and bariatric surgery on weight loss, liver function, body composition, insulin resistance, endothelial function and biomarkers of NASH in obese Asian adults (CGH-LiNASH)	NCT02654665	3	Ongoing since 2014	–	–
		Investigation of efficacy and safety of three dose levels of subcutaneous semaglutide once daily versus placebo in subjects with NASH	NCT02970942	2	Ongoing since 2016	–	–

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Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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