Review

JOURNAL OF HEPATOLOGY

Current efforts and trends in the treatment of NASH

Vlad Ratziu[1,*], Zachary Goodman[2], Arun Sanyal[3]

[1]*Université Pierre et Marie Curie, ICAN – Institute for Cardiometabolism and Nutrition, Hôpital Pitié Salpêtrière, Paris, France;* [2]*Center for Liver Diseases, Inova Fairfax Hospital, 3300 Gallows Road, Falls Church, VA 22042, USA;* [3]*Division of Gastroenterology, Hepatology and Nutrition, Department of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, VA, USA*

Summary

Of all the aspects of non-alcoholic fatty liver disease (NAFLD), the slowest advances have occurred in the therapeutic field. Thirty-five years after its formal description and after 15 years of intense scrutiny from researchers worldwide, there is still no approved drug for the treatment of non-alcoholic steatohepatits (NASH). In the meantime, progress in the understanding of pathophysiology, diagnosis – both invasive and non-invasive, epidemiology and even natural history have been substantial or, at times, spectacular. In contrast, hepatitis C virus (HCV) therapy underwent constant improvement and even before the great acceleration of the past few years, patients were already being offered approved therapies that were increasingly more efficient.

What then explains such a slow pace of therapeutic advances in NASH, and will this change in the near future? Here we will review commonly-held myths that have diverted attention from therapy of NASH, obstacles that have slowed down industrial development of drugs for this indication, and recent achievements that will create better conditions for drug development programs. We will also briefly review current knowledge of non-pharmacological and pharmacological management in this early era of NASH therapies.

Myths and misconceptions

Several long-held myths have considerably slowed the development of drugs for non-alcoholic steatohepatits (NASH). Because non-alcoholic fatty liver disease (NAFLD) implies the presence of steatosis, and steatosis was historically considered a benign lesion, many physicians did not perceive NASH as a disease of concern. It was seen as a generally benign manifestation of obesity or at most a complication of diabetes with questionable clinical relevance. Most practicing diabetologists did not see and still do not see their diabetic patients when they develop cirrhosis or liver-related mortality. This generated a lack of attention for NASH as a disease worth diagnosing, investigating and treating. Consequently, most of the new and innovative drugs intended to correct insulin resistance were developed for a diabetic indication. Yet observational studies of large cohorts of diabetic patients became available and showed that diabetic patients can die of liver disease [1,2]. Competing risk from other causes of death and the slowly evolving course of chronic liver disease explains why, in absolute values, liver-related mortality is considerably less frequent than death from cardiovascular or neoplastic causes. However, having diabetes still increases the risk of death from cirrhosis to the same or even a larger extent than that of death from the other associated diseases [3]. For instance, the standardized mortality ratio for liver cirrhosis (adjusted for multiple confounders) was 2.33 (CI 1.99–2.73) for men and 2.59 (2.15–3.12) for women, while that for ischemic heart disease was 2.11 (2.05–2.16) and 2.46 (2.39–2.53), respectively [4]. It may be anticipated that better prevention of cardiovascular death and improved screening programs for neoplasia will result in the emergence of increased liver-related mortality in a population of ageing diabetics. A precedent for this shift in the causes of death was witnessed during the AIDS epidemic when a better control of opportunistic infections allowed chronic viral hepatitis to emerge as a major threat. Even though patients with diabetes can have cirrhosis of different etiologies, non-alcoholic, non-viral cirrhosis accounts for most causes of cirrhosis-related death [3]. Equally relevant, death from primary liver cancer almost mirrors the rising risk and prevalence of cirrhosis in diabetics outlined above [5]. These findings are important as they demonstrate the existence of the unmet medical need for effective therapy in patients with NASH, a concept long overlooked by physicians other than hepatogastroenterologists from a tertiary care settings. Appreciation of this medical need is required before the considerable financial risk and human effort needed for successful NASH drug development programs will be undertaken.

Another misconception that is consubstantial to the view of NASH simply as a complication of diabetes, is that antidiabetic drugs, if successful in controlling diabetes will suffice to curb the course of NASH, hence making NASH-specific therapy unnecessary. This argument ignores the fact that most NASH

Keywords: NASH; Treatment; Drug development; Insulin sensitizers; Antifibrotic agents; Endpoints.

Received 10 February 2015; received in revised form 25 February 2015; accepted 28 February 2015

* Corresponding author. Address: Service d'hépatogastroentérologie, Hôpital Pitié Salpêtrière, 47-83 Bd de l'Hôpital, Paris 75013, France.

E-mail address: vlad.ratziu@upmc.fr (V. Ratziu).

patients do not have uncontrolled diabetes, and that the three most commonly used drugs in type 2 diabetes, namely metformin, sulfamides and insulin are totally ineffective for NASH. Similarly, since most NAFLD patients are overweight, do not eat healthy diets and do not exercise enough [6,7], many in the field believe that diet and lifestyle modifications but not sophisticated pharmacological approaches are a reasonable (and cheaper and safer) choice. By this reasoning, it would be better to spend the same effort but only a fraction of the resources necessary to bring drugs to the market in implementing these lifestyle changes in NASH patients. However, even in the context of a clinical trial, in which incentive and monitoring are maximized, patients are typically unable to sustain the assigned dietary goals and the initial weight loss [8]. Exceptions to this observation are rare, although occasionally young patients naive to medical intervention do achieve the desired weight loss with ensuing hepatic improvement. By contrast, most patients who seek hepatological advice have a long history of unsuccessful attempts at dietary and lifestyle changes [9]. The considerable effort and resources necessary to increase patient compliance are beyond the means and expertise of most hepatological centers [10], and therefore there is little chance for a better outcome than that achieved by specialists with expertise in nutrition, diabetes or endocrinology.

Finally, while these conceptual obstacles had started to subside, a different theoretical concern emerged as a potential deterrent to the development of specific drug therapy for NASH. Specifically, it was suggested that large clinical trials in NASH might be impractical because of the requirement of histological documentation, both for inclusion and for assessing of therapeutic efficacy. However, recent developments have again proved this wrong. Several phase 2b trials of reasonably large sample size have been initiated and recruited fully (Table 1), showing that the medical need and patient and caregiver expectations are high enough to overcome the hurdle of liver biopsy (Fig. 2).

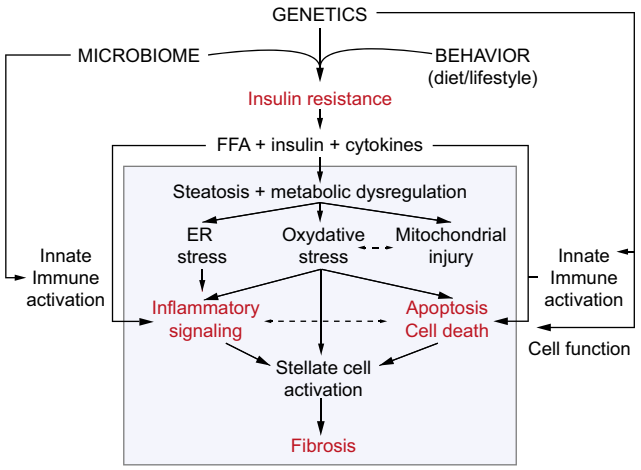

Fig. 1. Pharmacological targets for therapy of NASH.

Obstacles to drug development in NASH

Even when the myths surrounding drug development in NASH will have largely disappeared, real obstacles will still stand in the way. One of these stems from our current level of understanding of the pathogenesis of NASH. A multitude of potential pathogenic pathways along with their regulators have been described (Fig. 3), all of them able to alter the histologic and metabolic phenotype of a diet-induced insulin resistant rodent. Each one of these could be an attractive target for therapy. In fact, disappointingly, most of these potential pharmacological targets fail to materialize into human drug candidates either because of insufficient potency to curb the progression of human disease or because of alternate or duplicate pathways that rescue the NASH phenotype. PDE4 inhibitors [11], selective caspase inhibitors [12], resveratrol [13,14], omega 3 fatty acid preparations

Table 1. Recently completed and ongoing randomized controlled trails for NASH.

Agent	Treatment arms	Duration	Estimated enrolment (pts)	Population specifics	Clinicaltrials.gov identifier
Ethylicosapentate (EPA-E) [15]	600 mg *vs.* 900 mg *vs.* placebo, oral	52 weeks	243	Non-cirrhotics	NCT01154985
Obethicolic acid [120]	25 mg *vs.* placebo, oral	72 weeks	280	Non-cirrhotics	NCT01265498
Simtuzumab (GS 6624)	75 mg *vs.* 120 mg *vs.* placebo, intravenous	96 weeks	225	Cirrhosis	NCT01672866
Simtuzumab (GS 6624)	200 mg *vs.* 700 mg *vs.* placebo, subcutaneous	96 weeks	225	Advanced fibrosis without cirrhosis	NCT01672879
GFT 505	80 mg *vs.* 120 mg *vs.* placebo, oral	52 weeks	270	Non-cirrhotics	NCT01694849
Liraglutide	1.8 mg OD *vs.* placebo	48 weeks	50	Both diabetic and non-diabetic patients	NCT01237119
Losartan	50 mg *vs.* placebo	2 years	214	NASH with fibrosis	NCT01051219
Cenicriviroc	150 mg *vs.* placebo	2 years	252	NASH with fibrosis, no cirrhosis	NTC002217475
Aramchol	400 mg *vs.* 600 mg *vs.* placebo	1 year	240	NASH with prediabetes/diabetes and overweight/ visceral adiposity	NTC002279524

*www.clinicaltrials.gov accessed on February 9th 2015.

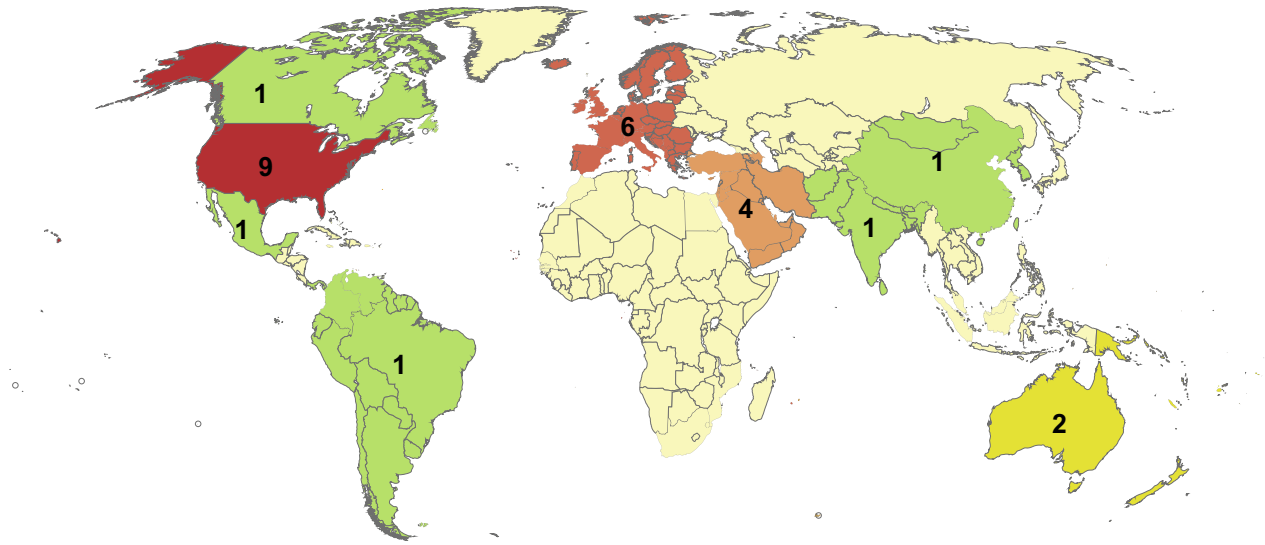

Fig. 2. Registered interventional trials for adult NASH. Open studies. Data downloaded from www.clinicaltrials.gov on Feb 9th 2015.

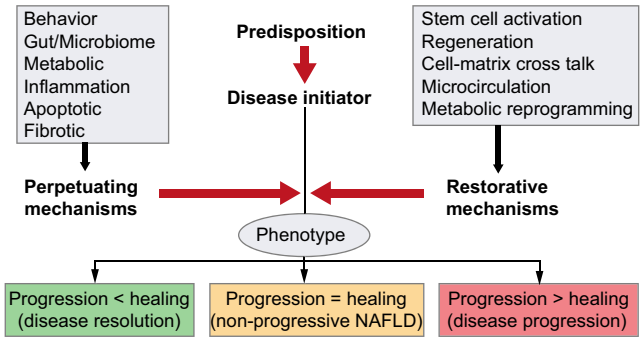

Fig. 3. A broad overview of the pathogenesis of NAFLD. The disease is usually initiated by either caloric overload (diet and lifestyle induced) or other potential mechanisms such as drugs e.g. tamoxifen. The threshold for developing a disease phenotype is modulated by genetic predisposition which involves both individual single nuclear polymorphisms (SNPs) and mutations but also the patterns of SNP distribution along specific metabolic and cellular pathways. Following disease initiating events, multiple cellular processes come into play. Some of these tend to perpetuate disease such as progressive inflammation, cell death and fibrogenesis. Tissue repair pathways also are activated that tend to restore tissue and metabolic homeostasis. The balance between disease progression pathways and tissue healing pathways determine whether progression to cirrhosis occurs. Behavior is a neglected area of research and can perpetuate disease if a healthy diet and lifestyle is not incorporated into an affected persons' way of life to remove disease initiating factors. Metabolic perturbations can drive both disease progression as well as tissue repair via metabolic reprogramming.

[15], anti-TNF alpha and probiotics, are examples of drugs that work well only in rodent models of NASH. Related to these failures is our inability to test the potency of these molecules in pre-clinical models that faithfully recapitulate in small animals both the spectrum of liver injury in humans (from steatosis to cirrhosis and hepatocellular carcinoma) and the associated metabolic conditions including insulin resistance. Some NASH models do not replicate insulin resistance and related comorbidities (the methionine/choline deficient model); others are genetic oddities that have no equivalent in regular human NASH and might be excessively pathway-dependent (MAT1, PTEN, NEMO deficient mice, ob/ob and db/db knockout mice lacking leptin and thus fibrogenesis); yet others do not fully progress to steatohepatitis and fibrosis (standard high fat diet or high sucrose diets). Although refinements of dietary models exist (Western diet, high fructose, trans fat, atherogenic diet models or a combination thereof) and allow steatohepatitis and early fibrosis to develop within an acceptable time frame of several months, their use for antifibrotic testing in the context of NASH is still not optimal. It follows that only limited predictions for human efficacy are achievable through pre-clinical models. Therefore this mandates early testing in humans, which is a risk multiplier in terms of financial and ethical commitments. To make things worse, most proof of concept trials in NASH need to rely on evidence of histological efficacy; the duration and size of such trials do not provide quick answers on whether a drug candidate for NASH should move forward. All this considerably complicates the drug development process.

Major breakthroughs and their impact on drug development

An important area of progress has been the identification and better understanding of the roles of the various morphological lesions in the natural history of NASH. Ludwig *et al.* [16], who coined the term "non-alcoholic steatohepatitis" defined it as a disease that mimics alcoholic hepatitis and may progress to cirrhosis. Subsequent publications with variable diagnostic criteria for NASH described a range of disease severity from inconsequential to rapidly progressive. This was clarified by Matteoni *et al.* [17], who reintroduced the term "non-alcoholic fatty liver disease", (used occasionally before this) to describe the spectrum of clinical and pathological severity in relation to progression to cirrhosis and mortality, which were limited to those whose liver biopsies had hepatocellular ballooning, Mallory-Denk bodies and/or fibrosis. Brunt *et al.* in 1999 [18] proposed a three-grade, four-stage system to characterize and stratify the histologic lesions. These advances were further refined in 2005 by the NASH CRN [19], which provided operational histologic definitions of NAFLD and NASH and a morphologic tool to measure histological changes (i.e. the NAS score). Both are crucial in the context of

clinical trials, as these rely on histology for inclusion and also for assessing treatment effects. Thus, the term steatohepatitis, defined histologically by the association of steatosis, lobular inflammation and hepatocyte ballooning with a predominant centrilobular pattern of distribution has come to be used for a morphological feature with clinical implications. It has been associated with a more severe clinical profile, more advanced insulin resistance and more advanced fibrotic disease than non-NASH NAFLD [20]. It has also been correlated with increased mortality and liver-related events [21–23], which provides justification for use as a selection criterion for participants in drug therapy trials. The NAS score may be used to characterize the lesions, but it must be remembered that as defined by the NASH CRN, [24] the diagnosis of steatohepatitis is a matter of pattern of injury and not of a score. Unfortunately, the NAS is not predictive of clinical outcomes [21,25,26], and therefore changes in NAS on therapy are probably not an adequate outcome surrogate. This of course does not mean that it cannot be used to measure the histological impact of different therapies; it simply may not be sufficient as a predictor of clinical benefit, and therefore its use for registration purposes is uncertain.

Recently a new histological classification was proposed by a panel of pathologists from the FLIP consortium. The FLIP algorithm and the SAF score were initially described and validated in a large population of morbidly obese patients undergoing bariatric surgery [27] and later in a NASH hepatological population [28]. Clinical correlates with the metabolic profile and disease severity have also been reported [29], although outcome studies are not yet available. This classification system uses a standardized definition of the elementary histological lesions and has been shown to increase agreement for the diagnosis of NASH between hepatopathologists with different levels of expertise [28]. It remains to be seen how this will be applied in clinical trials.

Another major advance is in the regulatory field (Table 2). Both the European and the American drug agencies now agree that NASH is a valid indication for therapy and as such, it can follow a regulatory path for drug approval. There is a need to develop therapeutics even in early stage NASH, especially in those patients at risk of progression [30]. Trial outcomes with clinical and regulatory value have been defined and are currently being used in several large trials of new drugs in NASH [30,31]. Given the high unmet medical need, the concept of accelerated approval followed by confirmation in outcome trials appears legitimate [30]. This is a big change from the previously held perception of NASH as a disease without a clear regulatory approval process. By ensuring technical feasibility of the registration pathway, it will certainly provide a big boost to all stakeholders in the industry-sponsored drug development programs. But most importantly, it will provide confidence to patients and caregivers that therapeutic options will be available to those who need them.

Table 2. Progress and continuing challenges facing the NASH pipeline.

Challenges	Progress
Multiple pathogenic mechanisms	Recognition of medical need
Imperfect animal models	NASH as an indication for therapy accepted
Non-invasive proof of principle trials	Operational pathological definition of NASH
Surrogates for hard outcomes	Agreed upon and achievable surrogate endpoints
Surrogates for response on therapy	Regulatory path increasingly clear

Management of NAFLD: what are the lessons so far?

Diet and lifestyle changes as a treatment for NASH

NAFLD patients have unhealthy dietary intakes characterized by overconsumption of fructose and soft drinks, lower consumption of fiber, overconsumption of meat, saturated fat and cholesterol, lower consumption of fish or omega-3 fatty acids or PUFA, and low consumption of some vitamins [6,32,33]. A high fructose consumption, possibly industrial fructose only (not fruit fructose) [34], increases the risk of fibrosis in NASH patients [35]. There seems to be some controversy, however, as to whether the excess risk is not in fact conferred by an excess caloric intake (irrespective of the type of sugars) [36,37] or confounded by an unhealthy lifestyle pattern that includes smoking, lack of exercise, diets rich in fat and poor in fiber etc [38]. Well designed, prospective studies accounting for multiple confounders are necessary to better establish the epidemiological basis for this association. Nonetheless, interventional data in animals have shown that while high fat diet alone only induces steatosis, high fat diet + high sucrose diets induce steatohepatitis, inflammation oxidative stress and fibrosis [39]. In overweight/obese individuals, dietary fructose specifically increases *de novo* lipogenesis, promotes dyslipidemia, increases insulin resistance and increases visceral adiposity [40]; other studies however did not confirm that fructose is metabolically more deleterious than other sugars like glucose [41].

There are very few RCTs of dietary interventions on liver injury in NASH patients. In a well conducted but small RCT, 32 NASH patients were randomized to receive complex, intensive lifestyle intervention or basic education about a healthy lifestyle (controls) over a 48-week period [42]. The active arm experienced higher weight loss, more frequent resolution of steatohepatitis (67% *vs.* 20%, $p = 0.02$), and a higher reduction in the NAS score, an aggregate score of steatosis and NASH histological activity ($p = 0.05$ only, possibly due to the small sample size). Only 40% of participants in the active group achieved a $\geqslant$10% weight reduction but, importantly, a post-hoc analysis revealed that a mere 7% weight loss (irrespective of the arm) was associated with histological improvement [42]. Those are important proof of concept results demonstrating that weight loss can result in histological improvement. However, more research is needed to answer this crucial question: is there a threshold of histological severity above which mild weight loss no longer suffices to improve the liver injury? In other words can severe, fibrotic NASH improve significantly with only diet and lifestyle measures? The only indication of liver disease severity in the two treatment arms of the trial by Promrat *et al.* is a mean NAS score between 4.4 and 4.9 and a mean fibrosis score of 1.4 to 1.7. However, it is well established that massive weight loss following bariatric surgery can result in spectacular histological improvement, including partial reversal of cirrhosis

[43,44]. In morbidly obese patients, bariatric surgery can improve histology across the board, including resolution of NASH in 75% of cases and reduction of fibrosis in 34% of cases after a long follow-up [45]. This level of improvement, however, cannot be achieved with diet and lifestyle measures. Interestingly, bariatric surgery might also correct the pro-inflammatory state associated with obesity [46]. Indeed massive weight loss reduces adipose tissue pro-inflammatory mediators such as TNFα and IL6, which results in reduced expression of hepatic SOCS3, thus improving hepatic insulin resistance and inhibiting hepatic inflammation [47].

The only other RCT of dietary intervention and exercise in NAFLD was conducted in type 2 diabetics and did not have histological endpoints [48]. It showed improvement in hepatic fat content as measured by magnetic resonance spectroscopy and a reduction in the incidence of NAFLD during follow-up. However, it is doubtful that this resulted in improvement of the other lesions of steatohepatitis including hepatic inflammation, since there was no difference in aminotransferase levels [48].

Despite the absence of well conducted trials in NAFLD, the relation between weight reduction and improvement in ALT [49] and at least some of the histological features of NASH [42,50] is now accepted. Even in the absence of specific NASH trials, a wealth of information on diets, hepatic fat and metabolic alterations exists and this can prove useful for NAFLD/NASH patients. A remarkable finding was that relatively small amounts of weight loss result in significant reductions in liver fat and hepatic insulin resistance improvement [51] although no data on other histological endpoints exist. The most important factor in dietary interventions seems to be caloric restriction. It is the main driver for weight loss and visceral adiposity, subcutaneous fat and liver fat reduction [52]. The macronutrient composition of the diet does not matter for the weight loss outcome, as long as weight loss is achieved, and this usually means that long-term compliance is key [52,53]. Some data point towards an early beneficial effect of a carbohydrate-deficient diet on hepatic fat and insulin sensitivity [54,55]. This might not make a difference however in the long-term when overall weight loss is achieved and insulin resistant sites other than the liver are studied [54]. Other studies did not confirm differences between low carbohydrate and low fat diets as long as they are both calorie restricted [56]. Recently it was suggested, within a randomized trial, that a Mediterranean diet which is very rich in monounsaturated fatty acids and rich in polyunsaturated fatty acids can reduce liver fat and improve hepatic insulin resistance even without weight loss [57].

Although in a clinical trial setting these dietary interventions might reach some of the assigned endpoints, most will fail both at the individual level and at the population level. These trials are largely an academic activity hardly applicable to real-life; patients will relapse once the intervention is over as they, or their physicians cannot sustain the major intervention efforts it requires. Weight loss among participants in diet trials will at best average 3 to 4 kg after 2 to 4 years [58] even less among poor and uneducated people who are hit hardest by obesity [59]. Even more worrisome is that individual treatments are powerless against an obesigenic environment that offers so many high-calorie foods and labor-saving devices [60]. Therefore, at a population level, only community interventions with total-environment changes might prove successful. In two small towns, a large community-based effort to prevent school children from being overweight has reduced the prevalence by half, a result no isolated dietary intervention can achieve in a sustainable manner [61].

Physical activity as an adjunctive therapy

Half of NAFLD patients are inactive and that includes almost a third that have virtually no physical exercise [62]. This has been observed both in the US and in European populations [7]. The benefits of exercise are well established and range from a reduction in the risk of type 2 diabetes, hypertension, dyslipidemia, the metabolic syndrome and insulin resistance to a reduction in all-cause mortality and in cancer mortality [63,64]. Meta-analyses have shown that exercise alone significantly reduces the hepatic fat content, a benefit occurring with minimal or no weight loss [65]. The effect on other histological lesions of NASH is unknown although preliminary data suggest an effect on hepatic markers of apoptosis [66]. Exercise also reduces visceral adipose tissue and plasma free fatty acids [67]. It reduces by a third the likelihood of having NASH [62] or advanced fibrosis in the context of NASH. Clearly, physical activity follows a dose-effect relationship [63] and vigorous (running), rather than moderate (brisk walking) exercise carries the full benefit, including for NASH and fibrosis [62]. Unfortunately NAFLD patients have a low compliance with physical exercise as they have high rates of fatigue which is associated with inactivity and daytime sleepiness [68]. Resistance training that promotes musculoskeletal fitness rather than cardiovascular fitness should also be implemented. It may be accessible to more patients, as it places less of a demand on the cardiorespiratory system [69]. In NAFLD, resistance exercise reduces liver fat (sometimes to the point where NAFLD resolves) and improves glucose control and insulin sensitivity and promotes fat oxidation, despite no weight loss and no impact on visceral fat [70]. Overall, NAFLD patients that benefit most from exercise intervention are those that have some level of cardiorespiratory fitness at baseline [71]. Those are actually a minority, since altered cardiorespiratory fitness is common in NAFLD patients [72]. Again, this casts doubt on the ability of non-pharmacological interventions to be effective in most NASH patients.

While engaging in physical exercise is beneficial, avoiding sedentary time is equally important. Sedentary time increases all-cause mortality independent of physical activity [73] and predicts higher levels of insulin resistance [74]; conversely, reducing sitting time improves insulin sensitivity [75]. No specific studies are available in NAFLD but since this only entails minimal disruption of daily activities by short bouts of walking it should be part of lifestyle changes in all NAFLD patients.

Akin to dietary measures it is also important to set realistic goals for exercise as well, since any engagement in physical exercise and any increase over previous levels of exercise is better than continuing inactivity [76]. Pragmatic approaches combining progressive increases in aerobic exercise and resistance training together with reduced sedentary time [77] are preferable and should be implemented on a case by case basis.

Where do we stand with pharmacological therapies?

An ideal drug candidate for NASH should reduce hepatic inflammation and liver cell injury, should correct the underlying insulin resistance and should have antifibrotic effects (Fig. 1). However, primarily "anti-NASH" drugs that have no direct antifibrotic effect could, theoretically, result in a subsequent reduction of fibrosis if a sustained resolution of NASH is achieved.

Review

Conversely, purely antifibrotic drugs with no anti-NASH activity and no interference with insulin resistance will leave the triggers for fibrogenesis intact. Therefore, even if an antifibrotic is effective, efforts to curb the underlying pro-fibrotic condition must be considered.

Insulin sensitizers

It is no surprise that most attempts to treat NASH have focused on insulin sensitizers. Insulin resistance is a near constant finding in primary NASH. It is the main driving force behind excessive fat accumulation in the liver but may also play a role in the initiation and perpetuation of steatohepatitis and fibrosis progression. The main source of free fatty acids reaching the liver is an uncontrolled release from insulin resistant adipose tissue. A current model for the pathogenesis of NASH is centered on lipotoxicity [78], which states that the influx of fatty acids and their derivatives through the liver induces apoptosis, oxidative stress, endoplasmic reticulum stress, activation of pro-inflammatory pathways and ultimately liver cell injury [79].

Of all tested drugs, glitazones are those with the best evidence-based data and also with the strongest pathogenesis-based rationale for treatment of NASH (reviewed in [80]). Glitazones promote differentiation of insulin resistant large pre-adipocytes into small, proliferative, insulin sensitive adipocytes [81–83]. Upon induction of lipoprotein lipase and of a large set of lipogenic genes [84], glitazones enhance fatty acid uptake and synthesis in the adipose tissue [85]; this diverts the non-esterified free fatty acid load towards adipocytes instead of other organs such as liver and muscle. Ultimately, inappropriate fat storage in organs other than adipose tissue is reduced, with subsequent improvement in insulin sensitivity despite the expansion in fat mass. Glitazones also upregulate adiponectin [86], an insulin-sensitizing and anti-steatogenic adipokine, which increases fatty acid beta-oxidation in liver and muscle [87]. PPARγ agonists also exert anti-inflammatory effects on Kupffer cells, which might be indicative of direct hepatoprotective effects.

Pioglitazone is the best studied pharmacological agent in NASH. The largest trial so far, the PIVENS trial, compared pioglitazone at a low dose of 30 mg/day *vs.* vitamin E (800 IU/day) *vs.* placebo for 2 years in patients without full-blown diabetes [88]. Pioglitazone improved all individual histological features (except for fibrosis) and achieved resolution of steatohepatitis – (currently considered the optimal surrogate endpoint in NASH trials [31,89]) more often than placebo. The histological benefit occurred together with ALT reduction and partial correction of insulin resistance [88]. It has been suggested [90], but not confirmed [91], that glitazones strongly improve adipose tissue insulin resistance, which correlates with the reduction in steatosis and necroinflammation [90]. Similar efficacy results were reported in two smaller randomized trials of shorter duration [92,93]. It is not clear whether the efficacy of pioglitazone depends on the degree of insulin resistance or the diabetic status. The optimal duration of therapy is also a largely unsettled issue. A prolonged, three-year therapy with rosiglitazone did not result in additional histological improvement beyond that obtained in the first year [94], suggesting a plateau effect of hepatic histological improvement once the maximum insulin-sensitizing benefit is obtained. This was not formally tested with pioglitazone. On the other hand, beneficial effects seem to be short-lived after treatment discontinuation. Both ALT and HOMA values return to baseline starting 3 months after discontinuation, and in the few patients with one year follow-up biopsies, steatohepatitis recurred despite on-treatment clearance [95].

The side effect profile of glitazones is of concern, particularly weight gain, which is not always reversible upon discontinuation. Bone fractures in women seem to be due to an increased rate of bone loss. Congestive heart failure is a rare complication, yet it warranted a black-box warning. Concerns about an increased risk of bladder cancer with pioglitazone [96,97] were not confirmed by recent data collected in one million type 2 diabetic individuals from six cohorts around the world [98]. Despite the safety and tolerability profile and while waiting for better options, pioglitazone can be used for NASH. In diabetic patients with an indication of therapy for NASH, a further reason to use pioglitazone is that this drug is also indicated for glycemic control.

Metformin is an oral biguanide approved for use in type 2 diabetes in which it acts as an insulin-sensitizing agent, reducing hepatic glucose production and increasing peripheral glucose utilization [99]. Metformin reduces the hepatic endogenous glucose production by activating AMP-activated protein kinase [100] but also by inhibiting the mitochondrial glycerophosphate dehydrogenase shuttle with subsequent changes in redox state [101]. Although metformin is a safe drug, it is not recommended for the treatment of NASH [102], as it has shown no effect on liver histology other than the occasional improvement in patients that lost weight. This inefficacy of metformin despite its insulin-sensitizing effect may be due to its weak anti-steatogenic effect and the lack of induction of circulating adiponectin compared to glitazones, although this has not been tested over long-term exposure [103]. Limited pre-clinical data support an anti-tumorigenic effect of metformin on liver cancer [104,105] but the clinical demonstration of a reduced rate of HCC in humans is only suggestive and limited to retrospectively collected data [105,106], which is subject to confounding by treatment assignment bias. In non-diabetic children with NASH, metformin improved ballooning but not aminotransferases, HOMA or any other histological outcome [107].

Among existing therapies for type 2 diabetes, incretin mimetics which are glucagon-like peptide-1 receptor (GLP-1R) agonists hold promise for the treatment of NASH. GLP, a peptide product of the L cells of the small intestine and proximal colon stimulates insulin secretion from the β cells and inhibits glucagon secretion from the α cells in a glucose dependent manner, but also enhances satiety and delays gastric emptying. GLP-1R agonists have a much longer half-life than natural GLP allowing a once daily administration. GLP-1R are functional in hepatocytes and *in vitro* studies have shown an induction in PPARα and γ expression upon GLP-1R agonist binding, which resulted in increased disposal of hepatocyte fatty acids by beta-oxidation and lipid export [108,109]. This was confirmed *in vivo* with improvement of steatosis in exendin-treated mice [110]. Moreover, GLP-1R agonists could improve hepatic insulin sensitivity [109] by increasing phosphorylation of key signaling pathways such as AKT and PKC-ζ [111]. In patients with diabetes, liraglutide at the high dose of 3 mg/day, currently FDA approved for the treatment of obesity, reduced ALT and showed a trend towards improvement of steatosis [112]. These effects were mainly mediated by weight loss and better glucose control [112]. Other potentially beneficial effects in humans for the treatment of NASH are reduced *de novo* lipogenesis, and improved adipose tissue lipolysis and inflammation [113]. Data on histological efficacy are eagerly awaited.

Novel therapies with insulin-sensitizing effects but also anti-inflammatory and antifibrotic actions are under development. Bile acids are now believed to play a crucial role in regulating liver and metabolic homeostasis. Their action is mediated through nuclear hormone receptors such as the farnesoid X receptor (FXR) and TGR5. FXR activation has a wide range of metabolic effects: it improves both glucose metabolism and peripheral insulin sensitivity [114]; it also reduces lipogenesis and enhances fatty acid β-oxidation [115]. Interestingly, FXR activation has also anti-inflammatory actions [116,117] with resultant protection against liver inflammation and fibrosis in the methionine/choline deficient model of NASH [118]. Obeticholic acid (OCA) is a synthetic bile acid with picomolar agonistic activity on FXR. A small randomized trial in type 2 diabetic patients with NAFLD showed an improvement in insulin sensitivity as measured by euglycemic clamp, a modest but dose-related weight loss, and a reduction in ALT levels [119]. Recently, the FLINT trial compared 25 mg of OCA *vs.* placebo over 72 weeks of therapy in non-cirrhotic NASH patients [120] and reported improvement in all lesions of steatohepatitis including fibrosis. The therapeutic phase of the trial was stopped early, partly because a preplanned interim analysis showed improved histology in more patients on OCA (50 [45%] of 110) than on placebo (23 [21%] of 109). Importantly, there was a reduction in fibrosis score (one stage) in 35% of OCA-treated patients *vs.* 19% in the placebo arm. These are encouraging data that deserve confirmation in larger trials. Side effects were pruritus and an increase in LDL cholesterol; studies are underway to fully understand whether the lipid changes are associated or not with increased cardiovascular risk.

Another innovative insulin sensitizer is GFT505, a dual PPARα/δ agonist that undergoes extensive enterohepatic cycling and is liver targeted [121]. PPARδ activation emerged as a potent metabolic regulator that induces hepatic fatty acid β-oxidation, inhibits hepatic lipogenesis [122], reduces hepatic glucose production and improves hepatic inflammation [123,124]. Human studies performed in abdominally obese, insulin resistant patients, with or without diabetes have shown that GFT505 improves hepatic and peripheral insulin sensitivity, dyslipidemia, inflammatory markers and liver function tests [125,126]. Animal data confirmed the hepatoprotective effects of GFT505 in dietary models of NASH or fibrosis with, in particular, a reduction in steatosis, hepatic inflammation and pro-inflammatory genes [127]. Importantly, this compound exhibited antifibrotic properties in fibrosis models that were independent of metabolic and insulin resistance abnormalities [127], thereby suggesting a universal antifibrotic potency in rodents. Based on these promising results, a large, phase 2b, randomized controlled trial is now underway in NASH patients. A good safety profile is anticipated from earlier phase 2a studies and, in particular, from the lack of PPARγ agonistic activity.

Hepatoprotective agents

Vitamin E is a fat soluble compound that is present in the phospholipid bilayer of cell membranes, where it protects from oxidative damage induced by free radicals. Vitamin E prevents liver injury by protecting against mitochondrial toxicity and blocking intrinsic apoptotic pathways [128,129]. It might also have non-antioxidant properties, for instance by altering cell signaling, gene expression [130] or down-regulating NF-kB-dependent inflammatory pathways [131]. Vitamin E has been tested in the above mentioned PIVENS trial at 800 IU daily. It significantly improved steatosis, inflammation and ballooning and induced resolution of NASH in 36% of patients (21% in the placebo group). The reduction in ALT was well correlated with histological improvement, and histological non-responders, for the most part, did not have a reduction in ALT [132]. The results were only partly reproduced in the paediatric TONIC trial where vitamin E failed to reduce aminotransferases, steatosis and inflammation but improved ballooning and cleared NASH in 58% of the patients (28% in the placebo group). These results stand in contrast to previous trials that were mostly negative in both adults and children. Concerns about long-term safety of vitamin E exist, mainly an increase in overall mortality [133], in the incidence of hemorrhagic stroke [134] and in that of prostate cancer in males older than 50 years [135]. However, the increased risk of prostate cancer only becomes significant after three years of exposure [135]. Vitamin E may be used in NASH patients with aggressive disease, although additional studies demonstrating its efficacy are needed before firm recommendations for use can be made. In non-diabetic children treated for 96 weeks, vitamin E induced resolution of NASH and improvement in ballooning more often than placebo although there was no effect on steatosis, inflammation or fibrosis [107]. Surprisingly, a 2 year study comparing vitamins E and C *vs.* tailored diet and lifestyle interventions did not show an advantage of the vitamin regimen perhaps because similar weight loss occurred in both groups [136].

Many other compounds have been tested for NASH with either inconclusive or negative results and most in small trials (reviewed in [137]). New compounds are in development in large multicentre, international RCTs either as anti-inflammatory agents (cenicriviroc a CCR2-CCR5 antagonist), metabolic modulators (aramchol, a fatty acid-bile acid conjugate) or antifibrotics (simtuzumab, a humanized, anti-lysyl-oxidase 2 monoclonal antibody).

Challenges for the near future

Probably the most urgent need in the field of NASH is the discovery of biomarkers that would help diagnose and monitor disease progression. In primary-care settings where numerous individuals are exposed to metabolic risk factors, biomarkers should identify those at high risk of NAFLD-related liver disease. In secondary- and tertiary-care settings, biomarkers should identify those with advanced/severe NASH. Not only will this help provide prognostic information but it will also select those in need for specific, liver-directed therapy. Equally important, biomarkers, or for that matter, imaging methods, should reliably monitor disease progression. This might require more than just the knowledge of fibrosis stage. Recent reports have documented the transition from NAFL to NASH, sometimes with advanced fibrosis [138,139]. This justifies long-term follow-up, even in patients without steatohepatitis at diagnosis, and especially if metabolic risk factors persist or worsen. Finally there is a strong need to predict the response to pharmacological or non-pharmacological interventions. Ongoing trials are already striving to identify companion tests for specific molecules that will eventually eliminate the need for histology to document efficacy. A timely prediction of response to drugs will shorten the timelines for completing clinical trials and will reduce useless drug exposure in patients that are non-responders.

Review

Whatever the fate of ongoing clinical trials, none of the molecules under active investigation are expected to provide significant improvement of NASH in more than a minority of patients. Therefore continuous research and discovery programs should aim at identifying new targets for therapy and eventually combine those that target synergistic pathways. Individualized therapy based on severity of disease and treatment response might be a reality as soon as anti-NASH and antifibrotic agents emerge.

Financial support

European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° HEALTH-F2-2009-241762 for the project FLIP.

Conflict of interest

Astra-Zeneca, Boehringer-Ingelheim, Galmed, Genfit, Gilead, Immuron, Intercept, Roche-Genentech, Tobira for VR. ZG has research support from Gilead Sciences, Galectin Therapeutics, Intercept Pharmaceuticals, Tobira Therapeutics, Conatus Pharmaceuticals, Fibrogen and Synageva. Dr. Sanyal has stock options in Genfit. He has served as a consultant to AbbVie, Astra Zeneca, Nitto Denko, Nimbus, Salix, Tobira, Takeda, Fibrogen, Immuron, Exhalenz and Genfit. He has been an unpaid consultant to Intercept and Echosens. His institution has received grant support from Gilead, Salix, Tobira and Novartis

References

[1] de Marco R, Locatelli F, Zoppini G, Verlato G, Bonora E, Muggeo M. Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. Diabetes Care 1999;22:756–761.

[2] Adams LA, Harmsen S, St Sauver JL, Charatcharoenwitthaya P, Enders FB, Therneau T, et al. Nonalcoholic fatty liver disease increases risk of death among patients with diabetes: a community-based cohort study. Am J Gastroenterol 2010;105:1567–1573.

[3] Zoppini G, Fedeli U, Gennaro N, Saugo M, Targher G, Bonora E. Mortality from chronic liver diseases in diabetes. Am J Gastroenterol 2014;109:1020–1025.

[4] Campbell PT, Newton CC, Patel AV, Jacobs EJ, Gapstur SM. Diabetes and cause-specific mortality in a prospective cohort of one million U.S. adults. Diabetes Care 2012;35:1835–1844.

[5] Seshasai SR, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, et al. Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med 2011;364:829–841.

[6] Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, et al. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. J Hepatol 2007;47:711–717.

[7] Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Zvibel I, Goldiner I, et al. Role of leisure-time physical activity in nonalcoholic fatty liver disease: a population-based study. Hepatology 2008;48:1791–1798.

[8] Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. N Engl J Med 2009;360:859–873.

[9] Dansinger ML, Tatsioni A, Wong JB, Chung M, Balk EM. Meta-analysis: the effect of dietary counseling for weight loss. Ann Intern Med 2007;147:41–50.

[10] Bellentani S, Dalle Grave R, Suppini A, Marchesini G. Behavior therapy for nonalcoholic fatty liver disease: the need for a multidisciplinary approach. Hepatology 2008;47:746–754.

[11] Ratziu V, Bedossa P, Francque SM, Larrey D, Aithal GP, Serfaty L, et al. Lack of efficacy of an inhibitor of PDE4 in phase 1 and 2 trials of patients with nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2014;12:1724–1730.

[12] Ratziu V, Sheikh MY, Sanyal AJ, Lim JK, Conjeevaram H, Chalasani N, et al. A phase 2, randomized, double-blind, placebo-controlled study of GS-9450 in subjects with nonalcoholic steatohepatitis. Hepatology 2012;55:419–428.

[13] Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai S, Schechtman Kenneth B, et al. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metab 2012;16:658–664.

[14] Chachay VS, Macdonald GA, Martin JH, Whitehead JP, O'Moore-Sullivan TM, Lee P, et al. Resveratrol does not benefit patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2014;12:e2096.

[15] Sanyal AJ, Abdelmalek MF, Suzuki A, Cummings OW, Chojkier M. No significant effects of ethyl-eicosapentanoic acid on histologic features of nonalcoholic steatohepatitis in a phase 2 trial. Gastroenterology 2014;147:e371.

[16] Ludwig J, Viggiano TR, McGill DB, Ott BJ. Nonalcoholic steatohepatitis. Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–438.

[17] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–1419.

[18] Brunt EM, Janney CG, DiBisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–2474.

[19] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.

[20] Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 2010;52:913–924.

[21] Younossi ZM, Stepanova M, Rafiq N, Makhlouf H, Younoszai Z, Agrawal R, et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. Hepatology 2011;53:1874–1882.

[22] Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865–873.

[23] Angulo P, Kleiner DE, dam-Larsen S, Adams LA, Bjornsson E, Charatcharoenwitthaya P, et al. Long-term prognostic relevance of liver histology in non-alcoholic fatty liver disease: the PRELHIN study. Hepatology 2014;60:226A.

[24] Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011;53:810–820.

[25] Ekstedt M, Franzen LE, Mathiesen UL, Kechagias S. Low clinical relevance of the nonalcoholic fatty liver disease activity score (NAS) in predicting fibrosis progression. Scand J Gastroenterol 2012;47:108–115.

[26] Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stal P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015. http://dx.doi.org/10.1002/hep.27368. [in press].

[27] Bedossa P, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. Hepatology 2012;56:1751–1759.

[28] Bedossa P. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60:565–575.

[29] Nascimbeni F, Bedossa P, Fedchuck L, Pais R, Lebray P, Poynard T, et al. Clinical validation of the FLIP algorithm and SAF score in non-alcoholic fatty liver disease. J Hepatol 2014;60:S348.

[30] Sanyal AJ, Friedman SL, McCullough AJ, Dimick L. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an american association for the study of liver diseases (aasld) – food and drug administration (fda) joint workshop. Hepatology 2015. http://dx.doi.org/10.1002/hep.27678. [in press].

[31] Sanyal AJ, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology 2011;54:344–353.

[32] Zelber-Sagi S, Ratziu V, Oren R. Nutrition and physical activity in NAFLD: an overview of the epidemiological evidence. World J Gastroenterol 2011;17:3377–3389.

[33] Ouyang X, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol 2008;48:993–999.

[34] Petta S, Marchesini G, Caracausi L, Macaluso FS, Cammà C, Ciminnisi S, et al. Industrial, not fruit fructose intake is associated with the severity of liver fibrosis in genotype 1 chronic hepatitis C patients. J Hepatol 2013;59:1169–1176.

[35] Abdelmalek MF, Suzuki A, Guy C, Unalp-Arida A, Colvin R, Johnson RJ, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1961–1971.

[36] Chiu S, Sievenpiper JL, de Souza RJ, Cozma AI, Mirrahimi A, Carleton AJ, et al. Effect of fructose on markers of non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of controlled feeding trials. Eur J Clin Nutr 2014;68:416–423.

[37] Sievenpiper JL, de Souza RJ, Mirrahimi A, Yu ME, Carleton AJ, Beyene J, et al. Effect of fructose on body weight in controlled feeding trials: a systematic review and meta-analysis. Ann Intern Med 2012;156:291–304.

[38] Chiavaroli L, Ha V, Kendall CW, Sievenpiper JL. Is industrial fructose just a marker of an unhealthy dietary pattern? J Hepatol 2014;61:172–173.

[39] Ishimoto T, Lanaspa MA, Rivard CJ, Roncal-Jimenez CA, Orlicky DJ, Cicerchi C, et al. High-fat and high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase. Hepatology 2013;58:1632–1643.

[40] Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 2009;119:1322–1334.

[41] Johnston RD, Stephenson MC, Crossland H, Cordon SM, Palcidi E, Cox EF, et al. No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. Gastroenterology 2013;145:e1012.

[42] Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. Hepatology 2010;51:121–129.

[43] Kral JG, Thung SN, Biron S, Hould FS, Lebel S, Marceau S, et al. Effects of surgical treatment of the metabolic syndrome on liver fibrosis and cirrhosis. Surgery 2004;135:48–58.

[44] Dixon JB, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic fatty liver disease: improvement in liver histological analysis with weight loss. Hepatology 2004;39:1647–1654.

[45] Lassailly G, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Raverdy V, et al. Effects of bariatric surgery on severe liver injury in morbid obese patients with proven NASH: a prospective study. Hepatology 2014;60:305A.

[46] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–867.

[47] Moschen AR, Molnar C, Geiger S, Graziadei I, Ebenbichler CF, Weiss H, et al. Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor expression. Gut 2010;59:1259–1264.

[48] Lazo M, Solga SF, Horska A, Bonekamp S, Diehl AM, Brancati FL, et al. Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. Diabetes Care 2010;33:2156–2163.

[49] Suzuki A, Lindor K, St Saver J, Lymp J, Mendes F, Muto A, et al. Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. J Hepatol 2005;43:1060–1066.

[50] Harrison SA, Fecht W, Brunt EM, Neuschwander-Tetri BA. Orlistat for overweight subjects with nonalcoholic steatohepatitis: a randomized, prospective trial. Hepatology 2009;49:80–86.

[51] Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. Diabetes 2005;54:603–608.

[52] Boden G. High- or low-carbohydrate diets: which is better for weight loss, insulin resistance, and fatty livers? Gastroenterology 2009;136:1490–1492.

[53] Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. Proc Natl Acad Sci U S A 2004;101:6659–6663.

[54] Kirk E, Reeds DN, Finck BN, Mayurranjan SM, Patterson BW, Klein S. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. Gastroenterology 2009;136:1552–1560.

[55] Browning JD, Baker JA, Rogers T, Davis J, Satapati S, Burgess SC. Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction. Am J Clin Nutr 2011;93:1048–1052.

[56] Haufe S, Engeli S, Kast P, Böhnke J, Utz W, Haas V, et al. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. Hepatology 2011;53:1504–1514.

[57] Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. J Hepatol 2013;59:138–143.

[58] Franz MJ, VanWormer JJ, Crain AL, Boucher JL, Histon T, Caplan W, et al. Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. J Am Diet Assoc 2007;107:1755–1767.

[59] Davis EM, Clark JM, Carrese JA, Gary TL, Cooper LA. Racial and socioeconomic differences in the weight-loss experiences of obese women. Am J Public Health 2005;95:1539–1543.

[60] Katan MB. Weight-loss diets for the prevention and treatment of obesity. N Engl J Med 2009;360:923–925.

[61] Romon M, Lommez A, Tafflet M, Basdevant A, Oppert JM, Bresson JL, et al. Downward trends in the prevalence of childhood overweight in the setting of 12-year school- and community-based programmes. Public Health Nutr 2009;12:1735–1742.

[62] Kistler KD, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. Am J Gastroenterol 2011;106:460–468.

[63] Wen CP, Wai JP, Tsai MK, Yang YC, Cheng TY, Lee MC, et al. Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet 2011;378:1244–1253.

[64] Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet 2012;380:219–229.

[65] Keating SE, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol 2012;57:157–166.

[66] Fealy CE, Haus JM, Solomon TP, Pagadala M, Flask CA, McCullough AJ, et al. Short-term exercise reduces markers of hepatocyte apoptosis in nonalcoholic fatty liver disease. J Appl Physiol 1985;2012:1–6.

[67] Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, et al. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. Hepatology 2009;50:1105–1112.

[68] Newton JL, Jones DE, Henderson E, Kane L, Wilton K, Burt AD, et al. Fatigue in non-alcoholic fatty liver disease (NAFLD) is significant and associates with inactivity and excessive daytime sleepiness but not with liver disease severity or insulin resistance. Gut 2008;57:807–813.

[69] Gordon BA, Benson AC, Bird SR, Fraser SF. Resistance training improves metabolic health in type 2 diabetes: a systematic review. Diabetes Res Clin Pract 2009;83:157–175.

[70] Hallsworth K, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, et al. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut 2011;60:1278–1283.

[71] Kantartzis K, Thamer C, Peter A, Machann J, Schick F, Schraml C, et al. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. Gut 2009;58:1281–1288.

[72] Church TS, Kuk JL, Ross R, Priest EL, Biltoft E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. Gastroenterology 2006;130:2023–2030.

[73] van der Ploeg HP, Chey T, Korda RJ, Banks E, Bauman A. Sitting time and all-cause mortality risk in 222 497 Australian adults. Arch Intern Med 2012;172:494–500.

[74] Helmerhorst HJ, Wijndaele K, Brage S, Wareham NJ, Ekelund U. Objectively measured sedentary time may predict insulin resistance independent of moderate- and vigorous-intensity physical activity. Diabetes 2009;58:1776–1779.

[75] Dunstan DW, Kingwell BA, Larsen R, Healy GN, Cerin E, Hamilton MT, et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. Diabetes Care 2012;35:976–983.

[76] St George A, Bauman A, Johnston A, Farrell G, Chey T, George J. Independent effects of physical activity in patients with nonalcoholic fatty liver disease. Hepatology 2009;50:68–76.

[77] Rodriguez B, Torres DM, Harrison SA. Physical activity: an essential component of lifestyle modification in NAFLD. Nat Rev Gastroenterol Hepatol 2012;9:726–731.

Review

[78] Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. Hepatology 2010;52:774–788.

[79] Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology 2012;142:e716.

[80] Kallwitz ER, McLachlan A, Cotler SJ. Role of peroxisome proliferators-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease. World J Gastroenterol 2008;14:22–28.

[81] Wu Z, Bucher NL, Farmer SR. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol 1996;16:4128–4136.

[82] Fajas L, Fruchart JC, Auwerx J. Transcriptional control of adipogenesis. Curr Opin Cell Biol 1998;10:165–173.

[83] Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev 1996;10:1096–1107.

[84] Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, et al. PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J 1996;15:5336–5348.

[85] Frohnert BI, Hui TY, Bernlohr DA. Identification of a functional peroxisome proliferator-responsive element in the murine fatty acid transport protein gene. J Biol Chem 1999;274:3970–3977.

[86] Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, et al. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes 2002;51:2968–2974.

[87] Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 2003;112:91–100.

[88] Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675–1685.

[89] Ratziu V, Bellentani S, Cortez-Pinto H, Day CP, Marchesini G. A position paper on NAFLD/NASH based on the EASL 2009 Special Conference. J Hepatol 2010;53:372–384.

[90] Gastaldelli A, Harrison SA, Belfort-Aguilar R, Hardies LJ, Balas B, Schenker S, et al. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. Hepatology 2009;50:1087–1093.

[91] Bell LN, Wang J, Muralidharan S, Chalasani S, Fullenkamp AM, Wilson LA, et al. Relationship between adipose tissue insulin resistance and liver histology in nonalcoholic steatohepatitis: a pioglitazone versus vitamin E versus placebo for the treatment of nondiabetic patients with nonalcoholic steatohepatitis trial follow-up study. Hepatology 2012;56:1311–1318.

[92] Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 2006;355:2297–2307.

[93] Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology 2008;135:1176–1184.

[94] Ratziu V, Charlotte F, Bernhardt C, Giral P, Halbron M, Lenaour G, et al. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial. Hepatology 2010;51:445–453.

[95] Lutchman G, Modi A, Kleiner DE, Promrat K, Heller T, Ghany M, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. Hepatology 2007;46:424–429.

[96] Ferrara A, Lewis JD, Quesenberry CP, Peng T, Strom BL, Van Den Eeden SK, et al. Cohort study of pioglitazone and cancer incidence in patients with diabetes. Diabetes Care 2011;34:923–929.

[97] Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP, et al. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. Diabetes Care 2011;34:916–922.

[98] Levin D, Bell S, Sund R, Hartikainen SA, Tuomilehto J, Pukkala E, et al. Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. Diabetologia 2015;58:493–504.

[99] Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002;137:25–33.

[100] Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167–1174.

[101] Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 2014;510:542–546.

[102] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005–2023.

[103] Tiikkainen M, Hakkinen AM, Korsheninnikova E, Nyman T, Makimattila S, Yki-Jarvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. Diabetes 2004;53:2169–2176.

[104] Bhalla K, Hwang BJ, Dewi RE, Twaddel W, Goloubeva OG, Wong KK, et al. Metformin prevents liver tumorigenesis by inhibiting pathways driving hepatic lipogenesis. Cancer Prev Res (Phila) 2012;5:544–552.

[105] Chen HP, Shieh JJ, Chang CC, Chen TT, Lin JT, Wu MS, et al. Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. Gut 2012;62:606–615.

[106] Zhang ZJ, Zheng ZJ, Shi R, Su Q, Jiang Q, Kip KE. Metformin for liver cancer prevention in patients with type 2 diabetes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2012;97:2347–2353.

[107] Lavine JE, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA 2011;305:1659–1668.

[108] Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, et al. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. Liver Int 2011;31:1285–1297.

[109] Mells JE, Fu PP, Sharma S, Olson D, Cheng L, Handy JA, et al. Glp-1 analog, liraglutide, ameliorates hepatic steatosis and cardiac hypertrophy in C57BL/6J mice fed a Western diet. Am J Physiol Gastrointest Liver Physiol 2012;302:G225–G235.

[110] Ding X, Saxena NK, Lin S, Gupta NA, Anania FA. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. Hepatology 2006;43:173–181.

[111] Gupta NA, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, et al. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. Hepatology 2010;51:1584–1592.

[112] Armstrong MJ, Houlihan DD, Rowe IA, Clausen WH, Elbrond B, Gough SC, et al. Safety and efficacy of liraglutide in patients with type 2 diabetes and elevated liver enzymes: individual patient data meta-analysis of the LEAD program. Aliment Pharmacol Ther 2013;37:234–242.

[113] Armstrong MJ, Hull D, Guo K, Barton D, Yu J, Tomlinson JW, et al. Effect of liraglutide on adipose insulin resistance and hepatic de-novo lipogenesis in non-alcoholic steatohepatitis: substudy of a phase 2, randomised placebo-controlled trial. Lancet 2014;383:S21.

[114] Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. J Clin Invest 2006;116:1102–1109.

[115] Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J Clin Invest 2004;113:1408–1418.

[116] Wagner M, Zollner G, Trauner M. Nuclear bile acid receptor farnesoid X receptor meets nuclear factor-kappaB: new insights into hepatic inflammation. Hepatology 2008;48:1383–1386.

[117] Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. Hepatology 2008;48:1632–1643.

[118] Zhang S, Wang J, Liu Q, Harnish DC. Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis. J Hepatol 2009;51:380–388.

[119] Mudaliar S, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, et al. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. Gastroenterology 2013;145:574–582.

[120] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015. http://dx.doi.org/10.1016/S0140-6736(14)61933-4. [in press].

[121] Staels B, Rubenstrunk A, Noel B, Rigou G, Delataille P, Millatt LJ, et al. Hepato-protective effects of the dual PPARalpha/delta agonist GFT505 in rodent models of NAFLD/NASH. Hepatology 2013;58:1941–1952.

[122] Qin X, Xie X, Fan Y, Tian J, Guan Y, Wang X, et al. Peroxisome proliferator-activated receptor-delta induces insulin-induced gene-1 and suppresses hepatic lipogenesis in obese diabetic mice. Hepatology 2008;48:432–441.
[123] Barish GD, Narkar VA, Evans RM. PPAR delta: a dagger in the heart of the metabolic syndrome. J Clin Invest 2006;116:590–597.
[124] Bojic LA, Huff MW. Peroxisome proliferator-activated receptor delta: a multifaceted metabolic player. Curr Opin Lipidol 2013;24:171–177.
[125] Cariou B, Zair Y, Staels B, Bruckert E. Effects of the new dual PPAR alpha/delta agonist GFT505 on lipid and glucose homeostasis in abdominally obese patients with combined dyslipidemia or impaired glucose metabolism. Diabetes Care 2011;34:2008–2014.
[126] Cariou B, Hanf R, Lambert-Porcheron S, Zair Y, Sauvinet V, Noel B, et al. Dual peroxisome proliferator-activated receptor alpha/delta agonist GFT505 improves hepatic and peripheral insulin sensitivity in abdominally obese subjects. Diabetes Care 2013;36:2923–2930.
[127] Staels B, Rubenstrunk A, Noel B, Rigou G, Delataille P, Millatt LJ, et al. Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Hepatology 2013;58:1941–1952.
[128] Soden JS, Devereaux MW, Haas JE, Gumpricht E, Dahl R, Gralla J, et al. Subcutaneous vitamin E ameliorates liver injury in anin vivo model of steatocholestasis. Hepatology 2007;46:485–495.
[129] Sokol RJ, McKim Jr JM, Goff MC, Ruyle SZ, Devereaux MW, Han D, et al. Vitamin E reduces oxidant injury to mitochondria and the hepatotoxicity of taurochenodeoxycholic acid in the rat. Gastroenterology 1998;114:164–174.
[130] Azzi A, Gysin R, Kempna P, Munteanu A, Negis Y, Villacorta L, et al. Vitamin E mediates cell signaling and regulation of gene expression. Ann N Y Acad Sci 2004;1031:86–95.
[131] Morante M, Sandoval J, Gomez-Cabrera MC, Rodriguez JL, Pallardo FV, Vina JR, et al. Vitamin E deficiency induces liver nuclear factor-kappaB DNA-binding activity and changes in related genes. Free Radic Res 2005;39:1127–1138.
[132] Hoofnagle JH, Van Natta ML, Kleiner DE, Clark JM, Kowdley KV, Loomba R, et al. Vitamin E and changes in serum alanine aminotransferase levels in patients with non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2013;38:134–143.
[133] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 2007;297:842–857.
[134] Schurks M, Glynn RJ, Rist PM, Tzourio C, Kurth T. Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials. BMJ 2010;341:c5702.
[135] Klein EA, Thompson Jr IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2011;306:1549–1556.
[136] Nobili V, Manco M, Devito R, Di Ciommo V, Comparcola D, Sartorelli MR, et al. Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: a randomized, controlled trial. Hepatology 2008;48:119–128.
[137] Ratziu V. Pharmacological agents for NASH. Nat Rev Gastroenterol Hepatol 2013;10:676–685.
[138] Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol 2013;59:550–556.
[139] McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis & clinical management. J Hepatol 2015. http://dx.doi.org/10.1016/j.jhep.2014.11.034. [in press].