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Time to Step-Up the Fight Against NAFLD

Over the past century, disease spectrums have undergone a world-wide epidemiological transition. Whereas many once-fatal infectious diseases (e.g., malaria, smallpox, and polio) have been eradicated in most countries benefiting from medical advances and new therapeutic developments, the incidence of metabolic disorders have sharply increased with the remarkable prevalence of overnutrition, and this sharp increase is threatening modern life. One apparent contributor to the epidemiological shift is the change of liver disease spectrum. The success of vaccines or therapeutic approaches make it possible to effectively cure or control the progression of chronic hepatitis B and C, which were the leading cause of cirrhosis worldwide; there has, however, been a tremendous increase in the prevalence of nonalcoholic fatty liver disease (NAFLD), which has become the leading cause of chronic liver disease afflicting more than 1 billion people worldwide.[1] Most recently, prominent journals, including *New England Journal of Medicine*, *Nature*, *Nature Reviews*, *Nature Medicine*, *Gastroenterology*, and HEPATOLOGY, have published serial outlook and perspective articles calling for more attention to the management of NAFLD.

NAFLD occurs in individuals with more than 5% hepatic steatosis (HS) that is not attributable to excess alcohol consumption or to other causes such as viral hepatitis or medications. Up to 30% of subjects with simple steatosis (nonalcoholic fatty liver; NAFL) progress to nonalcoholic steatohepatitis (NASH), as reflected by inflammation, hepatocyte ballooning, and, in its later stage, fibrosis.[1] All fatty liver disease was, at one time, thought to be induced by excessive alcohol intake, and the term NASH was only coined in 1980 by Ludwig et al. at the Mayo Clinic.[2] This report failed to attract sufficient attention at that time, and the concept of "NASH as a benign condition" endured until both clinical and epidemiological studies confirmed that NASH can be progressive and lead to liver-related morbidity and mortality after 10 years later.[3] Up to 20% of NASH patients may further progress to cirrhosis, resulting in a markedly increased risk for hepatocellular carcinoma (HCC) and liver failure.[4] Notably, NASH is correlated with a 10-fold increased risk of cirrhosis and HCC occurrence and contributes to a doubling of cardiovascular disease events. Moreover, NAFLD-related cirrhosis is currently the most rapidly increasing cause and second among indications for liver transplantation of adults in United States.[1] Now, more than ever, NAFLD has become a significant clinical and economic burden, and cutting-edge research is urgently needed to discover effective therapies for NAFLD.

The prevalence of NAFLD is still on the rise and, more alarmingly, the burden of NAFLD may, in fact, be much higher than is appreciated attributed to lack of recognition of the condition in broader medical community, as well as challenges in diagnosing this condition. Liver biopsy and blood aminotransferase levels are the most commonly applied diagnostic procedures for NAFLD, and biopsy-based pathology remains the only reliable standard to diagnose NASH.

Abbreviations: HCC, hepatocellular carcinoma; HS, hepatic steatosis; HSCs, hepatic stellate cells; IR, insulin resistance; MetS, metabolic syndrome; MetS-predisposed, predisposed to metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

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ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Hongliang Li, M.D., Ph.D.
Renmin Hospital of Wuhan University
Zhang ZhiDong Streat,
Wuchang Wuhan, 430060, China
E-mail: lihl@whu.edu.cn
Tel: +86-27-68759302

However, liver biopsy is invasive and has important limitations.[4] Clinicians also use transient elastography (TE) to evaluate liver stiffness as a metric of fibrosis or cirrhosis in advanced NASH. While it may be useful in detecting cirrhosis, TE yields unreliable results in many NAFLD subjects. In recent years, the noninvasive ultrasound, magnetic resonance imaging and elastography, have emerged as preferred approaches, with significantly greater accuracy. However, these imaging technologies are not routinely available and are unable to differentiate NAFL from NASH. The development of new noninvasive diagnostic approaches with reliable correlations to disease progression will largely facilitate both timely lifestyle interventions in subjects with mild or moderate NAFLD, as well as the development of new drugs to treat this common disease.

The lack of timely diagnosis of, and intervention against, NAFLD enables its sustained progression. NAFLD is a dynamic disease process with a complex pathogenesis, where HS, insulin resistance (IR), and inflammation interconnect in a vicious cycle.[5] However, the fundamental molecular etiology of NAFLD remains obscure and controversial. The traditional "two-hit" hypothesis suggests that HS, resulting from an imbalance in lipid flux, is the original driver of subsequent liver dysfunction, and that lipotoxicity contributes to the impaired responses to endogenous insulin, leading to inflammation, oxidative stress, and fibrosis. IR has also been considered as a primary contributor to NAFLD, leading to excess lipid synthesis and glucose production, which, in turn, promote chronic inflammation. However, this traditional concept of NAFLD pathogenesis has been challenged by the concept that "multiple parallel hits" may promote NAFLD and its progression. This concept is largely based on the heterogeneity of subjects.[1] For instance, a minority of subjects can go on to develop HCC from NASH without obvious cirrhosis whereas others may not progress at all and instead remain stable without suffering any liver sequelae. Moreover, different individuals may respond differently to identical interventions. The occurrence and progression of NAFLD are also influenced by age, sex, genetic susceptibility, smoking and dietary habits, which highlights the clinical importance of personalized and precision medicine.

Fibrosis is the most important predictor of the progression of NAFLD to more-severe liver injury and end-stage liver disease. The generation and activation of myofibroblasts that are proposed to be differentiated from hepatic stellate cells (HSCs) appears to be a pathogenic mechanism.[1] Inflammatory response and the subsequent transforming growth factor beta release from Kupffer cells, recruitment of macrophages, and their interaction with hepatocytes are key driving events in HSC activation and extracellular matrix deposition.[1] IR and lipid accumulation also contribute to fibrogenesis by incompletely characterized mechanisms. However, these understandings of fibrogenesis have been challenged by the evidence that fibrosis may have occurred at a very early stage of NAFLD without severe steatosis, inflammation, or IR. The complex pathogenesis of, and multiple mechanisms for, the progression of NAFLD makes it clinically important to clarify the fundamental events that may be targeted for NAFLD treatment. Indeed, systematic biological or "omics" approaches should help to delineate rational determinants of NAFLD and offer the hope of applying the precision medicine in clinical practice.

Application of animal models facilitates our understanding of the pathogenesis of NAFLD. An ideal NAFLD animal model should closely mimic clinical pathologies, including HS accompanied by inflammation, hepatocyte cell death, and fibrosis in the context of IR and a metabolic syndrome (MetS)-like disorder. These features should also be achieved within a reasonable time frame in an animal that is facile to breed and maintain in a laboratory environment. Currently, mouse models are most commonly used, with NAFLD being achieved by excess nutrition, selective nutrient deficiency, or genetic modifications. The methionine choline–deficient, conjugated linoleic acid, and choline-deficient L-AA diets have been used to induce liver fibrosis (LF). These diets induce NASH histopathological features in relatively short periods of time (i.e., several weeks), but they are complicated by weight loss and improved insulin sensitivity. These models thus fail to faithfully recapitulate the most common obesity-associated NAFLD in humans. In contrast to dietary deficiency, the high-fat diet (HFD) and the high-fat with high-cholesterol diet are more widely accepted because they induce obesity, HS, and IR. However, mice with excess nutrition do not readily develop hepatic fibrosis and the severity of established LF does not readily approach that which is observed in the advanced stages of human NASH. Mice with deficiencies in leptin (*ob/ob*) or the leptin receptor (*db/db*) spontaneously develop HS, but are resistant to fibrosis because the activation of HSCs is dependent upon leptin. Overall, it is generally appreciated that existing murine NAFLD models inadequately represent the complex heterogeneity of NAFLD phenotypes that are observed in patients.

Another major limitation of murine NAFLD models for basic research and clinical translation is the inherent differences in genetic profiling, anatomical structure, organ size, and pathophysiological characteristics between mice and human beings. Large animals with closer genetic relationships to humans may thus represent a more faithful model of human NAFLD. Ossabaw miniature swine, which harbor a "thrifty gene" that allows them to adapt to seasonal limitations in food, develop liver dysfunction and a MetS with close resemblance to human NASH in response to chronic food excess. Nonhuman primates may also naturally develop metabolic disorders. Monkeys predisposed to MetS (MetS-predisposed) have recently been used as a NASH model. These MetS-predisposed monkeys exhibit higher blood pressure and body weight than healthy controls. Importantly, moderate steatosis and inflammation developed in MetS-predisposed monkeys fed a normal chow diet, and they also exhibit clear increases in the hepatic expression of profibrotic genes.[5-8] When fed an HFD or even a simple high-carbohydrate diet, MetS-predisposed monkeys rapidly become obese and insulin resistant and develop hepatic inflammation with fibrosis.[5,9,10] The similarities between the pathophysiology, anatomy, and histopathological disease spectrum in nonhuman primate and humans suggest that the MetS-predisposed monkey is more representatively faithful to the clinical situation than murine models, and thus enable much more relevant studies of molecular mechanisms of NASH. However, large animal models have not been extensively applied in NAFLD research because of challenges in breeding and maintaining these animals in the laboratory setting. Moreover, these models do not lend themselves to the sophisticated genetic approaches that can be applied to rodents. Clearly, more work is required to develop animal models that will facilitate in-depth insights into NAFLD pathologies and identify molecular targets for therapy.

Lifestyle interventions consisting of diet, exercise, and weight loss remain the first-line therapy for NAFLD. However, only a minority of patients are able to lose 7%-10% of their body weight, which has been demonstrated as a threshold required to improve the majority of histopathological features of NASH.[4] Unfortunately, there are still no approved pharmacotherapies for NAFLD, notwithstanding extensive studies on its pathogenesis and associated efforts to develop new classes of medications.

We are now facing major challenges in tackling the alarming increase and soaring epidemic of NAFLD around the world. The considerable risk of NAFLD progression to advanced liver disease, along with metabolic impairments and cardiovascular disease, the unsatisfactory and invasive diagnostic procedures, and the lack of pharmacological approaches are important roadblocks for treating NAFLD. More than 1.7 billion people now have NAFLD, and the progression from NAFLD to cirrhosis or HCC occurs regularly. Yet, no drugs for the treatment of NAFLD are available in the clinic. It is estimated that major pharmaceutical companies, including Merck, Novartis, Gilead, and Allergan, have invested more than $5 billion to develop new drugs for NAFLD treatment. The market for NASH drugs has been predicted to rise to over $30 billion a year by 2025. The NAFLD-derived annual health care resource and economic burdens are more enormous—$103 billion in the United States alone.[4] These considerations establish reducing the incidence of NAFLD as well as early diagnosis and intervention as important priorities.

NAFLD now commands widespread attention from researchers and industry, but its alarming increase in prevalence demands still more attention from clinicians, society, and governments. It is indeed time to step-up the fight against NAFLD.

Xiao-Jing Zhang, Ph.D.,[1-3]
Zhi-Gang She, M.D., Ph.D.,[1-3]
Hongliang Li, M.D., Ph.D.[1-3]
[1]*Department of Cardiology*
Renmin Hospital of Wuhan University
Wuhan, China
[2]*Institute of Model Animal of Wuhan University*
Wuhan, China
[3]*Basic Medical School*
Wuhan University
Wuhan, China

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Author names in bold designate shared co-first authorship.