EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

NASH animal models: *Are we there yet?*

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Introduction

Given the obesity pandemic and the concomitant meteoric rise in nonalcoholic fatty liver disease (NAFLD) prevalence [1], there has been an urgent need to understand the pathogenetic mechanisms responsible for disease development and progression. This required the development of experimental models that mimic the human condition, as causative, mechanistic studies are many times more difficult or impossible to conduct in humans. Moreover, these animal models may serve as a powerful preclinical platform to study novel therapeutic strategies for this disease. The journey has presented us with a multitude of options including animals with naturally occurring mutations that inactivate key genes (e.g. *Leptin* gene in the *ob/ob* mice), animals with specific genetic manipulations, environmental, and dietary variations (Table 1) all with a common goal in mind; that of achieving the most relevance to the human condition. We do not wish to provide an exhaustive review of models available but rather walk the readers through the conceptual advances made in this field over the past two decades. Therefore, the question we would like to answer through this commentary is one most often asked by children on a long road trip – *are we there yet?*

The human condition

The first recognition of this entity was made in a group of 20 obese patients with many having co-morbidities of obesity such as diabetes [2]. The histological features defined the term nonalcoholic steatohepatitis or NASH that is characterized by the presence of hepatic steatosis, inflammation, ballooning degeneration of hepatocytes, and or fibrosis [3]. We now understand from longitudinal studies conducted that patients with NASH and fibrosis have a significant risk of progressing to cirrhosis and end-stage liver disease, while patients with hepatic steatosis alone tend to have a benign non-progressive condition [4]. The goal, therefore, is to best mimic NASH in an animal model that is both practical for experimental investigation and enjoys the defining features of the human condition including obesity, insulin resistance, steatohepatitis, and fibrosis (Fig. 1).

Early rodent models

The discovery of the *Leptin* gene in the *ob/ob* mouse by Friedman *et al.* in 1994 [5], validated the use of this animal model to study obesity and its co-morbidities, including NAFLD. A similar line of investigation has been performed on the *db/db* mouse which lacks the leptin receptor. Both these strains of mice have been very valuable for the study of hepatic steatosis and insulin resistance as well as the early stages of inflammatory changes in the liver but required the methionine and choline deficient (MCD) diet to develop NASH with fibrosis [6]. Indeed, this diet, which is one of the most widely used model of steatohepatitis, induces in mutant mice, as well as mice without these mutations, histological changes that mimic those of patients with severe NASH, including macrovesicular steatosis, lobular inflammation, and pericellular, perisinusoidal fibrosis. However, The MCD diet is not associated with overt systemic insulin resistance characteristic of patients with NAFLD, and the animals tend to lose weight rather than gain weight on this diet [7]. In order to avoid the pitfalls and limitations of these models, several groups have utilized environmental approaches mainly by using high caloric, "western type" diets. When given to C57Bl6 mice or Sprague Dawley rats, which are particularly sensitive to the effects of diet on weight and glucose regulation, these diets result in severe obesity, insulin resistance, and NAFLD [8,9]. Although, after prolonged feeding for up to six months, these "western type" diets may result in increased molecular markers of inflammation and fibrogenesis, they do not produce the typical histological changes of human progressive NASH such as hepatocellular injury and fibrosis. Thus, taken together, these models have been very valuable to study the early stages of NAFLD in its true humanistic context but were unable to provide a venue to study the severe features of NASH without resorting to the MCD diet.

The road to fructose

Recently, Brent-Tetri and colleagues provided a significant leap forward by giving sedentary non-genetically modified mice with a high long-chain trans-fat solid diet and high-fructose corn

Received 17 March 2011; received in revised form 27 April 2011; accepted 28 April 2011

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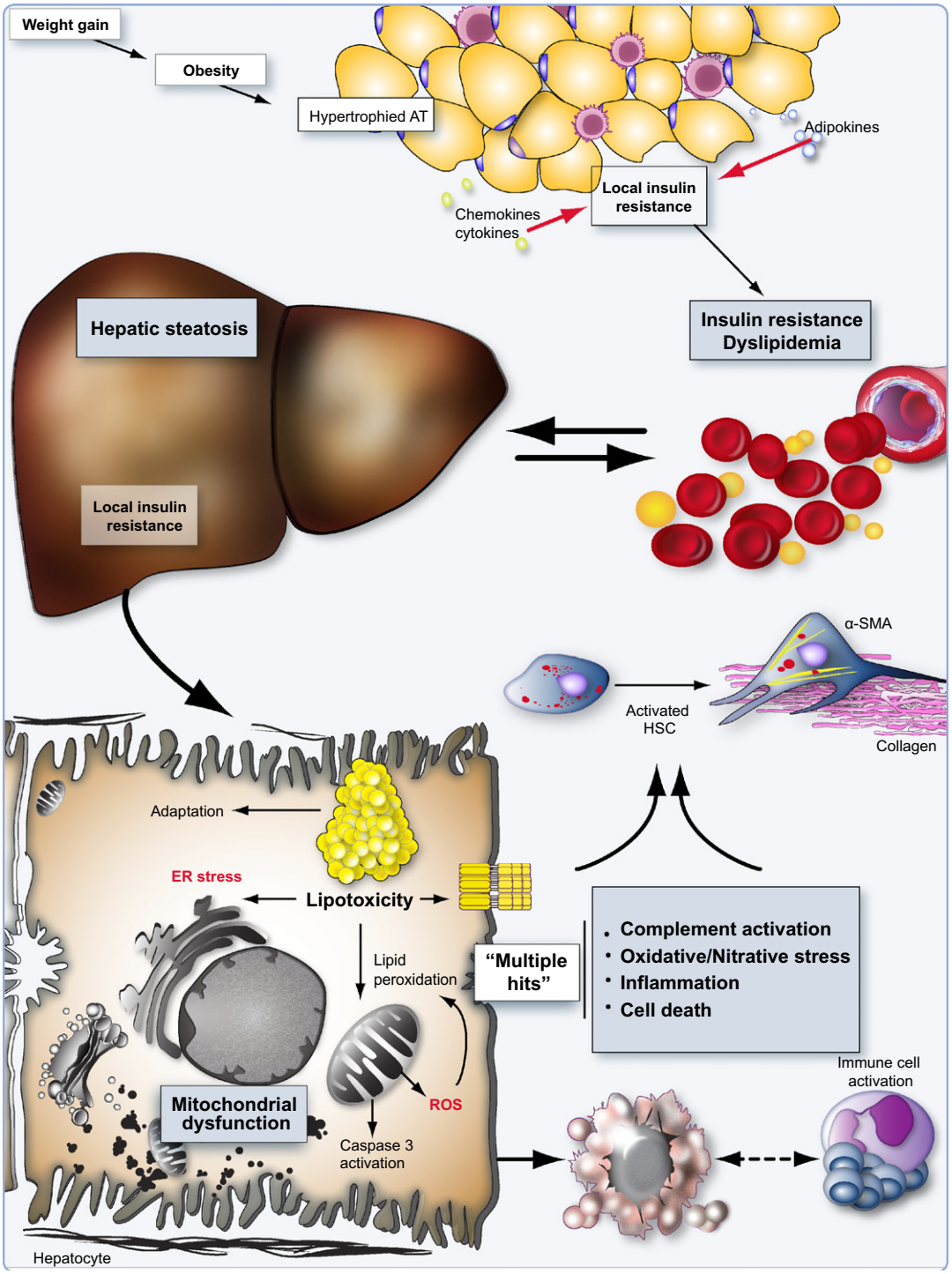

Fig. 1. Pathophysiological characteristics of a good animal model to study human NAFLD and NASH. Weight gain and obesity result in adipose tissue expansion and infiltration by macrophages with development of local insulin resistance and release of free fatty acids (FFA) into circulation. These events are thought to be critical for the development of hepatic steatosis which in turn contributes to local hepatic insulin resistance. Early in the disease process, the liver adapts to excess FFA; however, over time, these adaptive mechanisms fail, resulting to lipotoxicity. Lipotoxicity can trigger multiple deleterious pathways in hepatocytes including ER stress, upregulation of death receptors, and mitochondrial dysfunction. As a consequence, there is increased production of a wide variety of reactive oxygen, nitrogen, and lipid species, which dysregulate multiple redox sensitive signaling pathways leading to further increase in TG accumulation. The ensuing disruption of mitochondrial function triggers caspase activation and cell death; key "hits" in the progression to NASH. In conjunction, release of reactive species from hepatocytes can trigger activation of immune cells and convert the normally quiescent stellate cells to a fibrosis-inducing cell. This combination of events that are critical for the progression from steatosis to steatohepatitis to fibrosis is present to different extents in the various animal models that were more commonly used to study NAFLD and NASH. Abbreviations: AT, adipose tissue; HSC, hepatic stellate cells; ROS, reactive oxygen species; ER, endoplasmic reticulum; α-SMA, alpha-smooth muscle actin.

Table 1. Main characteristics of commonly used animal models of NAFLD and NASH.

NAFLD Model	Genetic Manipulation	Study Length (Weeks)	Obesity	Hepatic Insulin Resistance	Hepatic Inflammation	Hepatic Fibrosis
ob/ob	Y	4-8	Y	Y	N	N
db/db + MCD	Y	4-8	Y	Y	Y	Y
L-SACC1	Y	12	Y	Y	Y	N
PTEN liver k/o	Y	10-40	Y	Y	Y	Y
MCD Diet	N	8	N	Y	Y	Y
Lieber-DeCarli HF Liquid	N	3	Y	Y	Y	N
Tetri-ALIOS	N	16	Y	Y	Y	N
Kohli-HFHC	N	16	Y	Y	Y	Y

Leptin gene deficiency (*ob/ob*); leptin receptor deficiency (*db/db*), methionine and choline deficient diet (MCD), Phosphatase and tensin homolog (PTEN), Liver-specific inactivation of CEACAM1 (L-SACC1), High Fat (HF), HF Liquid (71% Fat), American Lifestyle Induced Obesity Syndrome (ALIOS), High Fat High Carbohydrate (HFHC).

syrup (HFCS) equivalent reconstituted gelatin water [10]. This combination of insults resulted in an obese animal with insulin resistance and severe hepatic steatosis with associated necroinflammatory changes in the liver. Again, given the importance of fibrosis as a predictor of severity and prognosis in NASH patients [4], having a rodent model that provides one with this feature in the appropriate obese and insulin resistant phenotype would have been ideal but was missing from this attempt. We have recently built on this further by combining the insults of a high calorie, predominantly medium chain of saturated fatty acids diet with a high-fructose enriched drinking water (HFHC) [11]. These cumulative hits to C57Bl6 mice over a 16-week period resulted in a phenotype of obesity, insulin resistance, increased hepatic oxidative stress, and most importantly a complete human NASH-like phenotype with steatosis, significant hepatic inflammation, and significant fibrosis. A similar paradigm to get to hepatocellular injury, inflammation, and fibrosis in NASH using fructose was subsequently reported in a rat model as well [12].

Thus, to answer the question of *"are we there yet?,* it has truly been a long trek and we may not be at the end of our journey, but with the advent of these more human-pathophysiologically relevant and practical rodent models we surely are on the right path!

Conflict of interest

The underlying research reported in the study was funded by the NIH Institutes of Health.

Financial support

This work was supported by NIH Grants (DK076852) and (DK082451) to A.E.F. and (DK084310) to R.K.

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