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White Paper

NAFLD/NASH: Accelerating Drug Discovery with an Improved Metabolic Dysregulation Model

The Challenge: Can we Provide Better Preclinical Tools for NASH Drug Development?

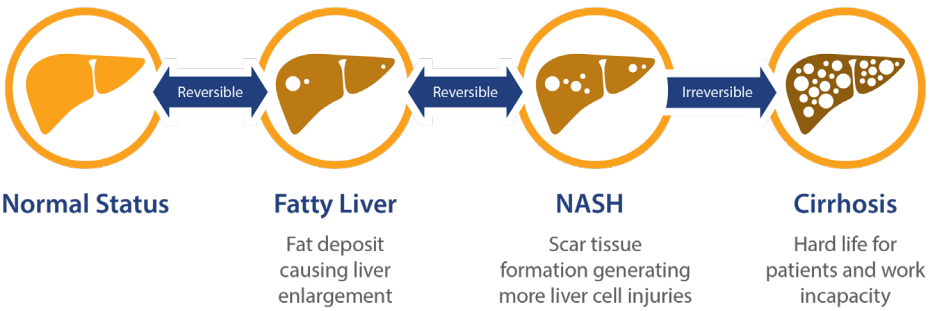
As nonalcoholic steatohepatitis (NASH) levels increase globally, and the race for a first approved NASH treatment continues, current preclinical research is hindered by a lack of models recapitulating both the metabolic context and appropriate pathology and/or liver injury observed in human disease. This often results in researchers requiring multiple models within one research program. This White Paper looks at the current status and tools available for NASH research, diagnosis, and treatment, and presents a preclinical model with improved translatability for potentially accelerating NASH drug development.

NASH: Defining the 'Silent' Liver Disease and Progression to Cirrhosis

Nonalcoholic fatty liver disease (NAFLD) and NASH are not uncommon and they are reversible. However, NASH is commonly known as a silent liver disease, leaving most people unaware they have a rectifiable liver problem and feeling generally well. Severe cases of NASH progress to cirrhosis, with permanent liver damage and loss of function occurring (Figure 1).

Progression takes places over many years, with patients beginning to show symptoms such as fatigue, weight loss, and weakness as the disease becomes more advanced. A person with cirrhosis experiences fluid retention, muscle wasting, bleeding from the intestines, and liver failure, with liver transplant being the only treatment.

Figure 1: The Progression of Fatty Liver Disease to Cirrhosis



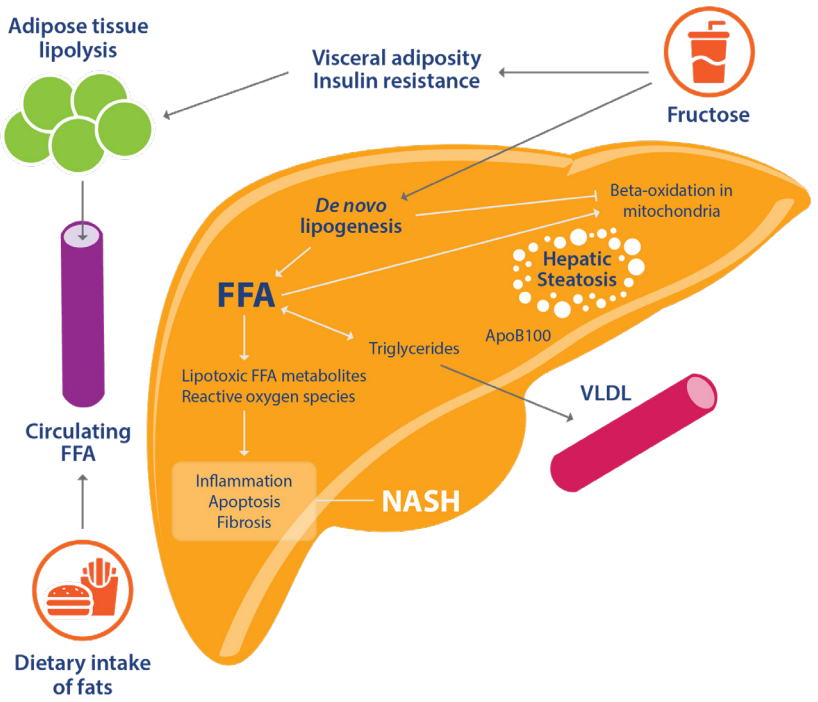
> "NASH is a serious public health issue - as a silent liver disease, it often progresses to a more severe, advanced stage before a patient realizes they are affected"

The Complex Pathogenesis of NASH

NASH is an extremely complex disorder (Figure 2). It is intertwined with patients having obesity, hyperlipidemia, insulin resistance, and glucose intolerance, as well as being linked with an increase in fructose intake and dietary intake effects. It has also been shown that inflammation plays a significant role in the pathogenesis of NASH. The first line NASH effects which are usually observed are elevated liver enzymes, often detected through routine medical testing.

> "An increased understanding of the complex NASH pathogenesis is essential to improving disease interventions"

Figure 2: The Pathogenesis of NASH

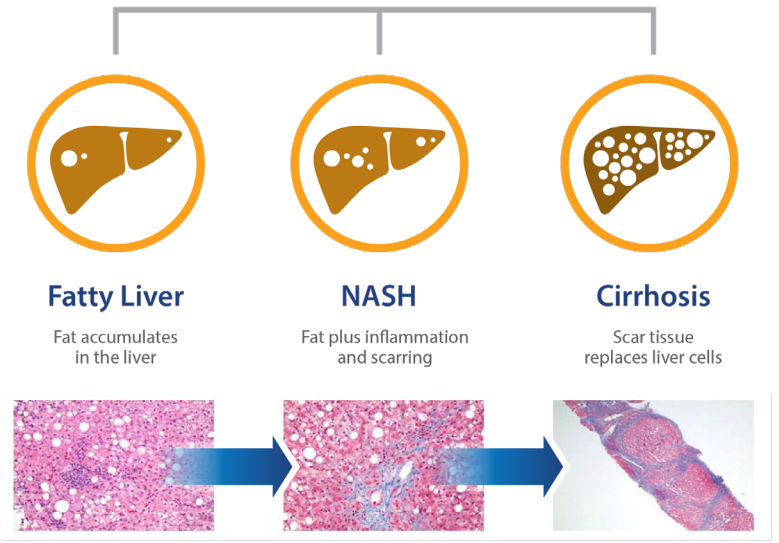


Current and Upcoming Tools for NASH Diagnosis

The elevations observed in liver enzymes (such as ALT or AST) are usually the first steps to a NASH diagnosis. Other background reasons for ALT/AST increases such as medications, viral hepatitis, or excessive use of alcohol need to be ruled out, before next steps are taken to confirm a diagnosis of NASH.

Noninvasive ultrasound imaging can be performed to confirm that there is fat accumulation in the liver. However, the only FDA agreed method for confirming a diagnosis of NASH is through an invasive liver biopsy. Histological markers which are assessed in biopsy samples are steatosis, inflammation, fibrosis, and ballooning which together make up the NAFLD activity score (NAS) which denotes the severity of each NASH case (Figure 3). Currently, there are no available blood tests or scans which can be used as a reliable alternative to biopsy to provide this detailed information.

Figure 3: The Spectrum of NAFLD



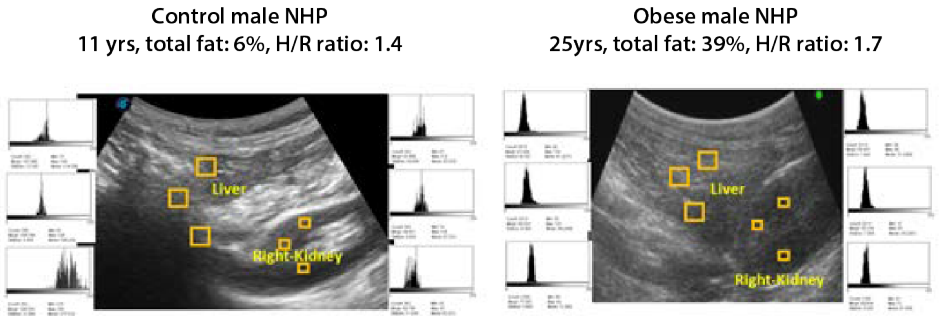
There are, however, non-invasive techniques on the horizon for NASH diagnosis, some of which are being utilized and trialed in a clinical setting, including magnetic resonance imaging-proton-density fat fraction (MRI-PDFF). Implementing these new applications would result in fewer liver biopsies being needed during patient diagnosis and treatment regimens, as well as potentially providing an earlier diagnosis, improving patient care during a disease with a long natural history.

These techniques include a noninvasive metabolomics based analysis from OWL. This *in vitro* analysis is performed on patient serum samples and has been shown to effectively diagnose the presence of NASH in patients[1,2,3]. This technology is also being used within clinical trials, where the tool can be used to stratify patient populations and to verify appropriate randomizations, which are critical for baselines especially in large studies.

Noninvasive sonography is another technique being employed to avoid liver biopsy. Preclinical studies using NHPs have utilized sonography to determine hepatic lipidosis (shown in Figure 4), with average echo intensity shown to be markedly different for obese versus control animals[4]. Combining these methods with gold standard biomarkers (such as liver enzymes, ALT, and AST levels) could provide excellent noninvasive tools for diagnosing the progression of NAFLD into NASH.

> “To improve patient quality of life throughout this chronic disorder, noninvasive techniques are required for NASH diagnosis and monitoring, to replace the invasive and subjective liver biopsy”

Figure 4: Noninvasive Sonography Determines Hepatic Lipidosis[4]



The Current Lack of NASH Treatment Options

Currently there are no therapies approved for the treatment of NASH. To prevent disease progression, physicians recommend lifestyle changes including:

- following a balanced and healthy diet
- exercising to reduce body weight
- avoiding alcohol
- avoiding any unnecessary medications.

Accompanying disease comorbidities, such as diabetes, dyslipidemia, or hypertension will also need to be treated. While these methods are all accepted as standards of care for NASH, none have been conclusively shown to prevent disease progression.

> NASH has no approved drug treatments, and standard of care lifestyle changes are not proven to prevent disease progression

Is the First Approved NASH Therapy on the Horizon?

The current raft of ongoing Phase III clinical trials in NASH have led many researchers to believe that the first approved therapy maybe on the horizon. However, the extended timelines of NASH, due to the chronic disease natural history, have resulted in a "sit and wait" timeline in NASH drug development. This reflects the conviction that the disease is so prolonged that even a very effective therapy may not show an observable effect for multiple years.

The three main trailblazers in Phase III clinical trials for NASH are:

- Genfit's elafibranor
- Intercept's obeticholic acid (OCA)
- Novartis/Allergan's cenicriviroc.

These treatments are now under the watchful eye of the NASH community not only for the first approval, but also as their clinical trials will be highly influential in establishing the standards in NASH clinical trial design and acceptable study endpoints (current Phase III clinical trials are summarized in Table 1). Competitors will soon be able to use these trials/approved drugs to visualize the market strategy for future NASH drug deliverables and costs.

> Study endpoints are the key differentiators in the ongoing Phase III NASH trials - Intercept are potentially the front runner due to NASH resolution or fibrosis improvement signifying a successful trial

Table 1: Summary of Ongoing NASH Phase III Trials
As of July 17, 2018.

Company	Agent and MOA	Phase III Trial	FDA Designation	Recruited Patients	Primary Endpoint	Estimated Completion Dates
Genfit	Elafibranor Dual PPAR α/δ agonist	RESOLVE-IT[5]	Fast Track	NASH patients with significant (F2) to advanced (F3) fibrosis	Resolution of NASH without worsening of fibrosis after 72 weeks of treatment	Primary completion: December 2021
Intercept	Obeticholic acid FXR agonist	REGENERATE[6]	Breakthrough Therapy	NASH patients with significant (F2) to advanced (F3) fibrosis	Resolution of NASH without worsening of fibrosis OR Achieving at least one stage of liver fibrosis improvement with no worsening of NASH after 18 months of treatment	Primary completion: 2019 Study completion: October 2022
Novartis/ Allergan	Cenicriviroc CCR2/CCR5 inhibitor	AURORA[7]	Fast Track	NASH patients with significant (F2) to advanced (F3) fibrosis	Achieving at least one stage of liver fibrosis improvement with no worsening of NASH after 12 months of treatment	Primary completion: July 2019 Study completion: July 2024

While all three of the Phase III trials have recruited a similar cohort of patients, the main study differences lie with the endpoints. Genfit's study is evaluating NASH resolution without worsening of fibrosis, whereas the cenicriviroc trial is based on the inverse endpoint – seeking liver fibrosis improvement without worsening of NASH.

The definition of NASH resolution which has been confirmed for NASH clinical trials emphasises the role of cell injury and inflammation, while keeping flexibility in the level of steatosis, i.e. the endpoint has a total absence of ballooning, an absence or even mild inflammation, but some steatosis can still be present.

The Intercept study stands apart in its endpoint selection. Initially, the study had co-primary endpoints of NASH resolution and fibrosis improvement. This was later amended to *"or"*, which now requires only one endpoint to be met for the trial to be considered successful. If both endpoints are reached this could differentiate OCA from other drug candidates in the field, leveraging the agent as first/best in class.

One further agent which is yet to advance to Phase III testing is MGL-3196 from Madrigal Pharmaceuticals, which is a THR β-selective agonist. Positive Phase II data has been released with the trial being of note as it was the first study to demonstrate a positive correlation between improvements in liver fat at 12 weeks via a noninvasive imaging technique (MRI-PDFF) with NASH histologic results via liver biopsy results at 36 weeks post-treatment[8]. This supports the continued development of the noninvasive NASH tools discussed above.

Current Preclinical NASH Models: No Ideal Model Available for All Disease Aspects

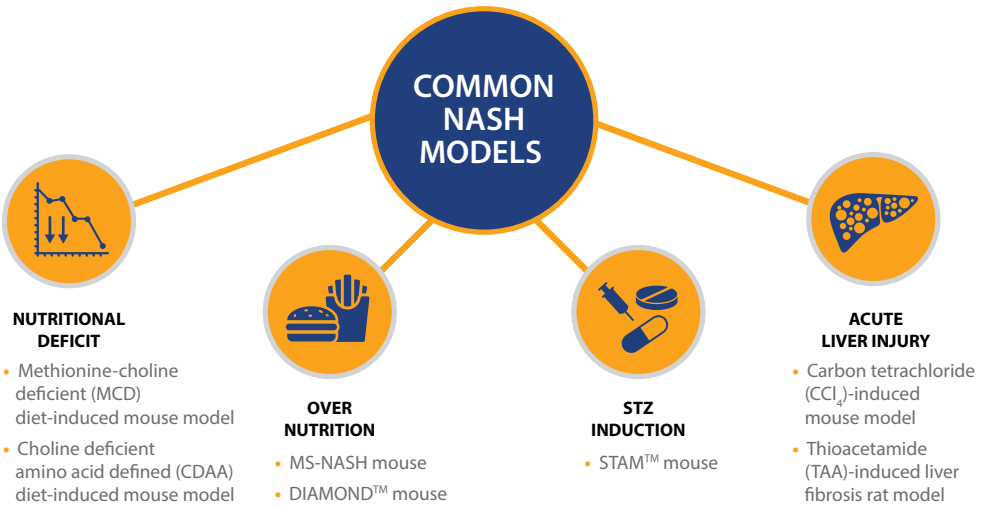
While a range of preclinical models are available for NASH studies, there is no standout model that is ideal for all of the aspects of the clinical signs of NASH e.g. over nutrition, insulin resistance, inflammation, and fibrosis. While some models may show the appropriate pathology and/or liver injury, they have inappropriate metabolic context, lacking the slow disease progression. Conversely, other models may show appropriate metabolic context, but have limited characterization and pathology.

An optimal animal model for NASH would ideally have the correct liver pathology within an appropriate metabolic context, providing a translational research tool that is relevant to human clinical research and studies.

Currently used NASH animal models include those shown in Figure 5, such as nutritional deficit models, over nutrition models which have high fat diet/Western Diet induced NAFLD/NASH, and STZ induction models. The STZ model may not have the appropriate NASH metabolic component, but it is highly useful for a range of research studies.

> “Current NASH animal models do not feature all clinical aspects of disease, no one model pairs up both the pathology and metabolic aspects of NASH”

Figure 5: Commonly Used NASH Animal Models



Acute liver injury models include the carbon tetrachloride (CCl_4)-induced mouse model which is proving to be a current popular model choice. It has historically been relied upon to have the acute injury required for liver injury and regeneration studies. Along with the thioacetamide (TAA)-induced liver fibrosis rat model, the acute liver injury models can be useful for certain aspects of liver injury and fibrosis studies, but they are not relevant for translational NASH research - lacking comorbidities and the metabolic components of the disease as compared with a nutritional deficit or over nutrition model.

While a selection of these models are providing positive preclinical data for progression to the clinic, it is still important to develop more translatable animal models which can address both the metabolic and liver fibrosis components of NASH.

The MS-NASH Mouse Model: A Validated and Improved Translatable Model for Drug Discovery

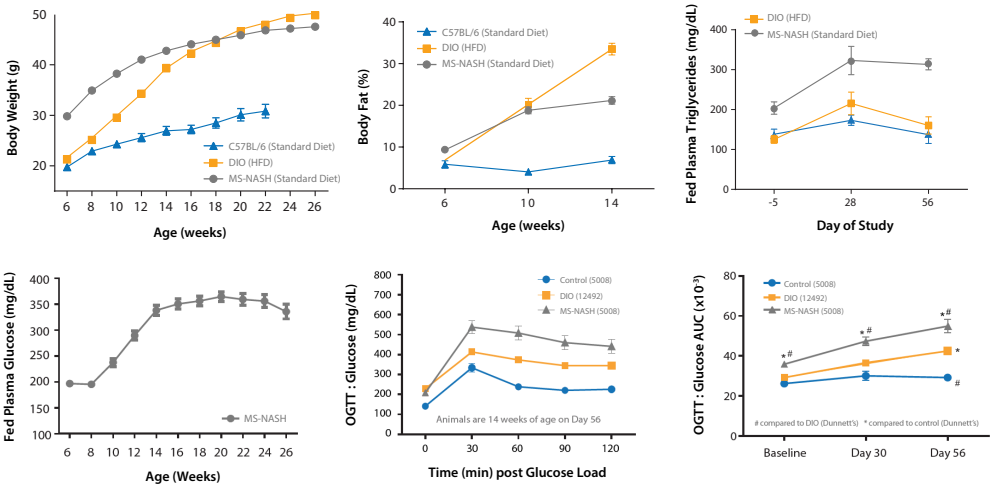
> "Spontaneously dysmetabolic rodent models provide an appropriate metabolic background for downstream NAFLD/NASH development, including being hyperglycemic"

The MS-NASH (formerly called FATZO) polygenic mouse model has a functional leptin pathway, and more closely mimics human obesity, metabolic syndrome, and type 2 diabetes (T2D), for more translatable obesity and T2D preclinical drug discovery[9,10]. Using a western diet and fructose in the drinking water, the MS-NASH mouse has been translated into a NAFLD/NASH model, providing an inherently dysmetabolic, obese, and diabetic mouse model for NAFLD/NASH drug development.

Figure 6 shows the development of obesity, dyslipidemia, hyperglycemia, and insulin resistance within the MS-NASH mouse. The model is unique in that obesity occurs spontaneously, without the administration of a high fat diet. The body weight of MS-NASH mice on a chow diet plateaus around 40-42 grams at 14-16 weeks of age.

Figure 6: MS-NASH Mice Develop Obesity, Dyslipidemia, Hyperglycemia, and Insulin Resistance

MS-NASH and B6 on standard diet compared to DIO model.



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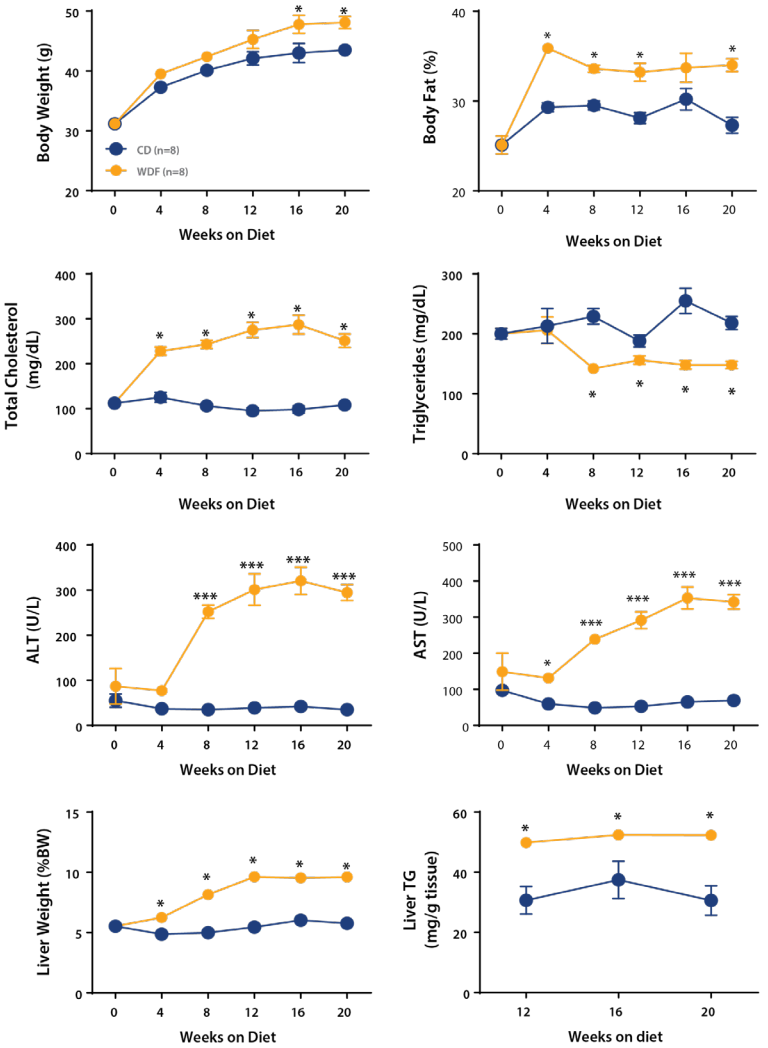
A key component to this animal model on chow is the development of hyperglycemia, which is missing in some other commonly used animal models. For example in DIO models, insulin resistance occurs, but without hyperglycemia which renders the models less than ideal for showing a window of efficacy during a glucose tolerance test.

NAFLD/NASH Development in MS-NASH Mice

When MS-NASH mice are placed on a Western diet plus fructose, the animals tend to become slightly heavier and have increased percent body fat, with the majority of the increase observed in the first four weeks on diet (Figure 7). Total cholesterol is also increased; however a decrease is seen in the levels of triglycerides circulating in the plasma. This is due to the triglycerides instead building up within the liver, with liver weights also rising, and both levels becoming significantly higher than control animals.

> MS-NASH mice on Western diet plus fructose develop many features of NAFLD/NASH, including striking elevations in ALT/AST providing a key window of opportunity for agent evaluation

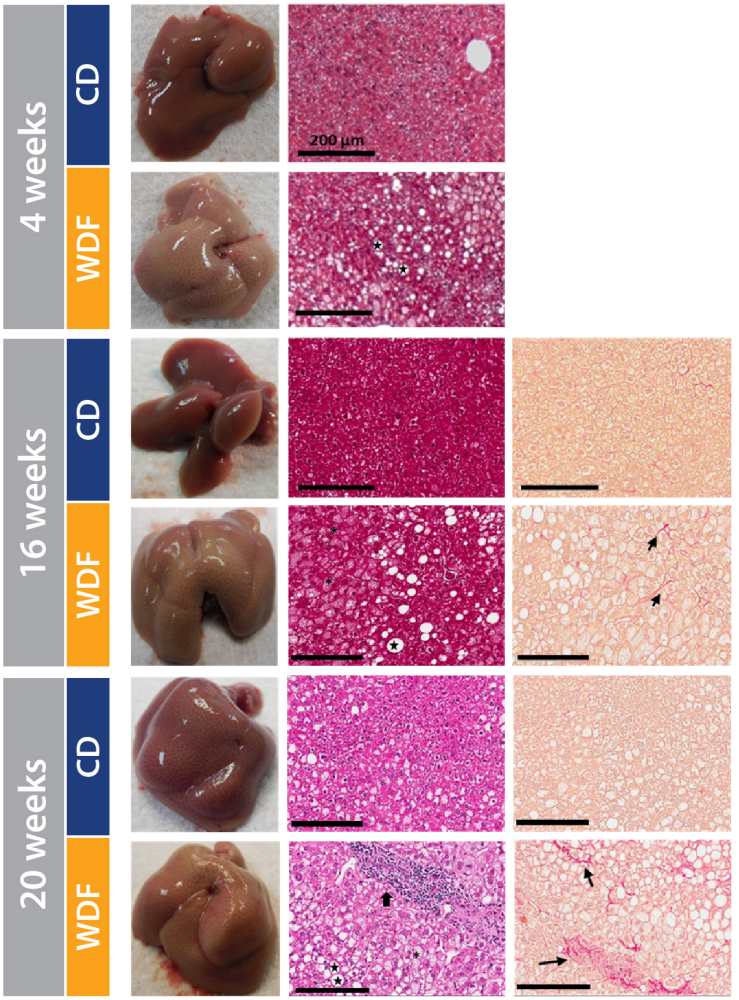
Figure 7: NAFLD/NASH Development in MS-NASH Mice



Another striking factor, which makes the MS-NASH mouse an attractive NAFLD/NASH model, is that significant increases in ALT and AST also occur (Figure 7), providing a window of opportunity for assessing effects and observing the efficacy of novel NASH treatments. These increases are observed within 8 weeks on the Western diet, and maintained across the 20 weeks on diet.

Histological changes are also observed in the livers of MS-NASH mice on diet (Figure 8). By 4 weeks on a Western diet, the livers start to become more light in color and opaque. By 20 weeks on diet, fat is observed accumulating in the liver.

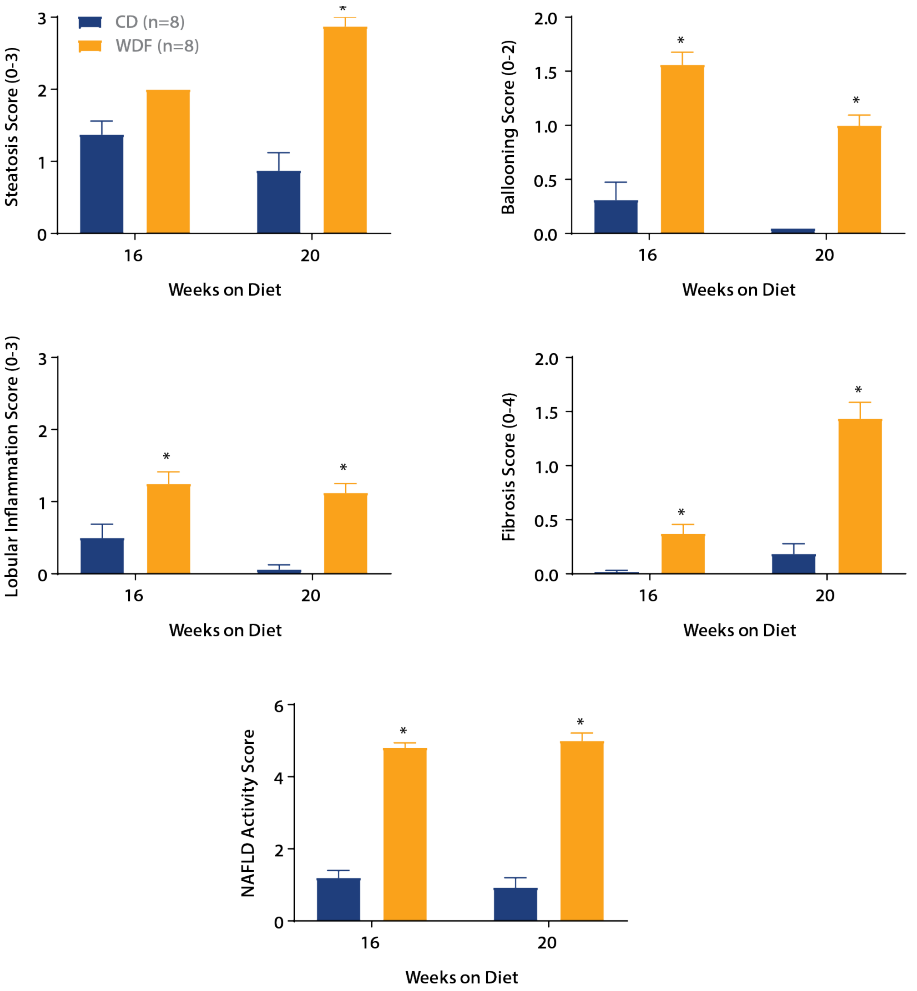
Figure 8: Histological Changes in MS-NASH Mice



> Fat accumulation in the liver and visible histological changes can be seen in MS-NASH mice by 20 weeks on Western diet plus fructose

Clinical scoring can also be performed on the livers of MS-NASH mice fed Western diets plus fructose vs control chow diet animals, looking at steatosis, ballooning, inflammation, and fibrosis (Figure 9). At 16 weeks on diet, steatosis is already increased with significant changes observed by 20 weeks. Ballooning, inflammation, and fibrosis are also significantly elevated at both 16 and 20 weeks on diet, which results in animals on the Western Diet having an overall NAS score over 4.

Figure 9: MS-NASH Mice Develop Clinically Relevant NASH Features



> Clinically relevant NASH features of the NAFLD Activity Score can be measured in this highly translatable model

Assessing NASH Therapeutics with the MS-NASH Mouse Model

To study the effects of NASH therapeutics on liver function, OCA was trialed in MS-NASH mice which had been on a Western diet plus fructose in the drinking water for 16 weeks, resulting in elevated ALT and AST at the start of treatment (Figure 10). Treating with 30mg/kg OCA did not significantly alter body weight or triglyceride levels, but these factors also did not worsen during therapy.

Total cholesterol levels improved over the course of OCA treatment, as did LDL levels, and both ALT and AST levels were significantly lowered, overall demonstrating signs of improvement in liver function.

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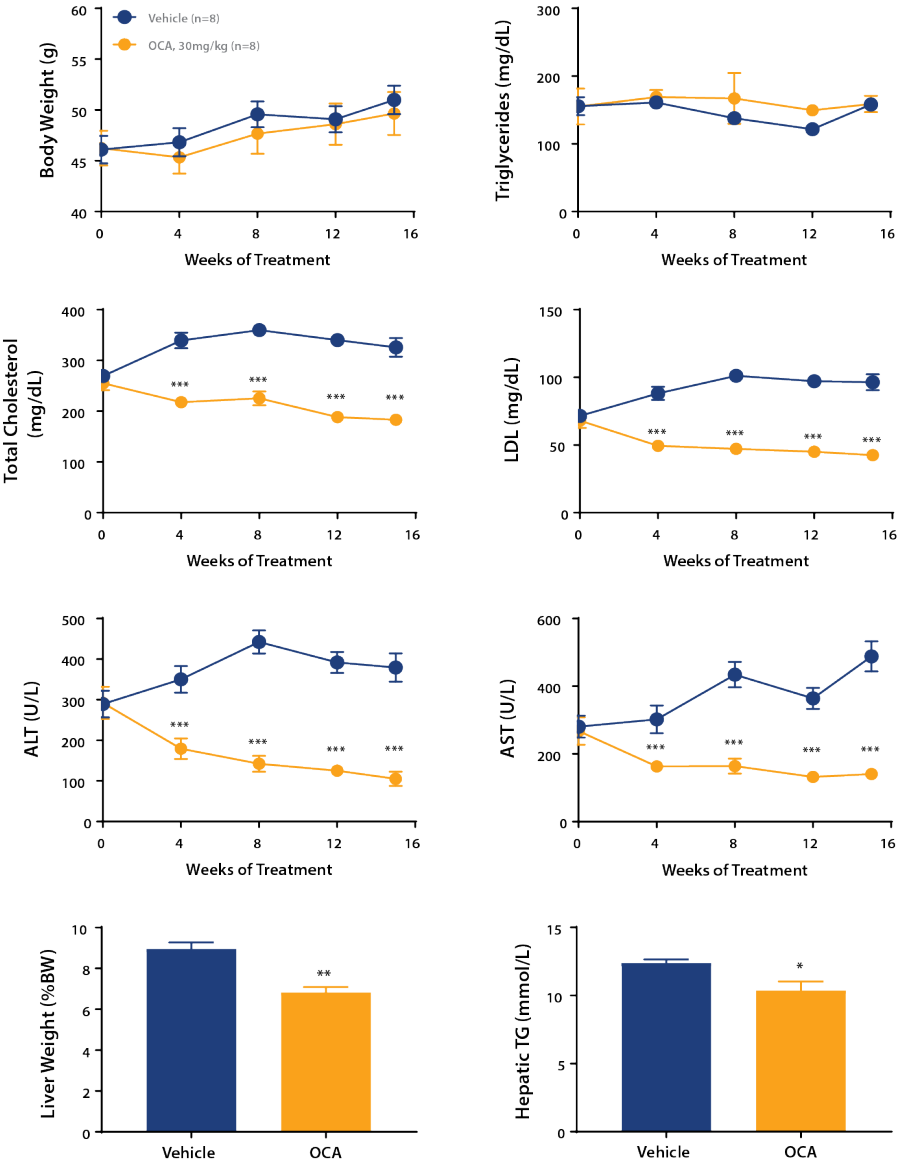
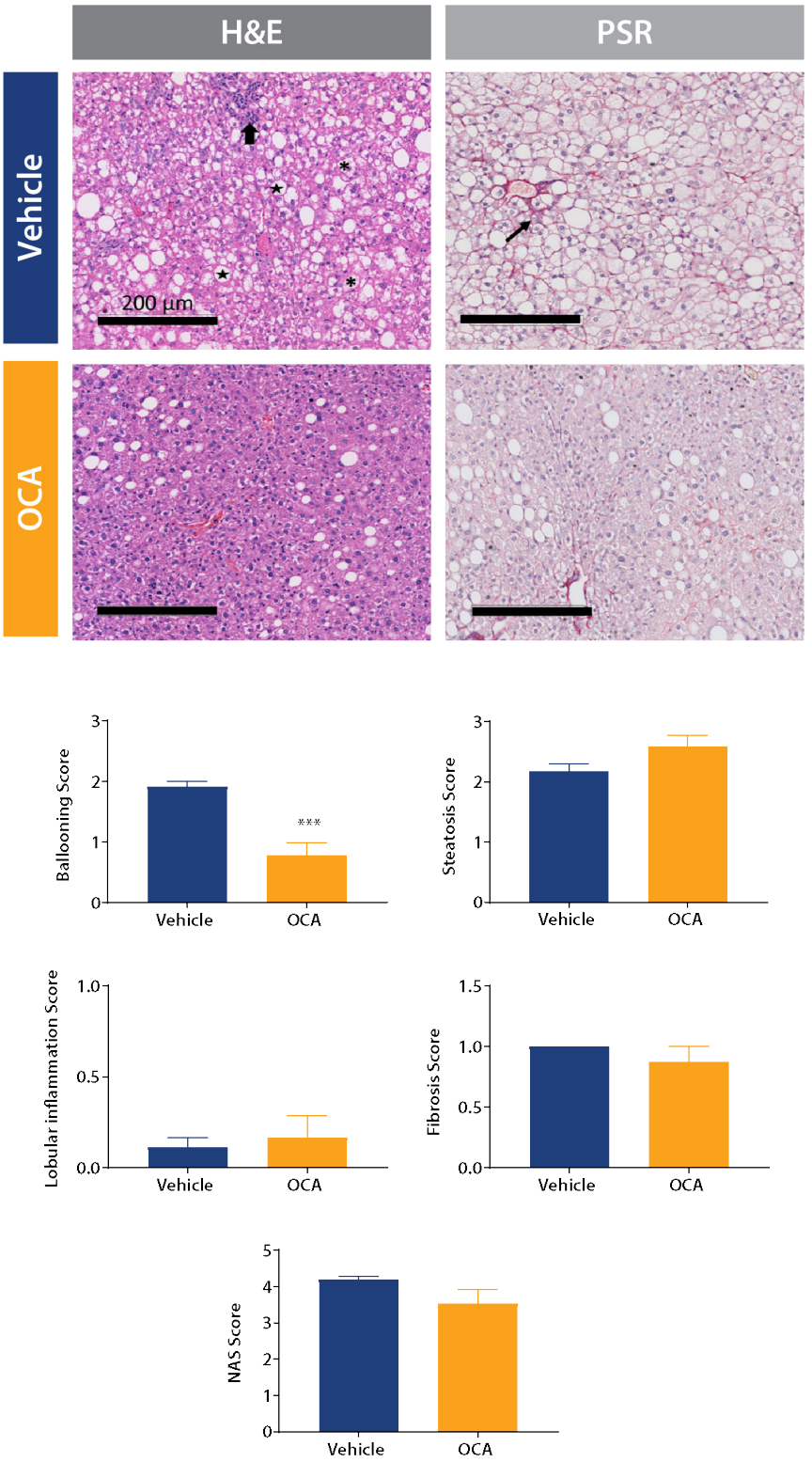
Figure 10: OCA Treatment Significantly Improves Liver Function in MS-NASH Mice with NAFLD/NASH



The effects of OCA treatment on liver histology was also assessed (Figure 11). A significant result was observed for ballooning, which was decreased compared with control following OCA administration. For the other indices trends rather than significant results were observed. For example, fibrosis did not become any worse following OCA treatment. The overall NAS score, therefore, failed to reach significance; however, the study did include several animals which no longer showed any fibrosis in the liver.

Figure 11: OCA Treatment Shows Trends for Improvement of Liver Histology Markers in MS-NASH Mice with NAFLD/NASH

> Novel agents induce clinically relevant improvements in liver function in the MS-NASH model, validating this platform for preclinical drug discovery



Summary

NASH is a chronic disease, characterized by fat in the liver, with inflammation and damage. As NASH levels rise around the world, regulatory approved treatments are urgently required – currently no NASH therapies exist and standard of care consists only of lifestyle changes.

While a number of therapeutic agents are in late stage trials, testing these and developing novel treatments is hindered by a lack of animal models which can truly recapitulate the complex and heterogeneous pathogenesis of the human disease. Models are required which address both the metabolic and liver fibrosis components of NASH, to save the use of multiple research models for different components on the disease.

The MS-NASH mouse model fed a Western diet plus fructose in the drinking water develops NAFLD/NASH within an inherently dysmetabolic, obese, and diabetic background with fibrosis observed by 20 weeks. Treatment of this model with OCA results in improvement in NAS score, with significant reduction in ballooning and successful return to liver function, measured through a significant decrease in levels of liver enzymes ALT and AST. Overall, the MS-NASH mouse provides a more translatable choice for NAFLD/NASH drug development.

> "To accelerate drug discovery, more translational rodent models truly recapitulating human disease can be added to preclinical programs, saving valuable time and resources for drug developers"

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