

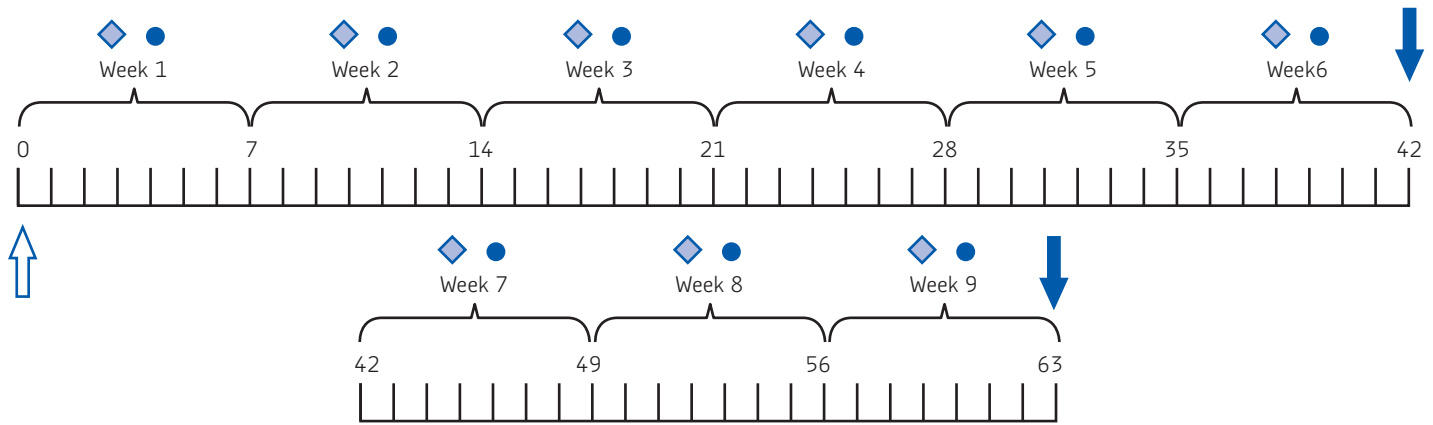
# Non-alcoholic steatohepatitis (NASH)

Non-alcoholic fatty liver disease (NAFLD) is a chronic condition affecting more than 30% of adults in the Western world that is characterized by accumulation of excess fat in the liver (hepatic steatosis) of people who drink little to no alcohol. While typically not considered a serious condition, NAFLD can develop into non-alcoholic steatohepatitis (NASH), which is a fatty liver accompanied by inflammation and various degrees of fibrosis that may develop into cirrhosis or HCC. There are no current FDA approved treatments for NASH, making pre-clinical drug development a top priority in the field. NASH can be modeled pre-clinically through diets, inflammatory results, use of transgenic mice or a combination thereof. Nutrient-deficient diet models used in NASH research include the Methionine and Choline Deficient diet (MCD) and Choline Deficient, high fat (CHDFD) diet. Both diets induce hepatic steatosis, liver damage and progressive fibrosis to varying degrees. MLM has validated multiple NASH diet models to offer clients for pre-clinical drug evaluation. Available readouts include longitudinal body weight and liver enzyme assessments and liver histopathology for fat content, inflammation, damage and fibrosis.

## Experimental Overview

Species Availability:	C57Bl/6
Study Duration:	MCD Deficient Diet-6-weeks CD1M Deficient Diet-9-weeks
Standard Assessments:	Liver histopathology (H&E or Masson's Trichome) ALT, AST liver assessment (weekly) Body weight (weekly)
Additional Assessments:	Proteomic/Genomic biomarkers IHC Flow cytometry

### NASH Diet Example Experimental Schematic



- Group allocation, initiation of special diet
- Body weight measurement  
Performed 1x/week
- Serum collection (early each week)  
Serum collected for ALT/AST measurement 1x/week
- Termination  
Mice euthanized  
Serum collection for potential additional biomarker analyses  
Liver collected and fixed for histological examination

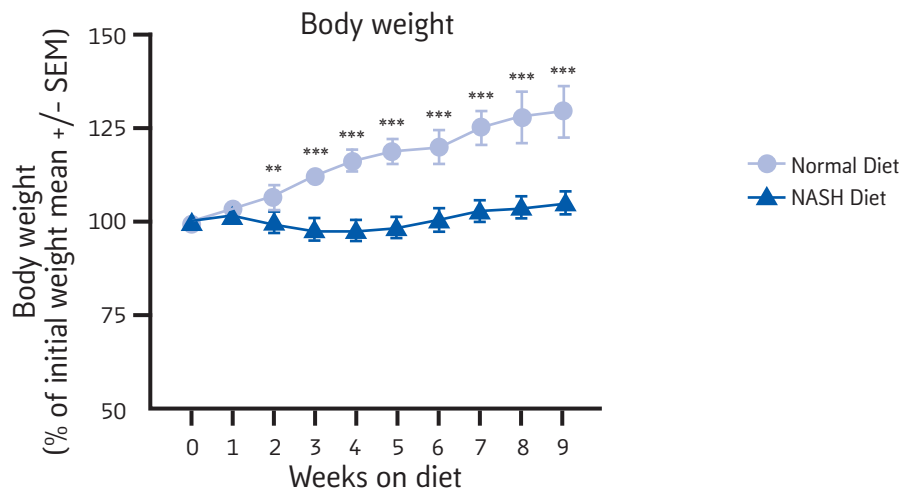
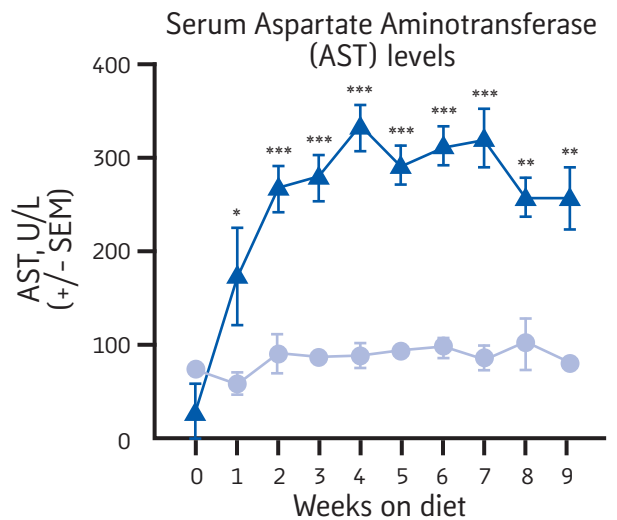
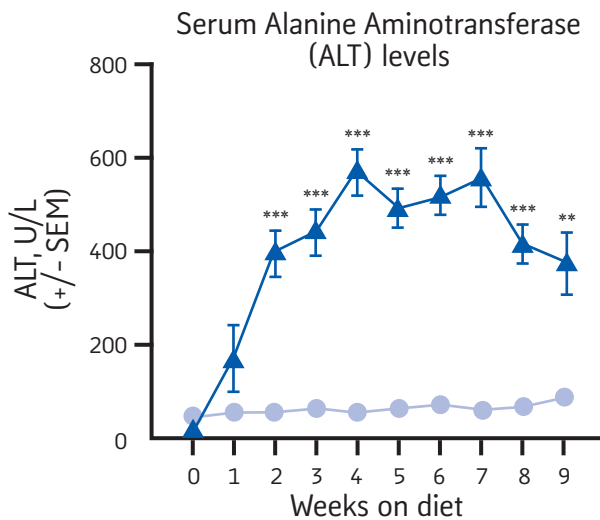
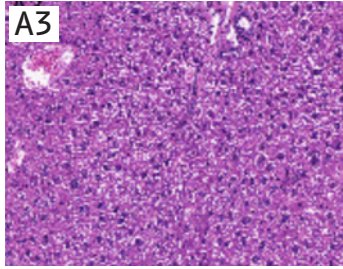
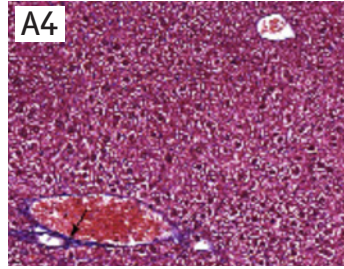
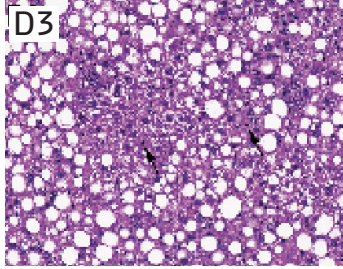
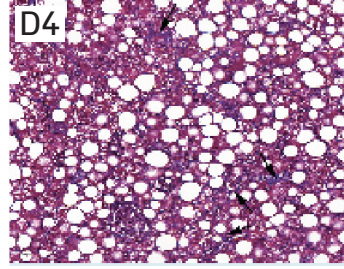
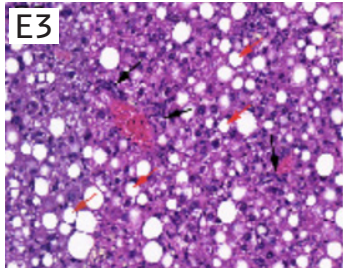
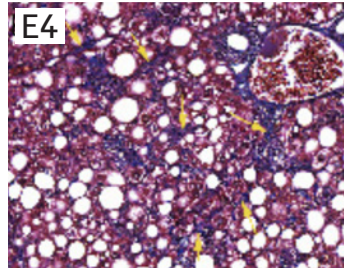
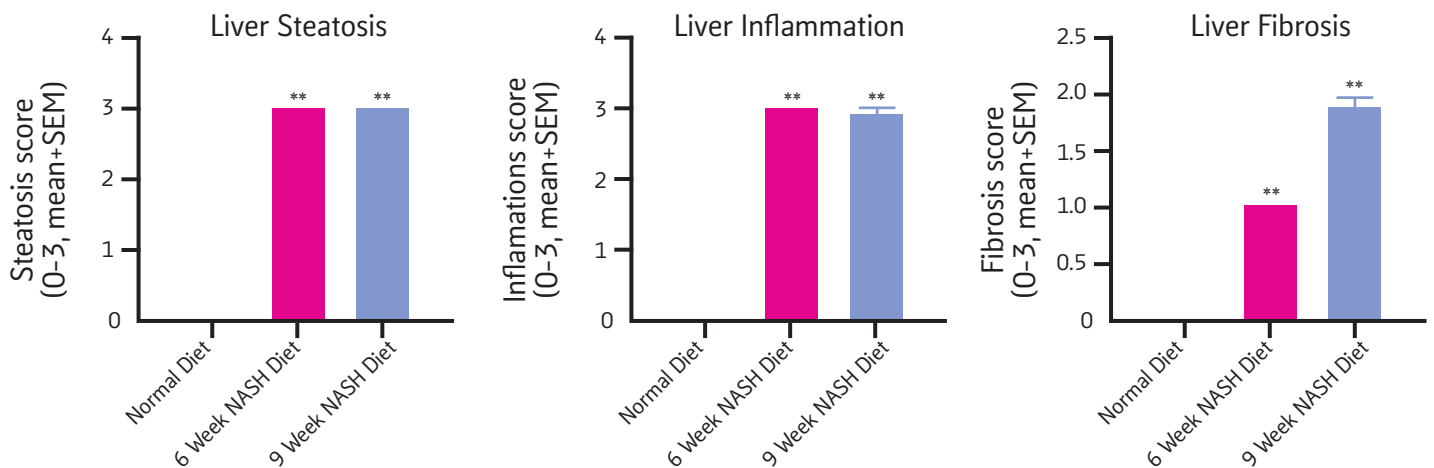


Figure 1. Graphs show elevated levels of AST/ALT, an indicative marker of liver disease. . \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001 using two-way ANOVA followed by Dunnett’s multiple comparisons.

### Histology Staining

	H&E Staining	M&T Staining
<p><b>Normal Diet Liver, 9 weeks</b></p> <p><b>A3. H&amp;E stain.</b> The vacuolation is of the glycogen-type (the pale areas have wispy outlines and the nucleus remains central).</p> <p><b>A4. MT stain.</b> Collagen stains blue and is limited to the walls of larger blood vessels and portal triads.</p>		
<p><b>NASH Diet Liver, 6 weeks</b></p> <p><b>D3. H&amp;E stain</b> of a severely affected liver. There are 2 replicating hepatocytes (arrows). Most hepatocytes in the field show macrovesicular lipidosi.</p> <p><b>D4. M&amp;T stain</b> of a severely affected liver. There is mild multifocal sinusoidal fibrosis (arrows).</p>		
<p><b>NASH Diet Liver, 9 weeks</b></p> <p><b>E3. H&amp;E stain</b> of a severely affected liver. Arrows point to oval cell hyperplasia. Red arrows: Hepatocytes showing macrovesicular lipidosi.</p> <p><b>E4. M&amp;T stain</b> of a severely affected liver. There is significant sinusoidal fibrosis.</p>		

**Figure 2.** Histological images of livers from Normal (A) and NASH diet animals (D&E). Images are 20x high magnification.



**Figure 3.** Graphical depiction of average scoring of the indicated parameters from histological assessment of mouse livers. \*\* indicates  $p < 0.01$  based on an Ordinary one-way ANOVA followed by Dunnett's multiple comparisons compared to the normal diet group.

## Our Clients Say ...

"The performance of your team far exceeded our expectations. The study was performed well and we appreciate all your input into the study design. Your responsiveness and feedback during the study and following in the data interpretation was extremely helpful to guide our next steps. That's something we don't find with every CRO."

*S.G., Toxicologist, Biotech Company*

"Of all the CROs that I have used over the years... MLM Medical Labs been one of the very best in terms of scientific knowledge, data quality, timelines, flexibility and personal contacts."

*O.B., Director of Therapeutics, Pharmaceutical Company*

"Throughout our relationship, you have been attentive to our needs and have completed exploratory pilot studies and three drug studies with professionalism and an understanding of tight biotech timelines that are unmatched by other CROs."

*D.Z., Director of Therapeutics, Biotech Company*

## About MLM Medical Labs

MLM Medical Labs is a leading specialty and central laboratory with comprehensive research services and diagnostic capabilities in Europe and the United States. Offering a range of standard and fully customizable analytical services across a variety of therapeutic areas, we add value at every stage of the drug development process from non-clinical/preclinical through phase IV clinical trials that serve to augment and accelerate research programs to their next stages and milestones. Each disease area is supplemented extensively by different models and batteries of *in vitro* and *ex vivo* analyses, offering answers to your therapeutics' effect on different parameters. With our strong reputation for scientific expertise, passionate approach to customer care, and adherence to quality data, we empower clients ranging from emerging biotech to Top Ten Global Pharma companies to reach confident clinical decisions that ultimately serve to improve patient lives.

If you'd like to discuss a particular study or speak with a scientist, please reach out to us!

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