# Characterization of a **Diet-Induced NASH** Mouse Model

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## ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) along with its more severe manifestation nonalcoholic steatohepatitis (NASH) is a growing public health threat. As there are currently no approved therapeutics to treat NASH, the need for preclinical animal models for drug discovery is great. A commonly used diet-induced NASH model consists of C57BL/6 mice fed a high fat, high fructose, high cholesterol diet with a large proportion of a trans fat called Primex (original AMLN diet, D09100301). With the FDA ban on trans fats, this diet is no longer available. We thus sought to validate whether a replacement diet using palm oil in place of Primex (D09100310) would induce NAFLD / NASH in C57BL/6NTac mice. Pilot production was aimed at identifying the best parameters for production of a consistent model. C57BL/6NTac male mice at the Murine Pathogen Free™ health standard were started on D09100310 diet at 5 or 6 weeks of age. Evaluation of weights just prior to diet administration compared to weights 17 weeks later identified a minimum weight threshold (15 g) required for best response to diet in terms of weight gain (n=92). Compared to C57BL/6J mice started on D09100301 diet at 12 weeks of age (n=6 per time point), C57BL/6NTac mice on D09100310 (n=8 per time point) gained weight more quickly and had similar liver weights. Both groups had similar blood glucose and non-esterified free fatty acid levels, but the C57BL/6NTac had reduced variance compared to the C57BL/6J group. Additionally, the C57BL/6NTac mice had higher transaminases, LDL, and triglycerides after 16 weeks on diet. At 16 weeks on diet, C57BL/6NTac mice (n=10) were obese, with enlarged, fatty livers. No evidence of liver tumors was seen by gross visualization. At 38 weeks on diet, C57BL/6NTac mice (n=8) displayed much higher body weight, liver weight, cholesterol, LDL, and transaminases compared to control mice on chow diet (n=4). The data support use of D09100310 as an appropriate diet for induction of NAFLD/NASH in C57BL/6NTac mice.

## RESULTS

Both C57BL6NTac mice fed D09100310 and C57BL/6J mice fed D09100301 become obese, with much higher weight gain compared to controls on chow. Both NASH groups had similar blood glucose and non-esterified free fatty acid levels, but the C57BL/6NTac had reduced variance compared to the C57BL/6J group (data not shown).

**Figure 1** Body weight gain for C57BL/6NTac mice fed D09100310.

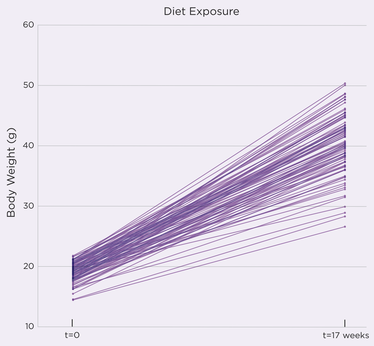



**Figure 2** Body weight gain by time on diet for (A) C57BL/6J fed D09100301 or chow and (B) C57BL/6NTac fed D09100310 or chow.


**Figure 3** Liver weight as a percentage of body weight, by time on diet.

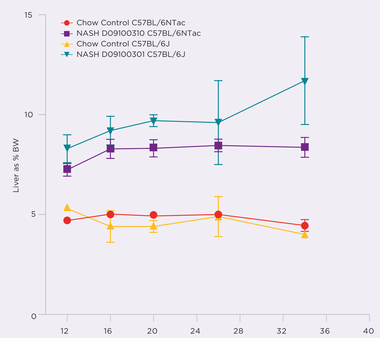



**Figure 4** Transaminases for (A) C57BL/6J fed D09100301 or chow and (B) C57BL/6NTac fed D09100310 or chow.

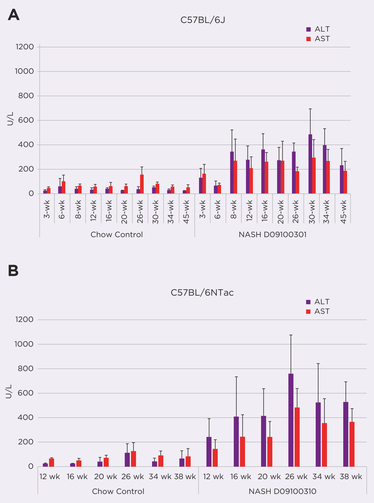
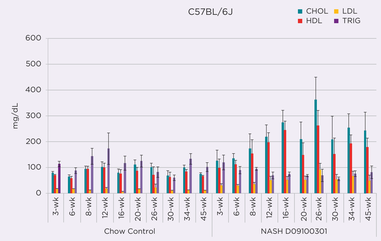



**Figure 5** Serum lipid profile for (A) C57BL/6J fed D09100301 or chow and (B) C57BL/6NTac fed D09100310 or chow.



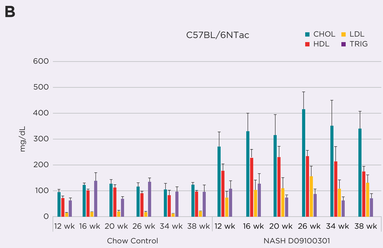




**Figure 6** Liver of C57BL/6NTac mouse on NASH diet D09100310 for 30 weeks displays moderate diffuse hepatomegaly with steatosis. Comparison between C57BL/6NTac mouse on (A) control NIH-31M chow versus (B) NASH diet D09100310 for 30 weeks. Images courtesy of Explora BioLabs.

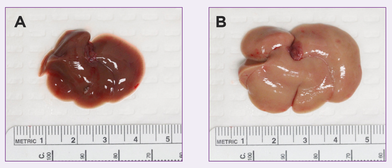
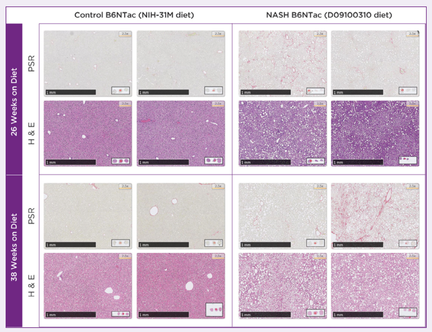



**Figure 7** Mean liver weights for C57BL/6NTac fed control chow or D09100310 for 30 weeks. Data provided by Explora BioLabs.




**Figure 8** Histopathology for C57BL/6NTac mice placed on D09100310 diet (NASH B6NTac) or kept on chow diet (Control B6NTac) from 5 weeks of age. Animals were on diet for 26 weeks (top set) or 38 weeks (bottom set). Picrosirius red (PSR) staining illustrates collagen I and III fibers and hematoxylin and eosin (H&E) staining illustrates steatosis. Two control and two NASH animals are shown for each time point, with PSR and H&E shown for the same individual animal. Different individual animals were used for each time point (i.e. data is not longitudinal by animal). Data provided by an anonymous pharmaceutical company.




## METHODS

For most experiments, C57BL/6NTac male mice at the Murine Pathogen Free™ (MPF™) health standard were placed on the D09100310 diet at 5 weeks of age at 4/cage. For the evaluation of minimum weight threshold, C57BL/6NTac male mice at the MPF™ health standard were placed on D09100310 at 6 weeks of age at 5/cage. C57BL/6J mice were placed on D09100301 diet at 12 weeks of age at 3/cage. Control mice were held at the same density as the NASH mice for each experiment and were fed a chow diet. Staining for histopathology for the C57BL/6NTac and C57BL/6J groups was done at different times.

## DISCUSSION & CONCLUSIONS

Weight gain was not well correlated with starting weight and we saw large variability in weight gain overall. We noted that mice below 15 g at start of diet displayed poorer than average weight gain, and thus a 15 g minimum start weight was selected for future commercial production. For the comparison between substrains and diets, the experiments were not perfectly controlled as these were comparisons to historical data. Thus, between the two groups, subtrain age at diet start and diet varied, making it difficult to tease out the contributions from any one parameter. Nevertheless, the data is sufficient to determine whether C57BL/6NTac mice on the D09100310 diet are an appropriate model for study of NASH. C57BL/6NTac mice on D09100310 are somewhat heavier than C57BL/6J mice on D09100301 on diet for the same length of time; this substrain difference has been observed for response to other high fat diets[1]. Most other parameters measured were similar between the two groups, with some differences noted such as less variability in non-esterified fatty acid levels and, after 16 weeks on diet, higher transaminases, LDL, and triglycerides in the C57BL/6NTac group. Compared to chow controls, C57BL/6NTac mice on D09100310 had higher bodyweight and liver weight as a % of bodyweight, and much higher cholesterol and LDL. They are obese, with enlarged fatty livers. By 38 weeks on diet, they display steatosis and liver fibrosis by histopathology. These characteristics make C57BL/6NTac mice on the D09100310 diet an appropriate model for NASH research, as they display NASH pathology in the context of metabolic syndrome.

## ACKNOWLEDGEMENTS

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**Reference:** 1. Hayward, et al. P3-372 A comparison of metabolic characteristics among C57BL/6NTac, C57BL/6J and C57BL/6JBOM diet-induced obese mice with environmental conditioning. Endocrine Reviews Volume 32, Issue 3 Supplement, June 2011.