Diets for Non-Alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disorders propagated by excessive lipid accumulation in hepatocytes, ranging from simple steatosis, steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma (1; 2). The most common animal models to study NAFLD are mice and rats (3; 4) fed various diets including high-fat diets (HFD), and methionine and choline deficient diets (MCD). Different variations of these diets can alter the extent and timeframe of NAFLD/NASH development. The manifestation of other metabolic diseases such as insulin resistance (IR)/glucose intolerance are also greatly affected by the diet choice. In this brief review, we describe several commonly used diet-induced approaches for rodent models of NAFLD, including the timeframes required, extent of disease development, and whether development of other metabolic diseases (including IR) occur.

Methionine and Choline Deficient (MCD) Diets

Among the different approaches for diet-induced NAFLD in rodents, MCD diets produce the most severe NASH phenotype in the shortest timeframe. MCD diets are formulated with the replacement of whole protein (such as casein) in purified diets with crystalline amino acids and the removal of both methionine and choline. These diets typically induce measurable hepatic steatosis (mainly macrovesicular) in rodents by 2-4 weeks, and if fed longer, this can progress to inflammation and fibrosis (5; 6). Within the context of a MCD diet, other dietary components also affect the NASH phenotype, including the carbohydrate source. Both fructose and sucrose are more capable of driving hepatic inflammation and liver injury than glucose sources (dextrose or corn starch) in mice (7; 8). The type of fat such as those high in polyunsaturated fatty acids (PUFAs) like corn oil can increase liver fat oxidation and inflammation more than sources high in saturated fatty acids (SFAs) like coconut oil or beef tallow in mice (9). In addition, fats such as olive oil (high in monounsaturated fatty acids; MUFAs) reduced liver triglyceride (TG) accumulation while fish oil (high in omega-3 PUFAs) reduced liver cholesterol in rats relative to rats fed butter fat (high in SFAs) (10). In Wistar rats, the addition of 1% cholesterol to a low-methionine (0.1%), no choline HFD (A16092003, 45 kcal% fat, mainly lard) accelerated development of fibrosis (~6 weeks) (11). A similar effect of added cholesterol was found in the context of a MCD diet in C57BL/6 mice fed 12 weeks (12).

Rats and mice fed MCD diets lose weight due to a vastly lower caloric intake, (13; 14; 15) display cachexia, are insulin sensitive, and have low fasting serum insulin, glucose, leptin and TG levels (15). To counter this effect, Matsumoto et al. (16) used a 0.1% methionine and no choline HFD (60 kcal% fat, mainly lard; A06071302) in C57BL/6 and A/J mice and found that this diet helped to maintain energy intake and body weight while increasing levels of liver fat accumulation, inflammatory markers, AST and ALT within 6 weeks. Furthermore, this diet increased fibrosis in C57BL/6J mice at week 6, and in A/J mice at week 9. Similar results were also observed by Chiba et al. (17) who used a similar diet containing 45 kcal% fat (A06071309), in C57BL/6 mice. Despite rescuing the mice from weight loss, no glucose intolerance was observed by Chiba et al. (17).

Similar to rats and mice, MCD diets can drive significant steatosis and fibrosis in male F1B hamsters within 8 weeks. However, unlike rats and mice fed a similar diet, hamsters gained weight during the study and had elevated plasma TG compared to animals on a grain-based diet (18).

Choline Deficient (CD) Diets

Typically, CD diets used in fatty liver disease studies tend to contain higher levels of fat (45-60 kcal%) and these diets can induce steatosis, inflammation and fibrosis without the reduction in body weight typically found when feeding MCD diets (19), making CD diets more appealing to some researchers. However, a CD lard-based HFD (45 kcal% fat, D05010402), improved glucose tolerance compared to a choline sufficient (CS) HFD in C57BL/6 mice fed for 8 weeks, and the glucose levels were similar to a lower fat purified diet with or without choline. Still, animals on D05010402 showed elevations in both fasting insulin and TG levels (20) and when compared to a grain-based diet, impaired glucose tolerance if fed longer (6 months) (21), suggesting that timeframe is important. Additionally, this shows that the lack of an appropriate CS low-fat matched control diet group may alter data interpretation. The development of steatosis and steatohepatitis are commonly observed in a fairly short timeframe (6-8 weeks) in CD, HFD fed mice (20), but fibrosis in mice consuming CD diets tends to be very minimal unless the diet is fed for a long time period (6-12 months) (21).

CD diets induce NAFLD and fibrosis in Wistar rats as shown in the publication by Takeuchi-Yorimoto et. al. (22). However in rats, significant steatosis occurs only when choline deficiency is combined with a HFD (60 kcal% fat, D05010403) as the low-fat CD diet (10 kcal% fat, D05010401) failed to induce any significant steatosis (23). This is unlike mice where the same low-fat CD diet (D05010401) also increased liver fat accumulation relative to the CS diet (20). These results suggest that the mechanisms involved with liver fat accumulation on CD diets may be different from those at work during MCD diet feeding, as MCD diets with lower-fat levels (~20 kcal% fat) can also induce NASH/NAFLD (24). Compared to CD diets, MCD diets also produce a much stronger NASH phenotype. After feeding either a CD or MCD diet to Wistar rats for 7 weeks, the MCD diet dramatically increased scores of liver inflammation, steatosis, ALT and fibrosis, all of which were minimal in rats fed a CD diet, similar effect was also found in mice consuming MCD or CD diets for 15 days (25; 26). However, the CD diet fed rats gained weight, had IR and higher plasma lipids than the MCD diet group (26).

High-Fat Diets (HFD)

The term 'HFD' encompasses a wide variety of diet formulas and fat type and levels (30 – 60 kcal% fat), as well as other compositional differences such as low or high sucrose or different forms (i.e. pellet or liquid). D12492 (60 kcal% fat, mainly lard) is commonly used in research for driving obesity and other metabolic disturbances, and this diet can induce hepatic steatosis and liver damage (as assessed by plasma ALT levels) in C57BL/6 mice (27) and rats (28) after 8 weeks. If fed longer (16 weeks), D12492 can induce hepatic inflammation, but only very mild fibrosis in mice (29) and rats (30). Furthermore, significant fibrosis may require up to one year with this diet (31). Another study using a diet with slightly lower-fat levels (45 kcal%, D12451) showed induction of steatosis and steatohepatitis after 6 months in C57BL/6 mice (32). NAFLD in mice was worsened (mild fibrosis) by addition of sucrose as shown in a different study that compared animals on a HFD (36 kcal% fat as mainly milk-fat) with a HF, high-sucrose diet (36 kcal% fat, 30 kcal% sucrose) (33). The type of fat in the HFD formulation also plays a role as palm oil in 45 kcal% fat diet increased liver TG, body weight and reduced insulin sensitivity more rapidly than other fat sources (olive oil, safflower oil) in C57BL/6 mice (34). Hamsters also develop a NASH phenotype when fed the 45 kcal% fat diet (mainly lard, D12451); this diet increased liver and plasma lipids (total cholesterol and TG), hepatic inflammation and steatosis score (histology) in golden Syrian hamsters fed for 10 weeks relative to a 10 kcal% fat diet (D12450B) (35). In addition, hepatic inflammation, as measured by levels of inflammatory cytokines like MCP-1, TNF-α, IL-1β and IL-6, was elevated in the HFD group (35).

It is important to note that when fed for equal lengths of time, HFD feeding results in about 10-fold lower liver fat levels and a milder NASH phenotype compared to what accumulates on an MCD diet (36; 37), highlighting an important difference between these dietary regimes. However, an advantage of using a HFD is that these mice will have features of metabolic disease including weight gain, IR and/or glucose intolerance.

Diet	Rodent Model	Body Weight	Fasting Plasma Glucose/ Insulin	Steatosis	Steato Hepatitis	Fibrosis	Time Frame (Fibrosis) #	References
Methionine Choline Deficient Diet (MCD)	Rats and Mice	↓	↓	+	+	+	4-8 weeks	14; 26; 37; 48; 49
0.1% Methionine Choline Deficient High-Fat Diet (CDAHFD)	Mainly Mice	↓*	No Change	+	+	+	6-12 weeks	16; 17; 50
Choline Deficient Amino Acid Diet (CDAA)	Rats and Mice	No Change	↑ Mainly Mice	+	+	+	4-8 weeks (rats) 12weeks (mice)	22; 51; 52; 53
Choline Deficient High-Fat Diet (CD)	Mainly Mice	↑	↑	+	+	+	12 months	20; 21
High-Fat Diet (HFD)	Rats and Mice	↑	↑	+	+	+ (Mild)	24 weeks (rats) 16 weeks (mice)	29; 32; 54; 55
High Fructose Diet (HFR)	Mainly Rats	No Change	↑	+	+	+	12 weeks	39; 40
High-Fat, High Fructose, High-Cholesterol Diet	Rats and Mice	↑	↑	+	+	+ Mainly Mice	20-30 weeks (mice)	30; 43; 46; 47; 56

The length depends on diet formula, length of study, species, strain and gender of the animal model.
* Compared to low-fat, choline sufficient group. Body weight of these animals typically remain unchanged compared to baseline.

High-Fat, High-Fructose, High-Cholesterol Diets

Studies in C57BL/6 mice fed a HFD or high-fat, high-fructose (HFHFr) diet (58 Kcal% fat) with high-fructose corn syrup in water for 16 weeks showed that fructose consumption was necessary for the progression from liver fat deposition to fibrogenesis (38). Although weight gain, body fat, IR and liver steatosis were similar between the two groups, the combination of high fat and fructose increased hepatic oxidative stress, inflammation, fibrogenesis and collagen deposition more robustly in mice (38). However, Kawasaki et al. found that a HFr diet (73 kcal% fructose) increased hepatic TG more than a diet with a similar level of sucrose (½ glucose and ½ fructose), a HFD (40 kcal% fat) or a HFHFr diet (40 kcal% fat, 41 kcal% fructose in diet) in Wistar rats. In addition, the HFr diet also promoted macrovesicular and microvescicular steatosis and lobular inflammation (39). A HFr diet (70 kcal% fructose) also was shown to induce NAFLD/NASH in the SD rat model (40), including increased hepatic ballooning, fibrosis, and inflammation.

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The addition of 1% cholesterol in a 33 kcal% fat diet (HFHC) increased weight gain, hepatic lipid accumulation, serum ALT levels, and liver fibrosis compared to animals on a low-fat diet (11 kcal% fat), the HFD (33 kcal% fat) and the high-cholesterol diet (11 kcal% fat, 1% cholesterol) in mice (41). In golden Syrian hamsters, adding 0.25% cholesterol to a HFHFr diet (40% fructose, 30% fat, 6-22 weeks) increased liver TG and cholesterol levels (42). Addition of 0.25% cholesterol to the HFHFr diet also worsened glucose tolerance and insulin sensitivity in these animals relative to the group that contained 0.05% added cholesterol. These data clearly indicate that dietary cholesterol exacerbates the effect of dietary fat and fructose, and is a major determinant of the severity of metabolic disturbances in the hamster model, similar to rats and mice.

When C57BL/6 mice were fed a diet containing 40 kcal% fat (Primex shortening, a combination of palm oil and partially hydrogenated soybean oil, of which ~18% was trans-fat), 22% fructose, and 2% cholesterol (AMLN diet, D09100301), they developed three stages of NAFLD (steatosis, steatohepatitis with fibrosis, and cirrhosis), as assessed by histological and biochemical methods, in ~30 weeks (43; 44). Animals on this diet also demonstrated metabolic dysfunction (i.e. increased total cholesterol, fasting insulin, HOMA-IR and lower adiponectin). This diet has also been shown to induce NAFLD/NASH in SD rats similar to mice; however, in the rat model, the NAFLD/NASH development (steatosis, inflammation, ALT) seems to progress in a shorter timeframe (~16-20 weeks) (30; 45). As the FDA has now banned the use of shortenings containing trans-fat, current evidence suggests that replacing Primex with palm oil (D09100310) or non-trans-fat Primex (D16022301) in the AMLN diet drives a similar phenotype in C57BL/6 mice (46; 47). Furthermore, the non-trans-fat Primex may worsen IR in the animals, which to our understanding has not been well characterized in the AMLN diet (47). The ability of these diets to drive symptoms of metabolic disease and all stages of NASH in rodent models provides researchers with a more human-like model of disease development, and contract research organizations and breeders a robust commercially available NASH model for drug discovery.

In conclusion, a variety of different purified diets are available to drive NAFLD/NASH in rodent models and individual components of these diets can be selectively manipulated to 'fine-tune' the phenotype to varying degrees along the NAFLD spectrum. While some have limitations in representing humans with NAFLD, they are useful tools for studying the pathogenesis and progression of NAFLD/NASH, and uncovering potential treatment targets. Nevertheless, purified diets have been integral in helping researchers identify different mechanisms and study in detail the NAFLD/NASH spectrum in animal models.

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