



Fatty Liver Disease

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NAFLD (Non-Alcoholic Fatty Liver Disease)

A Brief Scientific Literature Review- December 2012

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NAFLD encompasses a spectrum of disease states, from steatosis (fatty liver) to non-alcoholic steatohepatitis (also called NASH; steatosis with inflammatory changes) followed by progression to fibrosis and cirrhosis and hepatocellular carcinoma (27). Excess liver fat is believed to be a manifestation of the metabolic syndrome (14) and not surprisingly NASH is associated with obesity, insulin resistance, dyslipidemia and type II diabetes in humans (2). Most obese adults have hepatic steatosis and at least one-third of these individuals will eventually develop worsening NAFLD (1, 3). Therefore, the prevalence of NAFLD will likely rise with obesity rates.

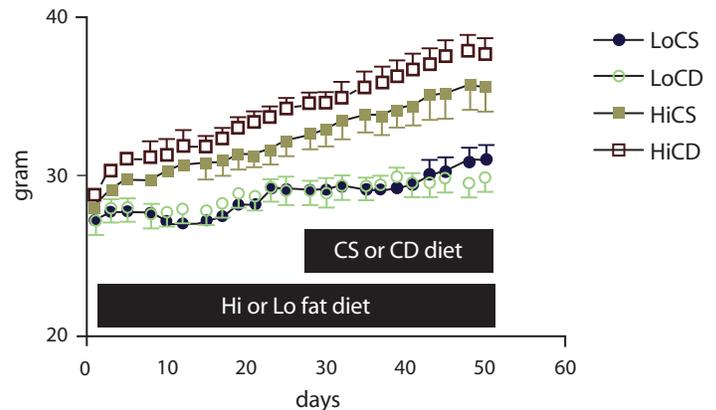
As with many human diseases, fatty liver in rodents is also diet-inducible. Here we briefly summarize three such dietary protocols for inducing fatty liver: a methionine and choline-deficient (MCD) diet, a choline-deficient diet (CD) or a high-fat diet (HFD). These terms do not define a specific diet formula and of course there is overlap in these diet types. Different dietary approaches produce different severities of disease along the NAFLD spectrum and likely work by unique mechanisms. Therefore it is important for the researcher to choose the diet that best suits the needs of their study.

MCD diets

Of the dietary approaches discussed here, MCD diets produce the most severe phenotype in the shortest timeframe. Used for over 40 years, MCD diets will quickly induce measurable hepatic steatosis (mainly macrovesicular) in rodents by 2-4 weeks and this progresses to inflammation and fibrosis shortly thereafter (22, 26). Fat levels in MCD diets can vary, though typically they contain about 20% fat by energy. The mechanism for steatosis includes impaired VLDL secretion due to lack of phosphatidyl choline synthesis (11). MCD diet-induced NASH is reversible in rats by switching to a diet with sufficient methionine and choline (15).

Importantly, unlike human or other diet-induced rodent models of NAFLD, rodents fed MCD diets lose weight (due to a vastly lower caloric intake) and do not become insulin resistant (9, 19). Since most humans with NASH are obese and insulin resistant, this represents an important difference in how MCD diets model human NASH.

Within the context of an MCD diet, other dietary components affect the NASH phenotype. Sucrose is an important component of the MCD diet, since replacing it with corn starch greatly reduces liver fat accumulation, inflammation and injury, likely through



Effects of high-fat diet and CDD on body weight. Twelve animals were fed a low-fat diet D12450B (10% calories as fat; Lo), and 12 were fed a high-fat diet D12451 (45% calories as fat; Hi) for 8 weeks. During the last 4 weeks, one-half of the animals in each group (n = 6) received a CDD (CD), whereas the other one-half (n = 6) continued on a diet supplemented with choline (CS). Data are expressed as means \pm SE. Total weight change was compared by two-way ANOVA with post hoc Tukey's test. Animals on a high-fat diet gained significantly more weight than those on a low-fat diet (HiCS vs. LoCS, $P = 0.001$). A CDD (CD) had no effect on weight gain. Graphic representation - for details see reference (15).

reductions in sucrose-induced de novo lipogenesis and triglyceride synthesis (17). In a follow-up study, Pickens et al. showed that despite inducing the same overall level of hepatic fat accumulation, fructose was more effective than glucose at inducing hepatocellular injury in mice fed MCD diets for 21 days (16). Not surprisingly, the source of dietary fat can also alter the phenotype. For example, relative to saturated fat, polyunsaturated fats increase liver fat oxidation, and induce expression of proinflammatory genes leading to inflammation though this does not necessarily correlate with increased liver damage (12). Also, relative to butter fat, olive oil reduced liver triglyceride accumulation while fish oil reduced liver cholesterol levels (8).

CD diets

Like MCD diets, CD diets also tend to contain higher levels of fat, though it is often difficult to know the specifics since unfortunately authors rarely publish the details of the diets. CD diets induce steatosis, inflammation and fibrosis over 10 weeks without any difference in body weight compared to the control group (6). This lack of weight loss makes CD diets more appealing to some researchers. When both CD and MCD diets were fed to rats for 7 weeks, the MCD diet group had higher scores of liver inflammation and steatosis than the CD group. However, the CD fed rats gained weight, were insulin resistant and had higher plasma lipids than the MCD group (25).

The mechanisms involved with liver fat accumulation on CD diets may be different from those at work during MCD diet feeding (11). Interestingly, choline deficiency in the context of a lard-based HFD (45% of calories) was shown to improve glucose tolerance compared to the choline sufficient group in mice (18).

High-fat diets (HFD)

HFD are well-known to increase body weight, body fat and induce insulin resistance in rodent models. HFD can also increase liver fat levels quite rapidly (within days) as well as hepatic insulin resistance before significant increases in peripheral fat deposition occur (23). Chronically, HFD-induced liver fat accumulation may not follow a linear progression and liver fat levels may actually decrease, then increase again during prolonged HFD feeding (7). When fed for equal lengths of time, HFD feeding results in 10-fold lower liver fat levels compared to what accumulates on an MCD diet (20). In general, HFD feeding does not produce liver fibrosis and only mild steatosis as compared to MCD diets (2), thus highlighting an important difference between these dietary regimes. It is important to remember that the term 'HFD' encompasses a wide variety of diet formulas and diets of different composition can be expected to alter the liver phenotype in various ways.

Cong et al. (5) took an interesting approach by modifying a very HFD (60% of calories) to simultaneously contain low levels (but not zero) of methionine and choline. C57BL/6 mice were fed the diet for 23 weeks and developed obesity, insulin resistance, dyslipidemia as well as liver steatosis, inflammation and fibrosis. While the study design is weakened by the lack of a high fat, choline and methionine replete control group, it seems plausible that longer-term feeding of HFDs containing lower than normal levels of methionine and choline allow for the development of NASH without the issues of weight loss. This idea of modifying so-called 'standard' HFDs is powerful since it allows the researcher to 'fine-tune' the phenotype to meet their needs.

Along these lines, in the LDLR (-/-) mouse, adding cholesterol to a HFD increases liver fat levels, signs of liver damage and produces macro- and microvesicular steatosis similar to that seen in human NASH, compared to HFD alone (24). Vitamin D deficiency within a HFD worsened NAFLD versus HFD alone in Sprague Dawley rats (21). When medium chain triglycerides replaced long chain triglycerides in a 35 kcal% fat diet, steatosis was prevented in rats (13). In C57BL/6 mice, animals consuming both HFD and fructose/sucrose enriched drinking water developed hepatic fibrosis while a group consuming HFD alone did not (10).

As with other diet diseases, it appears possible to influence liver disease using a HFD while mice are still in utero. Bruce et al. found that feeding a HFD to dams during gestation and lactation made offspring more susceptible to developing NASH when fed a HFD from weaning (4).



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