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Diets to induce metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH)

DIETARY FACTORS TO STUDY MASLD/MASH IN RODENT MODELS

The incidence of metabolic dysfunction-associated fatty liver disease (previously nonalcoholic fatty liver disease) is increasing with the global rise in the prevalence of metabolic syndrome and is the most common form of liver disease in the United States [1,2]. While the pathogenesis of simple steatosis to MASH and hepatic cirrhosis is not fully understood, evidence suggests progression from MASLD to MASH requires multiple "hits" or "insults" to the system from both a metabolic and hepatic standpoint [3,4]. However, the consensus is that there are three defining features of MASH which are described as steatosis, inflammation, and ballooning of hepatocyte cells.

Of those phenotypes, inflammation and ballooning are the drivers of MASH activity that lead to liver degradation, scarring, and more severe phenotypes like fibrosis and cirrhosis [5].

Animal models of MASLD and MASH are promising tools for researchers investigating the transition from simple steatosis to inflammatory, progressive fibrosis. Diet manipulation is an effective tool in producing rodent models of MASLD and MASH. Key dietary factors that determine the progression of MASLD from simple steatosis to MASH, fibrosis, cirrhosis, and hepatocellular carcinoma are summarized in the table below.

MASLD/MASH key dietary factors

DIETARY FEATURE	ROLE IN MASH/NASH DEVELOPMENT		REFERENCE(S)
	METABOLIC	HEPATIC	
Sucrose/Fructose	Obesity and metabolic syndrome	Promotes lipid synthesis and steatosis without fibrosis	4, 28-30, 41
High Fat	Obesity, dyslipidemia and metabolic syndrome	Induces steatosis	28,31
Trans-fat	Promotes insulin resistance	Promotes steatosis, inflammation and injury	10,32
Palmitic Acid/ Palm Oil	As part of a high fat diet: weight gain with metabolic syndrome	Induce hepatocyte lipid accumulation and pro-inflammatory cytokine production *in vitro*	33-35, 41, 42
Cholesterol	Hypercholesterolemia	Hepatic fat deposition and inflammation when fed in the context of a high fat diet	36,37
Cholate	Slows growth rates preventing the development of obesity and metabolic syndrome.	Enhances cholesterol absorption, inflammation, and fibrosis	25, 27, 36
Methionine	Deficiency causes weight loss. No development of metabolic syndrome.	Deficiency decreases S-adenosylmethionine (SAM) which limits glutathione, an important antioxidant, promoting inflammation and fibrosis. Limited SAM also impairs phosphyatidylcholine synthesis leading to lipid accumulation	28,38
Choline	Limited effects on growth rates with severe restriction. Choline deficient high fat diets can lead to obesity and metabolic syndrome.	Deficiency interrupts phosphyatidylcholine synthesis and normal methionine metabolism limiting hepatic fat export leading to lipid accumulation	38-40

MASLD is a progressive disease beginning with simple steatosis that can develop to MASH as soon as 12 weeks with a custom MASH inducing diet. Dietary methods to induce MASH, in rodents, can be split into two common categories: 1) diets fed for longer periods of time (>9 weeks) to induce obesity, metabolic syndrome, with mild MASH or 2) short-term (3-8 weeks) feeding strategies using nutrient deficient diets (for example methionine, choline) to induce hepatic features of MASH without inducing metabolic symptoms like obesity or insulin resistance.

When choosing dietary features to induce MASH pathologies, one must consider the animal model, time frame, and desired disease outcome. More complete descriptions of dietary MASH models are included on the following pages with example diets.

DIET OPTIONS FOR INDUCING OBESITY, METABOLIC SYNDROME, AND MILD MASH

GAN and AMLN diets with trans-fat or palm oil

Prior to the 2018 FDA ban on trans-fats, diets containing Primex shortening were used to reliably induce metabolic and liver histopathological changes in rodents. Following 2018, modifications to fat sources had to be made. The AMLN diet (uses a trans-fat containing hydrogenated vegetable oil very comparable to Primex; **TD.180939**), or a modified version known as the GAN diet (replaces trans-fat source with an equivalent amount of palm oil; **TD.200591**) contain ~40% kcal from fat (20% fat by weight) with high levels of simple sugars (22% fructose, 13% sucrose) and 2% cholesterol.

The GAN diet induces obesity, insulin resistance, hepatic inflammation, and steatosis in mice with disease characteristics that are considered highly comparable to human MASH phenotypes [6], with steatosis developing after 12 weeks of feeding. Severe MASH phenotypes, including hepatic ballooning and fibrosis, occur after 20 weeks of feeding [7-9]. In addition to feeding a GAN/AMLN diet, providing a glucose/fructose mixture in the drinking water is commonly cited in the literature, and may further promote MASH development [10-12].

GAN AND AMLN DIETS WITH TRANS-FAT OR PALM OIL	
TD.200591	40% Kcal Fat (Palm Oil, 2% Chol, 20% Kcal/Fruct)
TD.180939	40% Kcal Fat (HVO, 2% Chol, 20% Kcal/Fruct)

FPC diet: trans-fat, palmitate, cholesterol diet

FPC (trans-fat, palmitate, cholesterol) are popular MASH diets that include features of both the "Western" and American Lifestyle-Induced Obesity Syndrome (ALIOS) model diets to develop more severe metabolic and hepatic phenotypes of MASH including hepatic steatosis, inflammation, and fibrosis in an accelerated time frame. Key features of FPC diets like TD.160785.PWD and TD.190142 (pellet form) include high sucrose (~34% by weight), 1.25% added cholesterol and 52% kcal from fat with fat sources including milkfat fat, palmitic acid and hydrogenated vegetable shortening to provide trans-fats. FPC diets intentionally contain a lower methionine and choline content to exacerbate these phenotypes.

Like the GAN/AMLN model, FPC diets result in obesity after 14 weeks of feeding; more severe MASH phenotypes including fibrosis are seen after 16 weeks [13, 14]. In addition to feeding an FPC diet, providing a glucose/fructose mixture in the drinking water is commonly cited in the literature, and may further promote MASH development [10-12].

FPC (TRANS-FAT, PALMITATE, CHOLESTEROL) DIETS	
TD.160785.PWD	52 kcal/Fat Diet (C16:0, HVO, AMF, Choline/Met)
TD.190142 (pellet)	52 kcal/Fat Diet (C16:0, HVO, AMF, Choline/Met)

Western diets with saturated fats and added cholesterol

Western, or "fast food" diets are frequently used to develop MASH phenotypes with metabolic syndrome and obesity. Diets like **TD.120528** contain 40-45% kcal from milkfat (a fat source high in palmitate) with added cholesterol (primarily 1.25%) and high sucrose (>30%). Dietary palmitate and cholesterol have both previously been associated with the progression from simple steatosis to MASH [15]. **TD.02028** is a modification of the western diet with increased cholesterol and 0.5% cholic acid. Cholic acid enhances cholesterol absorption, exacerbating inflammation and fibrosis.

These diets can induce obesity, metabolic syndrome, and simple steatosis within 12 weeks of feeding [16, 17]. Increased hepatic inflammation, early fibrosis, and hepatic ballooning have been observed within 20 weeks of feeding [18].

In addition to feeding a western diet, providing a glucose/fructose mixture in the drinking water is commonly cited in the literature, and may further promote MASH development [10-12].

WESTERN DIETS WITH SATURATED FATS AND ADDED CHOLESTEROL	
TD.120528	42% Kcal/Fat Diet (Incr. Sucrose, 1.25% Chol.)
TD.96121	21% MF, 1.25% Chol. Diet
TD.02028	21% Milkfat (1.25% cholesterol, 0.5% cholic acid)

High Fat diets to induce uncomplicated MASLD

For MASLD only, similarly formulated diets with either no added or low-level cholesterol (i.e. ~0.2%) can be used; expect initial phenotype development in approximately 6 weeks of feeding. These high fat diets typically contain 40-60% kcal from fat.

Simple sugars, like sucrose or fructose, can be supplemented via diet or water to progress the fatty liver phenotype. Diets can be in pellet or powder/dough form depending on the formula.

High fat diets that include milkfat, lard, or hydrogenated vegetable shortening (HVO), with high sucrose (21-26% by weight), and the option of added cholesterol are associated with increased insulin resistance and hepatic inflammation. Commonly with these models, rodent activity in cages is limited by feeding diet in dishes within the cage and remove the feeding grid from the cage. For more information on diets to induce uncomplicated MASLD, contact us specifically on this topic.

HIGH FAT DIETS TO INDUCE SIMPLE FATTY LIVER DISEASE	
TD.08811	45%kcal Fat Diet (21% MF, 2% SBO)
TD.06414	Adjusted Calories Diet (60/Fat)
TD.88137	Adjusted Calories Diet (42% from fat)
TD.06303	22% HVO Diet
TD.120330	22% HVO + 0.2% Cholesterol Diet
TD.130885	ALIOS with Added Sugar

DIET OPTIONS FOR INDUCING MORE SEVERE HEPATIC MASH WITHOUT METABOLIC SYNDROME OR OBESITY

Methionine/choline deficient (MCD) diets

Expression of MASH phenotypes in a shorter time frame, without metabolic syndrome or obesity can be achieved with a methionine and choline devoid (MCD) approach. MCD diets are amino acid defined rodent diets deficient in methionine and choline, with high sucrose (>40% by weight) and ~10% corn oil by weight. The polyunsaturated fat in corn oil promotes hepatic lipid oxidation while dietary sucrose is necessary for hepatic lipid accumulation and oxidation [19, 20]. Methionine and choline deficiency decreases fat oxidation and export of fat from the liver. Expect that animals will lose weight (up to 40%) on this type of diet, so careful monitoring is recommended [19, 21].

Short-term feeding of MCD diet results in steatosis, increased serum alanine aminotransferase (ALT), inflammation, and hepatic fat oxidation within 3 weeks with fibrosis development reported after 6 weeks of feeding [22-24]. Modifications of our MCD diets with adjusted methionine, choline, cholesterol and/or fat levels are available upon request.

To mitigate weight loss seen with the methionine and choline deficient diet, a high fat diet (60% kcal from fat) with reduced methionine (0.1%) and no choline can maintain animal body weight without metabolic syndrome (CDAA Diet). Feeding the CDAA results in insulin-resistance, inflammation, increased ALT and TD, upregulated cytokine production, hepatic steatosis, and ballooning within 12 weeks [25, 26]

METHIONINE AND CHOLINE DEFICIENT DIETS	
TD.90262	Methionine/Choline Deficient Diet
TD.240263	CDAA Diet 0.1% Met (60/Fat, Lard, O)

Control Diets

The choice of control diet is dependent on your specific research goal. Many researchers choose to compare their MASLD/MASH diet-fed animals to animals fed a natural ingredient, grain-based diet (also referred to as standard diet or chow). These diets differ in the source and level of nutrients as well as in the presence of non-nutritive factors.

Depending on what your main comparisons are, it may be suitable to have a grain-based diet as your control/reference group. However, making such comparisons limits inferences to dietary patterns versus a specific dietary component. In some cases, such as those studies feeding amino acid defined diets like the MCD model, a matched control diet is recommended given the very different formulations and protein sources of grain-based diets.

When making inferences about specific nutrients within the diet an ingredient matched, low fat control diet may be necessary. There are many options with different levels and types of fat in addition to different types of carbohydrate ranging from sucrose (highly refined and digestible) to corn starch (refined, but more complex) to resistant starch (refined, but not fully digestible). A very basic purified control diet would be AIN-93M (TD.94048) or AIN-93G (TD.94045). AIN-93 diets have a moderate amount of sucrose at ~10% with fat from soybean oil providing a healthy fatty acid profile.

Emerging MASH models

Dietary models of MASLD/MASH continue to evolve with the goal of more accurately recapitulating both the metabolic and hepatic symptoms of human disease. A Teklad nutritionist can work with you to formulate new diets to investigate novel dietary models of MASLD/MASH. To speak with a specialist, please reach out to us via email (askanutritionist@inotiv.com) or our LiveChat feature on our MASH/NASH webpage.

Contact a nutritionist at
askanutritionist@inotiv.com
for additional information, control diet recommendations or for a diet consultation.

COMPARISON OF LONG-TERM FEEDING STRATEGIES TO INDUCE MASLD

	GAN/AMLN		FPC		WESTERN	
	TD.200591	TD.180939	TD.160785.PWD	TD.190142	TD.120528	TD.96121
PROTEIN SOURCES (%)						
Casein	20.0	20.0		14.0	19.5	19.5
Casein, "Vitamin-Free" Test			14.0			
Supplemental Sulfur	0.3	0.3			0.3	0.3
CHO SOURCES (%)						
Fructose	22.5	22.5				
Sucrose	12.5	12.5	34.0	34.0	40.5	34.0
Corn Starch						15.0
Maltodextrin	12.5	13.0	11.9	11.9	7.5	
FAT SOURCES (%)						
Hydrogenated Vegetable Oil		15.5	19.0	19.0		
Palm Oil	15.5					
Soybean Oil	2.5	2.5				
Lard	2.0	2.0				
Anhydrous Milkfat			6.0	6.0	21.0	21.0
Palmitic Acid			4.0	4.0		
Cholesterol	2.0	2.0	1.25	1.25	1.25	1.25
Kcal/g	**4.5**	**4.5**	**5.0**	**5.0**	**4.5**	**4.5**

KEY CONSIDERATIONS WHEN CHOOSING A RODENT DIET TO INDUCE MASLD/MASH

Diets to induce obesity, metabolic syndrome and mild MASH:

- Require long term feeding (3-12 months) to induce mild fibrosis
- Often add a glucose/fructose solution to the drinking water and promote sedentary behavior by removing overhead cage feeders

Diets to induce more severe hepatic MASH:

- Often do not recapitulate metabolic symptoms associated with MASH such as obesity or insulin resistance. Some models can induce weight loss
- Commonly fed for 3–12 weeks to induce hepatic inflammation and early fibrosis

Dietary MASH models **continue to evolve** with the goal of recapitulating both metabolic and hepatic symptoms common to human disease. New diets can be formulated in order to investigate novel dietary models of MASLD/MASH.

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