Am J Physiol Heart Circ Physiol 329: H974–H988, 2025.
First published September 6, 2025; doi:10.1152/ajpheart.00359.2025

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
HEART AND CIRCULATORY PHYSIOLOGY

REVIEW

Guidelines in Cardiovascular Research

Guidelines for diet-induced models of cardiometabolic syndrome

Timothy M. Sveeggen,[1] Pooneh Bagher,[1] Hiroe Toba,[2] Merry L. Lindsey,[3] Rebecca H. Ritchie,[4] Verónica J. Miksztowicz,[5,6] and Germán E. González[6,7]

[1]*Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska, United States;* [2]*Laboratory of Clinical Pharmacology, Division of Pathological Sciences, Kyoto Pharmaceutical University, Kyoto, Japan;* [3]*Department of Biomedical Sciences, School of Graduate Studies, Meharry Medical College, Nashville, TN and Research Service, Nashville VA Medical Center, Nashville, Tennessee, United States;* [4]*Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia;* [5]*Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Bioquímica General y Bucal, Buenos Aires, Argentina;* [6]*Laboratorio de Patología Cardiovascular Experimental e Hipertensión Arterial, Instituto de Investigaciones Biomédicas (UCA-CONICET), Facultad de Ciencias Médicas, Universidad Católica Argentina, Buenos Aires, Argentina; and* [7]*Departamento de Patología, Instituto de Salud Comunitaria, Universidad Nacional de Hurlingham, Buenos Aires, Argentina*

Abstract

Cardiometabolic syndrome (CMS) encompasses a cluster of metabolic abnormalities, including obesity, insulin resistance, dyslipidemia, and hypertension, that collectively increase the risk of cardiovascular disease and type 2 diabetes. Animal models are widely used to study CMS, with diet-induced models being the most physiologically relevant. A lack of reporting standards and variability in dietary composition, feeding duration, and macronutrient content across studies hinder reproducibility assessment and translational impact evaluation. We provide standardized guidelines for the consideration, preparation, and use of diets in experimental models of CMS. Advantages and limitations of different diet-induced models are compared with genetic and pharmacological approaches, emphasizing replicability to human metabolic dysregulation. Key components include macronutrient composition, fat source selection, feeding duration, strain susceptibility, and sex differences. In addition, we outline husbandry practices, including environmental factors, bedding material, and enrichment conditions, which can affect metabolic outcomes. Standardization is essential for enhancing experimental reproducibility and facilitating cross-study comparisons. These guidelines serve as a framework for researchers to optimize dietary interventions in CMS models, improving the predictive value of preclinical findings for translational applications to aid in the development of targeted therapeutic strategies.

cardiometabolic syndrome; cardiorenal; high-fat diet; husbandry; nutrition

INTRODUCTION

Cardiometabolic syndrome (CMS) is defined as a group of interrelated metabolic disorders that collectively increase the risk of cardiovascular disease and type 2 diabetes mellitus (T2DM). Clinical manifestations of CMS include obesity, insulin resistance and glucose intolerance, dyslipidemia, atherosclerosis, hypertension, and a systemic proinflammatory state (1). CMS is defined by the simultaneous occurrence of at least three of the following conditions that increase the risk of adverse cardiovascular outcomes: abdominal obesity, elevated plasma glucose and blood pressure, raised circulating triglycerides, or reduced high-density lipoprotein (HDL) cholesterol levels (1, 2).

CMS is reproduced in animal models through genetic manipulation (3, 4), pharmacologically induced (i.e., toxin) models (5–7), and diet-induced models (8–10). Although genetic strategies have been a key instrument for understanding CMS, there are important limitations in reproducing the multifactorial nature of human CMS. Pharmacologically induced models of CMS in rodents often follow a different time course and etiology to that evident in humans (5–7). Diet-induced strategies more faithfully replicate the contributing dietary patterns of humans and more closely mimic the human syndrome. Table 1 provides a summary of the metabolic features of rodents compared with humans (11–14).

Despite widespread use of diet-induced models in studying CMS, there is a lack of standardized dietary protocols. The literature describing diet and outcomes is scarce and with variable composition across diets used, there is a limited understanding regarding mechanisms associated with CMS. Variations in macronutrient composition, fat sources,

Correspondence: G. E. González (germangonzalez@uca.edu.ar).
Submitted 19 May 2025 / Revised 16 June 2025 / Accepted 26 August 2025

Table 1. *Comparative metabolic features between rodents and humans*

Feature	Rodents	Humans	Research Implications
Basal metabolic rate (11)	∼8 times higher	Lower	Alters energy expenditure and drug pharmacokinetics
Protein turnover (12)	∼10 times faster	Slower	Affects tissue remodeling and treatment response
BA/cholesterol (13)	High BA synthesis, low LDL	Lower BA synthesis, high LDL	Impacts lipid-related CMS and atherosclerosis
Fasted lactate-glucose metabolism (14)	∼2 times more than glucose	Similar	Uncoupling of carbohydrate-driven mitochondrial energy generation from glycolysis

BA, bile acid.

and feeding durations across studies hinder reproducibility, making it challenging to compare findings and draw definitive conclusions, which limit the translational relevance of these models. To address this gap, we propose a set of standardized dietary guidelines to enhance the consistency, reliability, and applicability of diet-induced rodent models in preclinical research, focusing on models of obesity, diabetic cardiomyopathy, and hypertension.

Here, we provide comprehensive guidelines specifically for the preparation and use of dietary interventions in experimental models. There are several animal models for inducing CMS, and these guidelines focus on optimizing diet composition, animal model selection, and husbandry conditions to ensure reproducibility and translational relevance in rodents. Standardizing experimental protocols will facilitate comparisons across studies, improve the predictive value of preclinical models, and ultimately contribute to the development of more effective therapeutic strategies for CMS. In the following sections, we outline specific dietary formulations, methodological considerations, and best practices for husbandry conditions, providing a comprehensive framework for researchers seeking to optimize diet-induced models.

MODELS OF DIET-INDUCED OBESITY

Obesity is a multifactorial chronic disease recognized as a worldwide health problem (15). The main characteristic of obesity is expansion of adipose tissue, which is an independent risk factor for metabolic and cardiovascular diseases including dyslipidemia, diabetes mellitus, atherosclerosis, and cardiac steatosis (16). Adipose tissue has multiple functions, including storage of triglycerides as an energy reserve when intake exceeds expenditure (17). Once fat levels exceed adipocyte storage capacity, neutral lipids are released and accumulate in other cells and tissues including the heart.

The presence of lipid inclusions within cardiomyocytes is called cardiac steatosis and is associated with both obesity and diabetes mellitus. Deposition of intracellular ectopic fat can lead to metabolic cardiomyopathy and heart failure (18). Lipid accumulation in the myocardium is a result of an imbalance between uptake and utilization of free fatty acids. Excessive uptake of free fatty acids activates their oxidation and induces lipotoxicity leading to cardiac dysfunction (19). When the oxidative capacity of myocardial mitochondria is exceeded, cardiac physiology changes include an increase in left ventricle (LV) mass and load, a decrease in septal wall thickening, and a decline in diastolic function. In cardiac steatosis, different lipotoxic intermediates such as diacylglycerol and ceramides accumulate within cardiomyocytes (20). Toxic metabolite accumulation within cells may result in inflammation, reactive oxygen species generation, altered Ca^{2+} signaling, and mitochondrial failure.

In cardiac steatosis, the high-fat diet (HFD)-induced obesity model is commonly used (21–24). When using the HFD model, critical considerations include the duration of high-fat feeding and the diet composition. Typically, the duration of diet ranges from 15 wk to as long as 1 year. Commercial rodent diets usually contains ∼10% fat, with a fat composition of 42%–60% considered HFD for rodents (25). To accelerate the onset of obesity and its associated metabolic consequences, animals can be fed a diet containing 60% fat (26).

Table 2 provides a comparative list of HFD dietary formulas, including content and percentages of the three main fatty acid types, source, duration of diet, animal strain, and a summary of the study results (31). A combination of high-fat diet and sugar-enriched drinking water is a widely used and effective strategy for inducing CMS in rodent models. Wistar rats develop obesity, insulin resistance, dyslipidemia, and hypertension when fed high-fat chow and 30% sucrose water for 26 wk, whereas C57BL/6J mice responded more strongly to dietary fat alone (27). The addition of 5% sucrose to a high-fat diet significantly worsened hepatic steatosis and increased liver triglyceride levels in rats fed for 90 days (28). Prolonged intake for 48 wk of a high-fat/high-sucrose diet induced endotoxemia and intestinal permeability, linking gut-derived inflammation to CMS (29). Glucose-enriched water in combination with high-fat feeding for 20 wk caused greater oxidative stress and insulin resistance than fructose, underscoring the importance of sugar type in metabolic outcomes (30). Together, these findings support the robustness of combined high-fat diet with enriched water, and that species-specific responses and duration of exposure should be carefully considered in experimental design.

The most significant difference among HFDs is the varying content of the three main fatty acids types: saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Dietary fatty acids can influence obesity grade, as diets with greater saturated fat content, including lard and palm oil, produce adipose tissue and liver inflammation and increase oxidative stress and alter the gut microbiome (25, 32). Because lard is a typical animal source of fat and often used in human diets, it is frequently used in HFD experiments. Consuming saturated fats in excess has a negative impact on a variety of organs, including the heart.

The development of cardiac steatosis induced by diet depends on the rodent model strain and sex. Since certain strains of mice are resistant to diet-induced obesity, selecting the right strain is critical for obtaining reproducible results. Different strains of mice show different phenotypic characteristics in growth rate, tendency of obesity, and ability to regulate blood glucose and lipids. For C57BL/6J mice,

Table 2. *Summary of high fat diet formulations and experimental outcomes in rodent models*

Diet	Fat, %	Fatty Acid Profile	Fat Source(s)	Strain/Species	Feeding Duration, Weeks	Outcomes
High fat + sucrose (27)	30%	Saturated fatty acids + monounsaturated fatty acids	Lard + 10% sucrose	Wistar rats and C57BL/6J mice	26	Metabolic syndrome: obesity, dyslipidemia, glucose intolerance
High fat + high sucrose (28)	60%	Predominantly saturated fatty acids	Lard + 10% sucrose	C57BL/6J mice	23	Non-alcoholic fatty liver disease with metabolic syndrome traits
High fat + high sucrose (29)	25%	Unspecified	Animal fat + 65% sucrose	Sprague-Dawley rats	48	Intestinal endotoxemia, insulin resistance, obesity, dyslipidemia, hyperglycemia
High fat + high glucose (30)	11%	Soybean oil, lard	Lard + 10% glucose	Wistar rats	20	Insulin resistance, dyslipidemia, increased adiposity
High fat (24, 31)	18%	Lard, high oleic sunflower oil	16% lard + 2% high oleic sunflower oil	C57BL/6J mice, Wistar rats	14–20	Dyslipidemia, hypercholesterolemia

only a proportion of mice develop significant obesity in response to HFD, with males more susceptible than females (33). Outbred mice from China (Kunming) and Switzerland [Institute of Cancer Research mice (ICR)] gain more body weight than popular inbred strains (C57BL/6 and BALB/c) when fed the same HFD, with all strains developing visceral obesity and steatosis (34, 35).

The most common rat strains used for research include Sprague-Dawley (SD) and Wistar rats (36, 37). SD rats develop metabolic dysfunction-associated steatotic liver disease and dyslipidemia under a high-fat diet for 12 wk, even without obesity, indicating a strong metabolic response to dietary fat (36). SD rats exhibited more severe dyslipidemia and cardiac pathology compared with Wistar rats fed for 4 wk, indicating strain-specific susceptibility to high-fat diet-induced metabolic and cardiovascular alterations (37).

Although biological sex differences significantly influence the development of obesity and associated cardiovascular remodeling in animal models, there is a critical gap in the literature. Most studies were historically performed in male rodents, leaving female-specific data underrepresented. Both male and female animals undergo cardiovascular remodeling after HFD exposure; however, the mechanisms and cardiac manifestations differ between sexes. For instance, male C57BL/6J mice develop heart hypertrophy and intramyocardial artery thickening after 12 wk on a high-fat, high-sucrose, and high-salt diet, whereas females exhibit increased adiposity without significant cardiac hypertrophy (38). Male Wistar rats display increased heart weight relative to tibia length following a high-fat, high-sucrose diet, whereas females show more pronounced glucose intolerance and elevated cholesterol levels (39). Male mice develop systolic and diastolic dysfunction after 4 mo on a high-fat, high-sucrose diet, whereas females maintain normal cardiac function despite similar metabolic challenges (40). These studies underscore the importance of including both sexes in research to fully understand sex-specific responses to dietary-induced cardiometabolic stress (1, 41).

Table 3. *Commercial vs. noncommercial diets*

Commercial Diet	Noncommercial Diet
Higher cost/weight of feed	Cost effective
Less comparable to human diet	More translational
Ready to use	Needs to be prepared

When selecting HFD for cardiac steatosis, the first consideration is whether to use commercial or noncommercial diets. Commercial HFDs are typically formulated by replacing calories from a carbohydrate source with calories from a fat source (42). Although commercial diets have the advantages of being more reliable in composition and quality of preparation, different HFDs from different companies will differ in fat percentages and fat sources. Furthermore, commercial animal diets do not exactly reproduce the diets consumed by humans and can be expensive. Noncommercial diets have some advantages in that they may better reproduce CMS observed in humans and can be produced at a lower cost. A comparison list of diet source is provided in Table 3. Although commercial diets provide reproducibility and quality control, their composition often diverges from human dietary patterns. In contrast, noncommercial diets allow greater flexibility to mimic human dietary composition but introduce variability in preparation and nutrient composition, which may hinder interstudy reproducibility.

Guidelines for HFD preparation in rodents are provided in Table 4. In brief, standard rodent chow pellets are powdered and mixed with the selected fat and reformulated using unflavored gelatin (26). The addition of lard to prepare a

Table 4. *Guidelines for preparing high-fat diets for use in rodents*

1) Weigh the food pellet and grind to powder using a crusher machine (e.g., Atma Corp). It is important that the pellet come from the same brand and batch throughout the experiment to reduce variability. It is common for chow to vary in ingredients from one batch to another
2) Cut, weigh, and melt the source of saturated fat (e.g., lard) in the microwave and add to the ground pellet at 45%–60% final concentration
3) Measure the source of unsaturated fat (i.e., high oleic sunflower oil) and add it to the mixture
4) Dissolve unflavored gelatine in hot water and add it to the mixture. Knead until uniformly mixed
5) Roll out the dough on a tray, cutting it into pieces similar in size to the original pellet. Pack the diet in sealed bags and freeze at −20°C for storage for until 1 mo

To maintain the integrity of the diet, it should be prepared weekly. Diet for the control group(s) should be prepared under the same conditions, without the additional fats.

45%–60% HFD is the recommended experimental diet to study cardiac steatosis.

DIETARY AND SEX DIFFERENCES IN THE MODULATION OF THE GUT MICROBIOME AND IMPLICATIONS FOR CARDIOMETABOLIC HEALTH

Emerging evidence highlights the gastrointestinal tract as a critical interface through which dietary components exert systemic effects, particularly via modulation of the gut microbiota. High-fat and high-sugar diets are known to induce dysbiosis, increase intestinal permeability, and promote endotoxemia, thereby triggering low-grade inflammation that contributes to CMS through gut-liver and gut-heart axis signaling (43). Diet and the gut microbiota are closely intertwined, with microbial metabolites emerging as key regulators in CMS. Short-chain fatty acids produced from dietary fiber fermentation improve gut barrier integrity, modulate glucose and lipid metabolism, and attenuate inflammation, thereby offering protective effects against metabolic and cardiovascular dysfunction (44). Therefore, understanding the bidirectional interactions between dietary patterns and gut microbiota is essential for elucidating the mechanisms by which nutrition impacts cardiometabolic risk.

Following nutrient absorption in the gastrointestinal tract, dietary components and gut-derived metabolites are transported through the portal vein to the liver, making the liver the first organ exposed to postprandial metabolic signals. This anatomical and physiological positioning places the liver at the core of nutrient sensing and metabolic regulation. Metabolic dysfunction-associated steatotic liver disease, formerly known as nonalcoholic fatty liver disease, encompasses hepatic fat accumulation linked to metabolic disturbances and may progress to metabolic dysfunction-associated steatohepatitis, a state marked by inflammation, hepatocellular injury, and fibrosis (45). The progression from dysfunction to steatohepatitis is influenced by dietary excess, insulin resistance, and gut microbiota dysbiosis, all of which are common features in CMS (46). As the liver integrates gut-derived and dietary signals before systemic distribution, its role is central in the development of CMS and associated cardiovascular complications.

Sexual dimorphism significantly influences metabolic outcomes in rodent models, largely mediated by estrogen and estrous cycle dynamics. The ∼4-day estrous cycle in female rodents yields fluctuating levels of estrogen and progesterone, which modulate insulin sensitivity, lipid metabolism, and energy expenditure (47). Estrogen exerts protective effects on adipose tissue by preserving insulin signaling pathways and reducing inflammation during metabolic stress, such as high-fat diet exposure (48). In addition, female mice exhibit greater mitochondrial respiratory capacity in brown and subcutaneous adipose tissue compared to male mice, which may contribute to enhanced metabolic flexibility and resilience to diet-induced dysfunction (49). Although estrous synchronization can reduce interindividual variability in metabolic readouts, current consensus suggests that documenting estrous stage without synchronization is preferable, as cycle-induced variability reflects physiologically relevant differences rather than confounding noise (50). Therefore, the inclusion of both sexes with careful cycle tracking is encouraged to improve translational relevance in the development of CMS.

TOXIN- AND GENETIC-BASED MODELS OF DIABETIC CARDIOMYOPATHY

Two comprehensive reviews evaluating the available preclinical models of diabetic cardiomyopathy were independently published in 2022 (6, 7). Both predominantly focus on animal models of diabetic cardiomyopathy and provide guidance to preclinical discovery scientists regarding the best choice of model for studies. The extensive array of models available spans the range from spontaneously occurring diabetes that result from inherited mutations to those induced by exogenous manipulation, such as diet, toxins, or planned genetic modifications. Heather et al. (6) emphasized available models of T2DM, particularly in small laboratory animals. They also reviewed in vitro cell-based models and large animal models of the disease (6). Prakoso et al. (7) produced a broad overview of in vivo animal (primarily rodent) models of both type 1 diabetes mellitus (T1DM) and T2DM.

The most appropriate choice of diabetic model for studying cardiovascular complications of the disease depends on which aspects of the human condition the research team is targeting. Furthermore, the appropriate model can depend on if the goal is to obtain understanding of the mechanisms behind the disease or as part of a drug discovery for its cardiovascular complications. A summary of the more commonly used rodent models of T1DM and T2DM is provided in Table 5 (51–55).

Models of Diabetic Cardiomyopathy in T1DM

The two most widely used models of T1DM cardiomyopathy are nondiet-induced models: *1*) the streptozotocin (STZ)-induced T1DM rodent and *2*) the Ins2Akita diabetic mouse model (Akita mouse). To our knowledge, no diet-induced models specifically of T1D-induced cardiomyopathy have been described. STZ is an effective diabetogenic agent in both mice and rats (and potentially even in pigs and nonhuman primates). Originally developed as an antibiotic, STZ readily enters pancreatic β-islet cells via the glucose transporter GLUT2, owing to its structural similarity to glucose (56, 57). The result is loss of β-cells with subsequent deficiency of insulin and resultant marked hyperglycemia (58). A range of different combinations of dose and frequency have been reported for inducing T1DM, ranging from a single high-dose injection (up to 200 mg/kg) and small accumulative doses (56, 59–61).

The favored approach for inducing T1D-induced cardiomyopathy is that described by the Animal Models of Diabetic Complications Consortium (AMDCC), in which five sequential daily injections of lower-dose STZ are administered (50 mg/kg ip over 5 days in mice) (62). This achieves a robust, reproducible phenotype of diabetic cardiomyopathy with minimal animal loss (63–70). In contrast, a single dose of STZ (55 mg/kg iv) is sufficient to induce a reproducible T1DM cardiomyopathy phenotype in rats (51). The phenotype of cardiomyopathy evident in the STZ-induced T1DM rodent is commonly characterized by left ventricular diastolic dysfunction (readily

Table 5. *Rodent models of diabetic cardiomyopathy*

Model	Animal	Diabetes Type	Key Features
Toxin-induced (STZ only) (51)	Sprague-Dawley rats	1	Hyperglycemia, cardiac fibrosis, LV dysfunction
Toxin-induced (STZ only) (52)	Wistar rats	1	Myocardial apoptosis, diastolic dysfunction
Genetic (db/db mice) (53, 54)	C57BL/KsJ-db/db mice	2	Obesity, myocardial lipid accumulation, systemic fat mass, cardiac hypertrophy, fibrosis, and lipotoxicity; diastolic dysfunction
HFD + low-dose STZ (55)	Sprague-Dawley rats	2	Hyperglycemia, insulin resistance, LV hypertrophy, fibrosis, and diastolic dysfunction

db, diabetes; STZ, streptozotocin.

detected using Doppler flow echocardiography, tissue Doppler and cardiac catheterization, and Doppler echocardiography) (51, 63, 65, 71). LV systolic dysfunction, detected using cardiac imaging or catheterization, often develops later in the progression of T1DM (72–75). The phenotype is consistent with observed features evident in the human heart in both T1DM and T2DM. This, together with its reproducibility and relative ease of use (particularly suited to use in transgenic and knockout animals), accounts for its broad popularity as the model of choice when studying diabetic cardiomyopathy and its management (64).

Alloxan, similar to STZ, can also be used to induce T1DM in both small and large animal models. Alloxan has a similar mechanism to STZ, is also amenable to GLUT2-mediated uptake by β-cells (56), and has a similar phenotype of cardiac dysfunction (21, 76–84). Alloxan has a more limited range of administration to achieve T1DM and is extremely toxic, contributing to its less common use in discovery research (7, 56).

The Akita mouse model of T1DM is favored by researchers who wish to avoid the use of a toxin to study cardiovascular complications of the disease. T1DM results from loss of pancreatic β-islet cells secondary to a single base-pair mutation in Ins2 (85, 86). Hyperglycemia is evident from 5 wk of age, with dyslipidemia (86) and LV diastolic dysfunction preceding systolic dysfunction (87–93). The Akita mouse may show absent or inconsistent cardiac mitochondrial oxidative stress (88), and female mice do not exhibit a hyperglycemia into adulthood (93). Finally, although the spontaneous autoimmune nonobese diabetic (NOD) mouse model of T1DM develops diabetes analogous to that in humans (94, 95), its cardiomyopathy phenotype has been less well studied (96).

Of note, all the abovementioned animal models of T1DM have predominantly been studied in males. Historically this was due, at least in part, to the milder elevation in blood glucose levels seen in female rodents following STZ administration. Despite this, females are more susceptible than males to impaired diastolic function (97), establishing the suitability of this model for research studies in diabetic cardiomyopathy in females.

Models of Diabetic Cardiomyopathy in T2DM

Commonly used animal models of T2DM include spontaneous genetic mutation models of T2DM [e.g., db/db mouse and Zucker diabetic fatty (ZDF) rat] and models combining diet with exogenous STZ. Although dietary models of T1DM do not exist, genetic and nongenetic models can be coupled with dietary interventions, resulting in models of T2DM, which exhibit varying degrees of obesity, ranging from modest to marked.

The db/db mouse develops obesity and T2DM (with marked hyperglycemia) as a result of a spontaneous leptin receptor mutation impairing leptin activity (98). Cardiac remodeling and dysfunction are clearly evident in db/db mice (5, 87, 99, 100), with female mice importantly exhibiting at least the same levels of both hyperglycemia and cardiac dysfunction as males (69, 99, 101). Many consider this the animal model of choice for T2DM cardiomyopathy (6, 7).

The ZDF rat develops obesity and T2DM to a similar extent as the db/db mouse, again a result of a spontaneous mutation in leptin receptors (102, 103). The ZDF has impaired cardiac contractility and concomitant cardiac remodeling (90, 104–106). The more restricted availability of the ZDF rat likely accounts for its more limited use in cardiac research relative to the db/db mouse.

The lesser-known New Zealand Obese inbred mouse develops a similar systemic phenotype to both the db/db mouse and ZDF rat, exhibiting obesity, insulin resistance, and dyslipidemia with cardiac contractile dysfunction in both male and female mice (107, 108), warranting a broader use of the New Zealand Obese mouse model in research studies in diabetic cardiomyopathy.

In contrast, the ob/ob mouse develops metabolic syndrome due to the spontaneous obesity (ob) gene mutation (21, 109, 110). The ob/ob mouse is less useful as a model of diabetic cardiomyopathy than the db/db or New Zealand Obese mouse models and the ZDF rat, as its cardiac phenotype is relatively modest (6, 7, 54, 111). Other useful nongenetic animal models of T2DM invariably combine HFD, commonly ranging from 42% to 60% of energy derived from lipids with a milder course of STZ than used in studies of T1DM. Without the addition of STZ, rodent diets high in fat develop metabolic syndrome (112), but with a more modest and often variable cardiac phenotype, particularly in mice (113) and similar to the ob/ob mouse (6, 7).

The addition of STZ (or high dietary sugars) to models of diet-induced obesity overcomes the mildness of the observed cardiac phenotype, leading to the desired development of LV diastolic and systolic dysfunction (114–118). Frequency of STZ doses in these studies ranges from a single dose, to two or even three doses of STZ. The most reproducible (and most T2DM cardiomyopathy-like) of these is likely that described by Ussher and colleagues (119, 120). Their model in mice uses 60% energy from lipids for 10 wk with a single intraperitoneal STZ injection (75 mg/kg) at 4 wk. This model has been adopted by other groups as well (121).

The combination of HFD with five STZ doses induces hyperglycemia and impaired glucose and insulin tolerance with resultant LV systolic dysfunction (122). Of note, body weight and LV diastolic function data were not reported. A similar model in rats placed on a diet comprising 60% of energy from fat (high fat) supplemented with high sucrose for 2 wk, at which point rats are administered two low-dose STZ (35 mg/kg ip) over consecutive days (123). Both exhibit LV dysfunction and adverse cardiac remodeling and are potentially useful models for research targeting diabetes-induced cardiomyopathy. These models combining diet and STZ predominantly use male animals only; disappointingly, they remain largely uncharacterized in female animals, limiting their broader utility.

Table 6 summarizes the strengths and limitations of rodent models used in the study of dietary interventions in diabetes. We have focused on the most used rodent models of diabetes. For a more exhaustive list, we refer the reader to three comprehensive reviews (5–7), which also provide more expansive details and consider larger animal models of disease. For cell-based models of diabetic cardiomyopathy, particularly those based on human-induced pluripotent stem cells (iPSC)-derived cardiac two-dimensional (2-D) and 3-D preparations, the reader is referred to reviews from Heather et al. (6) and Phang et al. (125). Although the most well-characterized rodent models of T1DM and T2DM remain, both models of CMS follow a different time course and etiology to that evident in humans. We anticipate that the guidelines as presented here will encourage better characterization specifically of diet-induced rodent models of CMS in both sexes, to more acutely reflect the human condition.

MODELS OF DIET-INDUCED HYPERTENSION

A high carbohydrate diet can induce hypertension in experimental animals (126, 127). Normotensive Wistar rats fed a high-sucrose diet develop hypertension, and there is an even greater magnitude of blood pressure elevation in spontaneously hypertensive rats fed sucrose (128–130). In addition to sucrose, a high-fructose diet also induces hypertension in SD rats (131). Feeding SD rats with a diet containing 66% fructose increases systolic blood pressure ∼20 mmHg in 14 days. Rats fed a high-carbohydrate diet exhibit nonobese insulin resistance and dyslipidemia with normal or only mildly elevated blood glucose. Like humans, blood pressure in experimental rats is due in part to hyperinsulinemia that results in compensation to insulin resistance (132, 133). The mechanism includes sympathoexcitation, activation of the renin-angiotensin system, enhancement of renal sodium reabsorption, and increases in vascular tone and remodeling.

Clonidine, an α_2-adrenagic agonist and suppressor of sympathetic nerve activity, and bunazosin, an α_1-adrenoreceptor antagonist, prevent the development of hypertension in fructose-fed rats, with no effect on the development of hyperinsulinemia (131, 134). The AT_1 receptor blocker olmesartan suppresses an increase in blood pressure and urinary catecholamine excretion in fructose-fed rats, indicating that hypertension in these models is caused by renin-angiotensin-mediated sympathetic nerve activation (134). Furthermore, olmesartan, and not bunazosin or hydralazine, attenuates fructose-induced increases in LV weight to body weight ratio, suggesting that the local renin-angiotensin system rather than hypertension may contribute to cardiac hypertrophy (134).

Addition of a low-salt (0.07%) diet blunts blood pressure elevation in fructose-fed rats. Although insulin receptor number in kidneys is progressively decreased with dietary salt content in normal diet-fed rats, high-salt (7.5%) diet has no effects on insulin receptor number in fructose-fed rats. The loss of a negative feedback mechanism that limits insulin-induced sodium retention may be also an important factor for blood pressure elevation (135). Hyperinsulinemia caused by exogenous insulin treatment does not by itself induce hypertension (136, 137). Nitric oxide production through the phosphatidylinositol-3 kinase/Akt pathway, a central component of insulin signaling, is reduced in the vasculature of fructose-fed rats, but not in insulin-treated rats. Impairment of vasodilation and vascular remodeling by activating the mitogen-activated protein kinase pathway may contribute to fructose-induced hypertension.

Fructose, rather than glucose, is considered the primary carbohydrate component that induces insulin resistance (138). Thresher et al. (139) compared the effects of a purified starch diet (68% of total calories), a sucrose diet (68% of total calories), a fructose/glucose diet (34%/34% of total calories), or a fructose/starch (34%/34% of total calories)

Table 6. *Strengths and limitations of rodent models to evaluate dietary interventions in diabetes*

Model	Diabetes Type	Description	Advantages	Limitations
STZ-induced rat/mouse (7, 51, 63, 65–70)	1	STZ destroys pancreatic β-cells, leading to insulin deficiency	Simple, reproducible, dose-dependent response	Variability with strain/dose; possible off-target toxicity
NOD mice (94–96, 124)	1	Spontaneous autoimmune β-cell destruction	Closely mimics human T1DM immunopathology; useful for immunological studies	Variable onset; requires controlled conditions
ZDF rats (90, 102–106)	2	Genetic leptin receptor mutation causes obesity, insulin resistance, hyperglycemia	Polygenic; resembles human T2DM; predictable onset	Expensive; limited availability; requires specific diet
db/db Mice (21, 109, 110)	2	Leptin receptor mutation → obesity and insulin resistance	Well-characterized; early-onset hyperglycemia; widely available	Rapid disease progression may not reflect late-stage complications
Diet-induced models (7, 112, 113)	2	High-fat/high-sugar diets induce insulin resistance and glucose intolerance	Mimics environmental factors in human T2DM; modifiable variables	Variable disease induction; less consistent than genetic models

db, diabetes; NOD, nonobese diabetic; STZ, streptozotocin; T1DM, type 1 diabetes mellitus; ZDF, Zucker diabetic fatty.

for 5 wk in male Charles River Wistar rats. All three sugar diets similarly impaired glucose tolerance and hepatic and peripheral insulin action, whereas the purified starch diet had no effect. Fructose is more rapidly metabolized principally in the liver compared with glucose. Fructose is metabolized by fructokinase to fructose 1-phosphate, which is further metabolized to glyceraldehyde, glyceraldehyde 3 phosphate, pyruvate, and then acetyl-CoA. This reaction is rapidly and greatly caused by high fructose intake, which leads to de novo lipogenesis, hepatic accumulation of triglyceride, and formation of very low-density lipoprotein (VLDL), resulting in insulin resistance (140). Of note, fructose generates only mild hypertension (141).

Carbohydrates can be administered in the diet or drinking water. Administration of drinking water with 20% sucrose to female C57BL/6 mice during pregnancy and lactation periods increases body weight, fat accumulation in the liver, insulin resistance, dyslipidemia, and systolic blood pressure in 7- to 8-mo-old male and female offspring, with multigenerational effects observed through the second generation (142, 143). Similarly, maternal intake of 60% high-fructose diet induces hypertension in male 12-wk-old SD rat offspring (144, 145). These models provide useful tools to investigate mechanisms of prenatal hypertension. Moderate dietary intake of fructose (10%) throughout gestation and lactation increases sensitivity to subsequent dietary fructose in female offspring more than male offspring, whereas maternal excessive fructose exposure causes hypertension in both male and female pups (146). Table 7 summarizes the studies evaluating the effect of high-carbohydrate diets on hypertension.

Sex differences in response to being fed with a high-carbohydrate diet occur to both the individual rats and rats whose mothers were exposed. In contrast to males, female rats fed with high-fructose diet for 9 wk do not exhibit increases in insulin resistance, plasma triglyceride levels, and blood pressure, which are all increased by ovariectomy (147). Gonadectomy blunts blood pressure elevation in fructose-fed rats, indicating significant roles for androgens in the development of hypertension (148). Additional studies evaluating sex differences in response are certainly warranted.

HUSBANDRY CONSIDERATIONS FOR DIET-INDUCED MODELS

The phenotype of any animal model is the culmination of genetics converging with environmental influences. Therefore, special consideration needs to be given to the external factors that influence models of disease. This includes standard animal husbandry practices which, while critical for maintaining healthy and humane housing conditions, can introduce variability between studies performed at different institutions.

The basic elements of husbandry that can influence diet-induced rodent models include hormone-related sex differences, food and water sources, cage and bedding types, enrichment opportunities, and coprophagy (consumption of feces). Investigators should provide comprehensive details of their animal husbandry practices to minimize variability among studies, increase rigor, and improve data translation.

To assess current practices in reporting diet and cage information, we evaluated research articles published by the *American Journal of Physiology-Heart and Circulatory Physiology* in December 2024 (accessed May 12, 2025) (124, 149–158). Of the 11 research articles published, 8 (73%) used animals. Of the eight articles using animals, three (38%) reported details on diet, seven (88%) reported basic details of feeding, six (75%) reported access to water, and four (50%) reported some details of cage conditions. Most articles simply stated that animals were provided food and water ad libitum. Although this evaluation indicates that basic information is provided, we have provided a checklist of details that should be reported in general for most studies, and specifically for all diet-induced animal models (Fig. 1). We provide the experimental importance of clarifying these details in the experimental design. This will increase rigor and reproducibility in the field by ensuring consistency in reporting details.

Decisions regarding animal feed intuitively have direct influence on diet-induced models. Although great effort is made by feed manufacturers and regulatory agencies to ensure that animal feed is of good quality and meets dietary requirements, different sources of commercial feed vary in nutritional content and presence of contaminants, even

Table 7. *Rodent models of high-carbohydrate diet-induced hypertension*

Types of Diet	Duration, Weeks	Animals	Blood Pressure Response
50% Glucose 50% Sucrose 50% Starch	5	Male WKY and SHR rats	↑ SBP in ∼2 wk for SHR and ∼3 wk for WKY
66% Fructose	2	Male SD rats	↑ SBP ∼ 20 mmHg
0.07% (low), 0.3% (normal), or 7.5% (high) salt	3	Male SD rats	↔ SBP on low-salt diet ↑ SBP on normal and high-salt diets
	8	Male Wistar rats	↑ SBP by tail-cuff plethysmography ↔ MAP by continuous telemetry
60% Fructose	9	Male Wistar rats	↑ SBP in ∼6 wk ↔ SBP after gonadectomy
		Female Wistar rats	↔ SBP ↑ SBP after ovariectomy
	During pregnancy and lactation	Maternal SD rats	↑ SBP after 12-mo-old in male offspring
20% Sucrose in drinking water	During pregnancy and lactation	Maternal C57BL/6 mice	↑ SBP in 7- to 8-mo-old male and female offspring

↑, increase; ↔, unchanged; MAP, mean arterial pressure; SBP, systolic blood pressure; SD, Sprague-Dawley; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

Husbandry Variables Reporting Checklist

- ☐ **Food**
  - ☐ Manufacturer, Catalog #, Lot #
  - ☐ Formulation (if available)
  - ☐ Analysis Certifications (if available)
  - ☐ Sterilization Procedure
  - ☐ Feed Frequency (e.g. *ad libitum*)
- ☐ **Water**
  - ☐ Source, Manufacturer, Catalog #, Lot #
  - ☐ Analysis Certifications (if available)
  - ☐ pH
  - ☐ Mineral Content
  - ☐ Sterilization Method
- ☐ **Cage Environment**
  - ☐ Cage Type, Manufacturer, Catalog #
  - ☐ Cage Dimensions
  - ☐ Animal Density
  - ☐ Substrate/Bedding Material
  - ☐ Cage Change Frequency
  - ☐ Room Temperature
  - ☐ Light Cycle
  - ☐ Humidity
  - ☐ Enrichment Items (e.g. nestlets, wooden chew sticks)
  - ☐ Microisolation Technique
  - ☐ Decontamination methods (e.g. autoclave, spray disinfectant)
- ☐ **Special Considerations**
  - ☐ Fasting
  - ☐ Coprophagy Inhibition

Figure 1. Checklist for reporting husbandry conditions of diet-induced models. This list serves as a guide to help investigators thoroughly report the husbandry-related environmental conditions that may impact their data and/or reproduction of their work. Although not all items have been explicitly discussed in these guidelines, readers are encouraged to examine the work in other fields that have identified confounding variables due to husbandry (e.g., cage decontamination practices and light cycle) to determine relevance for their specific models.

between lots of the same manufacturer (42, 159, 160). In addition, rodent models are highly sensitive to varying diets, as the presence or absence of certain substances can exacerbate or lessen an observed phenotype. For example, spontaneous development of cancer in mice with multiple intestinal neoplasia varies significantly depending on the commercial feed given (161). Although formulations of these different feeds were comparable in basic macronutrient composition, they varied in fiber source and type, and may have differed in levels of contaminants, nonnutrients, and biologically active compounds (161).

Different commercial rodent diets vary in additional details, including carbohydrate composition (e.g., fructose vs. glucose) (42, 159, 160). These differences provide important considerations for specialty diets where nutrients, such as carbohydrates, are given in excess. In a high-fat diet mouse model supplemented with either high-fructose or high-glucose water, differential effects on mitochondrial function, fatty acid oxidation, and fatty acid and triglyceride synthesis were observed (162). As there are many variables in formulation and production of feed that could impact diet-induced models, thorough reporting of feed information is essential and will allow for future comparisons, including meta-analyses.

As with feed, water can be highly variable between sources. Institutions will have different water treatment and sterilization protocols (e.g., acidification, filtration, or hyperchlorination), which can impact pH and mineral content (163). Although few studies have explored the impact of different water properties on rodent models, there is evidence that it is an important variable to consider (163). In two studies using a nonobese mouse model for T1DM, changes in the microbiome occurred between mice given neutral (pH ∼7) or acidified water (pH ∼3.2) (164, 165). One of the studies also observed a reduction in diabetes induction with acidified water (164), whereas the other did not (165), indicating that further investigation is needed. As the importance and sensitivity of the microbiome are further explored, the properties of dietary water in diet-induced animal models are essential to report.

Like food and water sources, husbandry considerations involving cage environment can impact diet-induced models. Animal density (number of animals per cage area) can influence food intake, growth, fat deposition, and behavior (166–168). Greater animal density reduces cold stress, as more mice will increase the cage temperature (168). Cold stress is common in animal facilities maintained at room temperature (∼22°C) instead of a controlled, more thermoneutral temperature (∼30°C for mice and rats) (169, 170). The thermoneutral temperature ranges for mice and rats vary greatly and have been extensively reviewed elsewhere (171). In addition, the cage substrate/bedding present can influence thermoregulatory-sensitive variables, namely due to the volume of bedding used, as increased substrate allows for larger nest coverage and improved thermoregulation (172).

The type of bedding can also influence other aspects of rodent biology. Common types of bedding used in rodent research facilities include woodchips, bleached cellulose, and corncob pellets (173). The performance of these different

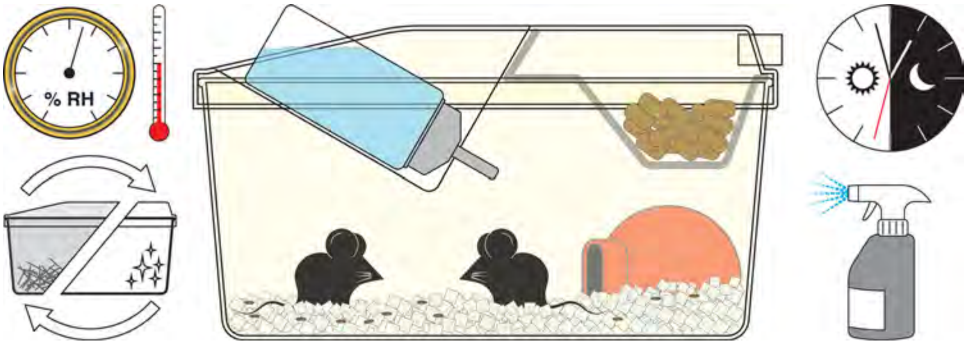

Figure 2. Recommended husbandry considerations for diet-induced models. Humidity and temperature, bedding conditions, water and food sources, cage details including presence and type of enrichment, circadian rhythms, light cycles, and disinfectant procedures are among the details that should be taken into consideration for proper experimental design.

beddings has been discussed by others (174–177); however, an often-overlooked factor pertinent to diet-induced models is the consumption of bedding. Rodents are known to ingest their cage bedding, due to their obligatory chewing and foraging behavior. Ingestion of nonfood materials is an essential consideration for diet-induced models, as the additional nutrition and fiber gained from consumption of bedding can drastically modulate the microbiome and a number of physiological parameters (178–180). Notably, the use of corncob bedding is now being cautioned against for diet-induced models as consumption of corncob bedding impacts feed efficiency (181), weight gain (178), hypertension (182), and fasting blood glucose (182, 183). Enrichment items, such as chewing blocks and nesting materials, are possible sources of supplemental nutrition, fiber, and dietary contamination. Thus, researchers should be cognizant of how enrichment items could affect their measured outcomes and should report specifics if used (e.g., type of enrichment and method of decontamination) (184, 185). Although more work is needed to evaluate the impact of enrichment items on diet-induced models, the presence or absence of enrichment could impact animal activity and well-being, which may impact certain models metabolically and behaviorally (184, 186).

Finally, it is vital to remember that rodents practice coprophagy, the consumption of feces, as a normal part of their behavior and digestion. Although the importance of coprophagy varies among species, mice and rats likely use the practice to maintain a healthy microbiome and improve nutritional intake (187, 188). Coprophagy is relevant to the translation of diet-induced disease models, particularly those dependent on fasting, as rodents will continue to recover nutrition, microbes, and microbial by-products through consumption of their own feces, even in the absence of food. Although methods exist to reduce coprophagy, such as using wire mesh cage bottoms and physical restriction (187–190), inhibition of coprophagy and the methods to do so can induce stress and alter the microbiome (187, 190), which in and of itself can impact diet-induced models. Thus, investigators should be mindful of this attribute of basic rodent behavior when designing their experiments and interpreting data and should report if they use methods for coprophagy prevention.

Although decisions in animal husbandry will directly impact scientific outcomes, it is unrealistic to establish a single standard of practice across institutions. Though husbandry can be a source of variation across studies, discovering the effects of environmental differences is a valuable part of the scientific process. To improve the rigor and reproducibility of studies with diet-induced models, researchers are best served to be thorough in reporting their methods including their husbandry practices (Figs. 1 and 2).

CONCLUSIONS

Here we provide comprehensive guidelines for the preparation and use of diets in experimental models of CMS. These guidelines will serve as a valuable companion for other recent guidelines published in American Physiological Society journals (41, 191, 192). We highlight the critical role of diet composition in inducing conditions such as obesity, insulin resistance, dyslipidemia, atherosclerosis, and hypertension, which closely mimic human CMS. We focused on diet-induced models, emphasizing the advantages and limitations of diet-induced strategies due to their physiological relevance. Specific diet formulations, including high-fat and high-sugar diets, were discussed in relation to their impact on CMS progression. In addition, the review explores animal strain selection, sex differences, and husbandry considerations to improve model reproducibility. These guidelines will help authors to standardize experimental methodologies and enhance the translational value of diet-induced CMS models.

GRANTS

This study received funding from the US National Institutes of Health under award numbers GM151274 and UC2MD019626 (to M.L.L.), R01HL155618 and R21OD037867 (to P.B.); from the US Department of Veterans Affairs under award number I01CX002780 (to M.L.L.); from the American Heart Association https://doi.org/10.58275/AHA.25IVPHA1462290.pc.gr.229780 (to M.L.L. and G.E.G.); from National Health and Medical Research Council of Australia under award number APP2029251 (to R.H.R.) and the Medical Research Future Fund of Australia under award number MRF2023754 (to R.H.R.); from Takeda Science Foundation under award number 2022060878 (to H.T.); from Agencia Nacional de Promoción Cientifica y Tecnologica de Argentina PICT 2019-02987 (to G.E.G.) and PICT 2018-03267 (to V.J.M.) and Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET) PIP 938 (to G.E.G.).

DISCLOSURES

Editor-in-Chief M. L. Lindsey has coauthored this article.

AUTHOR CONTRIBUTIONS

T.M.S., P.B., H.T., R.H.R., and V.J.M. prepared figures; T.M.S., P.B., H.T., M.L.L., R.H.R., V.J.M., and G.E.G. drafted manuscript; T.M.S., P.B., H.T., M.L.L., R.H.R., V.J.M., and G.E.G. edited and revised manuscript; T.M.S., P.B., H.T., M.L.L., R.H.R., V.J.M., and G.E.G. approved final version of manuscript.

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