Cardiometabolic Syndrome: An Update on Available Mouse Models

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Abstract

Cardiometabolic syndrome (CMS), a disease entity characterized by abdominal obesity, insulin resistance (IR), hypertension, and hyperlipidemia, is a global epidemic with approximately 25% prevalence in adults globally. CMS is associated with increased risk for cardiovascular disease (CVD) and development of diabetes. Due to its multifactorial etiology, the development of several animal models to simulate CMS has contributed significantly to the elucidation of the disease pathophysiology and the design of therapies. In this review we aimed to present the most common mouse models used in the research of CMS. We found that CMS can be induced either by genetic manipulation, leading to dyslipidemia, lipodystrophy, obesity and IR, or obesity and hypertension, or by administration of specific diets and drugs. In the last decade, the *ob/ob* and *db/db* mice were the most common obesity and IR models, whereas $Ldlr^{-/-}$ and $Apoe^{-/-}$ were widely used to induce hyperlipidemia. These mice have been used either as a single transgenic or combined with a different background with or without diet treatment. High-fat diet with modifications is the preferred protocol, generally leading to increased body weight, hyperlipidemia, and IR. A plethora of genetically engineered mouse models, diets, drugs, or synthetic compounds that are available have advanced the understanding of CMS. However, each researcher should carefully select the most appropriate model and validate its consistency. It is important to consider the differences between strains of the same animal species, different animals, and most importantly differences to human when translating results.

Keywords
► cardiometabolic syndrome
► obesity
► insulin resistance
► hyperlipidemia
► mouse model

Introduction

The cardiometabolic syndrome (CMS) was first described over 90 years ago by Kylin as a combination of hyperglycemia, hypertension, and gout.[1] Later on, Vague pointed out an associ-ation between abdominal adiposity and increased risk of type 2 diabetes and CVD.[2] CMS is now defined as a disease entity characterized by the simultaneous occurrence of at least three of the following medical conditions: abdominal obesity, raised plasma glucose, elevated blood pressure, raised triglycerides (TGs) or reduced high-density lipoprotein (HDL) cholesterol.[3] Additional components of CMS which are interconnected

through cytokine release from adipose tissue are (1) a proinflammatory state, suggested by elevated C-reactive protein (CRP) and (2) a prothrombotic state, characterized by increased plasma plasminogen activator inhibitor-1 and fibrinogen.[4] Microalbuminuria, increased inflammation/oxidative stress, endothelial dysfunction, nonalcoholic steatohepatitis, abnormalities in the coagulation system, and enhanced cardiovascular tissue renin–angiotensin–aldosterone system (RAAS) contribute to the pathophysiology of CMS.[5,6] The accumulation of the above metabolic and underlying risk factors promotes the development of atherosclerotic CVD and increases the risk for type 2 diabetes, stroke, and cognitive impairment.[6–8]

The prevalence of metabolic syndrome in United States has been estimated to be around 35% in both sexes of adults, whereas it increases to around 50% in the age group over 60 years old and particularly in women.[9–11] Increasingly high prevalence of CMS is seen in populations of different countries, confirming the global burden of the disease.[9,12,13] Several prospective cohorts and meta-analysis studies demonstrate that CMS is associated with increased relative risk for cardiovascular outcomes (twofold), diabetes (3.5–5-fold), and all-cause mortality (1.5-fold).[7,14] In a longitudinal cohort study of 1 million Chinese, the prevalence of cardiometabolic multimorbidity increased from 2.41 to 5.94% in 5 years, indicating the rapid progression of cardiometabolic disease.[15]

Because of the multifactorial nature of this syndrome and the lack of specific treatment,[16] it is essential to elucidate the exact pathophysiologic mechanisms.

Animal models have traditionally been used in research for CVDs in an effort to translate mechanistic and therapeutic findings into human disease.[17–19] Yet it is critical and rather challenging to choose the appropriate model that would best reflect the human pathophysiology, since animal models present only a fraction of the CMS features at a given time.

Rodent models and particularly mice have been the most popular animal models in the investigation of CMS. This is because of their obvious advantages over other animals, which include easy handling and relatively easy genetic engineering, short gestation and lifespan periods, and most importantly high genetic and physiological similarity to humans.[20] Moreover, they are responsive to experimental diets as they develop central obesity, hyperglycemia, and hypertension, some of the main characteristics of the CMS. The importance and popularity of mouse models are also reflected by the numerous studies that used mice as the reference animal. The database mouse genome informatics (MGI) has catalogued 169 mouse models matching the phenotype "metabolic syndrome," which are linked to 265 references. Moreover, genetic variation identified in mice has been shown to affect the corresponding human phenotypes.[21]

The aim of this review is to summarize the best characterized mouse models for CMS research and present newer models that have been described during the past 10 years. For this purpose, we conducted a PubMed search using the terms *cardiometabolic AND mouse models,* with a time filter of 10 years. This search returned 292 results, of which we selected the most appropriate ones using the following criteria: original research articles and those reporting the main aspects of CMS. Similar number (277) was obtained using Scopus as a search engine. To complement our search, we also investigated the MGI database; we found 169 mouse models linked to CMS syndrome and the most relevant models are included in this review.

Overview of the Best Characterized Mouse Models

There are three main ways of modeling CMS in mice. These are genetic manipulation, diet/drug-induced protocols, and the combination of any of the above.

Genetic CMS Mouse Models

The strategies of genetic engineering are focused on alteration of lipid metabolism, weight regulation, glucose homeostasis, blood pressure regulation, and the suitable combination of the above phenotypes.

Mouse Models of Dyslipidemia

Low-density lipoprotein (LDL) receptor (Ldlr) and apolipoprotein e (Apoe)-deficient mice are the two best characterized dyslipidemic models. Their hyperlipidemic profile is due to the absence of either Ldlr or Apoe, both of which participate in cholesterol clearance.[22,23] $Ldlr^{-/-}$ mice develop moderate hypercholesterolemia (total cholesterol ~250 mg/dL) on a normal diet with lipoprotein profiles similar to those of humans (i.e., elevated LDL) but they are very responsive to atherogenic diet (1.25% cholesterol diet), developing large atherosclerotic lesions and hyperlipidemia. Furthermore, when $Ldlr^{-/-}$ mice are placed on a diet with greater than 20% fat content, they also become obese, display insulin resistance (IR), and impaired glucose tolerance.[24] $Apoe^{-/-}$ mice develop a more severe hyperlipidemia, with an increase in plasma cholesterol levels and TG levels, which leads to spontaneous atherosclerosis on a normal diet.[23] In many cases, $Apoe^{-/-}$ mice do not become obese, nor do they develop IR, even on a high-fat diet (HFD).[25,26] However, there has been a case where $Apoe^{-/-}$ mice fed HFD (60% fat) for 17 weeks displayed increased body weight, glucose intolerance, and an increase in systemic inflammation, which indicates that modulation of the feeding protocol can have a significant biological effect.[27] In general, as summarized by the Jackson Laboratory and the Mouse Phenome Database which have phenotyped 8-week old male and female Apoe and Ldlr null mice against C57BL/6J mice after 6, 10, and 14 weeks of normal diet (6% fat), these transgenics develop a range of cardiovascular phenotypes. These include elevated plasma cholesterol and TG for both knockouts as well as increased LDL, TG, and ApoB levels in male $Ldlr^{-/-}$. Caution should be taken however when comparing absolute lipid concentrations between $Apoe^{-/-}$ and $Ldlr^{-/-}$ as various factors could lead to misinterpretations, such as the mechanism of function for each model and differences in the major plasma lipoproteins. Specifically, in $Apoe^{-/-}$ mice the accumulating particles are predominantly apoB48-containing cholesterol ester particles, whereas in $Ldlr^{-/-}$ the major lipoprotein is the ApoB100-containing LDL. Other

factors affecting the absolute lipid concentrations include sex, genetic variations, and differences in gut microbiota.[28]

Mouse Models of Obesity and IR

Visceral obesity is one of the requirements for CMS and has been positively associated with IR and hyperglycemia.[29] In agreement with human studies, most of the obesity mouse models manifest IR. Leptin deficiency is one of the gold standard models. A spontaneous mutation in leptin gene led to the description of *ob/ob* mice.[30] Leptin deficiency results in increased food intake, extreme obesity, and reduced energy expenditure. The effect on glucose metabolism is strain-dependent. A C57BL/6J background is characterized by mild hyperglycemia compared with severely diabetic *ob/ob* mice on the C57BL/KsJ background. Moreover *ob/ob* mice show increased HDL and reduced LDL, hence they are protected from atherosclerosis and they are also resistant to hypertension.[31,32] A single autosomal mutation in the leptin receptor[33] causes almost identical phenotypes to leptin deficiency in those mice (*db/db*) which develop obesity, hyperinsulinemia, hyperglycemia, and increased total cholesterol (predominantly LDL/HDL particles) in the absence of hypertension.[34] An additional model with altered leptin signaling is the POUND mouse generated by Charles River, which lacks all leptin receptor isoforms. When fed Purina diet they became hyperinsulinemic and hyperglycemic, which was accompanied by increased leptin and cholesterol levels.[35] Another model used in CMS research is the agouti yellow obese mouse (A^y/a). In the brain the secreted agouti protein antagonizes the binding of the anorexigenic α-melanocyte-stimulating hormone (α-MSH) to its receptor (melanocortin 4 receptor, MC4-R). α-MSH reduces food intake, increases energy expenditure, and regulates glucose metabolism, thus spontaneous mutations that occur in the agouti gene lead to disruption of these regulatory mechanisms. Agouti mice present adult-onset obesity, hyperinsulinemia, glucose intolerance, hyperglycemia, and hypertension, which establishes it as a useful model of CMS.[36,37] As mentioned above, hypothalamic MC4-R-mediated signaling plays a role in food intake and energy homeostasis; therefore, targeted disruption of MC4-R was used as an alternative approach. The human relevance of this model is supported by studies which revealed that *MC4-R* mutations are associated with hereditary obesity.[38,39] The *MC4-R* null mouse has a behavioral obesity syndrome characterized by hyperphagia, hyperglycemia, hyperinsulinemia, reduced metabolism, increased lean mass, and altered hepatic lipid metabolism.[40,41] It has been also shown that $MC4-R^{-/-}$ mice have elevated total plasma cholesterol and TG but tend to be hypotensive rather than hypertensive.[34,42] Cho et al from Yale University[43] created a polygenic obesity-induced diabetic mouse model (RCS10) by combining different obesity risk-conferring quantitative trait loci (eQTLs) from two different strains; NZO/HlLt and NON/Lt. This is a type 2 diabetes model accompanied by adult-onset obesity, hyperglycemia, IR, and increased hepatic, intramuscular and heart TG at 13 weeks of age; however, data of the effect on blood pressure are lacking.[43]

Obesity and Hypertension Models

Adiponectin is another protein that has been associated with obesity and obesity-related diseases in humans.[44] It is secreted by adipocytes and low levels are detected in obesity, which can be reversed during weight loss. *Adipo*$^{-/-}$ mice fed high fat-high sucrose diet for short periods (2–4 weeks) develop elevated glucose, insulin and free fatty acids, plasma TNF-a, and hypertension.[45,46] An established model of systemic lupus erythematosus (NZBWF1) was described by Ryan et al in 2006 as potentially important model to study obesity and IR. The NZBWF1 mouse phenotype showed increased weight, plasma insulin, but not glucose levels, increased plasma leptin, renal adipose tissue macrophage infiltration, and hypertension.[47]

Nonobese-Lipodystrophic Models

The lipodystrophic A-ZIP F-1 and the aP2-SREBP-1c mice are nonobese mouse models, with restricted adipose capacity concerning mainly the white adipose tissue (WAT) and may be used in the study of nonobese CMS and genetic forms of lipodystrophies.[48,49] The A-ZIP F-1 mice were generated by enhancing the expression of A-ZIP/F (dominant negative protein of C/EBP transcription factors that regulate growth and differentiation of adipocytes) by using an adipose-specific P2 enhancer promote. This model, which has complete deficiency of WAT, shows that fat ablation can cause liver steatosis and diabetes, with reduced leptin and elevated serum glucose, insulin, free fatty acids and TG, and elevated blood pressure.[48,50] The transgenic aP2-SREBP-1c mice, which overexpress sterol regulatory element binding proteins (SREBP) in adipose tissue, have decreased WAT but have hypertrophic brown adipose tissue containing cells resembling immature WAT. These mice are characterized by hyperglycemia, hepatic steatosis, and hyperinsulinemia potentiated by secreted frizzled-related protein.[49,51]

To better recapitulate the various aspects of the metabolic syndrome combinations such as obesity and hyperlipidemia, obesity and hypertension have also been employed and reviewed in detail by Kennedy et al.[34] ►**Table 1** summarizes the mouse models described above.

Diet-Induced CMS Mouse Models

Numerous dietary interventional studies have been conducted to study the pathophysiology of CMS. As with the genetic models these include either a single experimental diet or a combination of diets. The most known approaches are high carbohydrate, high sucrose, high fructose, and HFDs. The mode of action for each diet, with emphasis on the affected biochemical pathways, has been beautifully reviewed by Wong et al[52] and is beyond the scope of this review. Here we will present some key characteristics of the main experimental diets. As far as HFD is concerned, numerous modifications exist, with fat concentrations ranging from 20 to 60%, originating either from plants (e.g., corn, safflower, or olive oil) or animals (e.g., beef, tallow, and lard).[53] The common main outcome of HFD is the increased formation of TG following re-esterification of free fatty acids after lipolysis.[52] Interestingly, HFD-related outcomes are fat-dose-dependent.[54] Fructose is one of the monosaccharides alongside glucose and galactose and it is only an intermediary

Table 1 Genetic CMS mouse models and their metabolic phenotype

Mouse model	Genetic modification	Metabolic changes			Hypertension	Dyslipidemia	Source
		Obesity	Hyperglycemia	Insulin resistance			
ob/ob	Autosomal recessive mutation in leptin gene	✓	✓ (strain dependent)	✓	✗	↑Total cholesterol (LDL/HDL1)	30–32
db/db	Autosomal recessive mutation in leptin receptor gene	✓	✓ (age, strain dependent)	✓	✗	↑Total cholesterol (LDL/HDL1)	33
MC4-R−/−	Targeted disruption of MC4-R gene	✓ (exacerbated on HFD)	✓	✓	↓blood pressure	↑Hepatic TG, ↑serum FFA	40,41
Ay/a	Spontaneous mutations in agouti gene	✓ (delayed onset)	↓Glucose tolerance (HFD + HS-induced)	✓ (delayed onset)	✓	↑HDL	36,37
Ldlr−/−	Deletion of Ldlr gene	✓ (HFD-induced)	↓Glucose tolerance (HFD-induced)	✓ (HFD-induced)	✗	↑LDL	22,24
Apoe−/−	Deletion of Apoe gene	Generally resistant	✗	Generally resistant	✗	↑Total cholesterol, triglycerides, VLDL, LDL, L	23
NZBWF1	Cross between New Zealand black (NZB) mouse with the New Zealand white (NZW) mouse	✓	✗	✓	✓	–	47
Adipo−/−2–4 weeks on diet	Adiponectin knockout	✓	✓	✓	–	↑VLDL	45
A-ZIP/F-1	Overexpression of A-ZIP/F in adipose tissue	✗	✓	✓	✓	✓	48,50
aP2-SREBP-1c	Overexpression of SREBP-1c in adipose tissue	✗	✓	✓	–	↑Hepatic TG	49
RCS10	Combination of obesity risk loci on 2 strains; NZO/HlLt and NON/Lt	✓ (adult onset)	✓	✓	–	↑Hepatic and intramuscular TG	43
POUND mouse	Deletion of all leptin isoforms	✓	✓	✓	–	↑Total cholesterol	Charles River

Abbreviations: CMS, cardiometabolic syndrome; FFA, free fatty acids; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.
Note: ✓ and ✗ indicate the presence or absence respectively, whereas "–" indicates no data.

Table 2 Diet-induced CMS mouse models, the diet treatment, and their metabolic phenotype

Mouse strain	Diet treatment	Metabolic changes			
		Obesity	Hyperglycemia	Hypertension	Dyslipidemia
Male C57BL/6 J mice	High-fat diet, 8 wk	✔	✔	–	✔
Male AKR/J (AKR) mice	High-fat diet, 8 wk	✔	✔	–	✔
Male and female C57Bl/6J mice	High-fat diet (60%), 20 wk	✔	✔	–	✔
Male and female A/J mice	High-fat diet (60%), 20 wk	✔	✖	–	✔
Male C57BL/6 mice	High-fat diet, 16 wk	✔	✔	–	✔
Male C57BL/6 mice	High-fat diet, 40 wk	–	–	–	✔
Female C57Bl/6 J mice	High-fat diet, 24 wk	✔	–	–	✔
C57BL/6 mice (wild type)	High-fat diet, 13 wk	–	✖	–	✔
Ldlr$^{-/-}$ mice	High-fat diet, 13 wk	–	✔	–	✔
Female C57BL/6NTac mice	High-fat diet, 12 wk	✔	✔	–	✔
Male C57BL/6 mice	High-fat diet, 12 wk	✔	–	–	✔
Male C57BL/6 J mice	High-fat diet, 4 wk	✔	–	–	✔
Male C57BL/6 mice	High-fat diet, 16 wk	✔	✔	–	✔
Male NMRI mice	Fructose drinking water (15%), 10 wk Sucrose soft drink (10%), 10 wk Noncaloric soft drink, 10 wk	✔	–	–	–
		✖	–	–	–
		✖	–	–	–

Abbreviation: CMS, cardiometabolic syndrome.

Note: ✔ and ✖ indicate the presence or absence respectively, whereas "–" indicates no data.

Source: Adapted and reprinted with permission from Wong SK, Chin K-Y, Suhaimi FH, Fairus A, Ima-Nirwana S. Animal models of metabolic syndrome: a review. Nutr Metab. (Lond) 2016;13:65.

molecule during glucose metabolism. Chronic fructose consumption is an established way to induce increased energy intake, body weight, adiposity, hypertriglyceridemia, hyperlipidemia, hypertension, glucose intolerance, and decreased insulin sensitivity in laboratory animals and can be more effective than glucose or starch-feeding.[52,55] According to Wu et al, a low dose of fructose in drinking water (10%) is sufficient to induce CMS in animals.[24] As far as sucrose (table sugar)-enriched diet is concerned, it is widely used for induction of obesity, whole body IR, hyperglycemia, hypertriglyceridemia, and hypercholesterolemia. Amounts of sucrose supplementation range from 20 to 77%.[52,56] However, sucrose appears to be less effective than the equivalent amount of fructose in inducing CMS as it is a disaccharide consisting of 50% fructose and 50% glucose. Besides, mouse response to daily sucrose consumption is strain-dependent, affected in particular by polymorphisms in the *Tas1r3* sweet taste receptor gene.[57]

The effects of each diet or diet combination on mice are summarized in ►**Table 2**.

Drug/Chemically Induced CMS Mouse Models

Streptozotocin administration is widely used to induce diabetes in mice. The mode of action for streptozotocin is via destruction of the β pancreatic cells. Streptozotocin is a glucose analogue that accumulates in β pancreatic cells via the GLUT2 glucose transporter. Type 1 diabetes manifestation occurs in mice treated with Streptozotocin.[58] Glucocorticoid-induced

metabolic syndrome has also been reported. Glucocorticoids are hormones that regulate glucose homeostasis in various ways such as gluconeogenesis in liver, whereas in skeletal muscle and WAT they decrease glucose uptake and utilization by antagonizing insulin response. Therefore, mice treated with glucocorticoids display glucose intolerance and reduced insulin sensitivity. Apart from body weight gain, they present dyslipidemia, central, liver and skeletal muscle fat accumulation, and hypertension.[59]

Newer CMS Mouse Models Used in Cardiometabolic Research

Dietary Interventions

Diet-induced obesity models are repeatedly used to assess the effect of various factors on cardiometabolic phenotype. It seems that HFD administered for various intervals is one of the most preferable interventions used to induce CMS. This approach most frequently leads to weight gain, dyslipidemia, and IR. C57/BL/6J mice fed with 60% HFD for 11 weeks were used as a model of obesity to study the effects of angiotensin (1–7). These obese mice exhibited high blood pressure and reduced insulin sensitivity.[60] Similarly, Miranda et al utilized diet-induced obesity to investigate the effect of cannabinoid receptor 1 antagonists. In this study, mice were fed with 60% HFD for a longer period (16 weeks). HFD increased body weight, fasting glucose, and IR, but also promoted adipose tissue inflammation characterized

by inflammatory M1 macrophage infiltration.[61] Another study confirmed the HFD-induced M1–M2 imbalance in WAT and the abnormalities in glycemic indexes and lipid profiles.[62]

Guo et al[63] fed 2-month-old male C57BL/6 mice with either HFD (45% energy from fat) including 7% sucrose or low-fat diet (LFD; 10% energy from fat). Control mice fed LFD were compared with various HFD groups which were supplemented with one of three fatty acids (EPA, DPA, and DHA) or none, after 6 weeks of intervention. The HFD group with no addition of fatty acids showed gathered characteristics of the CMS, in that mice were significantly obese with increased liver weight and epididymal adiposity, fatty liver, increased hepatic total cholesterol, high serum glucose, alanine transaminase, reduced adiponectin, and IR. Additionally, upregulation of inflammatory genes in the TLR-4/NF-κB pathway was observed. Supplementation of long-chain omega-3 polyunsaturated fatty acids could reverse the above effects. Blood pressure was not assessed in this model.[63] Leptin levels were also elevated after 8 weeks of HFD in a different study.[64] Similar phenotypes were reported by several studies using HFD as a mean of mimicking the CMS.[65–69] CMS-related serum biomarkers were investigated in HFD (60%) fed mice for an extended period (36 weeks). Besides increased body weight, dysregulated lipid metabolism (high total cholesterol and TG), raised blood glucose, insulin, glycated hemoglobin (HbA1c), and brain natriuretic peptide, there were also abnormalities in renin–angiotensin system. This was suggested by high serum angiotensin-converting enzyme, angiotensin II (Ang-II), Ang-II type 1 receptor, and aldosterone. Cardiac remodeling was also observed in the HFD group compared with normal diet (10% fat).[70] A combination of diet and drug-induced CMS has also been tested. Here mice were maintained on 60% HFD for 5 to 15 weeks alongside nitric oxide synthase inhibitor L-NAME. This double hit model[71,72] developed several aspects of CMS, as evident by obesity, hyperglycemia, reduced insulin sensitivity, impaired glucose clearance (heightened fasting glucose, HbA1c), hypertension, and endothelial dysfunction. Various diet modifications have been also described, such as high fat (60%) diet with salt addition (7.25% NaCl), which was sufficient to cause most of the aspects of CMS including diabetes, hypertension, and secondary features such as cardiac dysfunction and vascular remodeling.[73] A combination of high fat (35%)–high sucrose (34%) long-term (4 months) feeding protocol caused body weight gain, impaired glucose homeostasis, increased hepatic and myocardial TG content, as well as cardiac dysfunction in male C57BL6R mice.[74] All the above studies indicate that HFD is a reliable and simple protocol that induces reproducible phenotypes. However, something that should be taken into consideration when it comes to HFD protocols is that mice may respond differently according to their genetic background[75,76] or their gut microbiota adaptation to HFD. This adaptation involves transcriptional changes in hepatic miRNAs leading to a divergent metabolic phenotype. Subsequently, one can claim that hepatic lipid metabolism is modulated according to the response to HFD.[77,78] Other limitations regarding HFD include disparities in phenotypes sometimes attributed to the modulation of the HFD protocol (i.e., duration of intervention, age of mice)[79] and the diet compositions particularly the type of fat and amount of carbohydrates,

which can sway the balance between protection and lipotoxicity,[80,81] sex-specific responsiveness to HFD[82] and strain specificity to sugar consumption,[57] behavioral effects[83] and intramyocellular lipid accumulation causing muscle defects.[84]

Although obesity-related complications such as IR, nonalcoholic fatty liver disease, and CVD share common pathophysiology mechanisms such as increased tissular lipid deposition, hypoxia, and low-grade inflammation, different lipid species and groups are implicated in the aforementioned processes.[85] For instance, in the case of atherosclerosis and CVD, it is widely accepted that the presence of ApoB-containing lipoproteins such as very low-density lipoprotein (VLDL), LDL, and lipoprotein-a is of determinant importance for the pathogenesis of such complications.[86] The adipose tissue, on the other hand, is predominantly characterized by the presence of triacylglycerols rather than cholesterol or phospholipids and, during obesity, specific triacylglycerol signatures, defined by different fatty acid compositions, are associated to the development of IR.[87] Cholesterol and especially cholesterol esters are considered important inducers of nonalcoholic fatty liver disease, independently of obesity and metabolic dysregulation.[88] Nevertheless, the progression of the disease into nonalcoholic steatohepatitis in both humans and mice depends on the existence of specific long-chain fatty acids that are toxic to the hepatocytes.[89] Considering that the different diets that are used in animal models to provoke CMS trigger the emergence of different metabolic signatures in vivo and finally simulate different CMS-related complications, and not all of them together, exceptional caution should be taken when animal models of CMS are used for the study of CVD.

Genetic Manipulations

In addition to diet-induced strategies, genetic mouse models have been extensively used as illustrated in the first part of this review. Genetic manipulations include either manipulation of known metabolic pathways or exploration of new genes that have been shown to be associated with human CMS. This approach allows researchers to dissect the genetic component of CMS, induce more severe phenotypes, and in some occasions shorten the study duration compared with long-term feeding protocols. Such an example is the human cholesteryl ester transfer protein (CETP), which has no homologous gene in mouse and has been found to be associated with human CMS.[90] CEPT is responsible for the exchange of TG and cholesterol esters between LDL and VLDL particles and HDL. CETP activity is associated with reduced HDL and increased risk of atherosclerosis. There is no mouse homologue of this gene, therefore overexpression of the human CETP has been applied to mimic the human conditions. In several studies, CETP overexpression is combined with Apoe deficiency conferring reduced HDL and reduced clearance of TG-rich lipoproteins. APOE*3-Leiden.CETP mouse is a model combining obesity, IR, and hyperlipidemia when treated with HFD and fructose. It is responsive to antidiabetic and lipid-lowering drugs evidenced by improved glucose regulation, IR, and dyslipidemia.[91] Moreover, transcriptome analysis revealed that these mice when fed HFD comprise distinct hepatic gene signatures and serum

miRNAs.[92] Hemizygous *CETP* expression combined with *Ldlr* haploinsufficiency was also used as a model that reflects the lipoprotein metabolism and the risk of cardiometabolic disease in humans. These transgenic mice have perturbed lipid profiles showing increased LDL and decreased HDL. Glucose was not altered as a function of *CETP* expression and blood pressure and body weight data were not available. Thus this model lacks some of the main components of CMS.[93]

The *Ldlr*-deficient mouse treated with HFD is still commonly used and seems to be quite consistent as far as the lipid phenotype, hepatic inflammation, and atherosclerosis development are concerned.[94–97] *Ldlr*$^{-/-}$ mice fed high fat sucrose enriched diet with 0.15% cholesterol for 12 weeks developed diet-induced obesity, increased fasting insulin and glucose levels, and dyslipidemia characterized by increased TG and total cholesterol. Accelerated liver inflammation was also detected by elevated expression of inflammatory genes such as *Emr1*, *Tnfa*, *Il-6*, *Ccl2* as well as susceptibility to atherosclerosis. However, blood pressure was not evaluated in this mouse model.[94] *Apoe*-deficient mice appeared less frequently in the literature search we conducted. Crossing of heterozygous aromatase-deficient mice (*Ar*$^{+/-}$) on the *Apoe*$^{-/-}$ background was used to generate metabolically altered mice (MetS-Tg). The double knockout animals displayed central obesity, increased body weight, elevated serum, and increased blood pressure. Glucose and insulin tolerance tests revealed glucose intolerance and IR. Detection of cytokines such as TNF-a, IL-6, and CRP indicates an inflammation state.[98] The same group reported almost identical results for MetS-Tg mice in a different study, which were characterized by increased body weight compared with wild types, no differences in plasma glucose but in glucose area under the curve, and higher mean arterial blood pressure.[99] The induction of hyperlipidemia has been traditionally achieved by time-consuming generation of knockout transgenics of *Ldlr* and *Apoe*. Recently, hyperlipidemia has been attempted via adeno-associated-virus-8 (AAV8)-mediated overexpression of *PCSK9*.[100] A single intravenous femoral injection of viral particles containing the human *PCSK9*(DY) gene, which is a gain-of-function mutation of *PCSK9* under the liver-specific promoter HCR-hAAH, led to stable hepatic expression of this mutant protein. Mice expressing the mutant *PCSK9* had increased serum cholesterol and particularly LDL from day 30 up to 1-year postinjection, under normal feeding conditions[100] and much earlier (day 7) when treated with HFD as reported by others.[101] Consistent with the elevated fraction of LDL, the hepatic expression of *Ldlr* was significantly reduced. HFD exacerbated the observed hyperlipidemia and atherogenicity. The potency of the adenovirus-mediated *PCSK9* transexpression was also confirmed in three different genetic mouse strains. This fast and efficient way of introducing hypercholesterolemia in mice without the need of genetic manipulation has since been employed by several groups.[102–104]

Genetic ablation of GC-A guanylyl cyclase-A (GC-A) receptor in β pancreatic cells is a novel genetic mouse model used for the investigation of the role of atrial and brain-type natriuretic peptides and its GC-A receptor in glucose homeostasis. The disruption of GC-A receptor in β-cells combined with diet-induced obesity (60% HFD) was sufficient to cause arterial hypertension, but not obesity (compared with wild types on HFD), increased area under the curve during glucose tolerance test which was maintained from 8 to 18 weeks of feeding, and raised blood insulin.[105]

Another genetic model utilizing the neuropeptide Y (NPY) was first described in 2008.[106] NPY is a co-transmitter expressed in the brain and released alongside noradrenaline in response to sympathetic stimulation. It is also expressed in neurons innervating the vasculature cardiomyocytes and endocardium, and is involved in physiological processes including vasoconstriction, cardiac remodeling, and angiogenesis. Overexpression of *NPY* in noradrenergic neurons of the central and peripheral nervous system leads to increased WAT weight (subcutaneous, epididymal, and retroperitoneal fat pads) but not overall body weight, increased liver TG, and fasting glucose. These changes occurred in the absence of modified diet. The relevance of NPY in cardiometabolic animal modeling is also supported by human studies, where genetic polymorphisms found in NPY receptors (Y1, Y2, Y5) are associated with cardiometabolic risk factors and early-onset CVD risk.[107,108] Deletion of protein kinase D2 (PRKD2) triggers hyperinsulinemia which precedes IR and precipitates metabolic changes. Increased β-cell insulin secretion was mediated by increased expression and activation of L-Ca^{2+} channels and subsequent high glucose and membrane depolarization. The authors have focused on insulin secretion regulation rather than lipid metabolism and blood pressure data which are not reported.[109]

Besides the known obesity-induced diabetes models, *ob/ob* and *db/db*, which are widely used with or without a diet combination,[110–113] a very recent study has designed a new mouse model, which recapitulates maturity-onset diabetes of the young (MODY).[114] MODY4 mouse is essentially a pancreatic and duodenal homeobox 1 (Pdx1) haploinsufficient animal. Pdx1 is a transcription factor that regulates β-pancreatic cell maturation. *Pdx1*$^{+/-}$ mice were further challenged by genetic inhibition of the stress-responsive IKK/NF-κB signaling pathway resulting in a double transgenic IKK2-DNPdx1 animal. Transgenics presented rapid onset of diabetes characterized by hyperglycemia, hyperinsulinemia, and loss of β-cell mass.[114] Although this is a model of diabetes, it could be potentially combined with high fat or other dietary intervention to introduce hyperlipidemia and/or obesity.

Other Models for CMS Simulation

The model of maternal separation and early weaning (MSEW) together with high fat feeding predispose female offspring to an early onset of cardiometabolic risk factors, including hyperinsulinemia, glucose intolerance, and hypercholesterolemia as well as increased weight and fat mass compared with MSEW and non-MSEW male counterparts. Both male and female MSEW mice fed HFD also exhibited an increase in blood pressure compared with controls, non-MSEW.[115] Other studies that link maternal feeding status and the cardiometabolic profile of offspring have been reported. Saad et al allocated pregnant C57BL/6J mice to fructose solution or water from day 1 of pregnancy. The fructose protocol promoted hypoglycemia as

indicated by intraperitoneal glucose testing in both sexes and higher mean arterial pressure compared with controls but had no effect on lipid levels. Moreover, there was a female-specific effect on various other parameters following fructose intake compared with the control group. Female mice had higher weights with visceral adiposity, hepatic fat accumulation, IR, and high serum leptin.[116] Similarly, another group concluded that maternal diet-induced obesity coupled with postweaning obesogenic diet worsened offspring hyperinsulinemia, hyperleptinemia, fat deposition, hypertension risk, and cardiac fibrosis.[117] A recent study has implicated maternal diet in offspring malfunctioning metabolism. Maternal high-fructose feeding caused multigenerational hypertension, which was characterized by increased mRNA expression of the RAAS genes, as well as the expression of renin in the kidneys. The authors conclude that high fructose intake during pregnancy activated the RAAS and epigenetically induced changes in the cardiometabolic system of the offspring.[118] While maternal nutrition status can affect the progeny, a different study reported that paternal cholestasis (10 weeks of 0.5% cholic acid-supplemented diet prior to mating) resulted in metabolic defects in male offspring. Male pups of cholestatic fathers further treated with western diet for 18 weeks had raised fasting insulin, hepatic TG content, and serum cholesterol levels compared with diet-matched controls. At 25 weeks of age, male offspring showed hypertension as well.[119]

The above studies suggest that the prenatal and perinatal periods are as important as the postnatal lifestyle for the onset of CMS and—as highlighted by others—it is important to understand the long-term effects of treatment during pregnancy as it can have significant effects on offspring.[120]

It is also worth mentioning that wild-derived inbred strains have been suggested to be informative about our knowledge of CMS. These inbred strains arose from inbreeding of wild mice caught around the world. Karunakaran and Clee have extensively reviewed the characteristic of these wild-derived species (Cast, Molf, PWD, PWK, Spret, WSB), which are generally obesity-resistant but they show a spectrum of abnormalities in lipid and glucose metabolism.[21]

Finally, in ►Table 3 we present additional mouse models that carry the main CMS components after searching the MGI database for mouse models under the phenotype metabolic syndrome.[121–134] Moreover, the MGI search confirmed already mentioned models such as HFD-induced, ob/ob, KKA^y/a, streptozotocin-induced, genetic strains (PWD, PCD1), NPY, and triple knockouts (ob/ob; Apoe$^{-/-}$ on ApoB100 background).

Conclusion

CMS is a global epidemic with increasing prevalence. Due to its complex etiology—a combination of genetic and environmental factors—and pathophysiology, it is challenging to

Table 3 Additional genetic CMS mouse models as reported in MGI database and their metabolic phenotype

Mouse model	Genetic modification	Metabolic changes	Source
GNB3/+	BAC transgenic mice that carry an extra copy of human GNB3	↑ Fasting plasma glucose, insulin, and C-peptide, triglycerides, cholesterol, phospholipids, and glucose intolerance	121
SNAP-25b	Targeted deletion of SNAP-25b	Hyperglycemia, liver steatosis, and adipocyte hypertrophy (HFD exacerbated)	122
Het-MλKO	Heterozygous muscle-specific PKC-λ knockout	Hyperglycemia, hyperinsulinemia, abdominal obesity, hepatosteatosis, hypertriglyceridemia, and hypercholesterolemia	123,124
Tyk2$^{-/-}$	Tyrosine kinase 2 knockout	Obesity, ↑ plasma insulin, cholesterol, and FFA	125
AdiposeENPP1-TG	Targeted overexpression of human ENPP1 in adipocytes	↑ Plasma FFA, triglycerides, glucose, insulin, fatty liver (HFD-induced)	133
Ankrd26	Partial inactivation of the Ankrd26 gene	Obesity, ↑ plasma insulin	126
PrRP-deficient mice	Targeted disruption of PrRP gene	Late-onset obesity and adiposity, ↑ blood glucose	127
Timo/Timo	Targeted disruption of Bdnf gene	Obesity, hepatic steatosis, ↑ leptin, insulin, glucose cholesterol, LDL cholesterol	128
Neil1$^{-/-}$	Targeted deletion of Neil1	Severe obesity, dyslipidemia, fatty liver disease, and tendency to hyperinsulinemia	129
GPCR12 KO	Deletion of GPCR12 gene	↑ Body weight, body fat mass, hepatic steatosis, dyslipidemia	130
RS1$^{-/-}$	Targeted deletion of RS1 gene	Obesity, ↑ total fat, serum cholesterol, and leptin	131
PLSCR3$^{-/-}$	Targeted deletion of PLSCR3 gene	↑ Abdominal fat, FFA, triglycerides, and leptin, ↓ leptin, insulin resistance, glucose intolerance	132
11β HSD-1 TG	Selective overexpression of 11β HSD-1 in adipose	Visceral obesity (HFD exacerbated) insulin resistance, hyperlipidemia, hyperphagia despite hyperleptinemia	134

Abbreviations: CMS, cardiometabolic syndrome; FFA, free fatty acids; HFD, high-fat diet; LDL, low-density lipoprotein; MGI, mouse genome informatics.

address effective therapies. Animal models have traditionally guided research and shed light on the involved mechanisms and candidate therapeutics.

In the first part of the review, we summarized the best characterized mouse models for CMS; the last comprehensive reviews focusing on CMS mouse models date back in 2010.[34,135] In the second part we reviewed the PubMed and MGI database and described mouse models that have been developed in the last 10 years. Also, we identified the models that are repeatedly used, confirming their utility and contribution to the field. We conclude that *ob/ob* and *db/db* mice are the most common obesity and IR models, whereas *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ are widely used to induce hyperlipidemia. These mice have been extensively used either as a single transgenic or combined with a different background with or without diet treatment. As far as diet-induced models are concerned, we found that HFD (40–60%) with modifications (sucrose, salt addition) is the preferred protocol, which generally leads to increased body weight, hyperlipidemia, and IR.

The significance of mouse as a proxy organism to study the human CMS has been extensively documented through numerous studies. The generation of genetically engineered mouse models and the idea of altered diet consumption date back to early 1990s. An important advantage is the availability of inbred strains, which provide a constant genetic background (within strains) that allows us to study the effects of various manipulations (gene–gene, gene–environmental) on CMS development. However, there are defined genetic differences among different inbred strains, which can alter their responsiveness threshold. In the context of CMS, it is proven that susceptibility to atherosclerosis, hyperlipidemia, hypertension, and diet adaptation varies among different mouse strains.[31,136–138] Thus, this should be taken into account when interpreting or comparing inter-study results. Furthermore, for diet-induced CMS the selection of proper control diet is crucial as cardiometabolic alterations were found to be underestimated in low fat diet-treated controls.[139] Another emerging issue that needs to be addressed is the impact of sex in the study of CMS. Traditionally, male mice are preferred over females because of the known development of a more severe disease phenotype and because of the concern that the estrous cycle induces variability in traits that complicate experimental designs; however, the latter has been debated.[140] The current scientific view encourages the study of both sexes since it can differentially affect metabolic phenotype and give ground to sex-based treatments of metabolic diseases.[141]

In conclusion, the plethora of genetically engineered mouse models, diets, drugs, or synthetic compounds that are available nowadays facilitates the research of CMS. However, each researcher should carefully select the most appropriate model for his/her research and validate the consistency of the chosen model. Overall, when translating discoveries across strains and species it is important to consider the differences between strains of the same animal species (e.g., not all mice are equivalent to C57/BL mice) and, most importantly, the differences between human and animal models.[142]

Conflict of Interest
None declared.

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