

Mouse models of the metabolic syndrome

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The metabolic syndrome (MetS) is characterized by obesity concomitant with other metabolic abnormalities such as hypertriglyceridemia, reduced high-density lipoprotein levels, elevated blood pressure and raised fasting glucose levels. The precise definition of MetS, the relationships of its metabolic features, and what initiates it, are debated. However, obesity is on the rise worldwide, and its association with these metabolic symptoms increases the risk for diabetes and cardiovascular disease (among many other diseases). Research needs to determine the mechanisms by which obesity and MetS increase the risk of disease. In light of this growing epidemic, it is imperative to develop animal models of MetS. These models will help determine the pathophysiological basis for MetS and how MetS increases the risk for other diseases. Among the various animal models available to study MetS, mice are the most commonly used for several reasons. First, there are several spontaneously occurring obese mouse strains that have been used for decades and that are very well characterized. Second, high-fat feeding studies require only months to induce MetS. Third, it is relatively easy to study the effects of single genes by developing transgenic or gene knockouts to determine the influence of a gene on MetS. For these reasons, this review will focus on the benefits and caveats of the most common mouse models of MetS. It is our hope that the reader will be able to use this review as a guide for the selection of mouse models for their own studies.

Introduction

The clustering of several metabolic abnormalities within an individual was first discussed by Dr Reaven in his Banting lecture in 1988 (Reaven, 1988). Although this clinical phenotype has been given different names over the years (insulin resistance syndrome, Syndrome X), it is now most commonly referred to as the metabolic syndrome (MetS). The constellation of metabolic abnormalities and their 'cut-off' points are still under discussion; however, the most recent consensus statement provided by the International Diabetes Federation (IDF) defines MetS as central obesity PLUS any two of the following: elevated plasma triglyceride (TG) levels (≥ 150 mg/dl), reduced high-density lipoproteins (HDL) (< 40 mg/dl for men and < 50 mg/dl for women), increased blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic), or increased fasting plasma glucose (≥ 100 mg/dl). By this definition, 20-25% of the world's population has MetS. Individuals with MetS have a twofold elevated risk of having a heart attack or stroke, and a fivefold increased risk of developing diabetes. Thus, it is crucially important that we combat MetS both in the clinic and at the bench.

This review article will focus on the primary mouse models of MetS in use today that are available commercially. Clearly, mouse models differ from human disease in many different aspects. Cut-off points such as waist circumference cannot be transferred from humans to mice. In addition, normal mouse lipoprotein profiles have

primarily atheroprotective HDL, whereas normal human lipoprotein profiles contain primarily atherogenic low-density lipoproteins (LDL) (Fig. 1A). Finally, the presence of hypertension in obese mice is inconsistent. Thus, no one mouse model can exactly mimic all aspects of human MetS. For the purpose of this review, we will define rodent MetS as obesity combined with dyslipidemia, hypertension and/or elevated glucose levels. Other things that might be of interest to consider in mouse models of MetS are systemic inflammation, hepatic steatosis and albuminuria; however, these characteristics will not be covered in the current review. In general, obesity and insulin resistance (IR) coincide in mouse models; however, there are exceptions to this, i.e. lipodystrophy, which will also be discussed. We have attempted to include more extensive descriptions of the most commonly used models. We would also like to refer readers to the Jackson Laboratory Mouse Phenome Database (<http://phenome.jax.org/pub/cgi/phenome/mpdcgi?rtn=docs/home>), which contains invaluable phenotypic data on many different mouse strains.

Models of obesity and insulin resistance

The IDF criteria for defining MetS requires the presence of visceral obesity, with a waist circumference of > 94 cm in males and > 80 cm in females. In humans, central obesity is positively associated with peripheral IR leading to hyperinsulinemia (Banerji et al., 1999). Furthermore, a fasting plasma glucose concentration of greater than 100 mg/dl is also recognized by the IDF as a defining characteristic of MetS. Through the use of mouse models, the pathophysiology by which obesity leads to the development of IR can be investigated. Similar to humans, elevations in abdominal fat and IR have been demonstrated in diet-induced and genetic mouse models of MetS.

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Table 1. Summary of obese mouse models of MetS

Model	Obesity	Hyperlipidemia	IR	Hypertension	Caveats	References
MODELS OF OBESITY						
Lep ^{ob/ob}	From weaning	↑ HDL (LDL/HDL1)	Yes	↑↓ blood pressure	No leptin signaling; strain differences; abnormalities with reproduction, HPA axis and thyroid axis	Ingalls et al., 1950; Coleman and Hummel, 1973; Nishina et al., 1994a
LepR ^{db/db}	From weaning	↑ HDL (LDL/HDL1)	Yes	↑↓ blood pressure	No leptin signaling; strain differences; abnormalities with reproduction, HPA axis and thyroid axis	Hummel et al., 1966
A ^{y/a}	Delayed onset	Slight ↑ HDL	Delayed	Yes	Tumor formation	Dickie, 1969; Tschop and Heiman, 2001
MC4-R ^{-/-}	Delayed onset; exacerbated following high-fat feeding; haploinsufficiency of MC4-R also seen in obese humans	ND	Yes	↓ blood pressure	–	Huszar et al., 1997; Tallam et al., 2005
MC3-R ^{-/-}	Delayed onset	ND	Protected	ND	↑ adiposity without an increase in body weight	Butler et al., 2000; Chen et al., 2000; Ellacott et al., 2007; Trevaskis et al., 2007
MODELS OF HYPERLIPIDEMIA						
LDLR ^{-/-}	HFD induced	↑ LDL	HFD induced	–	–	Ishibashi et al., 1993
apoE ^{-/-}	Generally resistant	↑ VLDL and LDL, ↓ HDL	Generally resistant	–	–	Plump et al., 1992; Zhang et al., 1992
MODELS OF OBESITY WITH HYPERLIPIDEMIA						
Lep ^{ob/ob} ;LDLR ^{-/-} and LepR ^{db/db} ;LDLR ^{-/-}	From weaning	↑↑ VLDL and LDL	Yes	ND	No leptin signaling; extreme hyperlipidemia	Hasty et al., 2001; Gruen et al., 2005
Lep ^{ob/ob} ;apoE ^{-/-} and LepR ^{db/db} ;apoE ^{-/-}	From weaning	↑↑ VLDL and LDL, ↓↓ HDL	Yes	ND	No leptin signaling; extreme hyperlipidemia	Gruen et al., 2005; Surmi et al., 2008
A ^{y/a} ;LDLR ^{-/-} Western diet feeding	Delayed onset	↑ VLDL and LDL	Yes	ND	Extreme hyperlipidemia	Coenen and Hasty, 2007; Nuotio-Antar et al., 2007
LDLR 3KO	From weaning	↑ VLDL and LDL	Yes	Yes	No leptin signaling; extreme hyperlipidemia	Lloyd et al., 2008
apoE 3KO	From weaning	↑ VLDL and LDL	Yes	Yes	No leptin signaling; extreme hyperlipidemia	Lloyd et al., 2008
apoE ^{-/-} 60% HFD	Over time on HFD	↑ VLDL	Yes	Yes	–	King et al., 2009
MODELS OF OBESITY WITH HYPERTENSION						
NZBWF1	Age onset	ND	Yes	Yes	–	Ryan et al., 2006
KKA ^{y/a}	Yes	–	Yes	Yes	–	Aizawa-Abe et al., 2000; Ohashi et al., 2006

ND=not determined; HFD=high-fat diet.

Many of the mouse models most commonly recognized in the study of obesity arose from spontaneous mutations. Among these are leptin-deficient (Lep^{ob/ob}), leptin receptor-deficient (LepR^{db/db}) and lethal yellow agouti (A^{y/a}) mice (Table 1). The abbreviations Lep^{ob/ob} and LepR^{db/db} will be used in this article as they bring in

elements of their traditional phenotypic, and their more recently discovered genetic, designations. Although their primary phenotype is obesity, these mice also display IR and some degree of dyslipidemia and, thus, can be considered models of MetS. Other spontaneous monogenic forms of obesity found in mice are the

tub and *fat* mutations (for a review, see Carroll et al., 2004); however, these mice are less well characterized and are not as commonly used for studies of MetS. We will also discuss two genetic knockout models of obesity, the melanocortin 4 receptor (MC4-R) and melanocortin 3 receptor (MC3-R) knockout mice.

Leptin-deficient mice

The $Lep^{ob/ob}$ model arose from a spontaneous mutation at the Jackson Laboratory (Ingalls et al., 1950), but it wasn't until 1994 that the gene mutated in $Lep^{ob/ob}$ mice was identified and named 'leptin' by Dr Friedman's group (Zhang et al., 1994). The $Lep^{ob/ob}$ mice are visually obese by 4 weeks of age, and their growth curves do not plateau even at 12 months of age. On a chow diet, $Lep^{ob/ob}$ mice can weigh greater than 100 grams, which is four times that of their littermate controls. The genetic background is an extremely important consideration for the use of $Lep^{ob/ob}$ mice in studying MetS. On all backgrounds, leptin deficiency results in hyperphagia, reduced energy expenditure and extreme obesity. On the C57BL/6J background, $Lep^{ob/ob}$ mice have mild hyperglycemia that is apparent transiently from 8 to 12 weeks of age. At this point, pancreatic β -cell compensation occurs and increased insulin levels bring glucose homeostasis under control (Genuth et al., 1971). By contrast, on the C57BL/KsJ background, $Lep^{ob/ob}$ mice develop hyperglycemia and diabetes, with blood glucose levels being sustained at about 400 mg/dl, accompanied by only temporary elevations in insulin levels and followed by β -cell failure (Coleman and Hummel, 1973). Because of the development of overt diabetes, $Lep^{ob/ob}$ mice on the C57BL/KsJ background reach their maximum weight at 3-4 months of age, after which they gradually lose weight and often die by 6 months of age (Coleman and Hummel, 1973). More recent studies of strain differences have shown that $Lep^{ob/ob}$ mice on the BALB/cJ background have a 35-40% reduction in weight gain with elevations in fed insulin and plasma TG levels, whereas $Lep^{ob/ob}$ mice on the FVB/N background have exacerbated IR compared with those on the C57BL/6J background (Qiu et al., 2001; Haluzik et al., 2004).

$Lep^{ob/ob}$ mice have elevated plasma cholesterol levels; however, the elevation is in HDL rather than very low-density lipoprotein (VLDL) or LDL (Fig. 1B; please note that the figure represents a $LepR^{db/db}$ mouse, however, the $Lep^{ob/ob}$ mice have a similar profile). The excess HDL in $Lep^{ob/ob}$ mice is larger than normal HDL, appears as a shoulder on the HDL peak (Nishina et al., 1994a), and has been labeled 'LDL/HDL1' (Silver et al., 1999). This LDL/HDL1 does not promote atherosclerotic lesion formation (Plummer and Hasty, 2008) and $Lep^{ob/ob}$ mice are actually protected from diet-induced atherosclerosis compared with C57BL/6J controls (Nishina et al., 1994b; Plummer and Hasty, 2008). Therefore, when using $Lep^{ob/ob}$ mice as a model for MetS, lipoprotein profiles should always be analyzed to determine whether changes in total plasma cholesterol levels are due to modulation of VLDL, LDL or HDL.

$Lep^{ob/ob}$ mice also have pronounced metabolic abnormalities related to reproduction, the hypothalamic-pituitary-adrenal (HPA) axis and the thyroid axis. For example, $Lep^{ob/ob}$ mice are infertile owing to the deficiency of leptin and can thus be difficult to obtain in sufficient numbers for metabolic studies. The role of leptin in regulating the HPA axis is well established (reviewed in Malendowicz et al., 2007), and the absence of leptin in $Lep^{ob/ob}$ mice significantly impacts this important metabolic regulation system.

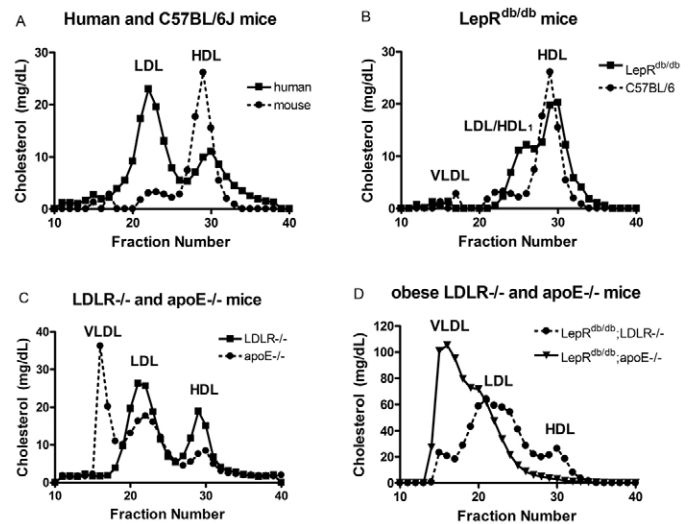


Fig. 1. Lipoprotein profiles different models. Lipoproteins were separated from plasma by running 100 μ l over a Superose 6 column at a flow rate of 0.5 ml/min in buffer containing 0.15 M NaCl, 0.01 M Na_2HPO_4 and 1 mM EDTA. Forty 0.5 ml fractions were collected, and the cholesterol concentration in fractions 10-40 was assessed. Typically, VLDL elutes in fractions 15-20, LDL in fractions 21-27, and HDL in fractions 28-34. (A) Lipoprotein profiles comparing a 'normal' human profile with a C57BL/6J profile. (B) C57BL/6J and $LepR^{db/db}$ mice. The LDL/HDL1 peak elutes in fractions 23-27. (C) Lipoprotein profiles of lean $LDLR^{-/-}$ and $apoE^{-/-}$ mice. (D) Lipoprotein profiles of obese $LDLR^{-/-}$ and $apoE^{-/-}$ mice.

These alterations in different neuroendocrine axes should be taken into account when selecting this animal model.

Leptin receptor-deficient mice

$LepR^{db/db}$ mice are deficient in the leptin receptor. As with $Lep^{ob/ob}$ mice, the obese and insulin-resistant phenotype of $LepR^{db/db}$ mice depends upon their genetic background, with mice on the C57BL/KsJ background developing diabetes and mice on the C57BL/6J background only becoming insulin resistant (Hummel et al., 1966; Coleman, 1978). The metabolic profiles of $LepR^{db/db}$ and $Lep^{ob/ob}$ mice are nearly identical. Both models are obese, hyperinsulinemic, hyperglycemic (depending on the age and strain), and have elevated total cholesterol levels with a predominance of LDL/HDL1 particles (see Fig. 1B and details in section above). In addition, the abnormalities with reproduction, HPA axis and thyroid axis are seen in both models. The primary difference between the two models is that the $LepR^{db/db}$ mice have dramatic elevations in circulating leptin concentrations that are proportional to their degree of adiposity, whereas the $Lep^{ob/ob}$ mice are absent in circulating leptin. This difference can be capitalized upon in certain studies where exogenous leptin can be added. In addition, tissue or bone marrow transplants can be performed to study the effect of leptin on other cell types (Bodary et al., 2002; Bodary et al., 2005; Surmi et al., 2008).

Agouti lethal yellow mice

Several spontaneous mutations leading to placement of the agouti (*a*) gene under the control of constitutively active promoters result in ectopic ubiquitous expression of the agouti protein. These mice

then display various coat colors and obesity phenotypes (Dickie, 1969). In the brain, the agouti protein competes against the anorexigenic factor α -melanocyte stimulating hormone (MSH) for binding to the melanocortin 4 receptor (MC4-R), acting as an antagonist of this signaling pathway (Yen et al., 1994). Thus, agouti mice display adult-onset obesity and IR owing to hyperphagia and hypoactivity (Tschop and Heiman, 2001), similar to what is seen in MC4-R^{-/-} mice (see below) (Lu et al., 1994; Fan et al., 1997; Huszar et al., 1997). The agouti yellow obese (A^{y/a}) mouse model is one of the most commonly used and is generally on the C57BL/6J or KK backgrounds, with the phenotype being more extreme in the KK strain. Lipoprotein profiles of A^{y/a} mice are similar to those of Lep^{ob/ob} and LepR^{db/db} mice, although the LDL/HDL1 peak is not as pronounced (unpublished data). Obese A^{y/a} mice are hypertensive, possibly owing to the ability of the agouti protein to inhibit α -MSH action in the brain (Mark et al., 1999).

The A^{y/a} mice express a number of characteristics that are ideal for MetS studies. They are fertile until about 4 months of age, making it easier to obtain offspring in large numbers. Also, the delayed onset of obesity and the presence of intact leptin signaling are useful traits for MetS. However, as with the Lep^{ob/ob} and LepR^{db/db} mice, they are resistant to atherosclerotic lesion formation on a high-fat diet (Nishina et al., 1994b). It should also be noted that these mice are susceptible to tumor formation.

Melanocortin 4 receptor (MC4-R) null mouse

It is becoming clear that changes to homeostatic networks in the central nervous system (CNS) can have profound effects not only on weight regulation, but on other aspects of metabolism. For example, studies by Stafford and colleagues showed that modulation of neuropeptide Y (NPY) signaling in the brain profoundly influences hepatic TG production rates, resulting in an increase in VLDL secretion (Stafford et al., 2008). It has also been shown that an increase in brain glucose concentration can reduce plasma TG levels (Lam et al., 2007). The mechanism by which changes in CNS signaling modulate hepatic TG storage versus secretion may be mediated by the central melanocortin system (Nogueiras et al., 2007).

The MC4-R is expressed in a number of nuclei in the rodent brain that are associated with autonomic and neuroendocrine pathways (Mountjoy et al., 1994). The central melanocortin system is known to mediate many of the actions of the adipokine leptin and plays a crucial role in the central regulation of energy homeostasis (for a review, see Cone, 2005). Strikingly, alterations in the *MC4R* gene are the most common monogenic cause of obesity known in humans (Vaisse et al., 1998; Yeo et al., 1998; Hinney et al., 1999; Mergen et al., 2001; Marti et al., 2003), including recent genome-wide association studies (Meyre et al., 2009; Willer et al., 2009). Furthermore, mice that are deficient in MC4-R expression show many of the same phenotypic characteristics as humans with *MC4R* gene mutations (Farooqi et al., 2000; Farooqi et al., 2003; Greenfield et al., 2009). The MC4-R-deficient mouse has a behavioral obesity syndrome characterized by hyperphagia, hyperglycemia, hyperinsulinemia, hypometabolism, and increased lean mass and linear growth (Huszar et al., 1997). Circumventing the hyperphagia by restricting the food intake of the MC4-R^{-/-} mice to the same level as wild-type controls (pair-feeding), reduces the adiposity in these animals;

however, they still remain significantly heavier than their wild-type counterparts (Ste Marie et al., 2000) highlighting the role of the hypometabolism in this phenotype. The hyperinsulinemia in this model is partially independent of obesity, as young MC4-R-deficient animals have been shown to have elevated circulating insulin levels prior to the onset of obesity (Fan et al., 2000). Despite their profound obesity in adulthood, the MC4-R-deficient animals are not hypertensive, but tend to be hypotensive (Tallam et al., 2005). Finally, another notable feature of the MC4-R null mouse is a profound sensitivity to high-fat feeding, which exacerbates the hyperphagia, obesity and hyperinsulinemia (Sutton et al., 2006).

Although MC4-R null mice develop obesity and hyperglycemia/hyperinsulinemia typical of MetS, little is known about dyslipidemia in this model. Circulating TGs and non-esterified fatty acids (NEFAs) appear normal (Albarado et al., 2004); however, as might be expected for an animal with such profound obesity, these animals show ectopic storage of fat in the liver, i.e. hepatic steatosis (Sutton et al., 2006). To the best of our knowledge, the serum lipoprotein profile of these animals has not been established, but one might anticipate that they would show a pattern similar to the A^{y/a} mouse, as both models share the MC4-R signaling deficiency.

Melanocortin 3 receptor (MC3-R) null mouse

In addition to the MC4-R, the MC3-R is the other centrally expressed melanocortin receptor that plays a role in regulating energy homeostasis, although its function is not as well studied. Genetic deficiency of the MC3-R results in a unique metabolic phenotype in mice, which is characterized by an increase in adiposity in the absence of substantial increases in body weight, food intake or alterations in glucose homeostasis (Butler et al., 2000; Chen et al., 2000). As with the MC4-R^{-/-} mouse, little work has focused on the lipoprotein profile in these animals. Despite the elevated adiposity seen in this model, animals are relatively resistant to many of the negative consequences of obesity, such as IR and hepatic steatosis, even when high-fat feeding further increases their adiposity. The resistance to the development of MetS in this model despite obesity is mediated, at least in part, by a reduced inflammatory response to obesity (Ellacott et al., 2007; Trevaskis et al., 2007); thus, this model may be useful for the study of obesity in the absence of MetS.

Summary of obese mouse models of MetS

The Lep^{ob/ob}, LepR^{db/db} and A^{y/a} mice are the three most commonly used spontaneously mutant obese mouse models. They display IR and can even develop diabetes depending on the background strain. In addition, A^{y/a} mice have intact leptin signaling and display a delayed onset obesity that can be amplified by high-fat feeding, making them a good model for human obesity. The MC4-R-deficient mouse model is similar to the A^{y/a} mice and is important because obesity in humans can be a result of mutations in the *MC4R* gene. However, with regards to plasma lipid levels and blood pressure, all three models fall short of an ideal model for MetS, and investigators should recognize this before choosing them for MetS studies.

Models of hyperlipidemia

According to the IDE, plasma TG levels of >150 mg/dl, or plasma HDL levels of <40 mg/dl in males and <50 mg/dl in females, are

two components of MetS. Visceral obesity and IR are strongly correlated with the development of dyslipidemia (Semenkovich, 2006). Increased adiposity along with elevated insulin levels can lead to an elevation of NEFAs, which impact lipoprotein metabolism. With the advent of gene targeting strategies in mice, it has been possible to generate mice which are deficient in genes that are known to influence lipoprotein metabolism. Most mouse models are hyperlipidemic (elevated VLDL and LDL) rather than dyslipidemic (elevated TG and reduced HDL); however, they are still useful for studies of MetS. Many different hyperlipidemic models have been developed; however, this review will focus on the two that are most commonly used for studies of hyperlipidemia and atherosclerosis.

Low-density lipoprotein receptor-deficient mice

One of the most severe forms of human hyperlipidemia, familial hypercholesterolemia, occurs due to mutations in the low-density lipoprotein receptor (LDLR), which results in elevated levels of the atherogenic lipoprotein LDL. Thus, the first mouse model of hyperlipidemia developed was the LDLR^{-/-} mouse (Ishibashi et al., 1993). These mice develop moderate hypercholesterolemia (total cholesterol ~250 mg/dl) on a chow diet with lipoprotein profiles similar to humans (i.e. elevated LDL) (Fig. 1C). On a high-fat/high-cholesterol 'Western-type' diet containing 21% fat and 0.15% added cholesterol, LDLR^{-/-} mice develop severe hyperlipidemia and extensive atherosclerosis (Ishibashi et al., 1993). Furthermore, when LDLR^{-/-} mice are placed on a diet with greater than 20% fat content they also become obese and display IR (Wu et al., 2006). Thus, the LDLR^{-/-} mouse model can be particularly useful when studying diet-induced obesity and IR in the presence of hyperlipidemia.

Apolipoprotein E (apoE)-deficient mice

The apolipoprotein E (apoE)-deficient mouse is another commonly used model of hyperlipidemia. ApoE is a ligand found on remnant lipoproteins that is recognized by various receptors in the liver. In humans, apoE deficiency, or the presence of mutant forms of apoE, results in type III hyperlipidemia characterized by the presence of elevated VLDL lipoproteins and an early age onset of atherosclerosis (Mahley, 1988). Unlike their LDLR^{-/-} counterparts, apoE^{-/-} mice develop a more severe hyperlipidemia (total cholesterol ~350 mg/dl), characterized by elevations in VLDL and reductions in HDL (Fig. 1C), which leads to spontaneous atherosclerosis on a chow diet (Plump et al., 1992; Zhang et al., 1992). In many cases, apoE^{-/-} mice do not become obese, nor do they develop IR, even on a high-fat diet (Gao et al., 2007; Hofmann et al., 2008). The reason that apoE^{-/-} mice are protected against obesity and IR in some studies is not entirely clear; however, there is some evidence that apoE may modulate adipocyte TG storage (Yue et al., 2004; Huang et al., 2006).

Despite other studies showing that apoE deficiency can protect against obesity, our laboratory has shown that specific dietary modulations may potentially make apoE^{-/-} mice useful for studies of MetS. Specifically, we found that apoE^{-/-} mice fed a high-fat diet (with 60% of the calories from fat) for 17 weeks have increased body weight and atherosclerosis (King et al., 2009). These high-fat-fed apoE^{-/-} mice are glucose intolerant and also display elevated systemic inflammation, as measured by increased serum amyloid

A concentrations in the plasma. Thus, apoE^{-/-} mice can be used as a model of MetS under the proper dietary conditions. In addition, other genetic modulations, such as placing them on the Lep^{ob/ob} or LepR^{db/db} background (described below), also allow for their use in MetS studies.

Models of obesity with hyperlipidemia

Obese mouse models such as A^{y/a}, Lep^{ob/ob} and LepR^{db/db} have increased total plasma cholesterol levels; however, this is the result of increased HDL rather than increased VLDL and LDL levels (Nishina et al., 1994a; Silver et al., 1999; Silver et al., 2000; Gruen et al., 2005; Gruen et al., 2006; Coenen and Hasty, 2007). The increase in HDL probably accounts for the resistance of these obese mice to atherosclerotic lesion formation (Nishina et al., 1994b). By contrast, human MetS is associated with obesity, increased levels of TG-rich VLDL and reduced levels of HDL. To generate obese mouse models with hyperlipidemia, our laboratory and others have crossed the Lep^{ob/ob}, LepR^{db/db} and A^{y/a} mice onto LDLR^{-/-} and apoE^{-/-} backgrounds. The models described below are all on the C57BL/6J strain.

Lep^{ob/ob};LDLR^{-/-} and LepR^{db/db};LDLR^{-/-} mice

To develop a model that better reflects MetS-related hyperlipidemia, we crossed Lep^{ob/ob} mice onto an LDLR^{-/-} background (Lep^{ob/ob};LDLR^{-/-}) (Hasty et al., 2001). These mice are obese and develop dramatic hypercholesterolemia characterized by elevated VLDL and LDL (Fig. 1D; please note that the figure represents a LepR^{db/db};LDLR^{-/-} mouse, however, the Lep^{ob/ob};LDLR^{-/-} mice have a similar profile), as well as hypertriglyceridemia. Furthermore, these mice spontaneously develop atherosclerotic lesions, and are thus very useful for studying the role of obesity in cardiovascular disease. Our group and others have used this model to study therapeutic treatments for MetS, as well as to study mechanisms of obesity-related hyperlipidemia (Mertens et al., 2003; Verreth et al., 2004; Hasty et al., 2006; Verreth et al., 2006; Coenen et al., 2007a). LepR^{db/db};LDLR^{-/-} mice have a phenotype identical to that of the Lep^{ob/ob};LDLR^{-/-} mice with the exception that they have very high circulating leptin levels (Gruen et al., 2006). We have also crossed the Lep^{ob/ob} and LepR^{db/db} mice onto an apoE^{-/-} background, and these mice are also obese, insulin resistant and hyperlipidemic (Gruen et al., 2006; Atkinson et al., 2008). The Lep^{ob/ob};apoE^{-/-} and LepR^{db/db};apoE^{-/-} mice have extreme elevations in VLDL and almost no HDL (Fig. 1D). Although these mice provide a better model for MetS than do Lep^{ob/ob} and LepR^{db/db} mice, because of their hyperlipidemia and susceptibility to atherosclerosis, the hyperlipidemia is quite extreme and the caveat remains that they are completely deficient in leptin signaling.

LDLR 3KO and apoE 3KO mice

Recently, Lloyd et al. crossed Lep^{ob/ob};LDLR^{-/-} and Lep^{ob/ob};apoE^{-/-} mice onto an apoB100 only background (named LDLR 3KO and apoE 3KO, respectively) (Lloyd et al., 2008). These mice are obese (>40 grams), hyperinsulinemic (>30 ng/ml), hyperlipidemic (total cholesterol >750 mg/dl and TGs >250 mg/dl) and hypertensive (systolic pressure >150 mmHg), as measured by the tail cuff method. Interestingly, the apoE 3KO mice are diabetic by 9-10 weeks of age, whereas the LDLR 3KO mice are not. This may be

because of their apoE versus LDLR genotype, or the fact that the apoE 3KO mice were only 74.6% C57BL/6J, whereas the LDLR 3KO mice were 94.7% C57BL/6J in the original report. One unique aspect to these mice is that they only express apoB100 (and not apoB48) from their livers. In humans, apoB48 is expressed solely from the intestines and apoB100 is expressed solely from the liver, whereas, in rodents, both forms of apoB are expressed in the liver. Thus, expression of only apoB100 from the livers of these mice provides a lipoprotein metabolism setting which is similar to that seen in humans.

A^y/a;LDLR^{-/-} and A^y/a;apoE^{-/-} mice

To develop a model of MetS with delayed onset obesity and hyperlipidemia, we crossed the A^y/a mice onto an LDLR^{-/-} background (Coenen et al., 2007b; Coenen and Hasty, 2007). In contrast to LDLR^{-/-} mice, the A^y/a;LDLR^{-/-} mice are obese, have an increased fat mass, and have slightly elevated plasma cholesterol and TG levels. Furthermore, when these mice are placed on a Western diet they become even more obese and hyperlipidemic, and also develop IR. The hyperlipidemia appears to be the result of both increased hepatic TG production and decreased VLDL clearance. The A^y/a;LDLR^{-/-} mice do not develop overt hypertension; however, on the Western-type diet they develop a fatty liver. Thus, these mice seem to have many different aspects of MetS and, to date, appear to be one of the best models for use.

The A^y/a and apoE^{-/-} mice have also been crossed to generate A^y/a;apoE^{-/-} mice (Gao et al., 2007). Interestingly, the deficiency of apoE protects A^y/a mice from obesity and hepatic steatosis, and improved their insulin sensitivity. Thus, the phenotype of A^y/a mice differs in the presence of LDLR versus apoE deficiency.

Summary of obese hyperlipidemic mouse models of the MetS

By crossing models of hyperlipidemia onto obesity-prone backgrounds, it is possible to study several aspects of MetS simultaneously. The mouse models listed above are the most commonly used in this regard, and their use has shed light on the mechanisms by which obesity influences lipoprotein metabolism. In addition, these models have been useful in testing various agents for their therapeutic potential. One disappointment in the studies of these models is that the presence of IR does not appear to impact atherosclerotic lesion formation in most models unless there are concomitant changes in plasma lipid levels (Merat et al., 1999; Wu et al., 2006; Coenen and Hasty, 2007; and reviewed in Goldberg and Dansky, 2006).

Models of hypertension

Blood pressure is regulated by a complex interaction between the nervous system, kidney, and a variety of humoral and mechanical factors. All individuals with MetS do not have hypertension, and the mechanisms contributing to hypertension in specific patients with MetS are not well defined. However, clinical studies have demonstrated that individuals with hypertension as a component of MetS have increased atherosclerosis (Irace et al., 2005; Kawamoto et al., 2005). This section will focus on blood pressure effects in mouse models of MetS and diet-induced obesity. However, to date, the majority of studies investigating the role of obesity in hypertension have been performed in rat models of the

disease, and few studies have investigated the role of hypertension in mouse models of obesity. Other issues include limitations in the methods used to measure moderate increases in blood pressure in mice, which will be addressed below. Given its role in hypertension, we have focused on the renin-angiotensin system (RAS) and then summarized the effects of alterations in the major adipokines, leptin and adiponectin, on hypertension as a component of MetS.

Models related to the renin-angiotensin system

In humans, a role for the RAS in the development of obesity, obesity-related hypertension and other metabolic perturbations is well established. Furthermore, angiotensin II (AngII), a potent vasoconstrictor, and other components of the RAS are present in adipose tissue (for a review, see Cassis et al., 2008). Clinical data suggest that angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) should be considered as the first line treatment for hypertension in obese patients (Masuo et al., 2001; Kintscher et al., 2007). Moreover, clinical data also suggest that ARBs improve insulin sensitivity, further demonstrating their utility in treating MetS in humans as well as in animal models of MetS.

The mRNA for the precursor of AngII, angiotensinogen, is increased in the visceral adipose tissue of mice fed a high-fat diet (Rahmouni et al., 2004). Moreover, C57BL/6J mice with diet-induced obesity and hypertension exhibit a reduction in visceral adipose tissue expression of angiotensin converting enzyme 2, an enzyme that catabolizes AngII (Gupte et al., 2008). Blockade of the AngII type 1 (AT1) receptor in these mice abolishes hypertension, as determined by telemetry (Gupte et al., 2008). Similarly, AT1 receptor deficiency decreases diet-induced increases in body weight and adiposity, and improves IR with a concomitant reduction in blood pressure, as measured by the tail cuff method (Kouyama et al., 2005). AngII also mediates biological effects through the AT2 receptor, which has been suggested to decrease AT1 receptor-mediated increases in blood pressure. Interestingly, mice that are deficient in the AT2 receptor are also protected against the development of diet-induced obesity and insulin resistance, as well as hypertension as measured by tail cuff (Yvan-Charvet et al., 2005). Thus, both clinical and animal models demonstrate an important role of the RAS in many aspects of MetS, as well as a role in obesity-related hypertension.

Models of obesity with hypertension Diet-induced obese models

Decisions regarding the use of models of hypertension can be complicated, primarily owing to inconsistencies in demonstrating hypertension in different experimental obese mouse models. This is most likely to arise from differences in background strain, age, gender, modes of inducing obesity (e.g. diet versus monogenic), and the method of measuring hypertension. For example, in C57BL/6J mice that are fed the same high-fat diet, diet-induced obesity can have differential effects on systolic blood pressure when measured by radiotelemetry and the tail cuff method (Gupte et al., 2008; Police et al., 2009; Symons et al., 2009). It is possible that the magnitude of the blood pressure increases (10-15 mmHg systolic pressure) in studies using radiotelemetry in obese mice may not be within the range of sensitivity of tail cuff methods.

Lep^{ob/ob} and LepR^{db/db} mice

Clinical data demonstrate increasing leptin concentrations with increasing blood pressure in non-obese individuals with essential hypertension (Agata et al., 1997). However, although the chronic elevations in leptin observed in obese individuals would be expected to result in enhanced sympathetic activity, which contributes to hypertension, obese individuals are generally resistant to the actions of leptin. Leptin correlates positively with adiposity and increases sympathetic activity and blood pressure when infused chronically into lean rodents (Shek et al., 1998). Thus, mouse models with the absence of intact leptin signaling pathways would be expected to have low blood pressure. In fact, Lep^{ob/ob} mice (on the C57BL/6J background) are hypotensive (Mark et al., 1999), whereas studies in LepR^{db/db} mice are conflicting, reporting that the mice are both hypotensive and hypertensive, as measured by telemetry in both reports (Bodary et al., 2007; Su et al., 2008). Transgenic mice that overexpress leptin are protected from obesity; however, they are hyperleptinemic and hypertensive. Thus, these animal models suggest that there may be selective leptin resistance, i.e. resistance to the metabolic effects while preserving the effects of leptin to enhance sympathetic activity.

NZBWF1 mice

NZBWF1 mice with systemic lupus erythematosus are a model of MetS with hyperleptinemia. These mice have age-onset obesity, increased visceral adiposity, increased plasma leptin concentrations, altered glucose tolerance and hypertension, suggesting that they are also a model of MetS with hypertension (Ryan et al., 2006). Interestingly, treatment of the NZBWF1 mice with a thiazolidinedione (TZD) reduced blood pressure, however it did not alter insulin sensitivity (Venegas-Pont et al., 2009).

KKA^{y/a} mice

The KKA^{y/a} mouse is a model of MetS with age-onset obesity, hypertension and IR (Aizawa-Abe et al., 2000; Ohashi et al., 2006). Interestingly, overexpression of adiponectin in KKA^{y/a} mice results in decreased blood pressure without altering IR. Treatment of KKA^{y/a} mice with bis(allixinato)oxovanadium, an antidiabetic drug, also decreased blood pressure, as measured by tail cuff and enhanced insulin sensitivity; however, it did not alter adiponectin concentrations (Adachi et al., 2006). Clinical studies have

demonstrated that administration of the AngII receptor blocker, telmisartan, increases adiponectin, decreases body weight, and improves insulin sensitivity with a concomitant reduction in blood pressure in patients (Makita et al., 2008; Yamada et al., 2008). Although telmisartan is an ARB, it is also a partial agonist for peroxisome proliferator-activated receptor (PPAR)- γ . Taken together, these data suggest that adiponectin may play a central role in obesity-related hypertension in humans with MetS and in mouse models of MetS. Other studies have also demonstrated that treatment with ACE inhibitors and other ARBs increases adiponectin concentrations in individuals with MetS, however, the effects on blood pressure were not presented (Tian et al., 2009).

Models of metabolic syndrome without obesity (lipodystrophy)

Adipose tissue functions primarily to store TGs and release NEFAs, as well as to secrete an array of adipokines and hormones. In humans and animals, dysregulation of adipose tissue function can lead not only to obesity, but also to lipodystrophy, resulting in increased circulation of lipids and inflammatory adipokines. This can result in progression of IR and the storage of fatty acids in organs such as the liver or muscle. Several mouse models have been developed with a focus on disruption of adipocyte function leading to lipodystrophy (Table 2). These models exhibit a number of markers of MetS, including hyperlipidemia, IR and ectopic lipid accumulation.

Adiponectin-deficient mice

Adiponectin is synthesized and secreted by adipocytes. Low circulating levels of adiponectin are strongly associated with MetS in obese humans (Arita et al., 1999). Adiponectin/ACRP30-knockout (adipo^{-/-}) mice on a C57BL/6J background have relatively normal metabolic parameters on a normal chow diet; however, they do display an increase in VLDL-TG levels (Maeda et al., 2002; Oku et al., 2007). The phenotype of adipo^{-/-} mice varies in different reports. When placed on a high-fat/high-sucrose diet for 2 weeks, adipo^{-/-} mice develop elevated plasma levels of tumor necrosis factor- α (TNF- α), glucose, insulin and free fatty acids levels (Maeda et al., 2002). By contrast, adipo^{-/-} mice on a high-fat/high-sucrose diet for 7 months do not develop IR because of enhanced beta oxidation in muscle and liver (Ma et al., 2002). Moreover, following

Table 2. Summary of non-obese and lipodystrophic models of MetS

Model	Description	Body weight	Hyperlipidemia	Hepatic steatosis	IR	Hypertension	References
aP2-SREBP-1c	Overexpress SREBP-1c in AT	↓	↑ TG	Yes	Yes	ND	Shimomura et al., 1998
Adipo ^{-/-} 2-4 weeks on diet	Adiponectin knockout	↑	↑ VLDL	No	Yes	Yes	Maeda et al., 2002; Ouchi et al., 2003
Adipo ^{-/-} 7 months on diet	Adiponectin knockout	↑	↑	ND	No	ND	Ma et al., 2002
A-ZIP/F-1	Overexpression of A-ZIP/F in adipose tissue	↓	↑	Yes	Yes	Yes	Moitra et al., 1998; Ablamunits et al., 2006; Lamounier-Zepter et al., 2008

ND=not determined; AT=adipose tissue.

a high-fat/high sucrose diet for 4 weeks, *adipo*^{-/-} mice have a higher systolic blood pressure and impaired endothelium-dependent vascular relaxation (Ouchi et al., 2003). Similarly, when maintained on a high-salt diet for 3 weeks, these mice develop hypertension, which is prevented by adiponectin treatment (Ohashi et al., 2006). When fed a 50% high-fat diet (Zhou et al., 2008), or on a choline-deficient L-amino-defined diet (Kamada et al., 2007), *adipo*^{-/-} mice develop liver steatosis that is reversed by adiponectin treatment (Zhou et al., 2008). In a separate study, Nawrocki and colleagues also developed *adipo*^{-/-} mice and, in their studies, these animals developed hepatic but not peripheral IR when placed on a high-fat diet (Nawrocki et al., 2006). In a third *adipo*^{-/-} model, Kubota et al. described increased plasma insulin, glucose and serum TG levels following feeding a 32% high-fat diet for 10 weeks. These mice also developed an increased thickness of the femoral arteries, suggesting that they are prone to develop cardiovascular disease (Kubota et al., 2002). Thus, in general, *adipo*^{-/-} mice display some aspects of MetS when placed on a high-fat diet in the presence or absence of obesity.

Transgenic aP2-SREBP-1c mice

Sterol regulatory element binding proteins (SREBP) are a family of nuclear transcription factors that regulate cholesterol and fatty acid metabolism. In mammals there are three isoforms of SREBP: SREBP-1a, SREBP-1c and SREBP-2. Transgenic aP2-SREBP-1c mice that overexpress SREBP-1c in adipose tissue have a reduced body weight and elevated plasma TG, glucose and insulin levels; they also have very low levels of adipokines such as leptin and adiponectin (Shimomura et al., 1998). They develop liver steatosis owing to an influx of plasma TG (Horton et al., 2003). Treatment of these mice with leptin prevents liver steatosis (Shimomura et al., 1999; Asilmaz et al., 2004). Currently there is no data on how a high-fat diet affects other metabolic parameters in these mice.

A-ZIP/F-1 mice

A-ZIP/F-1 mice were generated to investigate the role of the transcription factors CCAAT/enhancer binding protein (C/EBP) and activator protein-1 (AP-1) in the development and function of white adipose tissue (Moitra et al., 1998). C/EBP α , β and δ are a family of transcription factors that play a role in the growth and differentiation of adipocytes. A-ZIP/F-1 mice were developed by enhancing the expression of A-ZIP/F in adipocytes by using an aP2 enhancer promoter. Overexpression of A-ZIP/F inhibits the binding and function of B-ZIP proteins to both the C/EBP and AP-1 transcription factors. Adult A-ZIP/F-1 mice have an increased body weight compared with littermates. These mice are devoid of white adipose tissue and have reduced levels of brown adipose tissue, and thus are a model of lipodystrophy. A-ZIP/F-1 mice develop liver steatosis, which accounts for the increased body weight. These mice are also hyperglycemic, hyperinsulinemic, hyperlipidemic and hypertensive (Moitra et al., 1998; Ablamunits et al., 2006; Lamounier-Zepter et al., 2008).

Conclusions

There are many different naturally occurring and gene-targeted mutations in mice that lead to obesity and other metabolic defects associated with human MetS. Care should be taken when choosing an animal model for MetS studies, taking into account the diet used,

as well as the degree to which they develop obesity, hyperlipidemia, IR and hypertension. Although there is no perfect animal model of the human disease, each of the mouse models described have specific attributes that make them useful for studying both the mechanisms of development of MetS, as well as potential therapies.

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COMPETING INTERESTS

The authors declare no competing financial interests.

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