



Review Article

A Systematic Review on Animal Models of Metabolic Syndrome

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Metabolic syndrome (MetS) is a disorder comprising central obesity, dyslipidemia, raised blood pressure, insulin resistance. The MetS is one of the most important challenges to public health and biomedical research. The risk of developing MetS in human depends on synergy of both genetic and environmental factors. Animal models for the study of metabolic syndrome are diet induced, genetic models, chemical induced, diet and chemical induced. These models will help to determine the pathophysiological basis for MetS and how MetS increases the risk for other diseases. There are several spontaneously occurring obese mouse strains that have been used for decades and that are very well characterized. High-fat feeding studies require only months to induce MetS. Genetic model 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. In this review we analyze the data about models of MetS in rats.

Keywords: Metabolic syndrome, Obesity, High fat diet, Genetic model.

1. INTRODUCTION

MetS is also called insulin resistance syndrome, syndrome X, polymetabolic syndrome, deadly quartet, and civilization syndrome. It is a grave public health problem and is becoming increasingly widespread throughout the world¹. Metabolic syndrome includes central obesity, insulin resistance, elevated blood pressure, impaired glucose tolerance and dyslipidaemia². International Diabetes Federation (IDF) defines MetS as central obesity and 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),

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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 MetS³. Metabolic syndrome is a collection of various conditions, thus it does not have a single cause. Contributing factors for the features of MetS can be hereditary or environmental. Due to MetS multifactorial nature, selecting an adequate experimental model that best represents the pathophysiology of MetS in humans can be rather challenging⁴. The experimental models of MetS are valuable tools in the study of its pathogenesis, prevention and therapy. There are many models in rodents and other experimental animals. Since non-rational nutrition with high caloric intake is considered one of the leading causes for MetS in humans, the animal models frequently rely on manipulating the dietary intake⁵. The widespread occurrence of metabolic syndrome in humans means that there is an urgent need to study relevant causes and progression of the signs. These studies require viable animal models that adequately mimic all the aspects of the human disease, developing all major signs of metabolic syndrome, especially obesity, diabetes, dyslipidaemia, hypertension and possibly fatty liver disease and kidney dysfunction. Rodents have been used for many years as models of human disease, especially hypertension, diabetes and obesity^{6, 7}. This review will examine whether the existing rodent models for components of metabolic syndrome mimic the range of changes in humans and are therefore suitable to evaluate potential treatments for human metabolic syndrome.

Models for Metabolic syndrome

I) Diet induced metabolic syndrome:

Diet plays an important role in growth and development as a source of nutrition, but the composition of the diet decides its nutritional status. The modern diet, especially in Western countries, is rich in carbohydrates such as fructose and sucrose as well as saturated fat. This increased caloric intake has been associated with many diet induced complications including metabolic syndrome, cardiovascular diseases and nonalcoholic fatty liver disease^{8,9}.

1) Carbohydrate enriched diet

The increased prevalence of obesity and MetS, and related complications correlate with the growing carbohydrate consumption in recent years. Thus, our attention has been focused on high-carbohydrate diets as models for studying these conditions. Use of refined carbohydrates, such as HFCS (high fructose corn syrup) and sucrose, are associated with increase of body mass, rise of the level of circulating triglycerides, and development of insulin resistance in humans and animals¹⁰. Basic dietary models applied in experimental conditions include sucrose, fructose and glucose ones taking into account variations depending on the type of carbohydrate included. The increased fructose intake, for example, is considered one of the main factors in the development of obesity and the metabolic syndrome. A study on the various effects of sucrose, fructose and glucose

in rats. It was found that the groups receiving sucrose and fructose solutions increased substantially their body mass and had reduced glucose tolerance. The animals receiving granulated sugar had the biggest growth per consumed kilocalories and significantly increased retroperitoneal fatty tissue¹¹.

2. Fructose induced MetS

A fructose based meal for rats does not cause the same level of satiety as a glucose based meal for humans. When fructose and glucose were added to the drinking water of rats, different effects were observed. Acute administration of fructose was insufficient to stimulate insulin and leptin release or to inhibit ghrelin release¹².

A) Induction with 60% to 70% kcal fructose

Among the MetS criteria, this method induces insulin resistance (IR) and hypertriglyceridemia. The most appropriate animal to meet these criteria is the Golden Hamster, also called the Syrian Hamster, *Mesocricetus auratus*. After a 2 wk diet with 60% to 70% fructose, there was development of both IR and hypertriglyceridemia, demonstrating the occurrence of MetS. Supplementation of the feed of Sprague-Dawley and Wistar Albino type rats with 60% fructose (F60) was also associated with arteriopathy of the proglomerular vessels, cortical vasoconstriction, glomerular hypertension, and an increase in kidney hypertrophy. Samples were taken at the end of 6 to 8 wk of this diet¹³.

B) Induction by adding 10% fructose to drinking water

The addition of 10% fructose (F10) to the drinking water of Sprague-Dawley or Wistar Albino rats resulted in hypertension at the end of week 1. When the rats' systolic blood pressure, plasma uric acid (UA), and TG levels were measured at the end of week 8, there were no major increases. The rats did not differ significantly from controls in body weight, plasma glucose, or urinary albumin excretion. Clinical data indicate a parallel relationship between increased fructose consumption and weight gain. MetS was induced in association with elevated hypertension, hyperuricemia, and hypertriglyceridemia resulting from the F10 supplement in the drinking water. MetS is commonly induced in animal models with fructose. Most of the fructose metabolized in the liver is converted into glucose, glycogen, carbon dioxide, and lactate; the digestion, absorption, and metabolism of fructose differs from that of glucose in ways that make energy consumption likely to increase, resulting in an increased likelihood of obesity, diabetes and MetS. Worldwide HFCS and sucrose consumption have increased recently, elevating the risk for MetS. To elucidate the underlying mechanism of MetS in animal models, carbohydrates (especially 60%–70% kcal sucrose and fructose) have commonly been used. A MetS model using a 60% to 70% sucrose or fructose diet might not be realistic except for fruitarians; but in the following part of the review, we survey the information in the current literature about MetS induction with sucrose/fructose¹⁴.

3. Sucrose induced MetS

Induction with 60% to 70% kcal Sucrose

Male Wistar-Albino type rats were put on a 60% to 70% kcal sucrose diet (high starch diet [SD] or a high sucrose diet [HSD]) for 8 week (wk) to examine the changes in IR, TG concentrations, and liver fatty acid composition. The starch and sucrose diets had similar effects in terms of body weight, body fat rate, and liver fatty acid composition. A decrease in muscle glycogen synthesis was found in weeks 2, 5, and 8 of the starch diet. IR developed in the liver before it was seen in the muscles. This difference was attributed to the changes in TG concentrations in those tissues. Although a starch diet elevated the liver and plasma TGs, it was not reported to cause significant changes in the fatty acid composition of the total liver phospholipids. The male Sprague-Dawley type rats were found to have both IR and hypertriglyceridemia at the end of a 2 wk diet. Unlike older rats, those weaned on to a diet with high sucrose do not develop hypertriglyceridemia as their simple sugar intake is different. However, a high-fat diet causes IR in both young, weaned rats and adult rats¹⁵.

4. High Fat Diet Induced MetS

A) Induction with 45% to 60% fat and other fat sources

This kind of diet produces hyperglycemia and obesity, which are MetS criteria. The most important animal model used in these studies is the C57BL/6 mouse model. There were marked differences in body weight between groups fed on high and low fat diets in a 1wk diet program. Hyperglycemia takes about 4 wk to develop and is then followed by MetS¹⁶.

B) Induction by adding fat to feed (48% kcal)

In the ZDF rat model, groups were given various fat diets up to 48% kcal and were not subjected to any food or water restriction. Blood samples were collected from the animals in the morning after feeding. Among the male ZDF rats on the 12.3%, 16.7%, and 25.9% kcal fat diets, hyperglycemia developed, starting with the 23.3% kcal fat diet. The 12.3% and 16.7% kcal fat diets did not cause hyperglycemia in female ZDF rats, but hyperglycemia increased rapidly in the female rats on the 48% kcal fat diet. In light of these data, it was concluded that male ZDF rats were susceptible to diabetes on low-fat diets, whereas female ZDF rats developed diabetes only on very high fat (48% kcal) diets¹⁷.

C) Induction with fat (30% to 60% kcal) and other fat sources

In this model, where male Sprague Dawley type rats received high, medium, and low calorie fat supplements to their normal diet, a severe increase was observed in body weight within the first 6 wk. The 2 to 10 week diet program produced marked differences between diet induced obese (DIO) and diet resistant (DR) rats, and it was reported that the type of fat used could also influence weight gain¹⁸.

D) Induction with fat (32% kcal)

In this model, male Sprague Dawley type rats were exposed for 10 wk to a fat diet of 32% kcal. Systolic blood pressure, arterial hypertrophy, and plasma renin activity in rats

predisposed to obesity were doubled. Also, rats predisposed to obesity had twice the LDL-C levels and a 1.5 fold greater aortic wall deposition than obesity resistant rats, and more lipid peroxidation. The results of the study revealed that hypertension and renin-angiotensin system activation were associated with obesity. Also, obesity was shown to have detrimental effects on the kidneys and on lipid peroxidation. Although obesity is a risk factor for hypertension, the relationship between these two conditions is not completely understood. This model of obesity induced by diet is similar in hypertensive and obese individuals and is co-present with renal and vascular changes¹⁹.

5. Hypercholesterolemic MetS models

A) Induction with 0.05% to 1% high saturated fat and cholesterol

In these experiments, hamsters (*Mesocricetus auratus*) were divided into control and diet groups. Similar body weights and food consumption were noted at the beginning of the experiments. Similar plasma cholesterol levels also were noted after 2 wk of feeding. After 6 wk of diet, there was no significant difference between the total liver cholesterol (TC) concentrations. Free liver cholesterol and aortic TC concentrations rose, and atherosclerosis and hypercholesterolemia were reported, in the diet group; these are the criteria of MetS development²⁰.

B) Induction with 0.3% cholesterol and high-saturated fat

The control and diet groups comprised female guinea pigs (*Cavia porcellus*) (Hartley Guinea Pig) weighing between 250 g and 300 g. There were no differences in body weight, total plasma HDL-C, or LDL-C between groups. The 12 wk diet (0.33 g cholesterol/100 g food) also caused atherosclerosis and hypercholesterolemia²¹.

C) Induction with highly saturated fat, 1% cholesterol, and 0.25% cholic acid

In this study, C57BL/6J mice typically weighing 19 g were used. The diet group had higher total plasma cholesterol levels than the control group after the 4 wk diet. No statistically significant difference was noted between the body weights of the groups at the end of 18 wk. Mild atherosclerosis and hypercholesterolemia occurred after the 14 to 18 wk diet²².

D) Induction with 10% to 58% fat and other fat

A/J mice are resistant to obesity and do not become obese like C57BL/6 mice, even if their calorie intakes are similar. The mRNA found in the white adipose tissue (WAT) of A/J mice was seen to stimulate the expression of Uncoupling Protein 2 (UCP2). In the AKR/J mice, a high-fat diet increased the storage of fat through increased metabolism and there were differences in energy intake. These differences can be explained as follows: Although AKR/J mice on a high-fat diet generate more metabolic energy than SWR/J rats, when compared with C57BL/6 mice, they were reported to become obese after the high-fat diets²³.

E) Induction with 0.1% to 1.25% cholesterol

Male LDLR^{-/-} mice were fed a high-fat diet containing 1.25% cholesterol, 7.5% cocoa oil, 7.5% casein, and 0.5% cholic acid. Body weight increased more than in the control group and there was atherosclerosis and hypercholesterolemia. At the end of the week 12 of this diet, liver function tests showed no significant problems in the liver parenchyma or biliary system²⁴.

5) Combined High-Fat and High-Carbohydrate Diet

MetS inducing high-fat and high-carbohydrate models have various advantages. Increased levels of triglycerides are observed mainly in high fructose-fed rats whereas obesity is mainly achieved through a high-fat diet. They studied the decrease of insulin resistance of the heart and deterioration of myocardial contractility in rats. Another study traced changes in metabolism and cardiac activity during prolonged HFCD. The results showed significant lipid accumulation in the myocardium, left ventricular hypertrophy and morphological liver damage. Test animals did not present with elevated arterial pressure and no changes in the cardiac activity values at rest were recorded. In rats the combination of high-cholesterol and high-fructose diet is connected with an increase of cholesterol plasma levels, decrease of HDL-cholesterol and doubling the weight of the liver. In our study we investigated changes in time run to exhaustion during four month HFCD for inducing MetS in male Wistar rats. Time to exhaustion at 8th week, 12th week and at the end of the experiment was significantly reduced in comparison with controls. One advantage of the use of HFCD was that the cardiac tissue was damaged relatively rapidly, which allows for a detailed study of morphological, biochemical and functional features of the pathogenesis of cardiovascular changes, in addition of the metabolic ones²⁵.

6) Diet with High Content of NaCl and Fructose

These diets are used for provoking of hypertension in rats, but they can also lead to the development of MetS. The effect of increased salt intake using a diet with high content of NaCl (8% NaCl) for two weeks. No substantial change in body mass was achieved, but the values of systolic arterial pressure, plasma glucose levels, and liver gluconeogenesis increased. With high-fructose diets (60 en %), increased arterial pressure and signs of renal damage were observed even in the case of slightly increased intake of NaCl. Such dietary manipulations are mainly applied in the research of antihypertensive medications^{26,27}.

7) Diets Imitating Human Dietary Habits

Most people have improper dietary regimens characterized by high intake of fats, refined sugar and NaCl. The high energy density of foods, affordable prices and good flavour characteristics in addition to reduced physical activity lead to wide spread obesity and related diseases. In rats, the so called "Western diet" which is characterized by increased intake of saturated fatty acids, cholesterol, sugar and NaCl, affects glucose homeostasis, fat profile and adipocyte hormones. The use of tasty food in unselected Wistar rats

causes hyperphagia, increased energy intake, as well as brown adipose tissue growth, elevated body temperature and plasma leptin levels. Dietary manipulations offer great opportunities for studying the biochemical, genetic and physiologic mechanisms of obesity and the diseases related thereto. On the other hand, diets imitating dietary habits in people are diverse and it is not always possible to determine exactly which factor has had the highest effect²⁸.

8) Prenatal Dietary Manipulations

Prenatal dietary restriction leads to fetal retardation of the endocrine and metabolic status. Reduced intake of proteins during pregnancy causes reduction of the beta cellular proliferation and reduced size of the islets of Langerhans in the early ontogenesis. In rats HFD during pregnancy causes fetal insulin resistance, abnormal cholesterol metabolism and high arterial pressure. Prenatal dietary experiments are difficult to perform, could raise ethical questions with regard to the humane attitude towards animals, and should be considered for research projects only when use of other models is not possible²⁹.

2. GENETIC MODELS

Genetic animal models are imperative in order to investigate the pathogenesis of MetS caused by genetic factors. These genetic models of MetS are time saving because the duration for the development of MetS is significantly shortened compared to diet induced MetS.

1) Zucker rats (ZDF)

Obese Zucker rats (ZDF) are widely investigated and are among the best models for the study MetS. ZDF possess a (fa/fa) mutation and leptin receptor deficiency. These animals grow obese at the age of 3-5 weeks. At 14 weeks approximately 40% of their body is already composed of fat. Male animals develop diabetes mellitus. Female rats grow obese only, without developing diabetes mellitus, and have a long period of preserved insulin sensitivity. ZDF are characterized by hyperphagia, dyslipidemia, disrupted glucose tolerance, insulin resistance, hyperinsulinemia, increased expression of ghrelin, hypertension, endothelial dysfunction, proinflammatory and oxidative status. The obese Zucker rats develop severe obesity associated with hyperphagia, defective non-shivering thermogenesis and preferential deposition of energy in adipose tissue. By 14 weeks of life, body composition of the obese Zucker rats is approximately 40 % weight lipid. The affected rats develop hyperplasia and adipocyte hypertrophy. In addition to their characteristic obesity, obese Zucker rats present a range of endocrinological abnormalities. In reality, these animals are a widely extended model of insulin resistance, presenting very similar features to those characterising human metabolic syndrome. In fact, as well as resistance to the metabolic actions of insulin, these animals present dyslipidaemia, mild glucose intolerance and hyperinsulinaemia. Hyperinsulinaemia is detectable at 3 weeks and persists throughout the animals' lives, the islets of

Langerhans' hypertrophy moderately and increase in number. In addition, the animals present renal damage³⁰.

2. SHR (Spontaneously Hypertensive Rats)

Another genetic model is represented by the obese spontaneously hypertensive rats. They often have low body mass at birth. SHR milk has an altered electrolyte balance, lower protein content, and fatty acid content different from that of other breeds. Mature rats are characterised by obesity, hypertriglyceridemia and hypertension. SHR are predominantly used for studies of arterial hypertension and the related medication treatment³¹.

3. LCR (Low capacity runners)

LCR represent a genetic model of rats with low aerobic capacity. They develop hypertension, endothelial dysfunction, insulin resistance, hyperinsulinemia, visceral obesity, hypertriglyceridemia, and elevated plasma levels of nonesterified fatty acids³¹.

4. Obese spontaneously hypertensive rats/Koletsky rats

The obese SHR usually named Koletsky rats are considered an animal model with phenotypic features that strongly resemble metabolic syndrome X. This strain was originally established in 1970 by Koletsky and presents obesity, hypertension, hyperinsulinaemia, hyperlipidaemia and nephropathy superimposed on the background of SHR. The abnormal animal was derived by mating a female SHR of the Wistar – Kyoto strain with a normotensive Sprague – Dawley male. The obese rat appeared after several generations of selective inbreeding of hypertensive offspring of the original cross. The SHROB has monogenetic obesity superimposed on a hypertensive genetic background. The obesity mutation is a recessive trait, designated fak, which is a non-sense mutation of leptin receptor gene resulting in a premature stop codon in the leptin receptor extracellular domain. The SHROB carries two fak alleles, is leptin resistant and has circulating leptin levels 30 fold higher than its lean siblings. This mutation renders the SHROB incapable of central and peripheral responses to leptin. Animals can be identified as genetically obese at about 5 weeks of age. Body weight increases rapidly, and males usually attain weight of 750 – 1000 g when 7 – 12 months old. Although both sexes are involved, males are heavier than females at practically all ages. The rats uniformly develop hyperlipidaemia even though they are fed with standard diet, which was characterised by a marked triacylglycerolaemia and a moderate rise in plasma cholesterol. The animals exhibit hyperphagia and also have abnormal carbohydrate and protein metabolism. Hyperinsulinism is present in these rats and is associated with either normal or slightly elevated level of blood glucose. Spontaneous hypertension usually occurs at about 3 months of age. The arterial blood pressure rises progressively at 8 and 12 weeks of age, achieving more than 180 mm Hg, and rises progressively to 200 mm Hg between 20 and 30 weeks of age. These animals also develop premature vascular disease involving especially

abdominal arteries. Microscopically, the lesions occurred in these vessels simulate those of human atherosclerosis³².

5. Spontaneously hypertensive/N corpulent rats

The spontaneously hypertensive/N-corpulent rats are a substrain of Koletsky rats that has been developed and characterised as a model for noninsulindependent diabetes mellitus. It has been demonstrated that obese SHR/N-corpulent rats male rats have some metabolic and histopathologic characteristics similar to noninsulin dependent diabetes mellitus. Obese rats are hyperinsulinaemic, hyperlipidaemic, glucose intolerant and exhibit glycosuria and proteinuria. Hyperglycaemia is observed in obese rats following an oral glucose load or postprandially, but not in the fasting state³³.

6. Spontaneously hypertensive/NDmc-corpulent rats

The spontaneously hypertensive/NDmc-corpulent rats are an inbred subline of SHR/N-corpulent rats that also present obesity. This strain has also been used as an animal model for the metabolic syndrome. These animals are homozygous for the cp gene (cp/cp) and are hyperphagous and develop metabolic alterations, and they can be also named as (SHR-cp), whereas homozygous normal (p/p) animals are lean and hypertensive but not hyperlipidaemic and insulin resistant. The SHR-cp exhibit, in fact, metabolic and histopathologic characteristics associated with metabolic disorders in human subjects, such as increases in body and adipose tissue weights accompanying hypertension and hypercardia, diabetes and hyperlipidaemia³⁴.

7. Stroke prone – SHR fatty (fa/fa) rats

Stroke prone SHR (SHRSP) are a rat model that develops severe hypertension. SHRSP rats develop hypertension related disorders, such as nephropathy, cardiac hypertrophy and atherosclerosis, similar to human essential hypertension and 100 % die to stroke. As SHR rats, SHRSP is also a model of insulin resistance syndrome. In spite of SHRSP being a good model of hypertension and insulin resistance, SHRSP weigh less than their normotensive control, Wistar – Kyoto rats, and have reduced plasma total cholesterol and NEFA levels. A new animal model of the metabolic syndrome, by introducing a segment of the mutant leptin receptor gene from the Zucker line heterozygous for the fa gene mutation, into the genetic background of the SHRSP. Therefore, a new congenic strain, SHRSP fatty (fa/fa) rats, was derived by replacing the fa locus of chromosome from Zucker (fa/fa) rats. The SHRSP fatty rats are characterised by the spontaneous development of hypertension, obesity, hyperleptinaemia and several metabolic disorders such as hyperlipidaemia and hyperinsulinaemia³⁵.

8. Sterol regulatory element binding protein – spontaneously hypertensive rats

The relationship between the metabolic syndrome and nonalcoholic fatty liver disease has recently begun to attract considerable attention. In subjects with clinical features of the metabolic syndrome, the prevalence of nonalcoholic fatty liver disease can be very high even in the absence of

diabetes, obesity or abnormal liver enzymes. Moreover, 50 % of subjects with pure fatty liver and up to 90 % of subjects with nonalcoholic steatohepatitis have the metabolic syndrome according to Adult treatment panel III criteria. Although insulin resistance can be determinant of fatty liver, it has also been suggested that hepatic steatosis may play a role in the pathogenesis of the metabolic syndrome and promote insulin resistance in liver and skeletal muscle. Some investigators have further proposed that nonalcoholic fatty liver disease may be considered an additional feature of the metabolic syndrome. Therefore, the availability of animal models with hepatic steatosis, as well as insulin resistance, dyslipidaemia and hypertension, could be valuable for studying the pathogenesis and treatment of the metabolic syndrome and its relationship to nonalcoholic fatty liver disease. Sterol regulatory element binding proteins are transcription factors involved in the regulation of fatty acid and lipid metabolism and can activate the expression of multiple genes involved in the hepatic synthesis of cholesterol, TAG, fatty acids and phospholipids. This indicates hepatic steatosis and multiple biochemical features of the metabolic syndrome, including hyperinsulinaemia, hyperglycaemia and hypertriacylglycerolaemia in the absence of obesity. The sterol regulatory element binding protein-SHR model could therefore provide valuable opportunities for investigating pathogenetic mechanisms that may relate fatty liver disease to the metabolic syndrome³⁶.

9) Wistar Ottawa Karlsburg W rats

In 1995, a new inbred rat strain was developed, termed Wistar Ottawa Karlsburg W (WOKW) rats. These animals derived from a Wistar rat outbred strain of the Bio Breeding Laboratories (Ottawa, Ont., Canada). The WOKW strain provides a good animal model expressing the metabolic syndrome. It is especially useful because their metabolic syndrome is under polygenic control, as in human subjects, and not due to a single gene mutation. The dark agouti rats are usually used as control animals of WOKW. WOKW compared with dark agouti rats show hyperphagia, and are heavier and fatter. Segregating populations derived from this strain and inbred dark agouti rats have been successfully used to identify quantitative trait loci for major components of the metabolic syndrome, such as insulin resistance on WOKW chromosome 3 and hyper triacylglycerolaemia on WOKW chromosomes 4 and 6. The WOKW develops a nearly complete metabolic syndrome with obesity, moderate hypertension, dyslipidaemia, hyperinsulinaemia and impaired glucose tolerance. A cross sectional comparative study indicated that the WOKW rat begins to manifest the signs of the metabolic syndrome between 8 and 10 weeks of age. Very recently, the metabolic syndrome in WOKW rats has been also associated with coronary dysfunction. The dark agouti strain does not show any of these characteristics and has been considered as the control strain for the WOKW rats³⁷.

10) *ob/ob* (C57BL/6J-*ob/ob*) Mice

This was one of the first genetic models used for the study of diabetes. These mice inherited a monogenetic autosomal recessive mutation in the leptin gene on chromosome 6 and developed obesity, hyperinsulinaemia and hyperglycaemia after 4 weeks of age. They showed an increased body weight compared to their lean littermates at all ages. The presence of impaired glucose tolerance was found after 12 weeks of age. These mice developed left ventricular hypertrophy with decreased cardiac function at 24 weeks of age, cardiac fibrosis after 20 weeks of age and hepatic steatosis and inflammation at 12 weeks of age. Unlike humans with metabolic syndrome, these mice showed reduced blood pressure and did not develop dyslipidaemia even after the age of 36 weeks³⁸.

11. *db/db* (C57BL/KsJ-*db/db*) Mice

These mice have inherited an autosomal recessive mutation in the leptin receptor gene present on chromosome 4 leading to higher body weights than their lean littermates after 6 weeks of age. Fasting blood glucose concentrations were higher after 8 weeks of age and these mice showed increased plasma concentrations of triglycerides, total cholesterol and nonesterified fatty acids along with reduced HDL/LDL cholesterol ratio after 13 weeks of age. Hyperinsulinaemia and impaired glucose tolerance were observed after 12 weeks of age. In the heart, both infiltration with inflammatory cells and fibrosis were present after 12 weeks of age, although blood pressure was unchanged. These mice showed vascular endothelial dysfunction at 12 weeks of age and developed hepatic steatosis after 20 weeks of age. *db/db* mice failed to show hepatic inflammation and fibrosis³⁹.

12. Otsuka Long Evans Tokushima Fatty Rats

Otsuka Long Evans Tokushima Fatty (OLETF) rats have been used as a rat model of human diabetes and obesity. Pancreatic acini cells in OLETF rats were insensitive to the actions of cholecystokinin (CCK), which controls food intake, due to the absence of CCK-1 receptors. Male and female OLETF rats were similar in body weight to lean Long Evans Tokushima rats at the time of weaning but they became 30-40% heavier than age matched lean Long Evans Tokushima Otsuka rats after 20 weeks. Due to the lack of CCK-1 receptors, the average meal size and overall food intake were higher in OLETF rats. OLETF rats presented with high blood glucose concentrations after 18 weeks of age but they showed impaired glucose tolerance starting at 24 weeks of age. Plasma triglyceride concentrations in OLETF rats started increasing from 8 weeks of age but cholesterol concentrations were only slightly higher even after 40 weeks of age. After week 40 of age, OLETF rats showed diffuse glomerulosclerosis. Hearts from OLETF rats showed cardiac hypertrophy with left ventricular systolic and diastolic dysfunction. OLETF rats showed higher blood pressure compared to lean Long Evans Tokushima Otsuka rats after 14 weeks of age. After 34 weeks of age, OLETF

rats showed 5 times higher triglyceride deposition in liver compared to the lean Long Evans Tokushima Otsuka rats⁴⁰.

13. Goto-Kakizaki Rats

Goto-Kakizaki (GK) rats are nonobese and spontaneously diabetic. The occurrence of diabetes in these rats is an interaction of several events including presence of susceptibility loci for some diabetic traits, gestational impairment inducing decreased β -cell neogenesis and proliferation and loss of β -cell differentiation. These inbred rats were hyperglycaemic after 4 weeks of age with impaired glucose tolerance but they were lighter than the age matched Wistar rats. These rats developed cardiac hypertrophy and decreased systolic function at 20 weeks of age. There was no change in blood pressure even after 14 months of age. Plasma and liver lipid concentrations were higher in Goto-Kakizaki rats after 8 weeks of age compared to age matched Wistar rats. Goto-Kakizaki rats had higher urinary excretion of albumin and decreased creatinine clearance after 14 months of age along with increases in glomerular volume, basement membrane thickness and kidney weight. These genetic models consistently develop obesity and noninsulin dependent diabetes, but metabolic syndrome is a much broader constellation of pathophysiological changes, especially including hypertension⁴¹.

14. Cholesterol ester transfer protein (CETP) transgenic rats

CETP plays an inhibitory role in HDL mediated reverse cholesterol transport through the transfer of cholesteryl esters from HDL to VLDL and LDL, with implications for atherogenesis. The rat is a CETP deficient species in comparison to many other mammals, but the recent development of rats transgenic for simian and human CETP has permitted elegant studies of CETP function. In the CETP transgenic Fisher rat, a high-sucrose diet induced large increases in non HDL lipids with implications for reverse cholesterol transport and atherosclerosis. The CETP transgenic Dahl salt sensitive (Tg25) rat shows a marked dyslipidemia, compared to wild type rats, and advanced coronary artery lesions with a strong male to female bias. These early studies, which did not involve quantitation of cardiovascular dysfunction and disease, do indicate a promising strategy to advance the understanding of CVD. The development of transgenic rats in other CVD prone genetic backgrounds would permit definitive experimental studies not possible at present⁴².

3. CHEMICAL INDUCED Mets

MetS model induced by using chemical such as glucocorticoid, antipsychotic, alloxan and streptozotocin.

1. Glucocorticoid induced MetS

Endogenous glucocorticoids are naturally occurring stress hormones secreted by the adrenal glands. Glucocorticoids bind to its receptors (glucocorticoid and mineralocorticoid receptors) to exert their effects on different tissues. Apart from that, exogenous glucocorticoids are used as medicine to

treat a wide range of human diseases, such as autoimmune disease and cancer. It is also used to prevent rejection in organ transplantation. However, glucocorticoid treatment brings about undesirable side effects such as body weight gain, glucose intolerance, impaired calcium homeostasis, osteoporosis, cataracts and central nervous system effects [88]. Both endogenous and exogenous glucocorticoids have been used to develop MetS in animal models. Glucocorticoids cause MetS by acting directly on different tissues and organs (e.g. fat, liver, muscles, and kidneys) via several mechanisms: (1) glucocorticoids stimulate the differentiation of pre-adipocytes into mature adipocytes; (2) glucocorticoids increase lipolysis to release free fatty acids; (3) glucocorticoids increase proteolysis in muscle to increase free amino acids. Amino acid induced mammalian target of rapamycin complex-1 (mTORC1) activation causes phosphorylation of insulinreceptor substrate-1 (IRS-1), leading to the occurrence of insulin resistance; (4) glucocorticoids promote gluconeogenesis in liver and cause hyperglycemia; and (5) nonspecific binding of glucocorticoids to its receptor in the kidneys causes an increase in sodium retention, potassium excretion, water retention, and plasmavolume concomitantly with elevation of blood pressure. Using laboratory animals, glucocorticoid induced MetS has been done through various approaches, such as feeding, daily intraperitoneal injections, or surgically implanted glucocorticoid pellets⁴³. All these different routes of administration of glucocorticoids resulted in almost similar outcomes. Mounting levels of corticosterone enhanced food intake, weight gain, abdominal fat accumulation, severe fasting hyperglycemia, insulin resistance, impaired glucose tolerance, hypertension, dyslipidemia, as well as deposition of lipids in visceral adipose, hepatic tissue and skeletal muscle in animals. Meanwhile, the removal of corticosterone reversed all these adverse conditions⁴⁴.

2. Antipsychotic induced MetS

Antipsychotic drugs are medications used to treat neuropsychiatric disorders, for examples, schizophrenia, depression, and bipolar disorder. Antipsychotic drugs have been associated with a high incidence of MetS, evidenced by body weight gain, increased visceral fat, impaired glucose tolerance, and insulin resistance in animal studies. However, the exact underlying mechanism involved in antipsychotic induced MetS still remains an enigma. The proposed mechanism available currently is that the weight gain caused by antipsychotic treatment contributes to the development of diabetes and dyslipidemia. Latest evidence demonstrated that administration of the second generation antipsychotic, olanzapine, via intraperitoneal injection or oral gavage interacted with gut microbiota and caused body weight gain, increased plasma free fatty acids, infiltration of macrophages in adipose tissue, and deposition of visceral fat in both rat and mouse models^{45, 46}.

3) Alloxan and streptozotocin induced MetS

Alloxan and streptozotocin are structural analogues of glucose that enter pancreatic beta cells via the GLUT2 transporter. Single injections of alloxan or streptozotocin induce selective necrosis of pancreatic cells in rats, mice and rabbits as a model of type 1 diabetes. Chemically induced diabetic rodents show fatty liver and inflammation along with decreased ventricular contractility and function. In contrast to patients with metabolic syndrome, alloxan and streptozotocin induced diabetic rats are hypoinsulinaemic, do not gain weight and are usually hypotensive⁴⁷.

IV) High fat diet and chemical induced MetS

The High Fat Diet (HFD) along with 2% liquid cholesterol (3 ml/kg) was orally fed to rats for 3 weeks to induce metabolic syndrome. After 3 weeks of dietary manipulation, overnight fasted rats were injected intraperitoneally with STZ (40 mg /kg). The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. The body weight and biochemical parameters (Blood glucose, total cholesterol) were estimated 7 days after the vehicle or STZ injection, i.e., on 4 weeks of dietary manipulation in rats. The rats with blood glucose (>200 mg/dl), Total Cholesterol (>110 mg/dl), triglyceride (>150 mg/dl), change in body weight (8% of initial weight), Systolic Blood pressure (>130 mm/hg) and reduced HDL levels (<35mg/dl) confirmed presence of metabolic syndrome with diabetes. Thereafter the rats were either fed normal diet or HFD as per the protocol for 10 weeks⁴⁸.

V) Other animal models of MetS

Other animal models of MetS are available despite those typical laboratory rodent models, such as the use of guinea pig, swine, Nile rat, and Sand rat. A male Hartley guinea pig model of MetS was successfully developed by exposure to high-fat, high-sucrose or high-fat high-fructose diet for 150 days⁴⁹. Additionally, Ossabaw swine model of MetS was developed after fed with high-fat, high-cholesterol atherogenic diet, evidenced by obesity, elevated arterial pressure, glucose intolerance, and hyperinsulinemia. Nile rat (*Arvicanthis niloticus*) was introduced as a novel model of MetS that experiences onset of hyperglycemia, hypertension, dyslipidemia, and abdominal fat accumulation by age of one when rats were given laboratory chow diet. Sand rat (*Psammomys obesus*), found mostly in North Africa, spontaneously develops obesity and diabetes under laboratory diets. These MetS features have not been observed among the wild type of Nile and Sand rats⁵⁰.

4. CONCLUSION

The advantage of using animal models to study MetS is the ability to monitor histological, functional, biochemical, and morphological changes of MetS, which is difficult to conduct in humans. Chronic consumption of a high-carbohydrate, high-fat diet by normal rodents provides an adequate rodent model to mimic the human metabolic syndrome and for testing potential therapeutic

interventions. There are many different naturally occurring and gene targeted mutations in mice that lead to obesity and other metabolic defects associated with human MetS. All rat models included in this review could be potentially used to study the metabolic syndrome.

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