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Review Article

Experimental rat models to study the metabolic syndrome

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Being the metabolic syndrome a multifactorial condition, it is difficult to find adequate experimental models to study this pathology. The obese Zucker rats, which are homozygous for the *fa* allele, present abnormalities similar to those seen in human metabolic syndrome and are a widely extended model of insulin resistance. The usefulness of these rats as a model of non-insulin-dependent diabetes mellitus is nevertheless questionable, and they neither can be considered a clear experimental model of hypertension. Some experimental models different from the obese Zucker rats have also been used to study the metabolic syndrome. Some derive from the spontaneously hypertensive rats (SHR). In this context, the most important are the obese SHR, usually named Koletsky rats. Hyperinsulinism, associated with either normal or slightly elevated levels of blood glucose, is present in these animals, but SHR/N-corpulent rats are a more appropriated model of non-insulin-dependent diabetes mellitus. The SHR/NDmc corpulent rats, a subline of SHR/N-corpulent rats, also exhibit metabolic and histopathologic characteristics associated with human metabolic disorders. A new animal model of the metabolic syndrome, stroke-prone–SHR (SHRSP) fatty rats, was obtained 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. Very recently, it has been developed as a non-obese rat model with hypertension, fatty liver and characteristics of the metabolic syndrome by transgenic overexpression of a sterol-regulatory element-binding protein in the SHR rats. The Wistar Ottawa Karlsburg W rats are also a new strain that develops a nearly complete metabolic syndrome. Moreover, a new experimental model of low-capacity runner rats has also been developed with elevated blood pressure levels together with the other hallmarks of the metabolic syndrome.

Zucker rats: Obesity: Metabolic syndrome: Insulin resistance

The metabolic syndrome has been recognised in the medical literature for more than 80 years. The syndrome does not constitute one single illness. Instead, it can be defined as a group of health problems, caused by genetic and environmental factors, whose common fundamental pathogenic component is resistance to insulin. These problems may occur in one individual simultaneously or one by one, but their appearance together in one person is significant as these patients are more prone to CVD in general and to coronary disease in particular.

In its Third Panel of Adult Treatment, part of the National Program for Cholesterol Education, the U.S. National Health Institute gave a definition of the metabolic syndrome based on risk factors, which is straightforward to apply in epidemiological studies and daily clinical practice[(1)]. This definition does not require direct demonstration of resistance to insulin, which in clinical practice may be difficult to establish. The metabolic syndrome is assumed to exist when three or more of the following risk factors: abdominal obesity, high TAG, low cholesterol in the HDL, hyperglycaemia, while fasting and hypertension.

Being a multifactorial condition, different treatments should be used for the different patients with the metabolic syndrome, and it is impossible in the practice to develop animal strains that represent all the different patients with this syndrome. It is in fact nowadays a challenge to find adequate experimental models to study the metabolic syndrome, but some animal strains, and in particular some rat strains, with a profile of anomalies quite similar to those that characterise the majority of the patients with this syndrome, could permit nowadays to evaluate the drugs and lifestyle interventions to treat or prevent it. At the present moment, the most representative rat strain to study the metabolic syndrome seems to be the obese Zucker rats. These animals are mainly used as obesity experimental model, but they also present changes similar to those seen in human metabolic syndrome. Some experimental models different from the obese Zucker rats have also been used to study the pathogenesis, therapy and prevention of obesity, and some of them can also be used to study the metabolic syndrome. In the present review, we put forward a detailed account of the changes observed in the obese

Abbreviations: LCR, low-capacity runner rats; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone–SHR; WOKW, Wistar Ottawa Karlsburg W.

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Zucker rats, with particular regard to those which characterise the aforementioned syndrome, and we also present other rat strains with these abnormalities. Some of them derive from the spontaneously hypertensive rats (SHR).

The present review is finally focused on experimental rat models to study the metabolic syndrome, but it is also advisable to warn that other additional animal models, and in particular *Psammomys obesus* and some mouse strains, as the leptin-deficient (ob/ob) mice, the apoE-deficient mice or diet-induced obesity mice also present anomalies similar to those of the metabolic human syndrome and could therefore be used to study it.

Obese Zucker rats

Obese Zucker rats are the best known and most widely used animal model of genetic obesity. The *fa* mutation was discovered in 1961 by Lois Zucker in a cross between Merck M-strain and Sherman rats[2]. The animals that are homozygous for the *fa* allele, the *fa/fa* Zucker rats, better known as obese Zucker rats, become noticeably obese between the third and the fifth week of life. These animals present a mutation in the leptin receptor, which is the molecular base of their characteristic phenotype[3–5]. Leptin is produced by adipose tissue and plays an important role in the central regulation of energy balance[6]. This hormone is released into the circulatory system by the adipose tissue in proportion to the amount of lipids stored and acts in the brain on the leptin receptors, determining a decrease in food intake and an increase in energy expenditure[7–9]. A direct or indirect consequence of the lack of a leptin receptors-mediated counter-regulation is that obese Zucker rats display markedly elevated circulating leptin levels compared with their lean counterparts[10,11]. Old classical orexigenic peptides such as neuropeptide Y, galanin, orexins and melanin-concentrating hormone are upregulated in obese Zucker rats[12–15]. Concretely, this strain is characterised by an increased expression of ghrelin both at the peripheral and central levels[16,17]. This fact could be participating in the development of extra weight in the obese Zucker rats.

The obese Zucker rats develop severe obesity associated with hyperphagia, defective non-shivering thermogenesis and preferential deposition of energy in adipose tissue[3]. By 14 weeks of life, body composition of the obese Zucker rats is approximately 40 % weight lipid[18–20]. The affected rats develop hyperplasia and adipocyte hypertrophy[21].

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[18–25]. 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 damage[26].

At 17 d, obese Zucker rats can already be seen to eat more compared with lean animals from the same litter[27]. Hyperphagia is particularly apparent during the growth period of the obese animals, i.e. during the first 16 weeks of life[28]. Some pharmacological treatments, naloxone[29], *d*-amphetamine and fenfluramine[30], acarbose[31] and cholecystokinin[32] among others and dietary manipulations have succeeded in reducing hyperphagia in these animals to a varying degree, but have not managed to normalise the obese body composition. Lifelong food intake restriction results in a reduction in these animals' body weight, but the bodies of obese Zucker rats always continue to maintain a proportion of lipids of approximately 50 %. This percentage is much greater than the percentage of lipids found in the bodies of lean littermates (20 %)[33]. We also know that, when energy intake is reduced, these animals respond with a decrease in the number of fat cells rather than a decrease in the volume of these cells[34].

Different studies suggest that the activity of adipose tissue lipoprotein lipase activity, which is significantly correlated with enhanced TAG uptake by adipose tissue, is one of the candidates for the primary lesion produced by the presence of the *fa* gene in Zucker rats. The increase in this enzyme's activity may correlate with enhanced TAG uptake by adipose tissue[35]. Lipase lipoprotein activity, which controls lipid filling of adipocytes, is elevated in 12-d-old animals, in other words well before the animals can be visually identified as obese[36]. This change precedes other determining factors of obesity, such as enhanced liver lipogenesis and hyperinsulinaemia[37–39].

The amount of blood per unit of body weight in obese Zucker rats is lower than normal. The plasma of these animals is milky in appearance, as its fatty acid and cholesterol contents are ten and four times greater than normal, respectively. In reality, these rats present a hepatic overproduction of lipoproteins. The increase of lipids and lipoproteins in plasma is also one of the first anomalies to be observed in the rats[40–45]. They show an increase in VLDL and in HDL but although they present a decrease in the expression of hepatic receptors for LDL, they show no increase in LDL-cholesterol and cannot be used as a model of atherogenesis[46]. Like other rodents, they have larger amounts of HDL than LDL, but an increase in LDL-cholesterol can be induced in these animals by means of dietary supplements of saturated fats and cholesterol[47]. Thus the increase in TAG concentration in plasma exhibited by obese Zucker rats is due to the accumulation of VLDL, and the increase in cholesterol is due to the increase in cholesterol in the VLDL and HDL fractions. The increase in HDL-cholesterol is particularly manifest in the male rats[48]. In fact, in 1985, Lin described clear differences between obese males and females. This researcher showed that the increase in the serum cholesterol of obese females was caused principally by its high content of non-esterified cholesterol associated with VLDL. By contrast, in males, serum cholesterol was chiefly transported as esters of cholesterol with HDL.

These rat glucose levels are in reality normal or only slightly higher than normal. Therefore, these animals are not the best model to study the effective treatments to control alterations of glucose homeostasis. Nevertheless, some researchers have succeeded in identifying several vascular changes characteristic of diabetes in these rats[49]. The lipid profile of lean Zucker rats is similar to that of Sprague–Dawley[40,41] and Wistar[42] rats. These animals are sensitive to insulin, are normotensive and have a normal glucose tolerance.

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The link between obesity and hypertension has been recognised for some time. Several studies have reported conflicting results about whether obese Zucker rats are hypertensive compared with their lean controls[50–62]. Systolic arterial blood pressure in obese rats is lower than that in control lean rats of between 8 and 12 weeks of life. At 24 weeks, the phenomenon goes into reverse, and at 28 weeks, systolic arterial blood pressure in obese rats is significantly higher than in their lean counterparts. With these observations in mind, Kurtz *et al.* [53] indicated that obese Zucker rats could be considered a model of obesity and hypertension. These animals could constitute an experimental model in which hypertension was specifically associated with the genotype for obesity. The increase in arterial blood pressure in the obese animals is not due to an increase in renal Na retention[62]. The impaired vascular responses to acetylcholine that has been observed in some studies in the oldest obese Zucker rats indicate that endothelial dysfunction could justify, at least in part, the increased arterial blood pressure in these animals[63]. There is evidence for a local angiotensin II-generating system in adipose tissue[64–66], implying that the vasoactive component angiotensin II may be produced by adipose tissue. Angiotensin II is a powerful stimulus for the generation of reactive oxygen species in the blood vessels[67,68]. This increased oxidative stress may interact with NO function, leading to endothelial dysfunction[69]. Therefore, we can also assume that the increased proportion of adipose tissue in the obese Zucker rats, and consequently the increased production of angiotensin II and reactive oxygen species, could facilitate the development of hypertension and endothelial dysfunction in these animals.

Obesity is also associated with a state of chronic inflammation characterised by abnormal production of proinflammatory mediators[70], including TNF-α[71,72]and inducible NO synthase[73]. This inflammatory state is associated with a deficit of energy in the form of ATP[74,75]and simultaneous overproduction of fat and leptin, which is accompanied by leptin resistance in the brain[74,76]. Recent studies have shown that fat tissue is not a simple energy storage organ, but exerts important endocrine and immune functions. These are achieved predominantly through the release of several factors termed ‘adipocytokines’, which include several novel and highly active molecules released abundantly by adipocytes like above-mentioned leptin, as well as some more classical cytokines released possibly by inflammatory cell infiltrating fat like, TNF-α, IL-6, monocyte chemotactic protein-1 and IL-1[77]. In this context, TNF-α, a proinflammatory cytokine, is overexpressed in obesity and likely mediates insulin resistance in the major animal models of obesity[71], including obese Zucker rats[78]. Both research groups postulated that overexpression of TNF-α induces the activation of NADPH oxidase and production of superoxide anion leading to endothelial dysfunction in obese Zucker rats.

Obese spontaneously hypertensive rats

The SHR, a well-known experimental model to study hypertension, have been also proposed as a model of insulin resistance. These rats show hypertriacylglycerolaemia, abdominal obesity and hypertension[79,83]. In the background of SHR, different strains of corpulent SHR, such as obese SHR named, Koletsky rats, SHR/N-corpulent rats and SHR/NDmc-corpulent rats, seem to be even more adequate to study the metabolic syndrome than the SHR. The leptin receptor gene is also knocked out in these rats.

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[84,85]. This strain was originally established in 1970 by Koletsky[86–88] 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 *fa*^{k}, 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 *fa*^{k} alleles, is leptin resistant and has circulating leptin levels 30-fold higher that its lean siblings. This mutation renders the SHROB incapable of central and peripheral responses to leptin[89]. 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 that 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 this vessels simulate those of human atherosclerosis[88].

Spontaneoulsy 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 non-insulin-dependent diabetes mellitus[90]. It has been demonstrated that obese SHR/N-corpulent rats male rats have some metabolic and histopathologic characteristics similar to non-insulin-dependent diabetes mellitus[91,92]. 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 state.

Spontaneoulsy 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[93,94]. 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 (+/+) 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[95] accompanying hypertension and hypercardia[96], diabetes[97,98] and hyperlipidaemia[99].

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[100]. As SHR rats, SHRSP is also a model of insulin resistance syndrome[79,101]. 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. Very recently, Hiraoka-Yamamoto *et al.*[102] have produced 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 hyperinsulinaemia.

Sterol-regulatory element-binding protein–spontaneously hypertensive rats

The relationship between the metabolic syndrome and non-alcoholic fatty liver disease has recently begun to attract considerable attention[103–105]. In subjects with clinical features of the metabolic syndrome, the prevalence of non-alcoholic 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 non-alcoholic steatohepatitis have the metabolic syndrome according to Adult treatment panel III criteria[104]. 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[106–108]. Some investigators have further proposed that non-alcoholic fatty liver disease may be considered an additional feature of the metabolic syndrome[120]. 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 non-alcoholic fatty liver disease. Very recently, Qi *et al.*[109] have created a non-obese rat model with hypertension, fatty liver and characteristics of the metabolic syndrome by transgenic overexpression of a sterol-regulatory element-binding protein in the SHR rats. 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[110,111]. 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 syndrome.

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 BioBreeding 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[112]. The dark agouti rats are usually used as control animals of WOKW[113]. 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 hypertriacylglycerolaemia on WOKW chromosomes 4 and 6[114,115]. The WOKW develops a nearly complete metabolic syndrome with obesity, moderate hypertension, dyslipidaemia, hyperinsulinaemia and impaired glucose tolerance[114,116,117]. 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[113]. Very recently, the metabolic syndrome in WOKW rats has been also associated with coronary dysfunction[118]. The dark agouti strain does not show any of these characteristics and has been considered as the control strain for the WOKW rats[112,113].

Low-capacity runner rats

Very recently, Wisløff *et al.*[119] have generated an animal model of the metabolic syndrome. To obtain this model, rats were selectively bred based on their ability to perform on a treadmill endurance running task. Accordingly, rats that have a high intrinsic aerobic capacity and are capable of running comparatively long distances are classified as high-capacity runner rats and are bred together. On the other hand, rats with a low intrinsic aerobic capacity that are only capable of running relatively short distances are classified as low-capacity runner (LCR) rats and are bred with each other. Eleven generations of selective breeding resulted in elevated blood pressure in LCR rats when compared with

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Table 1. Abnormalities that characterise the different rat stains that could be used to study the metabolic syndrome

Animal strain	Abnormalities	References
Zucker	Obesity, hyperphagia, dyslipidaemia, mild glucose intolerance, insulin resistance and hyperinsulinaemia, hyperleptinaemia, increased expression of ghrelin, hypertension and endothelial dysfunction in aged animals, proinflammatory and oxidative status	3–5,16,17,18–26,40–45,63,78
Obese SHR	Obesity, hypertriacylglycerolaemia and hypertension	79–83
Koletsky rats	Hyperinsulinaemia and nephropathy, hyperphagia, altered carbohydrate and protein metabolism and premature vascular disease	84–88
N-corpulent rats	Hyperinsulinaemia, hyperlipidaemia, glucose intolerance, glycosuria and proteinuria	90–92
NDmc-corpulent rats	Hyperphagia, hyperlipidaemia, insulin resistance and hypercardia	93–99
Stroke-prone SHR fatty	Obesity, hypertension, hyperleptinaemia, hyperlipidaemia and hyperinsulinaemia	100–102
Sterol-regulatory element binding protein–SHR	Hypertension, fatty liver and hepatic steatosis, hyperinsulinaemia, hyperglycaemia and hypertriacylglycerolaemia	109–111
Wistar Ottawa Karlsburg W	Obesity, moderate hypertension, hyperphagia, insulin resistance, dyslipidaemia, hyperinsulinaemia and impaired glucose tolerance	113–117
Low-capacity runner	Hypertension, low aerobic capacity, endothelial dysfunction, insulin resistance and hyperinsulinaemia, visceral adiposity, hypertriacylglycerolaemia and elevated plasma NEFA	119

SHR, spontaneously hypertensive rats.

high-capacity runner rats. The LCR rats also show endothelial dysfunction, insulin resistance and hyperinsulinaemia, visceral adiposity, hypertriacylglycerolaemia and elevated plasma NEFA. Therefore, one advantage of this new experimental model is that elevated blood pressure in the LCR rats occurs together with the other hallmarks of the metabolic syndrome[119].

Conclusions

All rat models included in this review could be potentially used to study the metabolic syndrome. It is well known that this syndrome is not only one illness, but an association of health problems that are not coincident in all patients. The rat strains described in this review have a profile of anomalies quite similar to those that are present in the majority of these patients, but it is very important to exactly know the typical features or abnormalities of each strain, in order to correctly use them and to obtain the adequate information in the experimental trials. The obese Zucker rats have been extensively studied and are the best known animals to study the abnormalities present in the metabolic syndrome. More studies should be performed to characterise the other strains, in particular those that have been recently described as the LCR rats. Table 1 summarises the main characteristics of each one and could permit to adequately use them.

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