

PERSPECTIVES IN THE EXPERIMENTAL STUDY OF THE METABOLIC SYNDROME

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Manuscript received: November 2014

Abstract

The metabolic syndrome (MetS) is characterized by alterations of the glucose and lipid metabolism with long-lasting effects on the individual's health state. Research animal models resembling the human MetS symptomatology have been designed throughout time in order to better understand and treat it. Currently, both the genetic animal model and the diet animal model can be used. The research animal models provide the advantage of a homogenous study which is able to more easily underline and quantify the consequences of the variation of several parameters such as: diet, obesity, hypertension, insulin resistance etc. This review presents the main experimental animal models that can be used in the study of MetS.

Rezumat

Sindromul metabolic (SM) se caracterizează prin perturbări ale metabolismului glucidic și lipidic, cu repercursiuni pe termen lung asupra stării de sănătate a individului. Pentru a înțelege și trata mai bine SM, de-a lungul timpului au fost dezvoltate modele experimentale animale care într-o măsură mai mare sau mai mică, reproduc simptomatologia SM uman. La ora actuală se pot folosi atât animale modificate genetic cât și animale la care simptomatologia SM poate fi indusă cu ajutorul dietei. Modelul experimental animal conferă avantajul unui studiu omogen în care pot fi evidențiate și cuantificate mai ușor consecințele variației unor parametri precum: dieta, obezitatea, hipertensiunea arterială, rezistența la insulină etc. În acest articol sunt prezentate principalele modele experimentale animale care pot fi folosite în studiul SM.

Keywords: metabolic syndrome (MetS), research animal model, genetic animal model, diet animal model

Introduction

Although the repercussions of metabolic disorders (obesity, hyperglycaemia, hypercholesterolemia etc.) in the human health have been observed since 1950 [14], the concept of metabolic syndrome (MetS) was described for the first time, later, in 1988, by Reaven [26]. MetS has remained an important feature of developed countries health problems ever since [2, 17, 18]. The prevalence of MetS is currently estimated at 25% of the general population [35] with some important differences depending on sex, age, ethnic groups and geographical area [21]. Thus, it seems that MetS affects approximately 40% of the population in the USA, 30% in Europe, 27% in India and 10% in Japan [14, 21]. Lately, it has been also found that the number of children with MetS is constantly growing [6, 16].

Over the time, international expert groups have awarded several definitions to the MetS, the most important differences being given by the priority of symptoms and their accepted values (Table I) [17, 18, 34]. Although, currently, there is not an universal definition of MetS; in most cases it is

defined as a cluster of complex symptoms including: obesity (abdominal adiposity), insulin resistance (IR) with impaired glucose tolerance, dyslipidaemia (combination of low level of high-density lipoproteins cholesterol and high level of triglycerides) and hypertension [1, 9]. MetS is considered to exist when at least three of the mentioned factors are present [1, 5, 17, 18, 43]. Also, a proinflammatory and prothrombotic state [10], and an elevated oxidative stress level [31] are frequently associated to the metabolic perturbations [15]. The proinflammatory state is clinically recognized by high levels of C-reactive protein (CRP), interleukin 6 (IL-6), tumour necrosis factor-alpha (TNF α), and the prothrombotic state as increased plasma fibrinogen and plasminogen activator inhibitor-1 (PAI-1) [21, 35].

Generally, it is accepted that obesity and insulin resistance (IR) are the main factors responsible for the development of other metabolic perturbations and hypertension [6, 34]. Even if, initially, Reaven and the World Health Organization (WHO) considered that IR was the core feature of MetS, lately it was noted that abdominal obesity was

strongly correlated with all metabolic disorders [26]. In 2009 the international health organizations have tried to harmonize the diagnosis criteria of metabolic syndrome, maintaining the threshold for fasting glucose and blood pressure established in 2003-2005 by Association of American Clinical

Endocrinologists (AACE), American Heart Association (AHA)/National Heart, Lung and Blood Institute (NHLBI), International Diabetes Federation (IDF) (Table I) and introducing the threshold for waist circumference according to the geographical area of patients (America, Europe and Asia) [1, 17].

Table I
Metabolic syndrome diagnosis criteria [2, 6, 9, 17, 18, 43]

Diagnosis criteria for MetS	Obesity	Dyslipidemia	Blood pressure	Glucose	IR	Other
1. WHO (1998) (World Health Organization) Definition of MetS: IR or IFG, IGT, T2DZ + ≥ 2 other risk factors	BMI > 30kg/m ² WH r > 0.9 men > 0.85women	TG ≥ 150 mg/dL HDL-C < 35 mg/dL men < 39 mg/dL women	≥ 140/90 mmHg	T2DZ IGT, IFG	IR measured under hyperinsulinaemic euglycaemic conditions	microalbuminuria ≥ 20 µg/min albumin : creatinine ratio ≥ 30 mg/g
2. EGIR (1999) (European Group for the Study of IR) Definition of MetS: IR + ≥ 2 other risk factors	WC ≥ 94 cm men ≥ 80 cm women	TG ≥ 150 mg/dL HDL-C < 39 mg/dL men + women	≥ 140/90 mmHg	Fasting glucose ≥ 110 mg/dl (but no T2DZ)	Insulin > 95% IR	—
3. NCEP-ATP III (1999/2001) (National Cholesterol Educational Program Adult Treatment Panel III) Definition of MetS: ≥ 3 risk factors. Each factor has the same importance.	WC ≥ 102 cm men ≥ 88 cm women	TG ≥ 150 mg/dL HDL-C < 40 mg/dL men < 50 mg/dL women	≥ 130/85 mmHg	Fasting glucose ≥ 110 mg/dl	—	—
4. AACE (2003) (Association of American Clinical Endocrinologists) Definition of MetS: IGT, IFG + other risk factors.	BMI > 25 kg/m ²	TG ≥ 150 mg/dL HDL-C < 40 mg/dL men < 50 mg/dL women	≥ 130/85 mmHg	IGT, IFG (but no T2DZ)	—	Polycystic ovary Syndrome History of T2DZ or CVD
5. AHA/NHLBI (2005) (American Heart Association/National Heart, Lung and Blood Institute) Updated NCEP-ATP III criteria Definition of MetS: ≥ 3 risk factors. Each factor has the same importance.	WC ≥ 102 cm men ≥ 88 cm women WC Asian population ≥ 90 cm men ≥ 80 cm women	TG ≥ 150 mg/dL HDL-C < 40 mg/dL men < 50 mg/dL women	≥ 130/85 mmHg	Fasting glucose ≥ 100 mg/dl	—	—
6. IDF 2005 (International Diabetes Federation) Definition of MetS: abdominal obesity + ≥ 2 risk factors.	WC ≥ 94 cm men ≥ 80 cm women WC could be differently defined within various ethnic groups	TG ≥ 150 mg/dL HDL-C < 40 mg/dL men < 50 mg/dL women	≥ 130/85 mmHg	Fasting glucose ≥ 100 mg/dl or T2DZ	—	—

Body mass index: BMI, Waist: Hip ratio: WH r, Waist circumference: WC, Type 2 Diabetes mellitus: T2DM, Impaired glucose tolerance: IGT, Impaired fasting glucose: IFG, Triglyceride: TG, High density lipoprotein-Cholesterol: HDL-C

The aetiology of MetS is complex; the environmental (life style, unhealthy food, external pollution etc.) and genetic factors are both involved [18, 23]. The increased prevalence of MetS during the last thirty years has been frequently associated with high carbohydrate and high fat consumption in the diet of developed western countries [16, 23, 30]. The interaction between nutrition and the human genome should also be considered. The nutrients ingested could interact with transcription factors, influencing the gene expression and metabolic response, pathways which are involved in the health status [25, 30].

The MetS is not considered a disease itself [34]; however it can be considered a pre-diabetic state [13] and a major risk factor for the development of diabetes mellitus type 2 and cardiovascular diseases [15, 21, 33]. The MetS could also be associated with several systemic diseases such as the polycystic ovary syndrome [11], non-alcoholic hepatic steatosis, cholesterol gallstones, sleep disturbance and certain forms of cancer [8, 15, 33].

Research animal models used in the study of the metabolic syndrome

Taking into account the complexity of the MetS, the animal research model is still one of the most frequently used methods to both understand the physiopathological mechanisms within the MetS and test the efficiency of a drug substance in the context of this pathology. For this, a large number of species and subspecies are used, without currently using a model specifically designed for the MetS study [3, 32].

Although rats, mice and rabbits have a lipid metabolism different from that of the humans and though they could not spontaneously develop atherosclerosis and the typical cardiovascular diseases, they are the most widely used animals in the study of MetS [32, 38].

In order to simulate a symptomatology as similar as possible to the human one, animals on which the MetS is induced by different types of diets can be used or, in order to underline, more specifically, the importance of a certain factor, genetic animal model can be employed [32, 38].

The genetic animal model

There is, currently, a wide variety of genetic animal models in which the symptoms of the MetS manifest as a result of spontaneous mutations or following genetic manipulation [38, 42].

We will, next, characterize some of the most significant research models to be employed in the MetS study.

The genetic rat model

Fatty Zucker Rats or Obese Zucker Rats (*fa/fa*; *Lepr^{fa}* mutation) were described for the first time in 1961. This strain of rats presents a spontaneous mutation of the gene *fa* or fatty; a genetic mutation which determines a modification in the leptin receptor structure (ObR) [19]. At homozygous animals, for the gene *fa* (*fa/fa*) the affinity of leptin for specific receptors decreases, its plasmatic concentration increases 10-folds, that leads to severe obesity associated with hyperphagia [3, 32, 38]. The rats become obese around the third – fifth week of life and when 14 weeks old, their body is composed of approximately 40% lipids. These rats also develop an early moderate insulin resistance and hypertriglyceridemia and a little later, arterial hypertension representing, thus, an appropriate research model for the study of the MetS [3, 38].

Heterozygous animals (*Fa/fa*) and the homozygous normal animals (*Fa/Fa*) are lean and metabolically normal animals, being used in research study as the control group [32].

A more recent variant of Zucker rats is Zucker Diabetic Fatty (ZDF) Rats, a strain that is also frequently used in the study of type 2 diabetes mellitus [40]. These animals present, beside obesity, an early impairment of glucose metabolism (hyperglycaemia and hyperinsulinaemia) as a result of defective intracellular glucose-transporters: GLUT-2; GLUT-4 [32].

Spontaneously Hypertensive Rats (SHR) is the most used model in the experimental studies of high blood pressure [3, 24]. In normal conditions, SHR rats present a moderate MetS, but symptoms like insulin resistance and high plasma triglycerides level could be more obvious if the spontaneously hypertensive rats are fed with a sucrose enriched diet [3, 24].

After several generations of crosses between the SHR female rats and normotensive Sprague Dawley male rats, Dr. Simon Koletsky and colleagues obtained in 1973 a new strain: Spontaneously Hypertensive Obese Rats (SHROB) or Koletsky rats. Along with spontaneous hypertension, SHROB present a mutation of leptin receptor gene which determines a plasma leptin concentration 30-folds higher than in lean rats [3, 32]. Consequently, these rats develop obesity associated with hyperlipidaemia and hyperinsulinaemia from the 5th week

of life; the arterial blood pressure rises progressively only after 3 months of age. SHROB are considered to be an animal model with phenotypical features that strongly resemble the human metabolic syndrome [3, 32].

The genetic mice model

The transgenic mice model with deletion (knockout) of Apolipoprotein B ($\bar{\bar{}}$) or Apolipoprotein E ($\bar{\bar{}}$) characterized by a high plasma concentration of LDL-Cholesterol and LDL-oxidized is often used in the study of metabolic syndrome, atherosclerosis and cardiovascular diseases [32].

Obesity and metabolic syndrome symptoms in mice could be obtained as a result of some mutations affecting the leptin pathway. In this way, we can use mice in which the leptin receptor (ObR) has an incomplete structure (*db/db* mice), as well as mice with structurally modified leptin (*ob/ob* mice) [19, 39].

The diet animal model

Because rodents do not develop spontaneously MetS, specific diets were developed to induce the MetS symptomatology in animals. Depending on the type of regime, the composition and origin of food components, the food intake period, the species and the strain of rodents used, the symptoms of the metabolic syndrome are more or less developed [28, 38]. Starting from the human food habits associated with the onset of the MetS, two types of diets have been developed [4, 27, 29, 37]:

- high carbohydrate diet or western diet;
- high fat diet or diet-induced obesity (DIO).

High carbohydrate diet or western diet is frequently represented by a *fructose-enriched diet*, a diet which can reproduce the human MetS symptoms in rodents at a satisfactory extent. Fructose is a monosaccharide contained in many fruits and vegetables, with a glycaemic index lower than that of glucose, which does not induce an insulin secretion like glucose, representing, thus, a good alternative sugar for the diabetic patients. However, it seems that the frequent use of fructose as an additive in solid and liquid foods (soft drinks) is at the origin of dyslipidaemia, obesity, diabetes mellitus type 2 and hypertension in young adults [37, 41]. Rats fed with a fructose-enriched diet exhibit many features of the MetS, including an increase of insulin resistance, high plasma triglycerides and an increase of systolic blood pressure, without an excessive body weight gain [12, 20]. The most negative effect induced by high fructose intake seems to be hypertriglyceridemia, as a consequence of reduced triglycerides clearance [37]. As animal species fed with fructose enriched diet there can be used adult male Wistar rats, Sprague Dawley rats and adult male mice. The severity of

symptoms depends on the food's fructose concentration and food intake period [41]. The present research models show that fructose concentration may vary between 10% – 60%; fructose can be added in food (pellets) or in the drinking water for 1 to 4 months [12, 37].

Also, the same experimental studies mention that a sucrose enriched diet could induce the same metabolic alterations like fructose; however, the fructose-enriched diet remains the most relevant western diet model to induce MetS symptomatology in rodents [41].

High fat diet or DIO (diet-induced obesity) is a high density energy diet which provides 30 to 60% of the calories from fats, being widely used in rodents experimental models to induce obesity associated with insulin resistance, hypercholesterolemia, atherosclerosis etc [7, 39]. Its effects depend both on the quantity of food intake and on the type of fat used [39]. In this respect, it is widely known that saturated fatty acids (SFA) ingestion induces more weight gain and more metabolic alterations than a diet enriched in polyunsaturated fatty acids (PUFA) [27, 29]. Also, trans-fatty acids, especially the industrially produced trans-fatty acids, are more harmful than the cis-fatty acids [4]. The negative effect of this regime could be accentuated by addition of cholesterol [39]. A high plasma fatty acid level will be used by the liver for the synthesis of glucose (gluconeogenesis), very low density lipoprotein (VLDL) and triglyceride (TG) [22]. In addition to adipose tissue and plasmatic lipoproteins, ingested fatty acids can be found in cell membrane structures, influencing their fluidity. A high fat content diet negatively affects the sensitivity of insulin receptors and the expression of the intracellular glucose transporters (GLUT4), thus contributing to the insulin resistance of the peripheral tissues [22, 36].

It also seems that not only the type and amount of fat are important in the development of obesity but also the type of food proteins. It has been, thus, proved that, compared to a casein-high fat diet (animal proteins), a soya protein-high fat diet (vegetal proteins) induces in rats a weight loss as a result of a weaker stimulation of insulin secretion by soya proteins [39].

Conclusions

It can be stated that the metabolic syndrome encompasses a complex symptomatology, affecting glucose and lipids metabolism. It is considered a specific pathology in our days, having as aetiology the genetic modifications and the human diet habits. Though there are significant differences between the human and the animal metabolism, the research animal model is widely used in order to

better understand the MetS alterations, but also to find treatments that could be later applied on humans in order to reduce complications and prolong life expectancy.

Among the experimental animal models that can be used in the study of the MetS, the diet animal model is the one that resembles the most the etiology of the human MetS. On the other hand, the genetic animal model can also be used to underline and monitor a certain parameter of the MetS.

Acknowledgements

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

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