SUPPLEMENTAL MATERIAL

Veronika Tomankova, Pavel Anzenbacher, Eva Anzenbacherova.

Effects of obesity on liver cytochromes P450 in various animal models (doi: 10.5507/bp.2017.026)

ANIMAL MODELS OF OBESITY

The exact molecular mechanisms of obesity-related health problems are still unclear. To get a better knowledge of the pathological conditions in obese individuals, it is necessary to use suitable animal models[71,72]. There are several animal models of obesity, either rodent or non-rodent; however, mice and rats are perhaps the most popular species. For the study of obesity, two classes of genetic models are commonly used, monogenic and polygenic models. Besides genetic models, there are also non-genetic ones[73].

Genetic models – monogenic models

Monogenic models of obesity are characterized by a single gene that is missing or altered. The first gene connected with obesity characterized at a molecular level was the *agouti* gene cloned by Bultman et al. in 1992 (ref.[74]). The *agouti* gene (characterized in *agouti* rodents of both Americas) determines whether a mammal's coat is banded (*agouti*) or of a single color (*non-agouti*). Mice heterozygous for the *agouti* allele have yellow coats and exhibit a tendency to obesity. The gene is transiently expressed in follicular melanocytes. It induces the production of red or yellow pheomelanin pigment and inhibits the production of black or brown pigment. The model named as lethal yellow mutant mouse (A^y) is an *agouti* mutation that leads to ectopic overexpression. This animal model of obesity has been shown to be excellent for research. Mice with spontaneous ectopic mutation of the *agouti* gene have yellow colored coats. These mice are more prone to developing type 2 diabetes, exhibiting obesity, hyperphagia, hyperinsulinemia, slightly increased lean body mass and infertility[71,73,75].

Probably the most common animal models classified as having single-gene mutations in the leptin pathway are *ob/ob* mice (obese mice), *db/db* mice (diabetic mice) and Zucker diabetic fatty (ZDF) rats. The monogenic mutations include insensitivity to leptin due to mutation of leptin receptor or enormous resistance to leptin[73].

Nowadays, the obese *"ob"* gene is probably one of the most studied genes in research of obesity. The spontaneous mutation of the *ob/ob* mouse model is responsible for loss of single *"ob"* gene function. This mutation inhibits leptin secretion, that is, the synthesis of leptin (a protein hormone regulating energy balance by inhibition of hunger), is early terminated. The leptin gene is particularly expressed in white adipose tissue and influences central control of energy equilibrium[76,77]. The *ob/ob* mice exhibit hyperphagia, hyperglycemia and hyperinsulinemia leading to insulin resistance. These defects lead to early-onset morbid obesity with diabetes and the *ob/ob* mice are also infertile[73,75]. These mice exhibit one of the forms of obesity that can be treated by leptin administration[73].

Phenotypically similar to the *ob/ob* mice is the model of *db/db* mice, deficient in leptin receptor. This autosomal recessive *"db"* mutation leads to defective signaling of leptin. The *db/db* mice also exhibit morbid obesity and hyperphagia with consequent insulin resistance. Diabetes is developed in this model of mice and therefore these mice are often used to study type 2 diabetes[71,73].

Analogous to the *db/db* mice is the model of ZDF rats in which leptin receptor is generated but remains in the intracellular space[73]. There is mutation in the extracellular domain of the leptin receptor. ZDF rats are characterized by dyslipidemia and hyperglycemia leading to morbid obesity[71,75].

The above rodent models exhibit microvascular complications (e.g. diabetic retinopathy, neuropathy and nephropathy) similar to those observed in humans, being very important for testing experimental therapeutic approaches. However, mutation in leptin or in the leptin receptor gene only rarely occurs in humans[71,78].

Genetic models – polygenic models

Obesity in humans is, however, most probably mediated by multiple genes and not only by a single gene as in case of monogenic animal models. Therefore, polygenic obesity models better reflects the human obese phenotype[71].

The onset of obesity and diabetes in humans is primarily related to a diet containing large amounts of fat[79]. In obesity research, diet-induced obesity (DIO) models of mice and rats are very often used, mainly because of their greatest similarity to human obesity[80]. The DIO rodent models are classified as a polygenic mutation[73]. The most discussed factors essential for dietary obesity include hyperphagia, social factors, stress and others[81]. It is important to select the diet to be used during the experiment as well as the strain[80]. One of the most important DIO mice models, which are increasingly popular, is the *C57BL/6J* mouse strain. This mouse strain shows similarities to human metabolic syndrome as the *C57BL/6J* mice develop obesity, hyperglycemia, hypertension and hyperinsulinemia[71,79]. If their diet is restricted in fat, these mice remain lean[82]. Another model uses the Sprague Dawley rats as many of them become obese when exposed to high-fat diet[71,73].

Non-genetic models

Because of lower costs, better accessibility and also easier maintenance, non-genetic models are increasingly used instead of the genetic models[83]. Non-genetic models of obesity primarily include chemically- and surgically-induced ones[73]. Factors characterizing non-genetic models are increasing age, overweight, high caloric intake, sedentary lifestyle, central adiposity and low birth weight[84].

An animal model of obesity that results in lesion of the arcuate nucleus is monosodium glutamate (MSG) model. It uses subcutaneous administration of MSG to newborn animals to induce obesity. The MSG model is associated with dyslipidemia and insulin resistance. Animals with MSG-induced obesity are hypophagic[10,73,85].