Short review on the induction of obesity in laboratory animals

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Patil Rashmi, Bodhankar Subhash, Aswar Urmila[*]

ABSTRACT

Valid animal models are essential for successful screening of novel therapeutic strategies against all types of diseases. Development or selection of an animal model that resembles the human disease process and symptoms is dependent on a robust knowledge of the natural history and pathogenesis of the disease. The use of animal models thus becomes to understand the underlying physiological and genetic basis of energy regulation, taste and smell perception and food choice behaviour. Obesity is a chronic metabolic disorder which results from multiple etiologies like genetic, physiological, epigenetic and environmental factors. Animal models of obesity include direct measurement of food intake to long-term studies in animals exhibiting continuous overconsumption of food containing high calories and fat. It is of prime importance that we must choose the right models with high face, construct and predictive validity. Failing to select and use appropriate animal models impede successful discovery and development of safer and more potent therapeutics and prevent wastage of money and time. We have thus provided a short review on currently available animal models of obesity. We discuss different method of induction of obesity in laboratory animals as well as the transgenic animals used in anti obesity drug discovery.

Keywords: Animal models; Obesity; Transgenic animals; Antiobesity; Drug discovery

[*]Correspondence to:
Dr. Mrs Urmila M. Aswar, PhD, Associate Professor, Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Erandwane, Pune, Maharashtra, India- 411038
aswarurmila@gmail.com

INTRODUCTION

Obesity, defined as a body mass index (BMI) $>30kg/m^2$, is a significant health problem. In human BMI between 30 and 34.9, between 35 and 39.0, more than 40 is called as class I, class II and morbidity obesity respectively. Type -1 obesity caused by excessive eating habits, sedentary lifestyle and lack of physical activities is most common. Type-2 obesity caused by disease such as Cushing's syndrome, hypothyroidism, polycystic ovarian disease and insulinoma is observed in less than 1% population. Enlargement of fat cells in the body results in child and adult type of obesity. Abdominal and limb obesities mainly occur in men and women respectively. Obesity results from an imbalance of food intake, basal metabolism, and energy expenditure. Changes in the lifestyle, financial status, society, lack of physical activity, improvisation in transport mechanisms, increased as well as altered work shifts and the worldwide availability of nutrition have driven the obesity epidemic over recent decades. Modernization, urbanization and globalization of food markets are the important elements that have significantly contributed to the obesity. At an individual level, multiple endogenous or environmental causes could lead to obesity. However, in most cases, a combination of excessive caloric intake and availability of energy-dense meals is thought to be the main contributor to obesity.

There are different methods used in the clinic to assess obesity, anthropomorphic measurement includes total body fat composition, waist circumference and skinfold thickness measurements. Other more advanced methods are hydrostatic weighing, air displacement plethysmography, bioimpedance analysis, dual- energy X-ray absorptiometry (DXA) and quantitative magnetic resonance (qMR) measurement, ultrasonography is also used to measure the thickness of subcutaneous fat, muscle and intra-abdominal depth.[1] A precise assessment of body fat distribution can be obtained from Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) also.

Obesity is associated with substantial increases in morbidity, premature mortality, impaired quality of life and large healthcare costs.[2,3] More severe cases of it are associated with an endothelial dysfunction, increased incidence of sleep apnoea, asthma, gallstones, steatohepatitis, glomerulosclerosis and dyslipidaemia. The major comorbidities include type 2 diabetes, metabolic syndrome, hypertension, dyslipidaemia, myocardial infarction, stroke, certain cancers, sleep apnoea and osteoarthritis.[4,5]

Life style management, dieting does not produce a marked reduction. Bariatric surgery, gastric banding is effective however perioperative morbidity

Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed (to be) University, Pune, Maharashtra, India-411038.

Answers to the comments raised for our article entitled '**Short review on the induction of obesity in laboratory animals**' is given below. Kindly consider.

Sr.no	Reviewer A (Comments)	Explanation
1	Introduction 1st paragraph - The mention of 6 different types of obesity is not clear. Basically, I have not heard of such a classification system for obesity. The original reference link to this topic seems to be no longer working and no other proof for such a classification exists. Therefore, either the authors find a valid reference to these statements or delete these lines	Mentioned Lines were deleted.
2	Introduction 5th paragraph – The 1st sentence is not clear "………. As well as continuation of substance abuse". I would delete the last part of the sentence as it complicates the sentence.	The last part of sentence is deleted
3	Adding few lines on the advantages and disadvantages of each animal model would enrich the review significantly	Advantages and disadvantages is now added
4	Overall, I think more discussion needs to be added into each model. Some are just few sentences which is very short given the published informationout there	Discussion has been added along with conclusion.
5	Table 1 – Composition of high fat diet section is written inconsistently. Either use kcal/g or % or both throughout the table	Correction has been done. Now the values are mentioned in % for the diet which is available. In case of HFSS and Cafeteria diet, we could not find the reference that provides in percentage. Considering, these references as very recent, we have kept it as such.
6	Table1 – as per the author guideline, table should be double spaced	Correction has been done.
7	Add references to the Cafeteria diet-induced obesity section. Similarly,add references to any sentences other than that of the authors ownstatements throughout the manuscript	References are now added as suggested.
8	Title page – please refer to author guidelines to add missing info	Required correction is now done.
9	Reference section – Needs reformatting according to the journal style	References are now as per the journal style.
10	The manuscript requires thorough screening and correction for formatting, grammar and language errors.	Grammar and language correction now has been done.

Sr.no	REVIEWER B (Comments)	Explanation
1	Information on which models have been used previously for anti-obesity drug studies was provided	Relevant information has been given.
2	advantages/disadvantages of each model for use in anti-obesity drug studies	Advantages and disadvantages are now added for each animal model.
3	lack of referencing for statements and points made throughout the paper. The authors will need to include more references tofully support the information.	References are now added.
4	Developparagraphs of 5 to 6 sentences to discuss topics.	The changes are now considered.
5	A few sentences that were verbatim or very closely worded to sentences in the first reference,	The sentences have been removed.

and surgical complications are the associated drawbacks.[6,7]

Consumption of palatable foods, rich in fat and sugar is shown to activate the mesolimbic dopamine pathway which plays an important role in the development of excessive caloric intake. Psychostimulants that augments brain dopamine level has shown to induce anorexia therefore are used as supplements marketed to curb obesity. Amphetamine derivatives desoxyephedrine, phentermine and diethylpropion, rimonabant, sibutramine, fenfluramine and dexfenfluramine were among the earliest pharmacological agents used for weight loss and withdrawn from the market due to abuse liabilities and severe side effects. The current drug approved by FDA is orlistat which is a lipase inhibitor.[8] Lorcaserin, metformin, exenatide, liraglutide, semaglutide, amylin, pramlinatide and bupropion and naltrexone are some other antiobesity drugs.[9,10] Current drug discovery demonstrates, conventional monotherapies which are getting replaced with polytherapy.[11] Also, surge of weight reducing products from herbs and alternative and complementary medicine in markets as well as increased work out centres are evident.[12,13] Hence, it can be understood that the drug discovery in antiobesity sector is highly growing and lucrative. Animal models remain indispensable for discovery, validating and optimizing novel therapeutics for their safe use in humans. The laboratory animals share similar characteristics of human obesity and its co-morbidities be developed in the quest for novel preventions and/or treatments. The objective of this review is to describe different method of induction of obesity in laboratory animals as well as the use of transgenic animals in anti-obesity drug discovery.

SEX DIFFERENCE AND OBESITY

In human's differences exist between the two sexes in terms of energy expenditure and requirements as well as in fat metabolism and fat distribution.[14] Greater storage of fat in the lower body parts in females is because of lower basal fat oxidation and increased number of α2-adrenoceptors, while decreased α2-adrenergic sensitivity in the abdominal region. This may lead to more likely storage of fat in thigh region than the abdomen compared with men who have greater storage of fat in the upper body part. Moreover, women have more subcutaneous fat than men. In laboratory rats, males gain weight steadily throughout their lives while the body weight of female rats becomes stable in early adulthood.[15] Sex difference also persists in most rodent models of Type 2 Diabetes Mellitus (T2DM) and the Zucker rat (obese rat). As a result, female

rats are better models for studying obesity during adulthood since they are more like humans in their growth patterns.[16] In addition, subcutaneous fat is found more in females due to higher concentrations of estrogen and progesterone receptors in these depots whereas males have more visceral fat. Sex of the animals is also associated with the deviation in the cellular response of the adipose tissue to high-fat feeding diet. Previous studies have shown that when 10-day old Wistar rats were fed a cafeteria diet for 14 weeks, female rats exhibited more weight gain than their male counterparts. Studies on 12 h fasted rats demonstrated that sex difference rats had greater food intake with corresponding rise in ghrelin and decrease in leptin level. Administration of fat to NMRI mice for 14 weeks resulted in accumulation of fat in retroperitoneal and parametrial sites in females, and in subcutaneous depot in males signifying the relationship between fat cell hypertrophy and the level of lard in the diet specific to site and sex.[17] On the contrary, male mice and rats are considered as gold standards for studying dietary obesity because of the absence of estrous cycle in male rats. Estrous cycle of the female animals is repeated every 4–6 d and is observed to affect the food intake of the animal during this period.[18]

The present review discusses the different animal models used to study obesity. The aim of the review is to provide the details of diet, chemicals and drugs as well as various surgical models used in laboratory to induce obesity and allied biochemical changes like hypercholesteremia and hyperglycaemia so as to study anti-obesity drugs.

1. Diet-induced (hypercaloric diets) obesity
   1.1 High fat diet
   1.2 High fat high carbohydrate diet
   1.3 High fat high salt high sucrose diet
   1.4 Cafeteria diet
2. Maternal overfeeding
3. Drug induced obesity
4. Chemical induced obesity
5. Surgical model
   5.1 Ovariectomy
   5.2 Castration
   5.3 Ventromedial hypothalamic nucleus (VMH) lesion
6. Age related obesity
7. Stress induced obesity
8. Genetic models

1. Diet-induced (hypercaloric diets) obesity
High fat diet (HFD)

The physiological mechanisms considered to be involved in high-fat diet induced obesity are associated with overconsumption of high-fat diets due to

Table.no 1   Different diets with their compositions

Type of diet	Composition of high fat diet	Comment	Reference
High fat diet	54% basic feed, 15% lard, 15% sucrose, 4% milk powder, 3% peanut, 5% egg yolk powder, 1% sesame oil, 2% salt, 0.6% dicalcium phosphate, and 0.4% mountain flour	2 weeks of feeding with HFD made 2/3 of rats with significant increase in body weight gain. 8 weeks feeding with the diet showed marked rise in lipid profile	[43]
High fat high carb diet	condensed milk (39.5%), beef tallow (20%), and fructose (17.5%). powdered rat food (15.5 %) together with 25% fructose in drinking water	16 weeks administration showed significant increase in body weight, energy intake, abdominal fat deposition, abdominal circumference. Biochemical parameters demonstrated T2DM, hyperinsulinemia, dyslipidaemia, and increased plasma leptin. Cardiovascular signs included increased systolic blood pressure, endothelial dysfunction, fibrosis, hypertrophy of heart.	[44]
High fat high salt high sucrose diet	HFSS diet consisted of 8% NaCl (w/w) saturated fat (lard, 36.9% energy/kg), simple carbohydrates (sucrose, 27.3% energy/kg).	Increased visceral and subcutaneous adiposity after twelve weeks in female rats.	[45]
Cafeteria diet	The CAF diet (75 kcal/rat/day) comprised cookies (sweet or briny), milk chocolate, cereals, potato chips, processed meats, condensed milk with sugar, high-fat cheese (parmesan or provolone) provided in excess.	14 weeks of administration increased body weight and blood glucose level.	[25]

their low satiating effects, changes in the enzymes and hormones that are involved in energy balance, such as high-fat diet-induced hyperleptinemia and hyperinsulinemia accompanied by leptin and insulin resistance, and reduced suppression of ghrelin release.[19]

Diet induced obesity model usually takes longer time to develop obesity (about 3- 5 months)[20] (Refer table 1). There is existence of positive relationship between the level of fat in the diet and body weight or gain of fat. C57BL/6 mice are genetically more susceptible to HFD induced obesity (central adiposity), impaired glucose tolerance, and T2DM. The specific fatty foods used in the diets vary across studies, ranging from Crisco fat, lard and palm oil, beef tallow, peanut butter etc,[21] the human Western diet also known as cafeteria diet (high-fat, high-salt) is more useful in inducing obesity and other comorbidities than a conventional high-fat diet.[22] The method involves access of normal, lean rats or mice to diets high in fat over a period of 3-4 months. The most commonly evaluated parameters are weight gain, other physiological parameters involve altered blood pressure, biochemical parameters such as insulin resistance, glucose intolerance,

elevated plasma leptin, elevated total cholesterol, LDL and triglycerides.[23] HFD fed animals can be used to screen antiobesity, antihyperlipidemic and antidiabetic drugs. Advantages of HFD animal models is that it is inexpensive and it is based on type of diet involved. The model is appropriate for study of non-genetic lifestyle induced obesity. The disadvantage is the duration of diet regime which is around 16 weeks with delayed onset.[24]

1.4 Cafeteria diet-induced obesity

Cafeteria diets (CD) includes various palatable foods such as chocolate, peanuts, condensed milk, etc which mimics the western diet of humans. CD induced obesity mainly results from hyperphagia that may be attributed to increased energy expenditure due to diet-induced thermogenesis (DIT) resulting from sympathetic activation of brown fat. CD fed rats demonstrates rise in adiposity, hepatosteatosis and inflammation in white fat, brown fat, and liver. Cafeteria diet differs from palatable diet in terms of meal frequency as well as meal size. (Table 1). The model helps in closely monitoring nutrients composition as well as insulin resistance and hypertriglyceridemia can be rapidly achieved.

It helps in understanding the linkage between body mass, quantity of consumed fat and effect from different type of fat.

2. Maternal overfeeding and exposure to high fat diets

Obesity in mother is considered as a high-risk factor responsible for developing obesity in offspring. Maternal obesity with increased food or nutrient intake before and during gestation may cause obesity and glucose tolerance in adult offspring. Maternal overfeeding is demonstrated as a result in an amplified and prolonged leptin surge in neonatal rat offspring.[25] The model helps for controlling the epidemic of obesity. The model can be used to study the genetic factors and the perinatal environment impacting specific developmental stages. The main disadvantage of this model is that focusing on maternal diet and maternal obesity for programming of adult disease may have a negative impact on the obesity epidemic in successive generations.[24]

3. Drug induced obesity

Weight gain is a commonly observed as a side effect with many drugs especially antipsychotic and antidepressant medications. Decreased serotoninergic and dopaminergic activity and reduced sympathetic nervous system activity results in weight gain. The possible reason could be dopaminergic system works through reward hypothesis. Food and desire to eat food act as reward. The hypothalamus via releasing neuropeptides such as leptin, ghrelin, orexin, insulin and NPY regulates food intake. Most of the drugs interfere with these regulating peptides and brings obesity. Dry mouth due to anticholinergic effect increases intake of caloric beverages.[26] Some of the examples are antipsychotic agents like clozapine, quetiapine; Antidepressants such as tricyclic antidepressants, antimanic e.g. lithium, anti-convulsant e.g. valproate, carbamazepine. Sex hormones, antidiabetic agents, antimigraine and antihistaminergic drugs, glucocorticoids, β-adrenergic receptor blocker are the other agents which fall in the drug induced obesity category. The model is useful to study the correlation with other metabolic syndromes but exhibit delayed onset which is a disadvantage.

4. Chemical induced model

Mono Sodium Glutamate (MSG) induced obesity
MSG (4.0 g/kg body weight, s.c.) on 1st to 5th day of birth to the mice pups make them obese from 6th week onwards exhibiting vagal hyperactivity and sympatho-adrenal hypoactivity with resulting hyperinsulinemia and an increase of white fat. MSG can be administered (4-10 doses) subcutaneously or intraperitoneally, daily or alternatively to the neonatal rats to get obesity in their younger age.

In animals MSG increases the regular food intake and causes metabolic disorder which increases the glucose, triglyceride, insulin and leptin levels.[27]

Gold thioglucose is used to induce obesity in rodents. Mice when administered with single intraperitoneal injection of gold thioglucose (0.8 mg/gm or 30 -40 mg/kg) demonstrates obesity in 15 days.[27] Gold thioglucose administration produces change in capillary permeability thereby in adequate blood supply to this area, hence leading to necrosis in ventromedial portion of hypothalamus. The glucose moiety present in gold thioglucose is considered important for producing the lesion. The lesion due to necrosis causes hyperphagia and consequent obesity. It also indicates the presence of special glucoreceptor cells in the ventromedial hypothalamus that are involved in the regulation of food intake. There is increase in body lipid, body lipogenesis and triglycerides. The main disadvantage of this model is longer duration for induction of obesity and high mortality rate.

5. Surgical models
5.1 Ovariectomy in female rats

Preclinical studies in rat's population suggest that abrupt hormone deprivation caused by ovariectomy causes decline in estrogen level which leads to obesity and its metabolic sequelae. Ovariectomy produces attenuation of initial leptin levels followed by increase the same after seven weeks, called as leptin resistance. Ovariectomy studies in rats have demonstrated a relationship between bilateral ovariectomy that leads to obesity with that of leptin resistance, insulin resistance, adiposity and total and LDL-cholesterol level.[28] With the help of ovariectomy we can get better understanding of consequence of hormonal changes on obesity in women.[29] The disadvantage is that every animal has to undergo surgery and onset of obesity may vary.

5.2 Castration in male rat

Androgen the primary steroid hormone produced by male gonad plays an important role in fat homeostasis. Androgen deprivation leads to obesity resulting in abdominal adiposity, excess visceral fat, increase of hepatic triglyceride levels, increase of fasting blood glucose levels. Low testosterone levels are also associated with obesity. Castrated rats accumulate fat predominantly in the abdominal subcutaneous area.[30] Hypogonadism after castration has shown to produce abdominal obesity in high-fat diet (HFD)-in C57BL/6J mice.

5.3 Ventromedial hypothalamic nucleus (VMH) lesion

VMH is the main centre of brain for appetite control, satiety, adiposity and energy homeostasis.

It exhibits role in maintenance of reduced levels of triglycerides and cholesterol as well as inhibits development of Insulin Resistance. VMH lesion can be performed stereotaxically (Co-ordinates for rat brain: 1.6 mm posterior to the bregma, anteriorly; 0.5 mm lateral to the midsagittal line, transversely; and 0.2 mm above the base of the skull, vertically) with 1 mA of current intensity and 10 s duration. Post lesion significant rise in serum cholesterol and body weight is observed.[31]

6. Age-related obesity

Age is another important parameter that has profound impact on outcomes in obesity. In humans, body weight increases with age and peaks at about 55 years in both men and women. Aging has been associated with insulin resistance due to increase accumulation of intramuscular and intrahepatic fat. Ageing has been proposed to be an independent determinant of glucose tolerance, which progressively worsens with age. Progressive decline in androgens as well as other metabolic hormones also accelerate obesity in aging individuals.

The body weight of the C57BL/6J mouse, the most commonly used mouse strain for metabolic studies, increases with age, peak of obesity is observed at 9 months. The 22-month old C57BL/6J mice exhibit reduced lean mass and increased fat mass compared with young 3-month-old mice. Glucose tolerance get affected more in rats than in mice. Studies have demonstrated increased body weight, glucose, cholesterol and leptin in aging rats.[32]

7. Stress induced Obesity

In human and non-human primates repeated exposure to social stressors, results in increase in body weight, adiposity, and the intake of high calorie meals. Stress results in alteration in negative feedback mechanism due to repeated stimulation of the HPA axis and lead to metabolic changes[33] Many studies have shown that long-term stress increases food intake and promotes weight and fat gain in human subjects.[34] In addition, obesity is found to be associated with depression. It is previously reported that high food intake that corresponds with increased body weight in olfactory bulbectomy induced depressed rats.[35]

8. Genetic Models

The inbred C57BL/6J mouse strain is widely used as a model for Diabetes Induces Obesity (DIO) because it is prone to develop severe obesity, elevated adiposity, glucose intolerance and moderate insulin resistance. Because of different metabolic changes that are encountered during study as stated above, the researchers must carefully optimize their strain

selection on the basis demand of protocol and desired research findings. For example, for studying diabetes association with obesity, C57BL/6N strain but not C57BL/6J model may be the ideal choice asC57BL/6J strain rarely develops hyperglycaemia and islet atrophy when fed HFD, while C57BL/6N with HFD develops hepatosteatosis, hyperglycaemia and hyperinsulinemia.

C57BL/6J are the inbred strain susceptible to diet-induced obesity, type 2 diabetes, and atherosclerosis.[36]

Mice homozygous for the obese spontaneous mutation, Lep ob (commonly referred to as ob or ob/ob), are deficient in circulating leptin levels. They exhibit obesity, hyperphagia, transient hyperglycaemia, glucose intolerance, and elevated plasma insulin. They are also hypometabolic, hypothermic and subfertile.

The ob/ob mice suffer from pronounced diabetes mellitus marked by severe hyperglycaemia and atrophy of pancreatic islets, leading to premature death.[37] The ob/ob mouse model is mostly used to assess the potency of novel anti-obesity medications to overcome a strong hyperphagia-driven obese phenotype.

The db/ db mice exhibit high plasma insulin demonstrated at 10-14 days, they are leptin deficient and develops obesity at 4- 5 weeks. They exhibit polyphagia, proteinuria, glycosuria, polyuria, polydipsia, hyperinsulinemia despite of severe depletion of pancreatic β cells of islet. and are leptin receptor deficient.[38]

The sand rat, a diurnal gerbil (Psammomys obesus), is an outbred polygenic model of nutrition-dependent early-onset obesity with diabetic sequelae. In natural habitat, sand rat has access to low calorie, plant diet and is lean with normal blood glucose and body weight. In laboratory conditions and access to HFD, sand rat develops obesity and hyperglycaemia.[39]

The New Zealand Obese (NZO) mouse is another inbred polygenic strain that develops obesity at 4-5 weeks, it also exhibitsT2DM due to dysregulated leptin signalling, insulin resistance and glycaemic disturbances. Adiposity in the NZO mouse is driven by a moderate hyperphagia, reduced energy expenditure and reduced voluntary activity.[40]

The TALLYHO/Jng (TH) mouse is an inbred polygenic model of T2DM that exhibits moderate obesity. TH mice exhibit insulin resistance, abnormal pancreatic morphology and function. The pathogenesis of obesity and associated comorbidity resembles polygenic human.[41] Advantage of above model is that it occurs spontaneously. Disadvantage is that it is expensive and mutation in leptin receptor cannot be correlated in human because it rarely occurs.[24]

MACAQUES

Rhesus macaque monkeys belong to group of large laboratory animals that develop late-onset obesity.[42] Obesity develops over a period of about 10–15 years, mainly due to overeating. Macaque obesity resembles with that of metabolic changes observed in human metabolic syndrome such as increased intra-abdominal fat, elevated basal insulin, impaired glucose tolerance, and elevated serum triglycerides and cholesterol.

DISCUSSION

Obesity is characterised by high BMI (>30kg/m^2). The use of animal models has been the keystone of our understanding of the underlying physiological as well as genetic basis of energy regulation and food choice behaviour. Animal obesities can be differentiated not only by their eitiology but also by behavioural characteristics. In view of the number of diversities in animal obesity only one type of animal model cannot serve as general model for human obesity. Thus, future animal research should include different animal models such as genetics, dietary, VMH and surgical methods which will help in qualitative and quantitative evaluation. This review has discussed the various approaches of developing various animal models for research and development of novel anti-obesity drugs. The review aims at providing details of diet, chemicals and drugs as well as surgical models used in laboratory. The important factors that should be considered while designing the study are time as well as ease of developing the model, as it generally take two to three months for obesity to establish, type of diet fed to the animals as geographical diversity affects the food habit and type of food available in that area may differ. Hence, calculation of nutritive value of food may provide a better insight. The associated co morbidities such as type 2 diabetes, hypercholesteremia, atherosclerosis etc must also be considered. Biochemical evaluations of insulin, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides level, fatty-acid synthase (FAS), weight of adipose tissue and expression of metabolic enzyme levels are the imperative markers that can be used to determine the metabolic changes in the body.

CONCLUSION

The review has summarized different animal models for obesity considering the conventional animal models, animal models where obesity is due to lifestyle, genetically modified models and higher animals. The selection of relevant animal model is very crucial depending upon.

(i) Availability of diet or feeding manipulation and their role in development of obesity and relevant parameters, (ii) Selection of appropriate animal strain, quantification of biochemical parameters, (iii) Use of knockout rodent models so as to understand complexity with respect to neuronal circuits, food intake and energy balance. Other than animal models, surgical models can be used such as ovariectomy and castration which is more relevant to late onset diabetes.VMH lesion also, can be used to produce obesity as it is ultimate pathway for regulation of food in obesity.

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