A Comprehensive Insight into the Development of Animal Models for Obesity Research

By Amit Goyal, Anamika Gupta Dureja, Devinder Kumar Sharma & Kunal Dhiman

Abstract – Obesity, a multifactorial, metabolic disorder, involves complex interaction between genetic and environmental factors. With an alarming increase in the prevalence of obesity worldwide, it has become a major health care burden not just in terms of the increased risk of type 2 diabetes, cardiovascular morbidity, cholelithiasis, arthiritis and certain malignancies, but also in the economic costs to healthcare providers. The great similarity and homology between the genomes of rodents and humans make these animal models an important tool in unraveling the mechanisms involved in the etiology, prevention and treatment of obesity. This review summarizes the various approaches for the induction of obesity in the rodent models via genetic manipulation, hypercaloric diets and neuroendocrine perturbations.

A COMPREHENSIVE INSIGHT INTO THE DEVELOPMENT OF ANIMAL MODELS FOR OBESITY RESEARCH

Strictly as per the compliance and regulations of:

A Comprehensive Insight into the Development of Animal Models for Obesity Research.

Amit Goyal[α], Anamika Gupta Dureja[σ], Devinder Kumar Sharma[ρ] and Kunal Dhiman[ω]

Abstract - Obesity, a multifactorial, metabolic disorder, involves complex interaction between genetic and environmental factors. With an alarming increase in the prevalence of obesity worldwide, it has become a major health care burden not just in terms of the increased risk of type 2 diabetes, cardiovascular morbidity, cholelithiasis, arthiritis and certain malignancies, but also in the economic costs to healthcare providers. The great similarity and homology between the genomes of rodents and humans make these animal models an important tool in unraveling the mechanisms involved in the etiology, prevention and treatment of obesity. This review summarizes the various approaches for the induction of obesity in the rodent models via genetic manipulation, hypercaloric diets and neuroendocrine perturbations.

Keywords : Obesity; Animal models; Rodents; Hypercaloric diet; Genome.

I. INTRODUCTION

Obesity is a metabolic disorder characterized by an excessive accumulation of fat in the body to a sufficient magnitude which adversely affects the health of an individual. It is a direct consequence of perpetual imbalance between energy intake and expenditure with storage of extra calories in the form of triglycerides in the adipose tissue[1-2]. It is increasing probably, as a consequence of easily available hypercaloric diet and an increasingly sedentary lifestyle. Thus it can be appropriately termed as New World Syndrome or Disease of Civilization[3]. In obesity, there is an increase in intake of high fat and high energy food and a decrease in daily energy expenditure[4]. Diet and physical reduce appetite or to inhibit fat absorption[5]. However in exercise remain as main stay in obesity management; nonetheless antiobesity drugs may be required either to gastric balloon may be placed to reduce stomach volume and or bowel length, leading to earlier satiation and reduced ability to absorb nutrients from food[6-7].

Animal models of obesity not only allow us to investigate the basic mechanisms by which food intake is regulated but also act as tool for investigation of mechanism of antiobesity drugs. These models also provide significant insights into the etiology of human obesity consequently aiding in the development of pharmaceuticals for treatment of obesity. The animal models used for study of obesity either have a spontaneous origin or the result of experimental manipulation of the environmental or hypothalamic center that regulate food intake and energy balance or gene expression[8]. The rodent models are advantageous also in terms of their size, ease of handling, fast reproduction rate, shorter generation time availability of accurate and reliable metabolic tests[2,5,9]. This review summarizes the various approaches for induction of obesity in the rodent models via dietary manipulations, genetic interventions or neuroendocrinological perturbations.

II. DIET INDUCED MODELS

Diet induced obesity models are best suited and simplest obesity induction models and possibly the one that most closely resembles the reality of obesity in humans. Hypercaloric value diets varying between 3.7Kcal/g and 5.4Kcal/g have proved effective for induction of obesity[10]. The environmental conditions including temperature, duration of light and dark period, number of animals per cage or the feeding system used for the cages are important to develop these types of models[11]. Hence, to minimize the data variability, it is important to control the environmental conditions. Another factor that has to be considered is the age of the animals at which study is conducted. It is most effective to start high fat diet feeding at a young age, but it is also important to take into consideration that the energy balance differs in young compared to older animals. For example rats in their pubertal age rapidly gain lean mass and show completely different metabolic features compared to aged rats, which may in turn be losing lean mass and gaining fat mass. Another important variable is the duration of an obesity producing diet, that is the longer the feeding period, the greater the increment of bodyweight gain and presumably body fat.

a) High Fat Diet (HFD) Induced Models

Diet-induced obesity (DIO) has a late onset and is developed after feeding mice with high-fat diet which includes (powdered normal chow, 365 g; lard, 310 g; casein, 250 g; cholesterol, 10 g; vitamin and mineral mix, 60 g; dl-methionine, 03 g; yeast powder, 01 g; and NaCl, 01 g for 1.0 kg of diet)[12] for 10 weeks. Prolonged

Author α : Lecturer, Department of Pharmacology, Surya School of Pharmacy, Bapror, 140417, Punjab, India.
Phone :+919914100231 E-mail : goyal141186@yahoo.com.
Author σ, ρ, ω : Surya School of Pharmacy, Bapror, 140417, Punjab, India.

Exposure to HFD results in positive energy balance and obesity in certain rodent models that can be considered an adequate model of human obesity[13-14]. The male C57BL/6J mouse is the gold standard for a diet induce obese model. The C57BL/6J mouse develops obesity only when allowed ad libitum access to a high-fat diet whereas on a low-fat diet, C57BL/6J mice remain normal. In comparison to C57BL/6J, other strains such as the A/J mouse or the C57BL/KsJ are relatively resistant to these effects when fed a HFD[15]. The adipocyte hypertrophy and hyperplasia are responsible for obese phenotype in the C57BL/6J mouse. The outbred Sprague-Dawley rat is markedly more sensitive to HFD-induced obesity than other common rat strains. However, when outbred Sprague-Dawley rats are placed on a high-energy diet, only a subset of them overeat and develop diet induced obesity (DIO) whereas others remain lean (diet resistant, DR)[16-17]. The obesity prone subset of rats becomes obese, hyperphagic, hyperleptinemic, hyperinsulinemic, hyperglycemic, and hypertriglyceridemic[17]. The obese phenotype results from hyperphagia which is caused by an increase in meal size and increased energy efficiency[18]. The DIO rat displays a central resistance to circulating leptin indicating that reduced central leptin signaling may be involved in the etiology of hyperphagia in the obesity proneness. DIO rats exhibit a positive energy balance and a significantly higher respiratory quotient than DR rats, indicating a lower usage of fat as energy substrate. The physiological aspects of this model replicate many of the features observed with the human obesity syndrome: a polygenic mode of inheritance, a persistence of the phenotype once it is established, and dysregulated glucose homeostasis[19].

b) High Fructose Fed Animal Models

Fructose has several adverse metabolic effects, including hypertriglyceridemia, hyperinsulinemia and hypertension in rodents and induces moderate obesity[20]

The abnormalities and the disease progression in fructose fed rats resemble the human condition of metabolic syndrome, and are important risk factors for coronary heart disease. As to the metabolic mechanisms underlying the effects of dietary fructose the general notion is that hepatic intermediary metabolism is more affected by ingestion of fructose than of glucose. Fructose bypasses the phosphofructokinase regulatory step and enters the pathway of glycolysis or gluconeogenesis at the triose phosphate level, resulting in increased hepatic triglyceride production[21]. Recent findings also provide a novel hypothesis: Fructose raises uric acid, which in turn inhibits nitric oxide availability. Since insulin requires nitric oxide to stimulate glucose uptake it can be speculated that fructose-induced hyperuricemia may have a pathogenic role in promoting insulin resistance and metabolic syndrome[22].

c) Cafeteria Rats

The mixture of palatable commercially available supermarket food can act as a diet to stimulate energy intake in rodents. This diet is known as cafeteria diet, It is the combination of the high fat content with a high carbohydrate content[23]. These diets can be implicated in the development of obesity, leading to significant body weight gain, fat deposition and also insulin resistance[24]. It has been suggested that rats become more obese with cafeteria diets than with pure high fat diets, indicating a greater hyperphagia arising from the food variety, texture and palatability[25].

III. Venteromedial Hypothalamic (VMH) Nucleus Lesion

a) Monosodium Glutamate (MSG)

The ventromedial hypothalamic and arcuate nuclei is considered to be the area which controls the food intake and energy expenditure. The administration of monosodium glutamate to newborn rats is responsible for the destruction of the ventromedial hypothalamic and arcuate nuclei which leads to development of obesity. The subcutaneous or intraperitoneal route can be used for administration of MSG[26-27]. The dose that varies by 2-4 g/kg of body weight of the rat for 5 times every other day, during the neonatal period of rat causes obesity[28-31]. Overeating is not responsible for the obesity in neonatal MSG treated rodents. MSG obesity is associated with high level of corticosteroids[28]. The increase in the level of glucocorticoids is due to the chronic exposure of the adrenal gland to high serum levels of leptin, which occurs in rats treated with MSG[32-33].

b) Electrical VMH Lesion

The Electrical VMH lesion can be used to induce obesity. A bilateral destruction of hypothalamic nuclei, which leads to obesity can be caused by passing a current of 1.2 mA for 4 seconds, repeated thrice at an interval of 30-second each after adjusting the position of electrodes[34]. The stereotactic instruments can be used to cause injury with a single electrical current of 2.5 mA for 15 seconds by placing the tip of the rat nose 3.3 mm below the interaural line and positioning the tip of a stainless steel electrode 2.6 mm behind the bregma, 0.5-0.6 mm lateral to the midline and below the base of the brain and raised 0.5mm[35]. The irritative theory suggests that the hypothalamic nuclei gets destroyed due to the deposition of iron ions in the hypothalamus with the introduction of electrodes, the ablative theory is of the view that the cause of injury is electric current only. Studies were performed comparing electric injury with radiofrequency (without ion deposition) using the conventional technique and the results obtained were a lower index of obesity using radio frequency. Therefore, both mechanisms are involved in the development of obesity[36-39].

IV. OVARIECTOMY

The initial leptin level drops by the removal of gonads from female rats, which causes hyperphagia and marked weight gain. Seven weeks after ovariectomy, the leptin levels rise again reaching much higher levels that the preoperative ones. It is not known whether this increase is due to resistance to leptin, and could involve hypothalamic receptors[40-41]. More recent studies have tried to find changes in the expression of genes related to energy expenditure in ovariectomized rats to account for weight gain[42]. It appears that leptin and estradiol do not regulate themselves directly, because administration of these in intact female rats did not show that it altered either of them, and the reciprocal is true[43-44]. Therefore it is believed that there is a factor responsible for alerting the hypothalamus to the fact that estrogen production has ceased. A few studies speculate on the participation of neuropeptide Y. It appears to serve as a signal to the hypothalamus when the estrogen levels have dropped, since it would be raised after ovariectomy and would remain at the same levels if hormone replacement occurred in the female rats[41,45].

V. GENETIC MODELS OF OBESITY

The genetic models of obesity are very useful and can be easily developed. The use of these models to study obesity increased in the 1990s because of cloning and identification of the product of five different genes causing obesity. Furthermore, it was discovered that by crossing quantitative trait loci (QTL) with known genes, i.e., obese phenotypes vs identified genotype, the influence of quantitative gene loci, and its penetrance in the quantity of body fat and its distribution[46-47].

a) Monogenic Model Of Obesity

The diabetic (db/db), obese(ob/ob), Tubby (tub), "Agouti" yellow(Ay) and fat (fat) were first five monogenic models of obesity. Over a century ago The "Agouti" rat was described for the first time and it was the first obesity gene to be cloned and characterized at the molecular level in1992. Agouti is expressed in adipose tissue as well as in several other tissues in humans, suggesting that it could be involved in regulating the energy balance. The over expression of agouti in adipose tissue, by genetic modification in rats, results in increased body weight than the non genetically modified ones, without any change in the amount of intake. This suggest that the increase of fatty mass in these rats could be the result of changes in the energy expenditure[46-47] The gene of the obese rat (ob/ob) was cloned at the end of 1994, followed a year later by cloning the diabetic one (db/db). In experimental studies, it was found that a circulating factor of a normal rat or a db/db rat, when administered in an ob/ob rat, normalized its weight. But when this factor of a normal rat or an ob/ob was placed in a db/db rat, there was no weight change. These results strongly suggested that both genes were from the same metabolic pathway, and that db/db could be an ob/ob receptor, which was later proved. This factor was called leptin, i.e., a hormone produced by the ob/ob gene, that was responsible for communicating with the brain concerning the level of energy stored in adipose tissue in the form of fat[46-47].

b) Polygenic Models of Obesity

The body weight, adiposity and related metabolic traits shows significant variability in rodents, as with human beings[48].The high fat diet causes obesity and insulin resistance in inbred C57Bl/6J mice, but this strain remains non obese when fed on chow diet[49] Susceptibility to diet-induced obesity in this strain is polygenic and has been associated with hyperphagia and leptin resistance. AKR/J mice are also prone to diet induced obesity, however, unlike C57Bl/6J, AKR/J do not become hyperglycemic on a high-fat diet[50]. The SWR/J strain prefers carbohydrates and remains thin on high calorie diets[51]. Similarly, A/J mice are less prone to obesity[49]. As expected, these obesity-resistant mouse strains are less susceptible to hyperglycemia[49]. Obesity prone and resistant rats have also been bred in Sprague–Dawley and Fischer 344 genetic background[52] Together, these models facilitate the study of how diet and other environmental factors affect body weight, adiposity and metabolic disorders[53].

VI. TRANSGENIC MODELS OF OBESITY

a) Pro-Opiomelanocortin and Melanocortin Receptor Knockouts

Adrenocorticotropin (ACTH), α, β- and γ melanocyte stimulating hormone (MSH), and the opioid β-endorphin are the Pro-opiomelanocortin (POMC) derived peptides. Leptin, a soluble hormone secreted from the adipocytes, acts on the POMC neurons of the hypothalamic arcuate nucleus[54-55]. The acute anorectic effects of leptin 4 appears due to the activation of POMC neurons and its activation might also be involved in the stimulation of metabolism by leptin[56]. Five melanocortin receptors have been cloned (MC1–5R). Two of these receptors (MC3R and MC4R) are expressed in the CNS in regions involved in energy homeostasis. The development of MC3R and MC4R knockouts help us to know the roles of the neurons expressing these receptors as there is no specific melanocortin receptor agonists. Melanocortin-4 receptor knockout (MC4RKO) mice exhibit the same phenotype observed in Ay/a mice, notably obesity, hyperphagia, increased longitudinal growth and in some cases the development of type 2 diabetes[56-57]. Mice in which the Pomc1 gene has been inactivated, exhibit obesity and hyperphagia and mice lacking a functional Mc3r gene also exhibit increased adiposity[58-59].

b) Neuropeptide Y Receptor Knockouts

Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter found in the brain. The genetic approaches to the study of the role of NPY in energy homeostasis have included knockouts of the NPY receptor genes Npy1r[60-61], Npy2r[62], and Npy5r[63]. It has been shown that there are at least six receptors for . Surprisingly, for the receptors of an orexigenic all of the NPY receptor knockout mice developed so far exhibit a mild late-onset obesity. The Npy1r knockout mouse exhibited a 27% increase in mature bodyweight in females. The Npy2r knockout mouse exhibited mild late-onset obesity in response to a HFD, th greater sensitivity in the females[63].The Npy5r knockout mouse exhibited mild obesity with increased adiposity and hyperphagia[64].

c) Peroxisome-Proliferator-Activated Receptors(PPAR)

The PPAR are members of the steroid/thyroid/retinoid receptor superfamily that transactivate a variety of genes involved in the control of lipid metabolism65. The PPARα isoform is primarily expressed in liver, kidney, heart and skeletal muscle. The development of a mouse line with a disruption of the gene encoding PPARα has made it possible to determine the role of this receptor in vivo66. PPARα−/− mice develop late-onset obesity.

d) Steroid Receptor Knockouts

Estrogens are not just sex hormones, they play an important role in white adipose tissue regulation as estrogen replacement decreases white adipose tissue. ER knockouts exhibit an increase in white adipose tissue and reduced energy expenditure[67]. The ER knockout mice have helped to unravel the role of estrogen receptors in obesity[68]. Follicle stimulating hormone receptor is expressed on the granulose cells of the developing ovary[69]. Follicle stimulating hormone receptor knockouts (FORKO) retards ovarian development and causes chronic estrogen deficiency. Female FORKO develop obesity that is associated with an increased deposition of abdominal fat and that is reversed by estradiol treatment.

VII. β3-Adrenoreceptors

All three known subtypes of β-adrenergic receptors (β-ARs) are expressed in adipose tissue; however, the β3-AR appears to predominate in brown fat in the rodent. Mice lacking expression of the β3-AR (β3-KO) have normal body weight compared to WT mice, although they do exhibit modest increases in total body fat. Fed insulin and glucose levels and food intake are unchanged in the β3-KO mice; however, the increase in insulin and metabolic rate and decrease in glucose and food intake in response to a β3-AR agonist is eliminated. Compensatory mechanisms might operate in the β3-KO animals to maintain normal energy homeostasis and, in fact, the expression of β1-AR

mRNA is upregulated in brown and white adipose tissue in these mice.

VIII. Conclusion

As the incidence of obesity is progressing at alarming rate worldwide, there is a great need for relevant experimental models to provide a better understanding of the pathophysiology of this epidemic to facilitate its therapy and prevention. The multifactorial etiology of obesity provides substantial alternatives for induction of obesity in the experimental models. The animal models may be grouped into genetic, dietary and neuroendocrine forms depending on the origin of the obesity. Although the ultimate model for this human malady is man himself; nevertheless these models provide in valuable insight into the physiological mechanisms that control the energy homeostasis. This information further catalysis the future prospective research for identification of potential therapeutic interventions for obesity. The key factor governing the choice of the model may be either environmental or genetic, depending on the characteristics to be targeted for research.

References Références Referencias

1. Spiegelman, B.M. and Flier, J.S. Obesity and the regulation of energy balance. Cell. 104:2001; 531-543.
2. Imai, Y and Ahima, R.A. Rodents as genetic models of obesity. Drug Discovery Today: Disease Models. 2:2005; 165-174.
3. Popkin, B.M. and Doak C. The obesity epidemic is a worldwide phenomenon. Nutr. Rev. 56:1998; 106-114.
4. Labib, M. The investigation and management of obesity. J. Clin. Pathol. 56:2003; 17–25.
5. Rosenthal, N and Brown, S. The mouse ascending: perspectives for human-disease models. Nature Cell Biology. 9:2007; 993-999.
6. NICE 2006 p.10–11.
7. Imaz, I.,et al.. Safety and effectiveness of the intragastric balloon for obesity. A meta-analysis. Obes Surg. 18:2008; 841–6.
8. York, D.A. Lessons from animal models of obesity. Endocrinology & Metabolism Clinics of North America. 25:1996; 781-800.
9. Aitman, et al. Progress and prospects in rat genetics: a community view. Nature Genetics. 40:2008; 516-522.
10. Diemen, V.V., et al. Experimental model to induce obesity in rats. Acta Cirurgica Brasileira. 21:2006; 425-429.
11. Reuter. T.Y. Diet-induced models for obesity and type 2 diabetes. Drug Discovery Today: Disease Models. 4:2007; 3-8.
12. Srinivasan, K., et al. Combination of high-fat diet-fed

and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res. 52:2005; 313–320.

13. Gaiva, M.H., Couto, R.C., Oyama, L.M., Couto, G.E., Silveira, V.L., Riberio, E.B., and Nascimento, C.M. Polyunsaturated fatty acid-rich diets: effect on adipose tissue metabolism in rats. Br J Nutr. 86:2001; 371-7.

14. Lin, S., Thomas, T.C., Storlien, L.H., and Huang, X.F. Development of high fat diet induced obesity and leptin resistance in C57Bl/6J mice. Int J Obes Relat Metab Disord. 24:2000; 639-646.

15. Surwit, R.S., et al. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism. 44:1995; 645–651.

16. Sclafani, A., et al. Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. Physiol. Behav. 17:1976; 461–471.

17. Chang, S., et al. Metabolic differences between obesity-prone and obesity-resistant rats. Am. J. Physiol. 259:1990; R1103–R1110.

18. Farley, C., et al. Meal pattern analysis of diet-induced obesity in susceptible and resistant rats. Obes. Res. 11:2003;845–851.

19. Levin, B.E., et al. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. Am. J. Physiol. 273:1997; R725–R730.

20. Zavaroni, I., et al. Effect of fructose feeding on insulin secretion and insulin action in the rat. Metabolism 29:1980; 970–973.

21. Park, O.J., et al. Mechanisms of fructose-induced hypertriglyceridemia in the rat. Biochem. J. 282:1992; 753–757.

22. Nakagawa, T., et al. A causal role to uric acid in fructose induced metabolic syndrome. Am. J. Physiol. Renal. Physiol. 2006:290; F625–F631.

23. Rothwell, N.J., et al. The cafeteria diets as a tool for studies of thermogenesis. J. Nutr. 118:1988; 925–928.

24. Prats, E., et al. Energy intake of rats fed a cafeteria diet. Physiol. Behav. 45:1989; 263–272.

25. Kretschmer, B.D., et al. Modulatory role of food, feeding regime and physical exercise on body weight and insulin resistance. Life Sci. 76:2005; 1553–1573.

26. Bunyan, J., Murrell, E.A., Shah, P.P. The induction of obesity in rodents by means of monosodium glutamate. Br J Nutr. 35:1976; 25-39.

27. Shivshankar, P., Devi, SC. Screening of stimulatory effects of dietary risk factors on mouse intestinal cell kinetics. World J Gastroenterol. 11:2005; 242-8.

28. Dolnikoff, M., et al. Decreased lipolysis and enhanced glycerol and glucose utilization by adipose tissue prior to development of obesity in monosodium glutamate (MSG) treated rats. Int J Obes Relat Metab Disord. 25: 2001; 426-433.

29. de Carvalho, P.P., Vargas, A.M., da Silva, J.L., Nunes, M.T., Machado, U.F. GLUT4 protein is differently modulated during development of obesity in monosodium glutamate-treated mice. Life Sci. 71:2002; 1917-28.

30. de Mello, M.A., de Souza, C.T, Braga, L.R., Santos, J.W., Ribeiro, I.A., Gobatto, C.A. Glucose tolerance and insulin action in monosodium glutamate (MSG) obese exercisetrained rats. Physiol Chem Phys Med NMR. 33:2001; 63-71.

31. de Souza, C.T., Nunes, W.M., Gobatto, C.A., de Mello, M.A. Insulin secretion in monosodium glutamate (MSG) obese rats submitted to aerobic exercise training. Physiol Chem Phys Med NMR. 35:2003; 43-53.

32. Tokuyama, K., Himms-Hagen, J. Adrenalectomy preventsobesity in glutamate-treated mice. Am J Physiol. 257:1989; E139-44.

33. Perello, M., Gaillard, R.C., Chisari, A., Spinedi, E., Adrenal enucleation in MSG-damaged hyperleptinemic male rats transiently restores adrenal sensitivity to leptin. Neuroendocrinology. 78:2003; 176-84.

34. Shimizu, Y., Yamazaki, M., Nakanish, K., Sakurai, M., Sanada, A., Takewaki, T., et al. Enhanced responses of the chorda tympani nerve to sugars in the ventromedial hypothalamic obese rat. J Neurophysiol. 90:2003;128-33.

35. Dube MG, Xu B, Kalra PS, Sninsky CA, Kalra SP. Disruption in neuropeptide Y and leptin signaling in obese ventromedial hypothalamic-lesioned rats. Brain Res. 816:1999; 38-46.

36. King, B.M., Frohman, L.A. Nonirritative lesions of VMH: effects on plasma insulin, obesity, and hyperreactivity. Am J Physiol. 248:1985; E669-75.

37. King, B.M., Frohman, L.A. Hypothalamic obesity: comparison of radio-frequency and electrolytic lesions in male and female rats. Brain Res Bull. 17:1986; 409-13.

38. King, B.M., Daigrepont, P.M., Michel, R.E., Zansler, C.A., Ahmed, J.I., Walker, A., et al. Hypothalamic obesity: comparison of radio-frequency and electrolytic lesions in weanling rats. Physiol Behav. 45:1989; 127-32.

39. King, B.M. Ventromedial hypothalamic obesity: a reexamination of the irritative hypothesis. Neurosci Biobehav Rev. 15:1991; 341-7.

40. Chu, S.C., Chou, Y.C., Liu, J.Y., Chen, C.H., Shyu, J.C., Chou, F.P. Fluctuation of serum leptin level in rats after ovariectomy and the influence of estrogen supplement. Life Sci. 64:1999; 2299-306.

41. Meli, R., Pacilio, M., Raso, G.M., Esposito, E., Coppola. A., Nasti, A., et al. Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. Endocrinology. 145:2004; 3115-21.

42. Shimizu, H., Ohtani, K., Kato, Y., Tanaka, Y., Mori, M. Withdrawal of estrogen increases hypothalamic neuropeptide Y (NPY) mRNA expression in ovariectomized obese rat. Neurosci Lett. 204:1996; 81-84.

43. Pelleymounter, M.A., Baker, M.B., McCaleb, M. Does estradiol mediate leptin's effects on adiposity and body weight? Am J Physiol. 276:1999; E955-63.

44. Shimomura, K., Shimizu, H., Tsuchiya, T., Abe, Y., Uehara, Y., Mori, M. Is leptin a key factor which develops obesity by ovariectomy? Endocrinol J. 49:2002; 417-23.

45. Ainslie, D.A, Morris, M.J., Wittert, G., Turnbull, H., Proietto, J., Thorburn, A.W. Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. Int J Obes Relat Metab Disord. 25(11):2001; 1680-8.

46. Chagnon, Y.C., Bouchard, C. Genetics of obesity: advances from rodents studies. TGI. 12: 1996.

47. Marques-Lopes, I., Marti, A., Moreno-Aliaga, M.J., Martinez, A. Aspectos geneticos da obesidade. Rev Nutr. 17:2004; 327-38.

48. Barsh, G.S., et al. Genetics of body-weight regulation. Nature 404:2000; 644–651.

49. West, D.B., et al. Dietary obesity in nine inbred mouse strains. Am. J. Physiol. 262:1992; R1025–R1032.

50. Rossmeisl, M., et al. Variation in type 2 diabetes-related traits in mouse strains susceptible to diet-induced obesity. Diabetes. 52: 2003; 1958–1966.

51. Smith, B.K., et al. Increased flavor preference and lick activity for sucrose and corn oil in SWR/J vs. AKR/J mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281:2001; R596–R606.

52. Schemmel, R., et al. Dietary obesity in rats: body weight and body fat accretion in seven strains of rats. J. Nutr. 100:1970; 1041–1048.

53. Bell, C.G., et al. The genetics of human obesity. Nat. Rev. 6:2005; 221–234.

54. Leibel, R.L., et al. The molecular genetics of rodent single gene obesities. J. Biol. Chem. 272:1997: 31937–31940.

55. Cowley, M.A. et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411:2001; 480–484.

56. Marie, L.S. et al. A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proc. Natl.Acad. Sci.U. S.A. 97:2000; 12339–12344.

57. Huszar, D. et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:1997; 131–141.

58. Butler, A.A. et al. (2001) Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat. Neurosci. 4:2001; 605–611.

59. Chen, A.S. et al. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat. Genet. 26:2000; 97–102.

60. Kushi, A. et al. Obesity and mild hyperinsulinemia found in neuropeptide Y-Y1 receptor-deficient mice. Proc. Natl.Acad. Sci.U. S.A. 1998:95; 15659–15664.

61. Pedrazzini,T. et al. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. Nat. Med. 4:1998; 722–726.

62. Naveilhan, p, et al. Normal feeding behavior body weight and leptin response require the neuropeptide Y,Y2 receptor Nat. Med. 5, 1188-1193.

63. Marsh, D.J. et al. Role of the Y5 neuropeptide Y receptor in feeding and obesity. Nat. Med. 4:1998; 718–721.

64. Blomqvist, A.G. et al. Y-receptor subtypes – how many more? Trends Neurosci. 20:1997; 294–298.

65. Desvergne, B. et al. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocrinol. Rev. 20:1999; 649–688.

66. Lee, S.S.T. et al. Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol. Cell. Biol. 15:1995; 3012–3022.

67. Heine, P.A. et al. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc. Natl. Acad. Sci. U S A. 97: 2000; 12729–12734.

68. Stefan O M, and Kenneth S K. Estrogen receptors and endocrine diseases: lessons from estrogen receptor knockout mice. Anxiety and depression. 1:2001; 613-619.

69. Robker RL, Richards JS 1998 Hormonal control of the cell cycle in ovarian cells: proliferation versus differentiation. Biol Reprod 59:1998; 476–482.

Global Journals Inc. (US) Guidelines Handbook 2012