J. of Korean Oil Chemists' Soc.,
Vol. 30, No. 4. December, 2013. 622~634
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2013.30.4.622

Obesity, obesity-related diseases and application of animal model in obesity research An overview

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(Received December 8, 2013 ; Revised January 3, 2014 ; Accepted January 3, 2014)

Abstract : The multi-origin of obesity and its associated diseases made it's a complex area of biomedical science research and severe health disorder. From the 1970s to onwards this health problem turned to an epidemic without having any report of declining yet and it created a red alert to the health sector. Meanwhile, many animal models have been developed to study the lethal effect of obesity. In consequence, many drugs, therapies and strategies have already been adopted based on the findings of those animal models. However, many complicated things based on molecular and generic mechanism has not been clarified to the date. Thus, it is important to develop a need based animal model for the better understanding and strategic planning to eliminate/avoid the obesity disorder. Therefore, the present review would unveil the pros and cons of presently established animal models for obesity research. In addition, it would indicate the required turning direction for further obesity and obesity based disease research.

Keywords : Obesity, obesity-related diseases, animal models of obesity research.

1. Introduction

Obesity is a major health disorder in the present age. Having body mass index (BMI) more than 30 kg/m^2 is termed as obesity [1]. Research and investigation to explore the mechanism of obesity is one of the top priority areas of biomedical science research. Scientist are struggling to have a suitable solution to the obesity and diseases related to obesity such as hypertension, type 2 diabetes, sleep apnea, certain form of cancers, cardiovascular diseases, liver diseases including liver cirrhosis, premature death, cardiac arrest, infertility, polycystic ovary, respiratory dysfunction, lumbago, muscle weakness and many other diseases [2-3]. Moreover, the severity of obesity is not a single cause; several reasons of obesity have already been established and severe obesity might be the result of any single cause or the cumulative effect of more than one reasons. Defective genetic makeup followed by lack of leptin signaling, β-cells dysfunctions of pancreas and insulin resistance are proven causes of obesity and obesity-derived diseases; all these functions are not controlled by a single gene, it is controlled by more than one genes inherited through ancestors [4]. Beside this, over eating, in particular having food

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contained high animal fat and insufficient physical activity and exercise are also prominent causes of obesity, which is known as diet-induced obesity [5]. There are some other environmental and ambient factors severely affect the mechanism of obesity, these factors include lack of sleep and stressed condition, endocrine disruptions, little fluctuation of ambient temperature, decreased rate of smoking (as smoking directly affect appetite), consumption of medicines that enhances the metabolic activity, pregnancy at the later part of life (cause susceptibility to obesity in children), natural selection for higher body mass index (BMI) and assortative mating [6]. All of the findings related to the obesity are discovered through the animal study that is the animal model of obesity research contributed to point out the reasons behind obesity and also directed towards the strategic planning should be taken to avoid/eliminate the epidemic outbreak of obesity and its associated diseases. Meanwhile, many drugs and therapeutic treatment for human diseases have already been developed using various animal models. [7-12]. Though, different animal model of obesity research has been developed targeting specific research goals, but, neither all expected outcomes have been fulfilled nor the obesity epidemic has been eliminated yet. Therefore, the present review has been done to accumulate the established animal models of obesity research with the successful outcome of that model, to overview the trends of obesity outbreak and obesity research and finally to make interference over the future research directions related to obesity associated diseases and animal models development to enhance obesity research.

2. Current and future trends of obesity epidemic

There was no evidence of severe obesity problem before 1970s, the sudden prevalence of obesity since 1970s without any evidence of decreasing made it a hot concern in biomedical researches [13-14]. The National Health and Nutrition Examination Survey (NHANES) conducted on 2007-2008, showed 50% and 100% increase of obesity compare to 1988-1994 and 1976-1980 respectively [13]. The rapid increase in obesity trend resulted excess weight gain, poor health status, susceptible to complex diseases and increase cost of medicine and treatment. The estimated currency loss due to obesity is around 9% (over $147 billion/year) of total medical cost [15]. Recent studies forecasted that obesity prevalence would be increased by 33% with 130% increase in severity of obesity prevalence by 2030 [16]. If the projected future trends showed of obesity rates come true. If the powerful influences of environments (physical, social and economic environments that favor obesity) become more and more consolidated by the next two decades. That would further hinder the efforts of healthcare cost containment [16].

3. Animal model of human diseases and its classification

The idea of using animal model for the investigation of human diseases developed from need of conducting research as having donor human for experimental purposes is not possible and it creates ethical issues. Therefore, animal models for human diseases are being used since long back. The term animal model refers to the non-human living organism used for the research and investigation of human diseases. Animal models for human diseases enhance better understanding of particular human disease and to test the accuracy of newly developed drugs without having the risk of harming human being [17-18]. Establishment of animal model needs to select

an animal which meets the determined taxonomic equivalency to humans and the animal should have physiological similarities to human [17-18]. Success stories of drug and therapeutic treatment development for different human diseases using animal models is not little at all.

Animal models serving in research may have an existing, inbred or induced disease or injury that is similar to a human condition. These test condition are often termed as animal models of diseases. The use of animal models allows researchers to investigate disease states in ways which would be inaccessible in a human patient, performing procedures on the non-human animal that imply a level of harm that would not be considered ethical to inflict on a human. To serve as a useful model, a modeled disease must be similar in etiology (mechanism of cause) and function to the human equivalent. Animal models are used to learn more about a disease, its diagnosis and its treatment. For instance, behavioral analogues of anxiety or pain in laboratory animals can be used to screen and test new drugs for the treatment of these conditions in human. A 2000 study found that animal models (coincided on true positives and false negatives) with human toxicity in 71% of cases, with 63% for nonrodents alone and 43% for rodents alone [19]. Thus the animal models of diseases could be categorized in the following ways:

A. Spontaneous (naturally occurring in animals) models
B. Induced (by physical, chemical or biological means) models
C. Genetically modified models
D. Negative models
E. Orphan models

3.1. Spontaneous (naturally occurring in animals) models

The spontaneous models of diseases refer to the disease models established based on the naturally occurring genetic variants. Hundreds of inherited strains including rodents and non-rodents have been characterized and conserved as disease models that pose similar clinical conditions to human. For instance, the athymic nude mouse model for the study of hetero transplanted tumors and natural killer cells; the snell's dwarf mouse model for the study of pituitary and neural system; atherosclerosis in the squirrel monkey for the adipose tissue deposition around the inner wall of arteries; epilepsy in the Mongolian gerbils and diabetes in different strains of mice [20].

3.2. Induced (by physical, chemical or biological means) models

Induced models are those in which the disease or condition must be artificially produced in the animals, the clinical expression of disease or disordered could not be evident intermittently. For example, the use of metrazol (pentylenetetrazol) as an animal model of epilepsy [21]; Immunisation with an auto-antigen to induce an immune response to model autoimmune diseases such as experimental autoimmune encephalomyelitis [22]; Occlusion of the middle cerebral artery as an animal model of ischemic stroke [23]; Injection of blood in the basal ganglia of mice as a model for hemorrhagic stroke [24-25]; Infecting animals with pathogens to reproduce human infectious diseases; injecting animals with agonists or antagonists of various neurotransmitters to reproduce human mental disorders; using ionizing radiation to cause tumors; implanting animals with tumors to test and develop treatments using ionizing radiation; genetically selected (such as in diabetic mice also known as NOD mice [26]; various animal models for screening of drugs for the treatment of glaucoma; use of plasmodium yoelii as a model of human malaria [27-29].

3.3. Genetically modified models

The increase in knowledge of the genomes

of non-human primates and other mammals that are genetically close to human is allowing the production of genetically engineered animal tissues, organs and even animal species which express human diseases, providing a more robust model of human diseases in an animal model. The genetically modified mouse was first developed by Rudolf Jaenisch through the insertion of a DNA virus in to mouse embryo in the early stage and showing the inserted genes were presented in every cells [30]. Later in 1981 Laboratories of Frank Ruddle injected purified DNA into a single cell mouse embryo and showed the transmission of the genetic material to the subsequent generations [31-32]. The most common examples of genetically modified disease models are 1) Gene knockout animal, 2) Transgenic animal, 3) Chemically induced genetic modification. In case of knockout animal, the activity of one or more genes is removed, while the transgenic animal is produced by inserting the genes of interest to the target animal. These genetically modified animals are mostly used in disease models and researches of different diseases such as obesity, diabetes, heart disease, cancers, arthritis, substance abuse, anxiety, aging and also Parkinson disease.

3.4. Negative models

If a certain disease dose not develops to a particular animal species, breed or strain, following an experimental treatment (which causes disease in other animals) then that particular species, breed or strain is termed as negative model of that disease [20]. For example, human cholera normally restricted to a several species and the remaining uninfected species might be used as negative models for human cholera.

3.5. Orphan models

This term is used to explain a functional disorder that has not found in human yet and normally occur in other animal species, and which is recognized if similar human disease(s) get discovered later [20].

4. Keynote contribution of animal model in obesity research

It is a well established fact that the obesity develops from prolonged imbalance in energy intake and expenditure. Where, the resultant surplus energy is being deposited to the body in the form of adipose tissues predominantly in the subcutaneous and abdominal area. The proper understanding of the phenomena mediating obesity and obesity related diseases is the prerequisite for the development of strategies to overcome obesity and obesity derived diseases. Animal models provided that basic understanding of the parameters that coordinates the components of an individual's energy regulation. Animal models revealed different aspects of obesity development such as genomic influence, dietary influence, influence of the physical activity and exercise in clinical outbreak of obesity (Fig. 1). For example, the discovery of leptin [9], a cytokine hormone, derived from adipose tissue and regulates the satiety center, discovered from the genetically defect mutant mice (ob/ob mice and db/db mice) and also the leptin receptors [7-8]. Other predominant examples are the discovery of pancreatic hormone insulin [12], which was a result of working with dogs, and gut-derived satiety signals such as peptide YY [10] and cholecystokinin (CCK) [11] both discovered long before leptin, by work in animals. Beside this, animal models provided a milestone to the understanding of environmental influence on obesity such as epigenetics, effect of high-calorie and low-calorie diet and so. It also provided clues for the development of pharmaceutical agents used in the treatment of obesity. The explanatory flow diagram showed the application of animal model in human disease research and drug development (Fig. 2).

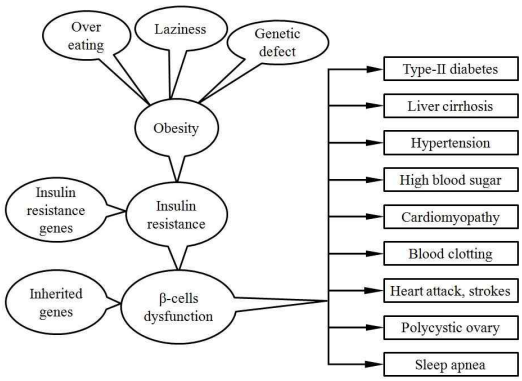

Fig. 1. The follow-diagram explaining the interactions among individual behavior, genetic inheritance, obesity and outbreak of obesity-related diseases.

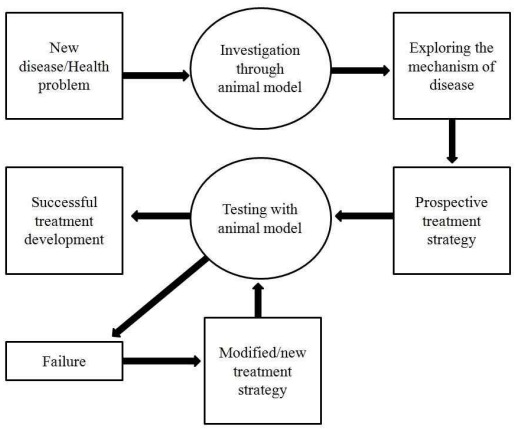

Fig. 2. Flow-diagram illustrating the principles of diseases etiology investigation and development of treatment/cures using animal model of human diseases.

5. Established animal models in obesity research

Various animal models have been developed to satisfy the research query of the particular researcher. Many models are made to study the genetic regulation of obesity; some are to reveal the influence of high energy rich diet in obesity and others are designed to investigate the relationship between obesity and different diseases (Fig. 1). Each model satisfied the particular research goal in partial or full, however, none of the model is enough to investigate everything related to obesity. In this review, we discussed the most famous animal models used in the obesity researches.

5.1. Mouse models in obesity research

Mouse is the most widely used laboratory animal for biological researches. The short gestation period, rapid generation interval, large litter size, small body size, ease of maintenance and physiological similarities established mouse as the most suitable animal model for human diseases. The presence of same obese gene and similar pattern of phenotypic expression of obesity and obesity related diseases in mouse helped to understand the mechanism of obesity and obesity derived diseases/disorders in human. Therefore, mouse model answered many unknown questions like monogenic influence to obesity, polygenic effect to obesity, diet induced obesity and also the obesity derived from lack of physical activity and exercise. In, monogenic mouse obesity model, the contribution of a single gene to the obesity is investigated. Whereas, polygenic mouse model deal with the obesity regulated by multiple genes. The effect and influence of high rich diet is studied under the diet induced obese mouse model. Similarly, the role of reduced physical activity and exercise on the obesity development is studied under the different mouse models. In this section the history, applications and limitations of famous mouse model has been discussed.

5.1.1. The agouti mutant mouse model

The agouti mouse model is one of the oldest and most famous mouse models in the field of obesity research, which has been developed at around one hundred years before. But, the genetic characterization was reported in the last decade of 20^{th} century [33]. The agouti mutant mouse model has been developed based on the expression of pigments, the expression

of agouti gene up regulates yellow/red color pigments and follicular melanocytes and down regulates brown/black pigmentation (Fig. 3) [34-36]. There are five dominant agouti mutations; however, the yellow mutant (A^y) is widely used mouse model for the obesity research [33]. The agouti gene expression is induced by the deletion of 120-170kb genomic DNA to make the mouse tissue specific control promoter deficient [37-38]. The agouti yellow mutant mouse (A^y) posses several distinctive phenotypic characteristics, these are 1) yellow coat color, 2) onset of obesity at maturity, 3) type-II diabetes, 4) accelerated linear growth, 5) hyperleptinemia, 6) infertility and 7) increased susceptibility to tumor [33]. Ubiquitous agouti expression through transgenic mouse showed similar phenotypic expression [39] and helped to unveil the molecular phenomena of agouti expression. The formation of excess adipose tissue due to agouti over expression without accelerating the food intake indicates the massive alteration of energy utilization and metabolism [40]. The obesogenic role of agouti is tissue specific as the transgenic agouti expression in skin did not induce obesity [41]. Interestingly, the agouti gene is also present in human, which is reported to be a regulator of type-II diabetes [42]. Thus, this agouti mouse model could be an important model to study the obesity and obesity related diseases in human [43-44].

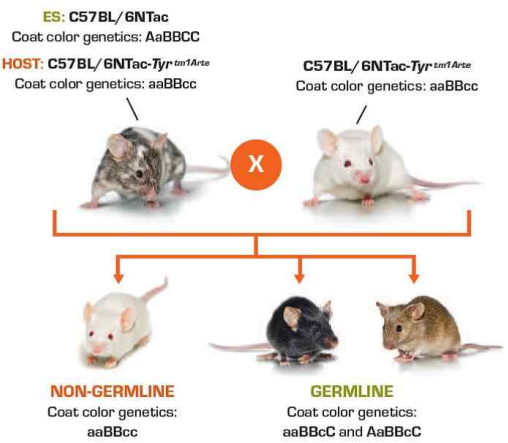

Fig. 3. The agouti mutant mouse model has been developed based on the expression of pigments.

5.1.2. Leptin Signaling Defects in Mice

Leptin signaling defects mouse is another famous monogenic mouse model for obesity research, where *ob/ob* and *db/db* models are vastly used. The *ob/ob* mutation is recessive; this model is named after obese (ob) gene and discovered by chance from the Jackson laboratory [45]. The obesity become visible with the increasing of age and it is reported to have three times larger mature body weight compare to the unaffected control littermates, however, neonatal mutant mice are normal. Though, the phenotypic features were clear but revealing the genetic relation with the observed phenotype took almost 50 years [9]. The leptin is found to be responsible for ob mutation; a single base pair deletion in the leptin coding region causes a premature stop codon [9]. Leptin gene is expressed abundantly in the adipose tissues, which is a key regulator of appetite (hunger and satiety). Thus, *ob/ob* mutation results unrestricted food intake followed by obesity (Fig. 4). This mouse model shows some obesity related diseases such as type 2 diabetes, insulin resistance and hyperinsulinemia.

The *db/db* mouse model was also reported by Jackson laboratory in 1966, where the term *'db'* stands for diabetes [46]. This *db* mutation is an autosomal recessive trait, encodes G-to-T point mutation in leptin receptor gene with the subsequent result of leptin signaling defect [47,7].The *db/db* .mouse showed intense fat deposition in the axillary and inguinal region, frank hyperglycemia at eight week of age and type-II diabetes [47,7].

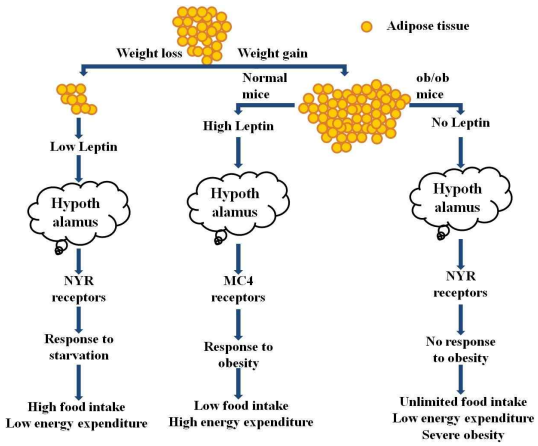

Fig. 4. Illustrating diagram explain the role and mechanism of leptin signaling in normal mice and ob/ob (leptin defecting) mice.

5.1.3. New Zealand Obese (NZO) Mouse

The New Zealand obese (NZO) mouse model exhibits most severe phenotypic expression among the polygenic mouse model of obesity, it resulted more than 40% fat accumulation of total body weight at only six months of age and starts gaining rapid body weight from the second month of age [48]. This mouse model results type-II diabetes only in male and the female is found to e normal. It assumed that this defect is related to the leptin gene though the genetically the leptin gene does not appear abnormal. Furthermore, NZO mice showed reduction in physical activity and exercise compare to the control littermates and also *ob/ob* mouse [49]. Therefore, the obesity in NZO mice is a cumulative effect of hyperphagia, low energy loss and limited exercise which is very nuch similar to the obesity pattern in human.

5.1.4. Tsumura Suzuki Obese Diabetes (TSOD) Mouse

Tsumura Suzuki Obese Diabetes (TSOD) Mouse was developed through selective inbreeding for obese and urine sugar positive colonies from two different strains of mouse. The TSOD mouse develops diabetes and obesity, however, diabetes does not reach to severe stage due to increased β-cell mass and maintained insulin secretion [50]. In contrast, Tsumara Suzuki nonobese (TSNO) mouse does not show obesity [50]. Polygenic obesity with hyperglycemia and hyperinsulinemia has been reported only in male TSOD mouse [50-51]. At the later age, TSOD mice are reported to show diabetic nephropathy and neuropathy [52].

5.1.5. M16 Mouse

The M16 mouse model was developed through long term selection for 3-6 week weight gain in an ICR background [53]. This is an outbred mouse model for polygenic obesity. The phenotypic expression for M16 mouse includes hyperphagia, hyperinsulinemia, and hyperleptinemia compared to ICR controls at 8 weeks of age [53].

5.1.6. Kuo Kondo (KK) Mouse

Another polygenic mouse model of obesity is known as Kuo Kondo (KK) Mouse model. It has been developed through selective inbreeding for large body weight in Japan [54]. Phenotypes of KK mouse model includes type-II diabetes, insulin resistance (outbreaks from the onset of puberty), hyperphagia, hyperinsulinemia at around two months of age [55-56]. The KK*Ay* mouse is widely used in experimental therapies for obesity and diabetes research , which has been developed from by transferring lethal yellow obese gene (*Ay*) to the KK mouse [57].

5.1.7. Transgenic mice models

With the discovery of different signaling pathways regulating weight and energy expenditure, a wide range of genes has been reported to be involved in this process. For the detailed investigation of obesity related diseases, transgenic mice have been developed with genes either over-expressed or deleted.

The most familiar transgenic mouse model

of obesity research has been developed using genes involved in the melanocortin pathway of weight regulation. The deletion of Mc4r gene in mice caused early-onset of obesity, non-insulin-dependent diabetes, and other obesity associated syndromes. The similar phenotypic expression of Mc4r gene knockout mouse to the yellow agouti mouse indicates that agouti expression inhibits Mc4r gene activity in hypothalamus and triggered to the subsequent outbreak of obesity. In continuation, knockout of Mc3r gene also resulted late onset of obesity, but appetite and metabolism remains intact [58] Mc3r transgenic mouse also reported to be susceptible to diet-induced obesity, in particular, the female mouse showed significantly increased body weight with high fat diet. The high fat diet induced obesity increased the ratio of adipose mass to lean mass, but overall body weight reported to be unchanged in Mc3r knockout mice like normal diets. Likewise, up-regulation of syndecan-1 gene reported to induce obesity having similar phenotype to melanocortin antagonism in the hypothalamus [59]. This might be due to the facilitation of *agouti*-related protein binding to Mc3r and Mc4r by syndecan-1, a membrane-bound heparan-sulfate proteoglycan.

5.1.8. Other mouse models used in obesity research

C57BL/6 is also referred to as "C57 black 6", "C57" or "black 6" mouse, which is a very common inbred strain of laboratory mice. They are widely used as genetic background for genetically modified mice in the obesity research of human being and well accepted due to the availability of congenic strains, easy breeding and robustness. C57BL/6 show characteristics black coat and high temperament. C57BL/6 mouse strains are widely used for the study of high-fat diet induced obesity [5].

The spiny mouse is the rodents within the *Acomys* genus. The mouse is similar in appearance to the common mouse of the genus *Mus*, spiny mouse are small mammals with bare, scaled tails. However, their coats are endowed with unusually stiff guard hairs act similarly to the spines to the hedgehog. The name of these mice also derived from this trait.

5.2. Rat models of obesity

Another familiar experimental animal of rodent family is rats, they are being used in the biological researches from long before and revealed different queries related to physiological regulations and drug development. In obesity research, several rat models have been used vastly to unveil the secrets related to human obesity. The most famous rat models of obesity research are being discussed in this chapter.

5.2.1. Zucker Fatty Rat (ZFR)

The Zucker Fatty Rat (ZFR) has been developed by L. M. Zucker and T. F. Zucke based on an autosomal recessive mutation in the *fatty* (*fa*) gene on chromosome 5. The appearance of obesity found at 5 weeks of age and also characterized by hyperphagia [60]. It shows insulin resistance with normal blood glucose concentration [61]. The Zucker diabetic fatty (ZDF) rats, is a substrain of Zucker Fatty Rat (ZFR) reported to have clinical outbreak of frank diabetes [62].

5.2.3. Wistar Fatty Rat

The Wistar fatty rat (WFR) has been developed by transferring the *fa* gene from ZFR (13 M strain) to Wistar Kyoto rats; this strains show poor glucose tolerance [63]. It exhibits early onset of obesity and obesity related diseases, at around 3 weeks of age. The phenotypic expression includes type 2 diabetes, hyperinsulinemia, and hyperlipidemia. Furthermore, WFR male shows metabolic abnormalities, but not in WFR females, which display only mild insulin resistance and some glucose intolerance [63]. The WFR rat is

vastly used for research related to type 2 diabetes as aged WFR displays diabetic complications including nephropathy and neuropathy [64-66].

5.2.4. Otsuka Long Evans Tokushima Fatty (OLETF) Rat

OLETF rats have been developed in Japan by the selection of spontaneously type 2 diabetic rats from the outbreeding of Long Evans rats in a closed colony of Charles River in Otsuka Pharmaceuticals in Tokushima [67]. All male OLETF rats display early outbreak of diabetes at 25 weeks of age and determined by oral glucose tolerance test, in contrast, only 30% of female OLETF rats develop diabetes even after 60 weeks of age [67-68] OLETF rats develop frank obesity [67]. OLETF rats are well suited for obesity and diabetes research.

5.3. Non-rodent models of obesity

5.3.1. Obese Monkeys

The Obesity models based on different monkeys such as macaques, rhesus monkey, and baboons could provide important information relevant to human obesity and obesity related diseases. Rearing of rhesus monkeys in indoor cages, exhibit increased rates of obesity and obesity-associated diseases [69-70]. Development of obesity in captive macaques highly age dependent, when food is provided on *ad libitum* [71]. It is rational that declined exercise increases the risk of obesity in these monkeys [70-71]; these monkeys develop type 2 diabetes and diabetic complications. Wild baboons are also reported to exhibit spontaneous obesity in a pedigreed colony [72]. Moreover, Japanese monkey, Macaca fuscata develop obesity in association with frank diabetes [73].

5.3.2. Dogs

The outbreak of obesity in domestic dog is found to be more extreme compare to the obesity in human. At the middle of 1980s 25-45% of domestic dogs presented at veterinary clinics were categorized as obese [74]. The physiological similarity of dogs to human being made them as a potential study model for the obesity and obesity related diseases in human [75]. The dog model of human obesity research could be able to unveil the genetic contribution to the problem [76].

In conclusion, the successful application of animal model in obesity research revealed many unknown question and directed towards the solution of human obesity and it related diseases. It opened the door of discovering many treatments, therapies, cures to get relief of obesity disorders. However, the most of the animal models used for the obesity research are small laboratory animals; it might be because of the feasibility of handling and running experiments. But, human being has a far larger life span compare to the mice, rat, rabbits and other laboratory animals. Thus, for the understanding of obesity and its interactions with aging, the small animal models might not give accurate observations. For the proper understanding of the mechanism of obesity and associated diseases in the later stages of life, for having accurate measure to avoid obesity in the old ages, the future research effort should be given to establish an animal model which had comparatively larger life span like human being. In continuation, instead of doing short-term study, it could be recommended to conduct long-term investigation to have a concrete understanding and checklist of safety measures to be taken at different stages of age to avoid the lethal effect of obesity. Moreover, the role of other genes in terms of obesity should be taken under consideration that might unveil a path to overcome the genetic effect of obesity (obesity derived from different genes inherited from ancestors).

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