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Review

Laboratory animals as surrogate models of human obesity

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Obesity and obesity-related metabolic diseases represent a growing socioeconomic problem throughout the world. Great emphasis has been put on establishing treatments for this condition, including pharmacological intervention. However, there are many obstacles and pitfalls in the development process from pre-clinical research to the pharmacy counter, and there is no certainty that what has been observed pre-clinically will translate into an improvement in human health. Hence, it is important to test potential new drugs in a valid translational model early in their development. In the current mini-review, a number of monogenetic and polygenic models of obesity will be discussed in view of their translational character.

Keywords: obesity; animal models; sibutramine; liraglutide; KK-A^y mice; ob/ob mice; Zucker rat; diet-induced obesity models

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Introduction

As the prevalence of obesity is rising along with its socioeconomic consequences, the quest to find new treatments or a cure is also increasing (http://www.who.int/mediacentre/factsheets/fs311/en/). Pharmaceutical treatment is one avenue that has been pursued, but currently there are only a limited number of compounds on the market because many have failed or been withdrawn because of side effects. Given that the development process from initial idea to marketed product typically requires more than 10 years and the attrition rate is notably high, it is important that the models used, whether *in vitro* or *in vivo*, are good surrogates for human obesity. Depending on the target in question, there are a number of models that can be applied. In the following pages, we will review the most widely used animal models in obesity research. We have categorized the different models into groups; rodent models are divided into monogenetic, polygenetic, and selectively bred diet-induced obesity (DIO) models, and finally we discuss the DIO pig model. To demonstrate the translational potential of the selected models, we have chosen two different model compound families that have been tested in human cohorts — sibutramine and liraglutide or other glucagon-like peptide-1 (GLP)-1 analogues[1, 2]. Sibutramine is a serotonin and norepinephrine re-uptake inhibitor that was developed for the treatment of obesity and has been on the market for the past 9 years, although in most markets it has been withdrawn because of undesirable side effects [3]. Liraglutide is a GLP-1 analogue currently in phase 3 clinical development for severe obesity after demonstrating positive results in phase 2 trials[2]. By choosing the best suited animal model for a particular study, it is possible to make a qualified assessment as to whether target X and/or compound Y will have an impact in clinical practice at a much earlier point.

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Monogenic models

KK-A^y mice

The inbred mouse strain KK was established in Japan and is a mouse model with peripheral insulin sensitivity and glucose intolerance[4]. Insulin resistance in the KK mouse is followed by moderate obesity[5, 6]. The occurrence of diabetes and obesity in KK mice is considered of polygenic origin. However, when the Agouti (A^y) mutation is introduced into the KK strain, the resulting KK-A^y mouse exhibits a more pronounced course of diabetes[7, 8]. The A^y gene is ubiquitously expressed in the KK-A^y mouse, and the agouti protein is thought to act as a melanocortin 4 receptor antagonist, thereby inhibiting the signals from alpha-melanocyte stimulating hormone (α-MSH) and affecting the regulation of energy balance[9, 10]. Thus, KK-A^y can be considered a mouse with a monogenic defect but in a polygenic background that results in a predisposition for obesity.

The body weight of KK-A^y mice usually reaches approxi-

mately 45 g at two months of age, which is characterized as moderate obesity. The body weight stabilizes by 4–5 months of age at approximately 50–60 g, with fat composing approximately 33% of the total body weight. Because obesity is partially caused by hyperphagia, food restriction seems effective in reversing obesity. The diabetes phenotype of KK-A^y mice exhibits as hyperglycemia, hyperinsulinemia, and glucose intolerance, although hyperglycemia declines after 1 year of age. In addition, an elevated pituitary growth hormone level in combination with glycosuria caused by glomerular lesions is often observed in this animal model[11–13].

The development of obesity and diabetes in KK-A^y mice has similarities to the development of human obesity and diabetes, and the strain has also been used in the early development of new experimental therapies. For instance, sibutramine has been tested in KK-A^y mice. Sibutramine acts to suppress calori intake and increase energy expenditure. Weight loss after sibutramine treatment has been shown in both humans and rodents[14, 15]. In KK-A^y mice, sibutramine treatment leads to a reduction in food intake; however, the sensitivity to sibutramine was less pronounced than in wild-type mice[16]. The weight-reducing properties of GLP-1 analogues have not, to the best of our knowledge, been tested in KK-A^y mice. However, exendin-4, a GLP-1 receptor agonist, increases insulin secretion and reduces glucose levels in KK-Ay mice[17, 18].

Ob/ob mouse as a model in obesity research

The *ob/ob* mouse is a monogenic model of obesity and diabetes due to a lack of leptin production. This mouse has been studied in many respects and is a commonly used model in obesity and diabetes research. The *db/db* mouse, which in many aspects resembles the *ob/ob* mouse, carries mutations in the leptin receptor and is usually found on the C57BLKS/J background[19]. This mouse is a model of more severe hyperglycemia and diabetes and will not be discussed in the present review.

The *ob/ob* phenotype was first discovered in mice in the Jackson Laboratory in 1949[20] and was crossed back to the C57Bl/6J background. The background of the metabolic phenotype was investigated by parabiosis studies with *ob/ob* mice and normal mice, which showed that the *ob/ob* mice exhibited reduced food intake and body weight to become phenotypically normal and led to the proposal that *ob/ob* mice lack a humoral satiety factor but still maintain sensitivity to this factor[21]. Based on these observations, considerable effort was put into finding this factor, which led to the positional cloning of leptin in 1994[22]. Furthermore, leptin was shown to be expressed in adipocytes, displayed increased levels in the obese and was reduced by starvation. Leptin mRNA is highly expressed in *ob/ob* mice, but the protein is not found in the plasma of these mice[23].

The *ob/ob* mutation in the C57Bl/6J background results in a phenotype with marked obesity, hyperinsulinemia, insulin resistance and relatively mild hyperglycemia. In contrast, the *ob/ob* mutation on the C57BKS background gives rise to the same phenotype as seen in *db/db* mice with the C57BKS background[24]. The identity of the genetic differences contributing to the different phenotypes is not well understood, but this observation underlines the importance of the background strain when considering mouse models of obesity and diabetes. Several colonies of *ob/ob* mice exist worldwide, each showing variations of the phenotype associated with the *ob/ob* mutation [25].

Obesity is the first observable phenotypic characteristic of the *ob/ob* mouse, whereas insulin resistance and hyperglycemia follow the development of obesity. Apart from increased energy intake, even on a chow diet, obesity is further increased in *ob/o*b mice because of a defect in thermogenesis in brown adipose tissue and therefore a larger deposition of ingested energy as fat[26, 27]. Furthermore, lipogenesis, especially hepatic, is enhanced in *ob/ob* mice, which also adds to the disposition for an obese phenotype[28].

Islet function has been extensively studied in the *ob/ob* mouse, displaying a large capacity for islet growth that results in hyperplasia and a large capacity for insulin secretion[29]. Based on these characteristics, the *ob/ob* mouse has been widely used for studies on insulin secretion in the prediabetic state and as a model of beta-cell stress and proliferation. For more detailed reviews of the phenotypic characteristics of the *ob/ob* mouse, see Lindström[30] and Chua *et al*[31].

Treatment with recombinant leptin in *ob/ob* mice results in an acute reduction of food intake and increase in glucose turnover with increased glucose uptake in brown adipose tissue and the brain, whereas chronic treatment dose-dependently reduces food intake and body weight and also results in an improvement in insulin sensitivity[32–34]. In humans with leptin deficiency, recombinant leptin administration has been shown to have the same profound effects on food intake and body weight as seen in *ob/ob* mice[35]. However, most obese humans do not have leptin deficiency; instead, they have hyperleptinemia and leptin resistance and thus generally do not respond with weight loss during recombinant leptin treatment. This finding underlines the fact that although the *ob/ob* mouse is indeed a valuable and useful animal model of obesity, it does not reflect the complete background of obesity in humans based on its monogenic cause of obesity and will therefore not always be predictive of the effects of pharmaceutical treatments in humans.

Treatment with sibutramine for 6 weeks in 6 week old *ob/ob* mice has been shown to reduce weight gain by 12%, but not to induce weight loss [36]. Because *ob/ob* mice are still growing at 6 weeks of age and thus do not have a stable body weight, it is not surprising that they did not lose weight in this study. However, this observation underlines the importance of choosing a model with a stable body weight if weight loss is the desired outcome of a study. Sibutramine treatment did not have a significant effect on food intake, but it did stop the increase in plasma nonesterified fatty acids (NEFA). Furthermore, the compound induced a decrease in plasma insulin and improved insulin sensitivity compared to vehicle-treated animals[36]. Treatment with sibutramine in humans usually results in a weight loss of approximately 8% and beneficial

effects on NEFA levels[1]. An acute study in *ob/ob* mice showed a dose-dependent reduction of food intake and body weight in this model during the first day after receiving liraglutide. However, 2 weeks of treatment with liraglutide at a dose of 100 μg/kg BID did not show significant effects on body weight in ob/ob mice, although effects on blood glucose levels were seen in this model[37].

Similarly, 2 weeks of exendin-4 treatment in *ob/ob* mice did not significantly affect body weight or food intake[38]. As was seen with sibutramine, another study with exendin-4 (10 or 20 μg/kg BID for 8 weeks) showed a dose-dependent reduction (20%–40%) of weight gain in the *ob/ob* model[39]. One explanation for the lack of effect on body weight in the study by Irvin *et al* could be that exendin-4 was only given once daily. Conversely, the study by Ding *et al* demonstrated an effect of a lower daily amount of exenatide, a synthetic form of exendin-4, indicating that other factors, such as the strain of *ob/ob* mice, could also contribute to the different results.

The lack of a consistent effect on body weight after GLP-1 receptor agonist treatment is in contrast to the robust weight loss that has been seen in humans after treatment with these compounds[40, 41], as well as to other animal models that are not monogenetic in origin.

The Zucker rat

The Zucker rat is a commonly used obese rat model. In 1961, it was discovered by TF ZUCKER and LM ZUCKER that an autosomal recessive mutation in the fatty gene (*fa*) resulted in the obesity seen in the Zucker rat. The homozygotes for the mutation (*fa/fa*) develop early onset obesity because of a defective leptin receptor[42]. The Zucker obese rat is hyperphagic and has reduced energy expenditure, leading to development of pronounced obesity at an early stage in life[43]. Under normal conditions, leptin produced from adipose tissue signals acts via the leptin receptor to reduce food intake. In the obese Zucker rat, this regulatory path is nonfunctional, and despite high levels of circulating leptin, the rats remain hyperphagic. Other orexigenic peptides, such as neuropeptide Y, galanin, orexin and melanin concentrating hormone (MCH), are also upregulated in the Zucker rat[44]. There is a preferential deposition of lipids in adipose tissue, and by 14 weeks of age, the Zucker rat has a fat percentage of 40%[45, 46]. Zucker rats develop insulin resistance in addition to obesity, but glycemic levels remain normal, and they do not develop overt diabetes[47]. In this aspect, the Zucker rat shares similarities with a large portion of the obese human population, the group who are both obese and insulin resistant but are not diabetic. The Zucker rat is therefore considered a good animal model for this metabolic syndrome.

The effect of sibutramine on food intake has also been examined in the Zucker rat. It was found that in lean Zucker rats, which are not homozygous for the *fa* mutation, sibutramine resulted in a significant reduction in food intake compared to the vehicle. In obese Zucker rats, this effect was totally absent at the dose tested (10 mg/kg)[16]. This finding is not in accordance with the effects of sibutramine seen in human clinical studies[1]. Thus, even if the obese Zucker rat is an animal model with several similarities to human obesity and metabolic syndrome, a discrepancy exists with respect to the pharmacological effect of sibutramine. For another class of pharmacological agents, the GLP-1 receptor agonists, the obese Zucker rat has been shown to be more predictive of the human situation with respect to regulation of appetite and body weight. Subchronic treatment of the obese Zucker rat with exendin-4 led to decreased fat deposition, reduced appetite and a reduction in weight gain[48]. Similarly, in humans, treatment with GLP-1 analogues leads to appetite reduction and weight loss[40, 41].

In a colony of outbred Zucker rats, a mutation rendering the diabetic rat was detected, which led to the development of the substrain Zucker Diabetic Fatty (ZDF) rat. Males more frequently show the diabetic phenotype than do the females. The females can develop diabetes if fed a high fat diet. However, the rate of diabetes development in males also depends on the diet[49]. The ZDF rat, especially the male, is widely used as an animal model for studies of anti-diabetic and anti-obesity drugs[50]. For example, liraglutide markedly attenuated the progress of diabetes in ZDF rats. Blood glucose was significantly reduced, and plasma insulin was 2–3-fold higher during a normal 24-h feeding period compared to vehicle-only treatment. Judged by pair feeding, approximately 53% of the anti-hyperglycemic effect was mediated by a reduction of food intake.

Polygenic models

Diet-induced obese rats and mice

Monogenic animal models of obesity are useful because the obesity and adiposity they develop is often severe, resulting in a distinct phenotype. Having a distinct phenotype might be of importance for certain aspects of obesity research. Furthermore, for pharmacological treatment, a clear phenotype with a large window for drugs to exert their effects is considered beneficial.

However, a common argument against the monogenic models in general and the monogenic models deficient in the leptin system in particular is that they are not representative of the human pathogenesis of obesity. Notably few cases of human obesity can be accounted for mutations in leptin or the leptin receptor[51]. The diet-induced obese (DIO) rat and mouse offer more human-like models, where the obesity is based on several factors, including an excess intake of calories. However, diet-induced obesity in rodents can be obtained by different means; there is a large variation both with respect to the content of the diet used, as well as the strain used.

In mice, C57Bl6/J is generally considered an obesity-prone strain in which pronounced weight gain, as well as hyperinsulinemia and sometimes also hyperglycemia is seen. This strain is also the most commonly used mouse strain for diet-induced obesity. A/J mice, on the other hand, are considered obesity resistant[52]. For a more elaborate examination of the obesity propensity of different mice strains, see the work by West *et al*[53,54]. There are also rat strains that are considered resistant

to high fat diet-induced obesity, such as S5B/PI and Lou/C, whereas others develop diet-induced obesity, such as Wistar, Sprague-Dawley, Long Evans and Osborne Mendel rats[55]. Thus, it is clear that the genetic background is of major importance for the body weight response to a high-fat diet.

The modern diet, especially in West, contains high levels of fat and carbohydrates such as fructose and sucrose. Different predefined mouse and rat diets for obesity induction can vary in the percentage of calories from fat and carbohydrates, as well as the source of fat or carbohydrates, all of which can result in minor differences in phenotypes.

For a diet containing 42% of the energy as fat, the source of fat (lard, olive oil, coconut oil or fish oil) has been shown to result in differential effects on body weight gain and glucose homeostasis in male Wistar rats[56]. The authors showed that the most pronounced manifestations of obesity and insulin resistance are seen when the fat source is lard (which contains comparable quantities of saturated fat and monounsaturated fat) or olive oil (monounsaturated fat) compared to coconut oil, fish oil or a chow diet. The relatively beneficial effect of fish oil (polyunsaturated fatty acids) on lipid and glucose homeostasis compared to other fat sources has been shown previously[57–59]. However, the observation that coconut oil, which contains saturated fatty acids, is less deleterious than lard and olive oil has not been corroborated in the literature. There are actually studies suggesting that diets containing more saturated fat are obesogenic and prone to inducing insulin resistance[60, 61]. It has been suggested that the response of the major hypothalamic neuropeptides regulating energy balance varies depending on the type of fat in the diet[60].

A diet high in fat and carbohydrates, either from fructose or sucrose, closely mimics the human diet. A high-fat and high-sucrose diet has been reported to result in a similar effect on body weight, abdominal fat, hyperinsulinemia and hyperglycemia as a high-fat-only diet in mice[52, 62]. In recent years, the role of fructose in the development of adiposity and metabolic syndrome has received significant attention[63]. In rodents, a high-fat and high-fructose diet has been shown to result in metabolic syndrome with obesity and changed body composition[64, 65]. In a study by Shapiro *et al*, it was shown that feeding SD rat with fructose caused leptin resistance, which led to an exacerbated weight gain when also given a high-fat diet, suggesting that fructose increases the propensity for obesity development[66].

Commonly used diets when inducing obesity are the 45% and 60% kcal energy from fat diet (D12451 and D12492, Research Diet), where the fat source is soybean oil and lard, and the difference between the two diets is the lard content. The pharmacological treatment of DIO rodents with anti-obesity agents, such as sibutramine and liraglutide, has been shown to have effects comparable to those reported in humans. Sibutramine reduces food intake, but there are conflicting data as to whether sibutramine has an effect on energy expenditure[67]. In DIO rats, sibutramine has also been shown to reduce body weight compared to placebo (9%) because of a reduction in food intake. This reduction was accompanied by a reduction in body fat[2]. A similar effect with a reduction in food intake and body weight for the GLP1 receptor agonists liraglutide and exendin-4 has also been established in DIO rats fed the 60% HF diet from Research Diet[68]. Also, in DIO mice fed a diet containing 45% calories from fat, liraglutide reduces energy intake and body fat[69]. Thus, diet-induced obese rodents can be considered a valid animal model that reflects the effects seen in humans during pharmacological treatment that affects appetite and thereby reduces body weight. With respect to the effect on obesity of agents that reduce body weight, the diet used is not of crucial importance as long as proper obesity is induced by the diet of choice. However, the extent to which the different diets are comparable with respect to the secondary outcomes of obesity medications, such as plasma lipids and glucose tolerance, needs to be further explored.

Cafeteria diet-induced obesity

Obesity in rats can also be induced with less standardized and predefined diets, such as the Cafeteria Diet, which means that the animals have a choice of various palatable energy-dense food items as an alternative to standard chow[70, 71]. The advantage to this approach is that the diet is palatable and the propensity to overeat is larger than that for a standardized, predefined high-fat diet. Furthermore, it is more similar to the human diet situation. However, the diet is criticized for being difficult to standardize with respect to nutritional content, and the animals might experience deficiencies in proteins or essential vitamins[72]. The model has not been as widely used for the development of obesity treatment as the DIO rat described above. At Novo Nordisk, we have employed a Cafeteria Diet DIO rat model in which the rats were fed chow *ad libitum* and were offered up to 20 g of candy per day. The candy was provided on a rotational basis and included chocolate biscuits, other kinds of chocolate and grape sugar to maximize the interest in the candy and to tempt the rat to eat more. Seventy-five percent of the calories were provided by candy intake because the DIO rat preferred candy over chow. This preference led to a 15%–20% higher weight gain in DIO rats than in chow-fed rats, which was attributable to an increase in fat mass[73]. Treatment with liraglutide, but not with vildagliptin, resulted in a significant reduction in body weight. Liraglutide, but not vildagliptin, reduced the total caloric intake. Interestingly, it also seemed to change food preference because a preferential reduction in candy intake and a relative increase in chow intake were seen after liraglutide treatment in candy-fed rats[73].

DIO-DR

Three decades ago, Dr Levin and co-workers refined the DIO model by selectively breeding Sprague Dawley (SD) rats for obesity[74]. Dr Levin took 100 normal SPD rats and subjected them to a high-fat diet (60% calories from fat). After several weeks on the diet, the animals could be divided into the following three groups: a low, middle and high BW group. Thereafter, the researchers mated rats that had increased their

body weight by a small or large amount in subsequent generations until total segregation was achieved, and the researchers started an out-breeding program. The individual groups were named diet resistant (DR) or obesity prone (DIO)[74]. The advantage of this process was that it was clear from the pup stage they could be absolutely sure that the pups from the DIO group would gain more weight and pups from the DR group would gain less weight when exposed to a high-fat diet. The increase in BW was predominantly caused by a general increase in fat mass and, to a much lesser extent, muscle mass, making the DIO rat a true diet-induced obese rat and not simply a "large" rat. Over approximately the past decade, Dr Levin, academic colleagues, and the biotech and pharmaceutical industry throughout the world have applied this model in target discovery and also as an important benchmark model[75–77]. The obesity in the DIO rat is not caused by a single gene but rather is truly polygenetic in nature. After 14–16 weeks on a high-fat diet (60% calories from fat, provided by Research diet) the metabolic parameters of the DIO rats reflect the altered diet with an increase in TG, insulin and lipids, which mirrors the human condition known as metabolic syndrome, making this model truly translational[75]. The model has shown its value already, as all approved drugs that have a marked effect on BW in humans have been shown to have a similar effect in the DIO rat. Sibutramine *po* for 3 weeks leads to a weight reduction of approximately 10%–13% of basal weight in this model, which is slightly more than what has been seen in clinical experiments. With respect to liraglutide, the weight loss in rats is approximately 10%, which is slightly more (12%)[75] than has been seen in 1-year liraglutide trials performed by Dr Astrup[2].

UCD-T2DM rat

One of the drawbacks of the DIO rodent models is that they develop hyperinsulinemia but not always hyperglycemia, thereby making them good models for obesity but not necessarily for type 2 diabetes. Havel and co-workers[78] have developed a polygenic rat model with adult-onset obesity, insulin resistance and late onset type 2 diabetes that maintains leptin signaling without dietary intervention. The model originates from crossing obese, insulin-resistant SD rats with lean ZDF rats.

Thus, the model is a cross between a model employing obesity and insulin resistance but with β-cells robust enough not to develop diabetes and a model with a β-cell defect that does not develop diabetes when insulin sensitivity is normal. By the F7 generation, it appeared that all animals were homozygous for the β-cell defect[79], and both sexes develop diabetes at 183±10 and 286±17 days of age, respectively (the incidence is 91.9% for males and 42.6% for females). The body weight and caloric intake are significantly higher for the UC Davis type 2 diabetes mellitus rat [UCD (University of California Davis)-T2DM] than the lean SD rat. The increased obesity and hyperphagia precede the onset of diabetes, and after the onset of diabetes, a slow loss of body weight is seen. Furthermore, early body weight has a significant influence on the age of onset of diabetes, which is consistent with the reported increased risk for T2DM with obesity. The UCD-T2DM rat is well suited for studies of pharmacological prevention of T2DM. Liraglutide has been tested for its ability to reduce body weight, as well as to prevent or delay the onset of diabetes in UCD-T2DM rats (0.2 mg/kg)[80]. This study included a group that was weight matched to the liraglutide group. This group was restricted to a food intake of 9% less energy/kg body weight than the animals that received liraglutide. The requirement of a 9% reduction in energy intake as compared to treatment with liraglutide to obtain similar weight suggests a beneficial component of liraglutide treatment on energy expenditure. The energy intake after liraglutide treatment was significantly reduced compared to vehicle-treated animals. Both liraglutide treatment and food restriction significantly delayed the onset of diabetes in the UCD-T2DM rat. However, liraglutide markedly lowered fasted plasma insulin compared to food-restricted rats, suggesting that improved insulin sensitivity was not caused by the effect on body weight alone. The large effect on the delay of the onset of diabetes after food restriction suggests that part of the beneficial effects of liraglutide is mediated via a reduction in energy intake and body weight. Furthermore, despite having a similar body weight, the liraglutide-treated animals had an even more pronounced reduction in body adiposity than did weight-matched animals.
NZO and NoncNZO mouse

The New Zealand obese (NZO) mouse is a polygenic model that develops hyperphagia and juvenile onset obesity, even when fed a low fat diet. Both subcutaneous and visceral fat is accumulated. Furthermore, the mouse develops type 2 diabetes, although at varying frequencies depending on substrain and gender[81, 82].

The NZO mice have been crossed with the nonobese nondiabetic (NON) mouse, which has impaired glucose tolerance and impaired β-cell insulin secretion capacity but still do not develop overt diabetes. The NONcNZO10/LtJ strain is a result of several crossings between NZO and NON mice. Both female and male NONcNZO10/LTJ mice develop obesity, but only males develop type 2 diabetes[83]. The disease inheritance of NONcNZO10/LtJ reflects the complex inheritance pattern of human obesity.

The NZO mice and the NONcNZO10/LtJ mice have primarily been used for pharmacogenetic studies. However, there are reports of pharmacology studies. For example, it has been shown that a β_3-adrenergic receptor agonist (CL316, 243) lowers body weight in these mice while increasing food intake and suppressing the development of diabetes in the mice[84]. Furthermore, rosiglitazone treatment leads to a body weight increase in NONcNZO/LtJ mice and in humans[84, 85]. The utility of the two models in pharmacological obesity studies as well as their predictivity in humans remains to be verified.

Tallyho mouse

The Tallyho mouse is also a model with moderate obesity and male-derived hyperglycemia with a polygenic origin[86]. A recent publication suggests that increased food intake, not

reduced energy expenditure, is the reason for the obesity in Tallyho mice as compared to C57BL6/J mice. When pair fed with C57Bl6/J mice, the Tallyho mice have the same rate of weight gain as the C57Bl6/J mice. Furthermore, the authors show that Tallyho mice have hypothalamic leptin resistance and upregulation of NPY mRNA levels[87]. There are, to the best of our knowledge, no publications in which Tallyho mice have been used in the pharmacological treatment of obesity; therefore, their utility and predictivity for human obesity treatment is unclear.

DIO minipigs

The obese Göttingen minipig is a relatively novel model; therefore, there are few reports on its metabolic status, as well as limited reports of pharmacological intervention studies.

Adult Göttingen minipigs have a body weight of 30 to 35 kg, and food restriction is necessary to maintain a lean phenotype. When fed *ad libitum* with normal pig chow, females have a food intake that is more than double that of the age-specific norms for this breed based on a restricted diet[88]. This increased food intake leads to massive obesity, and by 18 months of age, the pigs are two to three times the weight of animals fed restricted amounts of fat with percentage close to 50% because of their hyperphagic behavior. Thus, without food restriction, this model seems to have a normal development toward severe obesity. The hyperphagic element of this obesity seems similar to the food cravings observed in severely overweight humans, in contrast to the polygenic DIO rodent model, which shows moderate obesity and body fat. The degree of obesity measured by body weight, as well as excess body fat seen in the DIO pig model, closely resembles human obesity. However, one should be aware that the model is not well characterized, and although the obese minipigs become insulin resistant, similar to obese humans, they do not develop type 2 diabetes. Also, this model is resource intensive with respect to space, time and the quantity of compounds needed for studies.

In a pharmacological intervention study[89] with liraglutide, the effect on body weight loss was of the same magnitude as has been shown in human clinical obesity studies with this compound[2]. Six female Göttingen minipigs (Ellegaard Göttingen Minipigs, Dalmose, Denmark) aged approximately 18 months of age and with a mean body weight of 90.3±6.0 kg at the beginning of the study were used in this experiment. The minipigs had been fed *ad libitum* since weaning and this continued throughout the study.

Food intake was monitored continuously throughout the study, and body weight was determined twice weekly using a standard large-animal scale. In this three-period experiment (baseline, treatment and posttreatment follow-up), each animal was used as its own control. Liraglutide profoundly affected food intake with an overall reduction of 60% for the 7-week treatment period without any signs of desensitization. This approach meant that food intake came close to the level required for maintenance of normal body weight in these pigs. Food intake returned to pre-treatment levels within 4 days of termination of liraglutide treatment and, although variable, remained within the pre-treatment range for the remainder of the study. The effect on body weight was a reduction of 4.3±1.2 kg compared to a 7.0±1.0 kg weight gain during the 7 week pre- and post-treatment periods. The DIO Göttingen minipig model seems to resemble human obesity and also responds to a similar degree to obesity intervention treatment with liraglutide.

Conclusion

As described, there are a number of valid surrogate animal models of human obesity that can be utilized in the discovery and developmental process. An overview of the different models in this review is found in Table 1. That these models have translational character is evident from the data we have presented on sibutramine and liraglutide, although the number of models described is far from exhaustive. However, the human obesity phenotype is, for the majority of people, caused by the interplay between a long list of genes and the environment; therefore, the laboratory animal model that best reflects this is polygenetic dietary-induced models. The monogenetic models do have a role in terms of teasing out the mechanism and mode of action of these diseases, but the best surrogate models are the DIO. Over the past few years, a number of models using dietary manipulations (high-fructose/high-carbohydrate) have emerged, but they are left out of the current

Table 1. An overview of the different models of obesity.

	KK-A^y	*Ob/ob*	Zucker	DIO or Cafeteria diet or DIO DR	UCD-T2M	DIO minipig
Cause of disease	Monogenic (A^y) and polygenic (KK)	Monogenic	Monogenic	Induced and polygenic	Polygenic	Induced
Obesity	Yes	Yes	Yes	Yes	Yes	Yes
Hyperphagia	Yes	Yes	Yes	No	Yes	Yes
Hyperinsulinemia	Yes	Yes	Yes	Yes	Yes	Yes
Glucose intolerance	Yes	Yes	Yes	Yes	Yes	?
Hyperglycemia	Yes	Mild	No	No	Yes	No
Weight reducing effect of sibutramine	Marginal	Marginal	No	Yes	?	?
Weight reducing effect of GLP1 analogues	?	None or marginal	Yes	Yes	Marginal	Yes

review because further testing is required to validate these models. It is likely that these models will potentially lead to a model that is even more predictive of the human disease, leaving us with a more optimized tool that will potentially reduce the path to market and will eliminate the necessity of a number of animal studies.

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