# Considering our methods: Methodological issues with rodent models of appetite and obesity research

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Abstract: A large number of animal models are currently used in appetite and obesity research. Because the worldwide incidence of obesity continues to climb, it is imperative that animal models sharing characteristics of human obesity and its co-morbidities be used appropriately in the quest for novel preventions or treatments. There is probably no animal model, at least in rodents, that recapitulates all aspects of "common" human obesity and its comorbidities, but rodent models allow insight into specific mechanisms of disease or its consequences. Frequently used obesity models can be partitioned into different categories, the major ones being a) based on mutations or manipulations of one or a few individual genes or b) those in genetically intact animals exposed to obesogenic environments such as, e.g., being maintained on high-fat diets or being raised in small litters. Characteristics of these models include distinct phenotypes of obesity, hyperphagia or changes in energy metabolism, and frequent comorbidities of obesity, like hyperglycemia, insulin resistance or diabetes-like syndromes. This review which is based on a presentation given during the Annual Meeting of the Society for the Study of Ingestive Behavior in July 2017 points out some observations and characteristics of rodent models in obesity and diabetes research. The choice of rodent models discussed here is subjective and based on the author's own experience or on fruitful discussions with colleagues about the pros and cons of specific models. Hence, this review, by no means, is meant to give a complete picture of rodent models used in this type of research, but the review tries to bring up some issues which, in the author's mind, may also be relevant for models not discussed here. For example, by discussing specific mouse and rat models, similarities and differences between mice and rats will be discussed that need to be considered to interpret experimental findings cautiously and in the context of the respective animal model. Knowing which animal model to use means, knowing its limitations.

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# Considering our methods: Methodological issues with rodent models of appetite and obesity research

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ABSTRACT

A large number of animal models are currently used in appetite and obesity research. Because the worldwide incidence of obesity continues to climb, it is imperative that animal models sharing characteristics of human obesity and its co-morbidities be used appropriately in the quest for novel preventions or treatments. There is probably no animal model, at least in rodents, that recapitulates all aspects of "common" human obesity and its comorbidities, but rodent models allow insight into specific mechanisms of disease or its consequences. Frequently used obesity models can be partitioned into different categories, the major ones being a) based on mutations or manipulations of one or a few individual genes or b) those in genetically intact animals exposed to obesogenic environments such as, e.g., being maintained on high-fat diets or being raised in small litters. Characteristics of these models include distinct phenotypes of obesity, hyperphagia or changes in energy metabolism, and frequent comorbidities of obesity, like hyperglycemia, insulin resistance or diabetes-like syndromes. This review which is based on a presentation given during the Annual Meeting of the Society for the Study of Ingestive Behavior in July 2017 points out some observations and characteristics of rodent models in obesity and diabetes research. The choice of rodent models discussed here is subjective and based on the author's own experience or on fruitful discussions with colleagues about the pros and cons of specific models. Hence, this review, by no means, is meant to give a complete picture of rodent models used in this type of research, but the review tries to bring up some issues which, in the author's mind, may also be relevant for models not discussed here. For example, by discussing specific mouse and rat models, similarities and differences between mice and rats will be discussed that need to be considered to interpret experimental findings cautiously and in the context of the respective animal model. Knowing which animal model to use means, knowing its limitations.

## 1. Obesity models in research

A large number of animal models are used in obesity research. Use of a specific model should be done on the basis of the exact research question and not on the ease of availability of a specific model. The phenotype of obesity, whether it is associated with hyperphagia or a change in energy metabolism differs among the different models, and the same is true for frequently occurring comorbidities of obesity. Some of these models are also associated with hyperglycemia, insulin resistance or diabetes-like syndromes [1].

Animal models of obesity research can be classified into models developing obesity because of monogenic mutations which may, e.g., be spontaneous or engineered in a specific manner, or polygenic animal models. The best established models with monogenic mutations are those involving the leptin pathway, like the leptin deficient ob/ob mouse or various mice and rats with deficient leptin receptor function [2]. The knowledge generated with these models then lead to the generation of a large number of engineered mutants, often targeting the pathway downstream of the leptin receptor [1]. Animal models with spontaneous or engineered mutations can be extremely useful to study specific mechanistic aspects of eating controls, but they do not recapitulate "common" obesity in the human population very well because these mutations are only responsible for a small number of (often severely) obese people [3,4]. Diet induced models of obesity (DIO) are polygenic animal models that are believed to mimic common obesity better than genetically modified models [1].

The choice of rodent models discussed here is subjective and based on the author's own experience and on fruitful discussions with colleagues about the pros and cons of specific models. Hence, this review will not give a complete picture of rodent models used in this type of research, but the review uses some specific examples in an attempt to bring up some issues which, in the author's mind, may also be relevant for models not discussed here. In other words, we need to know the limitations of our animal models to interpret our data correctly; this is

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even more important if we want to translate our findings from rodents to humans.

## 2. Rats and mice in diabetes research – mimicking the aspect of pancreatic amyloid

Animal models of diabetes research in part overlap with models of obesity research. E.g., the leptin receptor deficient Zucker fa/fa, the Zucker diabetic fatty (ZDF) or the DIO rat may, under certain conditions, also develop some aspects of type 2 diabetes mellitus (for overview, see [1]). A type 2 diabetes like phenotype was also shown in the ZDSD rat which is based on cross-breeding of heterozygous ZDF$^{fa/wt}$ rats with DIO rats [5,6].

None of these models, however, recapitulates the most typical morphological change observed in type 2 diabetic humans, the deposition of pancreatic islet amyloid which results from misfolded islet amyloid polypeptide, or amylin. Amylin is only fibrillogenic in species that spontaneously develop the full picture of type 2 diabetes, i.e. in humans, other primates and cats, but not in rodents [7–9] (please note that the hormonal effect of amylin as a satiating hormone presumably is relevant in all species, but that its fibrillogenic, or amyloidogenic, properties are only relevant in some species, and under some conditions). Important aspects of islet amyloid in the pathogenesis of type 2 diabetes are the fact that deposits may already be detectable before the onset of clinical signs and that deposits only form when the amyloidogenic amino acid sequence of amylin is present. The toxic effect of aggregated amylin seems to be due to small oligomers that form intracellularly which then triggers a number of intracellular events eventually leading to pancreatic beta-cell destruction (for review: [9]). Because rodent amylin is not amyloidogenic, transgenic mice and rats that express the human form of amylin have been generated to study the pathophysiological relevance of amylin-derived islet amyloid deposits [8–10]. These rats and mice eventually develop diabetes mellitus due to beta cell failure [8,10] and therefore allow the investigation of an aspect of the diabetes pathophysiology [11] that cannot be studied with more “traditional” diabetes models. However, these animals typically do not develop another important aspect of type 2 diabetes pathophysiology, i.e. the insulin resistance in peripheral tissues.

Generally speaking, this example of a diabetes model underlines that we need to realize that most animal models that we use in obesity and diabetes research allow the study of specific aspects of the disease in humans, but that most models do not recapitulate all aspects of a human disease. We must know these limitations.

## 3. Polygenic models of obesity: Diet-induced obese (DIO) versus diet-resistant (DR) rodents

The best characterized rat DIO model is based on outbred Sprague-Dawley (SD) rats. When maintained on a low fat (chow) diet, the amount of carcass fat does not differ between DIO and DR rats. Nonetheless, DIO rats tend to be bigger. Importantly, when these rats are exposed to a high-energy diet (HE; 31% fat), rats with the DIO phenotype become obese while DR rats maintain a phenotype very similar to chow fed animals; hence, the latter group of rats resists the development of obesity, despite HE exposure [12]. When food restricted, DIO rats mainly loose adipose mass and are characterized by reduced leptin and insulin levels; when returned to ad libitum feeding, DIO rats regain adiposity similarly to never restricted DIO rats [12]. This DIO versus DR phenotype shares many characteristics with the common form of human obesity [12–15]. When the DIO and DR rats are bred selectively over several generations [14,16], the obese phenotype in DIO compared to DR rats becomes more distinct. In particular, the obese phenotype is also maintained if the selectively bred DIO rats are switched back to a low fat chow diet [13]. The DIO phenotype seems to be inherited in a polygenic fashion but the involved genes are largely unknown. The DIO phenotype, including its metabolic abnormalities like glucose intolerance, is even maintained when selectively bred DIO rats are backcrossed with obesity-resistant Fischer F344 rats, supporting the idea of a polygenic obesity trait in the DIO rats [17,18].

An important characteristic of the selectively bred DIO rat is that it develops leptin resistance, insulin resistance, hypertension and hypertriglyceridemia soon after the exposure to the HE diet, i.e. before overt obesity develops [14,17,19–21].

The extent of weight gain and ensuing obesity once exposed to HE diets in selectively bred DIO rats depends on the type of diet. DIO rats that had access to both the HE diet and liquid Ensure$^{R}$ gained more weight than rats exposed to HE only [13]. Further, when Ensure$^{R}$ exposed DIO are switched back to a low fat chow diet, their body weight decreases, but only to the level of HE exposed DIO rats, and not to the level of chow fed rats [13].

Interestingly, selectively-bred DIO rats can be protected from becoming obese when their nutrient supply is limited during the suckling period, e.g., by rearing them in large litters [22]. These animals do not develop leptin resistance, and their reduced body weight persists into adulthood [21,23].

Because selectively bred DIO rats have an inborn resistance to the behavioral and physiological effects of leptin and insulin, and because they show abnormal glucose sensing responses compared to DR rats, the two substrains of DIO versus DR rats are ideally suited to study interactions between genes and the environment in the development of obesity and diabetes [23,24].

Overall, the selectively bred DIO and DR rats are a very good polygenic model that recapitulates important features of common human obesity. In this sense, the DIO and DR rats bear clear advantages over monogenic obesity models that may only be relevant for a very small fraction of obese patients. The disadvantage may be that the DIO/DR model does not allow us to study the role of specific gene products.

## 4. Specific examples of genetic rat and mouse models with differing phenotypes

In the following, I will briefly address some mouse or rat models with alterations in specific signaling pathways to indicate certain issues that need to be taken into account when using these animals, including some surprising phenotypic differences that often remain unknown for their underlying mechanisms.

### *4.1. The Otsuka Long Evans Tokushima Fatty (OLETF) rat versus the CCK-1 receptor knockout (ko) mouse*

The OLETF rats develop a relatively mild form of obesity [25–27]. OLETF rats lack the cholecystokinin-1 (CCK-1) receptor which leads to overeating compared to their lean counterparts, the Long-Evans Tokushima Otsuka rat (LETO). As expected, based on the physiological role of cholecystokinin (CCK) to induce satiation and to control meal size, hyperphagia seen in these OLETF rats is due to an increase in meal size because of the lack of feedback signal to the caudal hindbrain [28]. As a consequence, the expression of the orexigenic neuropeptide Neuropeptide Y (NPY) in the dorsomedial hypothalamic nucleus (DMH) is increased. OLETF rats develop obesity independent of the diet that they are exposed to, but the degree of obesity compared to the LETO controls depends on type of diet consumed [29].

In stark contrast to OLETF rats, CCK1 receptor knockout (ko) mouse do not become obese on a normal diet [30]. They do not show hyperphagia on either low fat or high fat diets, and high fat feeding induces similar degrees of obesity in the ko and respective wildtype mice. The reason for the different phenotype of rats and mice lacking the CCK1 receptor, respectively, may be related to a difference in the counter-regulatory response of the expression of NPY and other neuropeptides in the hypothalamus [29].

The important point is that seemingly similar animal models with similar genetic defects may show different phenotypes, and the

underlying reasons for such differences may not always be known (please also see Section 6.1).

### 4.2. Mice with defects in glucagon-like peptide-1 (GLP-1) signaling: the preproglucagon (PPG) silenced mouse and the GLP-1 receptor ko mouse

Glucagon-like peptide-1 (GLP-1) is produced in enteroendocrine cells, in the endocrine pancreas and in specific neurons of the caudal hindbrain. Next to its incretin effect to potentiate glucose-induced insulin release, GLP-1 reduces eating [31]. In fact, a large body of literature about the eating inhibitory effect of GLP-1 is available [31–34], and GLP-1 analogues are successfully used in anti-obesity therapy (e.g., [35]). It is therefore interesting to see that animals that have a defect in GLP-1 signaling often do not show the expected phenotype. The PPG silenced mouse, i.e. a mouse that does not express any of the PPG derived peptides (GLP-1, GLP-2, glucagon, etc.) because the Gcg gene that codes for PPG, has a stop signal flanked by LoxTB sites, does not show marked differences in body composition [36]. Similarly, mice lacking the GLP-1 receptor in neuronal tissue or specifically in visceral afferent and efferent nerves have unaltered food intake and lean and fat body mass [37], similar to the whole body GLP-1 receptor knockout. Female GLP-1 receptor ko mice even seem to be protected from high fat diet induced obesity under certain conditions [38], the opposite of what would be expected from an animal model that lacks the capacity to respond to an important eating-inhibitory hormone.

The important point is that even though genetically modified models are extremely useful to study the effect of a given factor, the models may not always show the “expected” phenotype. Hence, we need to realize that these findings probably reflect the complexity of the system controlling eating, energy metabolism and body with a large number of redundant control points [39], and interactions that to a large extent may remain unknown.

### 4.3. NPY receptor deficient mice

Next to the complexity and interactions among different feedback signals, receptor complexity within a specific signaling system may also explain some findings reported from genetically modified mice that do not seem to reflect the expected phenotype. One such example is the NPY system. Several receptor subtypes mediate the effects of NPY and its related hormones, e.g., peptide YY (PYY). Within the central nervous system, NPY is a neuropeptide potently stimulating eating, and this effect has been associated with signaling at the Y1 and at the Y5 receptors [40]. Deletion of the Y1 receptor has no effect on spontaneous food intake, but it did result in reduced fasting induced food intake. In contrast, deletion of the Y5 receptor paradoxically even increased spontaneous and fasting induced eating. Only the deletion of both receptor subtypes lead to the expected hypophagia both under ad libitum feeding conditions and in response to fasting [41]. Whether the respective receptors are present on the same target neurons or whether different brain sites, expressing specific subtypes of Y receptors interact, remains unclear.

The point that I want to make is that the effectiveness or the effect strength of a given signal may depend on several receptors, or signaling pathways. Hence, it may be necessary to target them all to define a signal's role in the control of energy metabolism.

### 4.4. Mouse models of ghrelin deficiency

Ghrelin is a stomach-derived neuropeptide that acts on the growth hormone secretagogue receptor and that increases eating when given exogenously. Endogenous ghrelin levels increase before meal onset and typically decrease in response to eating. Ghrelin seems to act as a functional antagonist to leptin because generally speaking, it has actions in the hypothalamic feeding centers that are opposite to leptin. Nonetheless, its physiological role in the control of eating and body weight is less clear. Ghrelin ko mice have been generated but their phenotype does not match the expected picture. In other words, ghrelin ko mice exhibit a normal growth rate, body weight and food intake [42]. These findings contrast with animals lacking the enzyme ghrelin-O-acyl transferase (GOAT) which is necessary to produce biologically active acylated ghrelin. GOAT ko mice eat less and gain less weight when given access to high carbohydrate and high fat diets [43,44] which matches the predicted phenotype much better than the ghrelin ko mouse.

The important point is that these examples indicate that knockout models with similar targets do not necessarily result in the same phenotype. The exact reasons for these seemingly paradoxical differences can sometimes be resolved but often remain unknown.

### 4.5. Amylin knockout mouse

The last example given here is the amylin deficient amylin ko mouse [45,46]. Amylin is best known for its role as meal ending satiation hormone, and acute and chronic administration of amylin or its analogues have been shown to reduce eating and eventually body weight [47,48]. The amylin ko mouse would therefore be expected to exhibit hyperphagia and increased body weight gain. While this is in fact the case in young adult mice up to an age of approximately 4 months, food intake and body weight are similar in older animals [47,49].

This example indicates that an expected phenotype may be present in genetically modified animal models but that the expression of the phenotype may differ during different periods of the animals' life cycle. In other words, mechanisms that presumably compensate for the lack of a specific factor that is involved in the control of energy balance may also differ in their efficiency in different periods of an animal's life.

## 5. Role of energy expenditure in overall energy balance and the importance of brown adipose tissue

Energy balance is not only dependent on energy intake but obviously also on energy expenditure. Recent years have seen an important revival of research in energy expenditure when the presence and potential importance of brown adipose tissue (BAT) as a thermogenic organ in humans was (re)appreciated [50–52]. Two important points will be discussed here.

### 5.1. Energy balance in different environments

A large number of experiments using laboratory rodents is performed at ambient temperatures which are far below the animals' thermoneutral zone. This zone corresponds to the ambient temperate range where energy expenditure is minimal because the animal (or human) does not require additional energy for heat generation [e.g., shivering, physical activity, non-shivering thermogenesis via BAT activation] or heat disposal [e.g., sweating or panting]. In small rodents, the thermoneutral zone, at least when single-housed, is around 28–30 °C (e.g., [53]). It is therefore difficult to extrapolate findings of rodent experiments performed at 21–24 °C (presumably the range for the most frequent housing temperature) directly to humans because under most conditions, (dressed) humans live close to or within a temperature range corresponding to the thermoneutral zone. In contrast, single-housed rodents housed at 21–24 °C use a large component of their total energy expenditure to maintain a stable body temperature.

It is important to note that the situation may be different under group-housing conditions because animals show adaptive behaviors like nest building, huddling in groups, etc.; such behaviors are reduced at higher ambient temperature but the overall exact impact on energy homeostasis is not clear [54,55].

These few examples point out that energy balance in an individual animal depends on many factors. To draw meaningful conclusions from published work, we need to know these factors. Unfortunately, some

publications often simply do not report enough methodological details to draw meaningful conclusions about a potential impact on energy metabolism; apart from the examples given above, i.e., the single- versus group-housing aspect, factors like the availability of nesting material, the use of ventilated cages [56], and many others should considered.

### 5.2. Importance of brown adipose tissue in energy balance

A second important aspect is the relative role of BAT and of white adipose tissue (WAT) undergoing browning (or beiging) for energy balance. A recent study [57] investigated the absolute and relative changes of the expression of uncoupling protein-1 (UCP-1) which is one of the key markers for the thermogenic capacity of BAT or of browning WAT. While the relative increase in UCP-1 expression in WAT was much more dramatic than in classical BAT when mice were moved from an ambient temperature of 21 °C to 4 °C, total UCP-1 expression in BAT still largely outweighed that of inguinal WAT. A second important finding of that study was that UCP-1 expression in BAT was already increased dramatically at a "baseline" of 21 °C when compared to mice kept at 30 °C [57]. Browning of WAT, however, did not seem to be relevant for this temperature shift because UCP-1 expression in WAT was similar at 21 °C and at 30 °C. This study clearly indicated that first, one must not confound relative increases in UCP-1 expression (and hence thermogenic capacity) with the overall relevance for energy expenditure (which depends on the total amount of UCP-1 expressed). Second, for the potential extrapolation of findings to humans, the relevant temperature ranges must be considered; moving mice from 30 °C to 21 °C is probably a much more relevant comparison for the extrapolation to the human situation than a change of the ambient temperature from 21 °C to 4 °C in mice.

Overall, this study indicates that classical BAT may be the more important factor for total energy expenditure than the browning of WAT [57]. Finally, if present, browning in mouse and human WAT does not seem to occur in the same depot because it seems to be more prevalent in intraabdominal than in subcutaneous fat in people but the opposite pattern is observed in mice [58].

## 6. Mouse and rat models of bariatric surgery

Bariatric surgery, in particular the Roux-en-Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG), is the most efficient weight loss therapy currently available, and in particular because it also reduces obesity-related comorbidities [59–63]. Many research groups established rodent RYGB and VSG models which were instrumental in better understanding the mechanisms that contribute to weight loss and to the maintenance of lower body weight post-surgery (e.g., [53,64–73]). I do not want to recapitulate the underlying mechanisms in detail, but I want to point to some important differences across species when evaluating the different components of energy metabolism.

RYGB in rats leads to a strong decrease in body weight which is due to a decrease in food intake and, when compared to food restricted rats that are weight matched to RYGB, at least a relative increase in energy expenditure (e.g., [64,66,69,74–76]). In rats, eating after RYGB is decreased because of a reduction in dark phase food intake and in particular a decrease in the average nocturnal meal size which is only partly compensated by a higher meal frequency. Interestingly, light phase food intake may actually be increased in RYGB rats, and the size of average meals during the light phase may approach that during the dark phase post-surgery [69]. The latter may indicate that RYGB (or VSG) rats may not be able to ingest large meals because of some mechanic restriction, even though mechanic restriction does not seem to be a decisive factor for the overall decreased ingestion [77–80].

RYGB and VSG in mice typically also lead to a decrease in body weight (or at least a decrease in body weight gain), but this effect is usually not paralleled by a decrease in eating. Food intake may be decreased temporarily for a few days after surgery, but then rapidly reaches levels comparable to control animals. The lasting difference in body weight between operated and control animals seems to be due to a strong increase in energy expenditure after RYGB or VSG [75,81–86].

Hence, particularly in mice there is a fundamental difference to the human situation because the main factor for weight loss post-bariatric surgery in people seems to be a decrease in eating, even though changes in energy expenditure have been reported in some but not all studies [87–90]. In other words, even though the phenotype "weight loss" in RYGB or VSG operated mice may be similar to humans, the major underlying mechanisms may differ fundamentally. Rats seem to be intermediate between humans and mice.

### 6.1. General reflection about species-specific differences for the relative importance of eating versus energy expenditure

The differences discussed above may reflect a more general difference in the control of energy metabolism in small versus large animals. The smaller an animal, the larger its body surface (heat loss) relative to body mass (heat production), and vice versa. Hence, for a (small) mouse, it may be more effective to control its total energy balance by adapting energy expenditure while for a (larger) rat, the control of energy intake may be a more effective means to control whole body energy balance. The latter aspect of course is even more relevant for larger animals or humans, respectively.

## 7. Summary

This review is an attempt to sensitize the reader to certain aspects that must be considered when using animal models in appetite, obesity and diabetes research. Because the worldwide incidence of obesity continues to climb, it is imperative that these animal models which usually only share part characteristics of human obesity and its comorbidities, are used appropriately. There is probably no animal model, at least in rodents, that recapitulates all aspects of "common" human obesity and its comorbidities, but rodent models have the important advantage that they allow insight into specific mechanisms of disease or its consequences.

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