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Authors

Kleinert, Maximilian
Clemmensen, Christoffer
Hofmann, Susanna M
et al.

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Animal models of obesity and diabetes mellitus

Maximilian Kleinert[1–4], Christoffer Clemmensen[1–3], Susanna M. Hofmann[3,5,6], Mary C. Moore[7], Simone Renner[3,8], Stephen C. Woods[9], Peter Huypens[3,10], Johannes Beckers[3,10,11], Martin Hrabe de Angelis[3,10,11], Annette Schürmann[3,12], Mostafa Bakhti[3,5,13], Martin Klingenspor[14,15,16], Mark Heiman[17], Alan D. Cherrington[7], Michael Ristow[18], Heiko Lickert[3,5,13], Eckhard Wolf[3,8], Peter J. Havel[19], Timo D. Müller[1–3] and Matthias H. Tschöp[1–3]

Abstract | More than one-third of the worldwide population is overweight or obese and therefore at risk of developing type 2 diabetes mellitus. In order to mitigate this pandemic, safer and more potent therapeutics are urgently required. This necessitates the continued use of animal models to discover, validate and optimize novel therapeutics for their safe use in humans. In order to improve the transition from bench to bedside, researchers must not only carefully select the appropriate model but also draw the right conclusions. In this Review, we consolidate the key information on the currently available animal models of obesity and diabetes and highlight the advantages, limitations and important caveats of each of these models.

On the current trajectory, over half of the adult population in the United States will be obese by 2030, and similar increments are likely to occur in other developed countries[1,2]. With its many related medical complications, obesity is challenging smoking and drinking as the number one contributor to morbidity and mortality worldwide. The prevalence of one of the most devastating consequences of obesity, type 2 diabetes mellitus (T2DM), which is characterized as the progressive worsening of insulin resistance and compensatory increases in insulin secretion, is increasing at similarly alarming rates. While T2DM is clearly a multifactorial and complex disorder, there is no doubt that obesity-induced insulin resistance accelerates pancreatic islet exhaustion and thus the onset of T2DM[3].

Intricate environmental and genetic factors are at the root of the modern-day obesity crisis. The unprecedented availability of inexpensive palatable, calorie-dense foods coupled with a decrease in occupational physical activity has created an obesogenic environment. Nonetheless, a considerable portion of the global population remains lean, highlighting innate genetic components that influence a person's susceptibility to developing obesity. This genetic predisposition to obesity (and also to T2DM) is determined by the interaction of multiple risk genes that individually have only modest bearing[4].

Unravelling this complex interaction of nature and nurture is pivotal to develop strategies to prevent, reverse or ameliorate the harmful effects of obesity and T2DM. To tackle these objectives, researchers rely on diverse animal models that span multiple species and strategic scientific approaches. The first widely used animal model for metabolic research was the dog, with pioneering studies in the early 20th century including Ivan Pavlov's Nobel Prize work (awarded in 1904) on gastric secretions and the later discovery of insulin by Frederick Banting and Charles Best (Nobel Prize in 1923)[5]. For the past 2 decades, research that has used small rodents, especially mice, has been at the forefront of scientific advancements on obesity and diabetes mellitus. For example, rodent studies led to the discovery of the central actions on energy balance of leptin[6] and ghrelin[7]. In this Review, we highlight the advantages and limitations of the most commonly used animal models of research on obesity and diabetes mellitus.

Human obesity — hallmark manifestations

Obesity is characterized by a pathological excess of body fat that results from a persistently positive energy balance[8]. Comorbidities directly associated with excess body fat include T2DM, certain cancers and cardiovascular diseases including hypertension, coronary artery disease and stroke[9]. More severe cases of obesity are associated with an increased incidence of sleep apnea[10,11], asthma[12–14], gallstones[15], steatohepatitis[16], glomerulosclerosis[17], dyslipidaemia and endothelial dysfunction[18,19]. The metabolic complications linked to obesity are often attributed to systemic processes such as metabolic inflammation or cellular pathologies such as mitochondrial dysfunction[20]

[1]*Institute for Diabetes and Obesity, Helmholtz Diabetes Center at Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.*
[2]*Division of Metabolic Diseases, Department of Medicine, Technische Universität München, D-80333 Munich, Germany.*
[3]*German Center for Diabetes Research (DZD), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.*
Correspondence to M.H.T.
matthias.tschoep@helmholtz-muenchen.de

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Key points

- Development of safe and potent therapeutics is required to combat the obesity and diabetes mellitus pandemic
- Animal models remain indispensable for discovering, validating and optimizing novel therapeutics for their safe use in humans
- To improve the transition from bench to bedside, researchers must select the appropriate models, beware a myriad of confounding factors and draw appropriate conclusions
- Experimental procedures and conditions should be accurately detailed to improve the reproducibility and translation of findings in preclinical animal models
- Different animal models, ranging from non-mammalian models to non-human primates, each have distinct advantages and limitations

Author addresses

[1]Institute for Diabetes and Obesity, Helmholtz Diabetes Center at Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.
[2]Division of Metabolic Diseases, Department of Medicine, Technische Universität München, D-80333 Munich, Germany.
[3]German Center for Diabetes Research (DZD), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.
[4]Section of Molecular Physiology, Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, DK-2200 Copenhagen, Denmark.
[5]Institute for Diabetes and Regeneration Research, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.
[6]Medizinische Klinik und Poliklinik IV, Klinikum der Ludwig-Maximilians-Universität München, Ziemssenstr. 1, D-80336 Munich, Germany.
[7]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37212, USA.
[8]Chair for Molecular Animal Breeding and Biotechnology, Gene Center, Ludwig-Maximilan University München, Feodor-Lynen-Str. 25, D-81377 Munich, Germany.
[9]University of Cincinnati College of Medicine, Department of Psychiatry and Behavioral Neuroscience, Metabolic Diseases Institute, 2170 East Galbraith Road, Cincinnati, Ohio 45237, USA.
[10]Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.
[11]Technische Universität München, Chair of Experimental Genetics, D-85354 Freising, Germany.
[12]Department of Experimental Diabetology, German Institute of Human Nutrition (DIfE), Arthur-Scheunert-Allee 114–116, D-14558 Nuthetal, Germany.
[13]Institute of Stem Cell Research, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.
[14]Chair of Molecular Nutritional Medicine, Technische Universität München, TUM School of Life Sciences Weihenstephan, Gregor-Mendel-Str. 2, D-85354 Freising, Germany
[15]Else Kröner-Fresenius Center for Nutritional Medicine, Technische Universität München, D-85354 Freising, Germany
[16]Institute for Food & Health, Technische Universität München, D-85354 Freising, Germany
[17]MicroBiome Therapeutics, 1316 Jefferson Ave, New Orleans, Louisiana 70115, USA.
[18]Energy Metabolism Laboratory, Institute of Translational Medicine, Swiss Federal Institute of Technology (ETH) Zurich, CH-8603 Zurich-Schwerzenbach, Switzerland.
[19]Department of Molecular Biosciences, School of Veterinary Medicine and Department of Nutrition, 3135 Meyer Hall, University of California, Davis, California 95616–5270, USA.

and/or stress of the endoplasmic reticulum due to lipotoxicity[19,21]. Under conditions of obesity, the lipid storage capacity of the adipocytes is exceeded, resulting in adipocyte-derived fatty acids and cytokines leaking into the circulation[22]. Harmful lipid species then accumulate in ectopic tissues and cause local inflammation that contributes to the development of endothelial dysfunction, non-alcoholic fatty liver disease and insulin resistance[23–25]. In addition to fatty acids and cytokines, altered secretion of other adipokines (such as adiponectin) or lipokines also contribute to metabolic impairments in other tissues such as the liver or the pancreas[26,27].

Reliable methods to assess both the quantity of body fat depots and the distribution of body fat are available for humans as well as for animal models. Methods used in the clinic to analyse total body fat composition include a measurement of height with weight to calculate a BMI in addition to waist circumference and skinfold thickness measurements. Hydrostatic weighing, air displacement plethysmography, bioimpedance analysis, dual-energy X-ray absorptiometry (DXA) and quantitative magnetic resonance (qMR) are accurate and precise techniques to assess total body fat but are more costly than anthropomorphic measuring. Ultrasonography is an approach that allows clinicians to measure the thickness of subcutaneous fat, muscle and intra-abdominal depth. A precise assessment of body fat distribution can be obtained from CT and MRI[28].

Methods are also available to measure body fat in animal models (BOX 1). Investigators can dissect fat depots at termination of an experiment and weigh them. In addition, measurements of total body composition at termination can be made by whole-body chemical carcass composition analysis. While these methods are inexpensive, instruments also exist to measure the total body composition of living animals, allowing for longitudinal measurements and calculations of change. DXA provides a noninvasive approach to assess body fat and lean tissue contents. Data derived from DXA are validated using chemical analysis[29]. A DXA scan, however, can be conducted on only an anaesthetized animal and will likely disrupt feeding on those days. qMR is another precise method that compares well to chemical analysis methods[30]. qMR is rapid and does not require the animal to be restrained, avoiding use of anaesthesia. MRI can noninvasively be used to obtain separate fat and water images with quantification in anatomically distinct fat depots with animal models of obesity[31]. However, as with DXA, the animal must be anaesthetized during the measurement.

Successful weight-loss interventions must decrease food intake or increase energy expenditure — ideally both together. Although successful changes in lifestyle with a decrease in calorie intake and increased physical activity can meet these criteria in humans, such lifestyle changes typically result in only modest weight loss of 3–4% after 4 years[32]. Pharmacotherapy as an adjunct to lifestyle changes can further increase weight loss, but the efficacy of previous or current weight-loss pharmacotherapies is also only moderate, with weight loss typically in the range of 6–8% after 1–4 years[32–35]. The development

of novel pharmacotherapies with improved efficacy and sustained action is therefore a major challenge of global priority. In this regard, one promising avenue is based on recent advances in the engineering of unimolecular multiagonist peptides that simultaneously activate several key metabolic pathways, each having beneficial effects on metabolism[36–39]. Several of these multiagonist peptides are currently in clinical evaluation[40–42].

Box 1 | **Methodologies to measure body composition in models of obesity**

Body weight

- Whole animal body mass
  - Equipment: balance
  - Advantages: quick; accurate; no anaesthesia; suitable for linear measurements
  - Disadvantages: no assessment of body composition; low precision of live weight

Organ weight

- Target organ mass
  - Equipment: balance
  - Advantages: quick; accurate; tissue specificity
  - Disadvantages: precision depends on standardized dissection of organs or tissue depots; terminal measurements only; not for whole-body assessment

Carcass analyses

- Chemical analyses of the whole body
  - Equipment: oven; grinder; specialized instruments
  - Advantages: whole-body end points; total fat values considered the gold standard; provides ash mass as a readout for bone mineral density (BMD); whole-body water content
  - Disadvantages: impractical for large animals; low precision; terminal measurements only; provides total protein content rather than total lean mass

Dual-energy X-ray absorptiometry (DXA)

- Absorption of X-ray beams by tissues of different densities
  - Equipment: DXA instrument
  - Advantages: whole-body end points; BMD values considered the gold standard; provides total fat and lean mass; suitable for linear measurements; accurate; precise
  - Disadvantages: anaesthesia (disrupts feeding on procedure and day); time-consuming scan; exposure of animal and experimenter to X-rays

CT

- Absorption of X-rays by tissues of different densities by use of quantification software
  - Equipment: CT instrument
  - Advantages: whole-body end points; BMD values considered the gold standard; cortical and trabecular bone recognition; provides subcutaneous and visceral fat quantification; suitable for linear measurements; accurate; precise; quick
  - Disadvantages: anaesthesia; exposure of animal and experimenter to X-rays; low accuracy for lean mass

NMR relaxometry or quantitative magnetic resonance (qMR)

- NMR spectra of tissues
  - Equipment: whole-body NMR
  - Advantages: whole-body end points; provides whole-body fat, lean and free water mass; suitable for linear measurements; requires no anaesthesia; accurate; precise; quick
  - Disadvantages: No bone data; does not distinguish subcutaneous from visceral fat; lean mass is calibrated to lean chicken muscle

MRI

- NMR in a strong magnetic field
  - Equipment: MRI instrument within a room shielded for powerful electromagnetic forces
  - Advantages: high-resolution anatomical images based on water and fat distribution; quantification of total fat and lean mass; suitable for linear measurements
  - Disadvantages: anaesthesia (disrupts feeding on procedure and day); scan is time-consuming

The development of these and other novel pharmacological options to tackle the global 'diabesity' pandemic requires a solid understanding of the complex interactions of central and peripheral signal mechanisms that regulate systemic energy and glucose metabolism.

Preclinical animal models have provided considerable valuable information about obesity and T2DM (FIG. 1). Despite constant improvements and refinements in cell-based applications, careful metabolic assessment of compound effects in *in vivo* models is vital before drugs can be considered for clinical evaluation and commercialization. The choice of a specific method to measure body composition depends on the need for accuracy, precision, convenience, cost and safety. Of note, before selecting any of the animal models described in this Review, researchers could consider the method or methods that will be used to measure body composition, if that is an aim of the study.

Zebrafish, *Caenorhabditis elegans* and *Drosophila*

Two major advantages of using non-mammalian experimental organisms (and particularly metazoans) are their short lifespan, which expedites quantification of long-term and transgenerational consequences of obesity and diabetes, and the fact that investigators can perform high-throughput analyses on such organisms, as exemplified by the availability of whole-genome RNA interference (RNAi) libraries. For example, genome-wide genetic screens have identified novel candidates pertinent to the control of body fat accumulation and function[43,44] in the nematode *Caenorhabditis elegans*[45] and in the fruitfly *Drosophila melanogaster*[46]. Dietary induction of obesity is achieved in the roundworm *C. elegans*[44,47], in the fruitfly *D. melanogaster*[48] and in the zebrafish *Danio rerio*[49]. While all three of these species have been subjected to experimental calorie restriction, only *Drosophila* has been successfully exposed to different sources of macronutrients[50,51]. Food cue sensing and satiety are linked to the amphid neuron ASI and to transforming growth factor-β (TGFβ) signalling in *C. elegans*[52,53], resembling findings in mice[54]. Neuronal circuits controlling body fat storage have also been established in *Drosophila*[55]. To date, investigators have not identified many of the hormones known to regulate lipid storage in mammals in non-vertebrate species such as *C. elegans* and *Drosophila*, but the vertebrate zebrafish has mammalian-like ghrelin[56] and leptin[57] activity. Consistent with the latter, overexpression of endogenous agouti-related protein (Agrp) causes obesity in zebrafish[58].

At the intersection of energy storage and glucose metabolism, glucagon signalling seems to exist in *Drosophila*[59] and zebrafish[60], but published evidence in nematodes is lacking. By contrast, extensive evidence exists for orthologous insulin and insulin-like growth factor I (IGF-I)-like signalling peptides in all three species[61–63]. Consistent with these findings, insulin resistance can be induced in *Drosophila* by activating forkhead box protein O (Foxo)[64] or by a high-calorie diet[65]. In non-mammalian metazoans, several different cell types including neurons and hypodermic cells produce insulin and IGF-I-like signalling peptides.

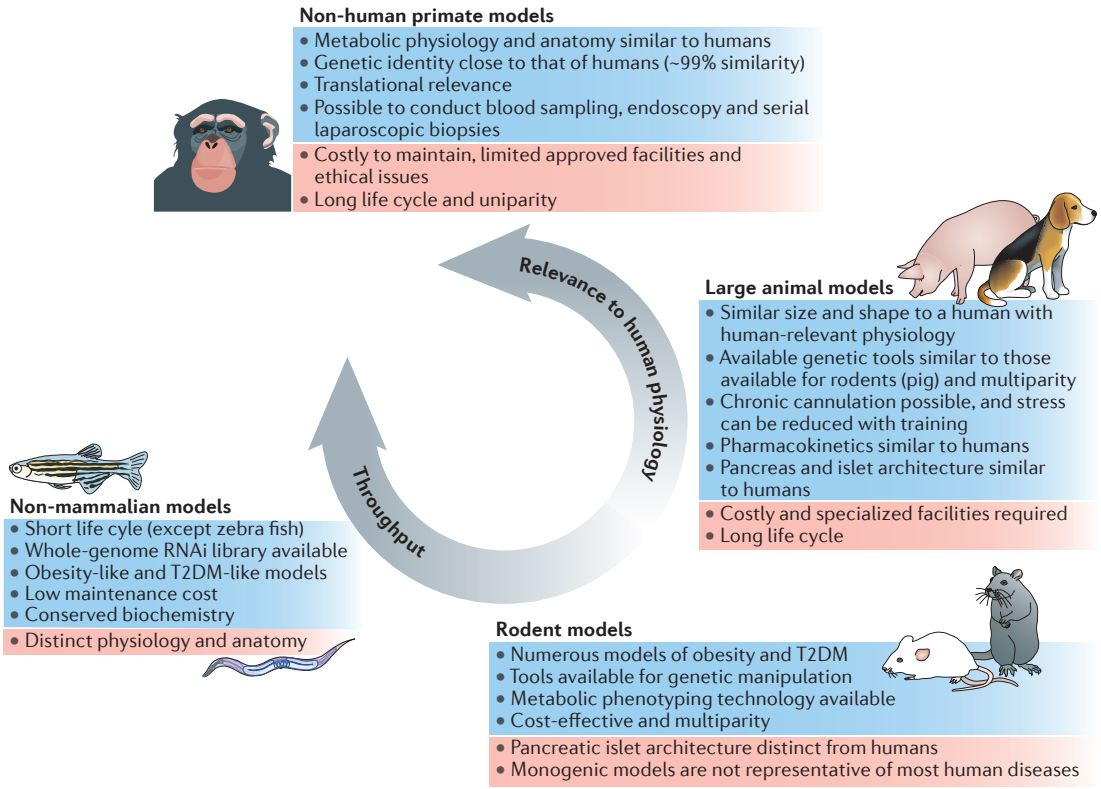

Figure 1 | **Key advantages and disadvantages of different classes of animal models used in obesity and diabetes research.** Obesity and type 2 diabetes mellitus (T2DM) research spans a wide range of animal models that have distinct advantages and shortcomings. As a general rule, non-mammalian models have the advantage of low maintenance cost, a short life cycle and the availability of diverse gene-editing tools. However, their translational value is limited given their distinct anatomy and physiology. By contrast, the physiology of large animal models including the dog and the pig, and especially that of non-human primates, closely resembles human physiology. However, the trade-off is that these species have high maintenance costs and especially long life cycles. Further, because each dam produces only a low number of offspring in her lifetime, these models are undesirable for large-scale, time-efficient experiments. Small rodents and especially the laboratory mouse represent a good compromise between throughput and translational physiology. Their physiology is closer to that of humans than non-mammalian models, and their small size, high fecundity and short life cycle, coupled with the relative ease of editing their genome, explain their popularity. RNAi, RNA interference.

Dedicated insulin-producing cells have been identified in zebrafish but not in *C. elegans* or *Drosophila*[60,66]. Similar to what occurs in mammals, β-cell differentiation from common precursors in zebrafish requires both Notch and pancreas transcription factor 1 (Ptf1) signalling[67–70]. Terminal differentiation has been obtained with a set of FDA-approved compounds[71] as well as with a bacterial protein named β-cell expansion factor A (BefA) that is also present in the human gut microbiota[72]. Ablation of β-cells in zebrafish[66] and ablation of insulin-producing neurons in *Drosophila*[73] cause hyperglycaemia — a comparable diabetes-like state has not been induced in *C. elegans*. Nevertheless, nematodes are used to study reductions in lifespan induced by glucose toxicity[47,74] as well as the induction of diabetic neuropathy via formation of methylglyoxal and advanced glycated end products[74].

Studies have investigated cardiac function[75] and memory impairment[76] in metabolically impaired *Drosophila* and zebrafish, respectively. Collectively, non-mammalian species possess multiple, but not all, pathways of body mass control and energy balance found in mammals, exhibit conserved features of insulin-like signalling and secretion, develop diabetes-like states when exposed to genetic or environmental (particularly nutritive) perturbations and are consequently valuable tools to initiate or refine the conceptual basis of more detailed analyses in higher organisms.

Amphid neuron
Sensory neurons found in the anterior head region of the nematode *Caenorhabditis elegans*.

Rodent models

Small rodents, including rats and especially mice, are the most widely used preclinical animal model to study metabolic disorders[77–80]. Being mammals, the physiology of mice and rats is closer to that of humans than non-mammalian species. In recent decades, the popularity of mouse models has surged to the point that currently ~60% of all preclinical animal research is conducted in *Mus musculus*[81]. This surge in usage can be explained in part by the many molecular genetic tools available to engineer targeted or non-targeted mutations, from single nucleotide exchanges to chromosomal rearrangements, for gene functional assays in mice. Additionally, standardized

methods and devices for phenotyping mice are abundant and continue to be improved. Finally, mice are small in size, have reproductive outputs of approximately 6–12 pups (depending on the respective mouse strain), have a moderately short reproductive cycle, reaching sexual maturity within 4–8 weeks of birth, and have a gestation period of just 3 weeks — all these characteristics make mice an economical choice for researchers. In the sections below, we consolidate key aspects and considerations for performing and analysing rat and mouse studies under the umbrella of obesity and diabetes research (TABLE 1).

Diet-induced obesity and insulin resistance

Diet-induced obesity has considerable face validity with human obesity[82] and consequently is a widely used paradigm to study the interaction of diet and genes in manifest obesity and insulin resistance. In a typical procedure, rats or mice are given free access to calorie-dense foods highly enriched in fats (also known as high-fat diets (HFDs)) or other ingredients of interest, such as sugar or sodium, and the progression of obesity and diabetes as well as their related comorbid sequalae is monitored. For example, assessments of the effects of certain drugs or genetic manipulations on the development of obesity (or reversal of obesity) or on the impairment of glucose metabolism may be superimposed on investigations into how obesity progresses in response to different diets. Although the diet-induced obesity (DIO) model recapitulates large parts of the pathogenesis of human obesity, including the slow progressive weight gain and the secondary development of insulin resistance, it does have shortcomings. DIO studies are time-consuming and costly, and although the proposition seems straightforward, numerous factors (described below) influence the outcome and interpretation of the results and their translational value.

Strain. Inbred mouse strains have genetic differences that influence their susceptibility to DIO and diabetes. The inbred C57BL/6J mouse strain is widely used as a model for DIO because it is prone to develop severe obesity, elevated adiposity, glucose intolerance and moderate insulin resistance[83,84]. Other inbred strains, including SWR/J and A/J mice, are less susceptible to DIO and related complications[85–87], making them especially intriguing models to study human obesity resistance.

By measuring insulin resistance as defined by HOMA, one report demonstrated the staggering breadth of strain variation by surveying over 100 unique inbred mouse strains after 8 weeks on a high-fat, high-sucrose diet[88]. The authors noted a remarkable 63-fold and 37-fold variation in the degree of insulin resistance between males and females across the strains, respectively[88]. Because of these findings, researchers must carefully optimize their strain selection (and other experimental factors) on the basis of their specific research questions. For instance, if a study is investigating the effects of a drug on overt diabetes end points, then the DIO C57BL/6J model is not the ideal choice because this strain rarely develops frank hyperglycaemia and/or islet atrophy when fed an obesogenic diet[89,90]. By contrast, the rather closely related but distinct C57BL/6N strain[91] develops hepatosteatosis, hyperglycaemia and hyperinsulinaemia following 3 weeks on a HFD[92].

The susceptibility of rats to DIO also depends on the respective strain[93]. The most prevalently used laboratory rat strains (Sprague Dawley, Wistar and Long–Evans) are outbred, meaning that they have considerable genetic variation, which investigators should take into account when determining sample size. Sprague Dawley rats, for example, exhibit a wide distribution in HFD-induced body weight gain[94], reminiscent of the genetic heterogeneity of the human condition.

The choice of inbred strain is also important when working with genetically modified mice. 129/Sv and FVB mice are the preferred strains in which to generate knockout models, and C57BL/6J mice are favoured for metabolic studies; however, ongoing large-scale projects aimed at mutating every protein-coding gene in the mouse are underway in C57BL/6N embryonic stem cells[95]. Investigators have already used this resource to produce mouse lines and to systematically phenotype them for a broad range of tests including hallmark parameters for diabetes and metabolism[96]. The ultimate aim of the project is to have a knockout mouse line for every gene in the genome (see: www.mousephenotype.org) to search for phenotyping data and availablemouse lines[97].

In contrast to these large multicentre studies, which have been performed on an inbred background, mixed strains resulting from insufficient backcrossing respond differentially in metabolic studies[98–101], and backcrossing for at least 8–10 generations is considered minimal to establish a sufficiently homogenous background. Investigators should confirm a genetic background by single nucleotide polymorphism (SNP) panel analyses, which are commercially available. As subtle genetic drift can influence experimental results, littermates are most often the best controls in metabolic research. While experimental control is paramount in order to obtain conclusive results, differences due to genetic drift and strain variation are informative, considering the complex polygenic nature of obesity in humans.

Sex. Male mice are more susceptible to DIO, whereby they develop obesity sooner and to a greater extent than female mice[102–104]. By contrast, in rats, DIO progression is more comparable between males and females[93,94,105]. Sex differences in mice and rats for phenotypes of diet-induced insulin resistance and glucose intolerance are more pronounced, with male mice and male rats being the most affected[105–109]. This sex difference largely persists in most rodent models of T2DM and the Zucker Diabetic Fatty (ZDF) rat provides one striking example. On normal rodent chow diets, male ZDF rats develop severe hyperglycaemia (~400 mg/dl) and hypoinsulinaemia (~1,000 pmol/l) by 4 months of age. By contrast, and rather remarkably, female ZDF rats maintain normal levels of glucose (~100 mg/dl) and insulin (~5,000 pmol/l) throughout their life, despite developing obesity to a similar extent as the males. Sexual dimorphism in glycaemia also translates to humans. Data suggest that

Table 1 | **List of selected rodent models potentially useful in obesity, insulin resistance and type 2 diabetes mellitus research**

Strain or method	Species	Diet	Obesity	Hyperglycaemia	IR	T2DM	Dyslipidaemia	Pathologic islet changes
Polygenic								
C57BL/6J	Ms	HFD	+	–	+	–	+	–
SWR/J	Ms	HFD	–	–	–	–	+	–
A/J	Ms	HFD	+	–	–	–	+	–
C3H/HeJ	Ms	HFD	+	–	+	+	+	–
DBA/2J	Ms	HFD	+	–	+	–	–	–
NZO	Ms	CHO	++	+	++	+	+	+
TALLYHO/Jng	Ms	SD, HFD	+	+	++	+	++	+
DIO-sensitive Sprague Dawley	Rat	HFD	+	–	+	–	+	–
DR Sprague Dawley	Rat	HFD	–	–	–	–	–	–
UCD-T2DM	Rat	SD, HFD	+	+	++	++	+	+
Sand rat	Gerbil	SD, HFD	+	–	+	+	+	+
Goto–Kakizaki	Rat	SD, HFD	–	+	+	+	+	+
Monogenic								
C57BL/6J–ob/ob	Ms	SD, HFD	++	–	++	–	+	–
C57BLKS/J–db/db	Ms	SD, HFD	++	+	++	+	++	+
Otsuka Long–Evans Tokushima Fatty	Rat	SD, HFD	+	+	+	+	+	+
fa/fa	Rat	SD, HFD	++	+	++	–	+	–
Zucker Diabetic Fatty	Rat	SD, HFD	++	++	++	+	++	++
Koletsky	Rat	SD, HFD	++	–	+	–	+	–
Experimental								
Low-dose streptozotocin	Ms, Rat	HFD	+	+	+	+	+	+
VMH lesion	Ms, Rat	SD, HFD	++	–	+	–	+	–

–, absent; +, mild; ++, severe; CHO, carbohydrate enriched diet; DIO, diet-induced obesity; DR, diet resistant; HFD, high-fat diet; IR, insulin resistance; Ms, Mouse; SD, standard diet; T2DM, type 2 diabetes mellitus; VMH, ventromedial hypothalamus.

women are generally less likely to suffer from diet-induced consequences of T2DM than men[110–113]. This finding is reflected in the global prevalence of T2DM, with women being less frequently diagnosed with T2DM than men[114]. Obesity, however, is more prevalent among women[115]. Sexual dimorphism in obesity complications is linked to the gonadal hormones (testosterone versus oestradiol and progesterone) and to their differential effect on fat distribution. Central adiposity, especially visceral fat, is detrimental to health, while fat accretion in the lower body in the form of subcutaneous fat might actually bestow protective effects[116]. Although the mechanisms of these depot-specific effects remain poorly defined, women and female rodents have more subcutaneous and less visceral fat than their male counterparts[117,118]. Removing the ovaries, thereby ablating endogenous oestradiol and progesterone production, masculinizes fat distribution and increases the susceptibly to diet-induced insulin resistance in female rats and mice[103,119,120]. In addition, evidence indicates that non-gonadal sex-chromosome-specific factors also have a role in the manifestation of metabolic differences. Using a mouse model known as the four core genotypes[121], one group found that sex chromosomes, independent from gonadal sex, have a role in adiposity, feeding behaviour, the development of fatty liver and glucose homeostasis[121]. Potential mechanisms responsible for the effects of sex chromosomes on the susceptibility to DIO and diet-induced insulin resistance might include differential gene dosage from X chromosome genes that escape inactivation and/or distinct genomic imprints on X chromosomes inherited from the mother or father[122–124].

The take-home message is that sex differences are the norm rather than the exception in obesity research. Analyses of genome-wide association studies (GWAS) support this notion, highlighting a sex-specific genetic blueprint that underpins the susceptibility to obesity and diabetes mellitus[88,125]. Therefore, researchers should heed the recent NIH initiative[126] to investigate (and report) both sexes in preclinical biomedical research. Currently, there is a lopsided reliance on using male rodents in preclinical research[127], especially in drug studies. Female rodents are explicitly avoided because

of a widespread, albeit unsubstantiated[128], assumption that the female oestrous cycle induces undesirable experimental variability; however, controlling for the oestrous cycle phases is of course important. In addition, males are preferred for their more pronounced disease phenotypes; yet, the human obesity and T2DM crises affect both men and women. Clearly, interventions that aim to prevent or reverse obesity and/or treat T2DM should thus be tested in both sexes in order to improve their translational value. By ignoring sex, we also risk discarding promising drug candidates, on the basis of their performance (or lack thereof) in males only, that potentially benefit females.

Age. Age is another factor that has a considerable effect on outcomes in obesity and T2DM research. In humans, body weight increases with age and peaks at ~55 years in both men and women. Ageing *per se* is associated with a redistribution of both the fat-free mass and the fat mass, with the latter increase starting at ~30 years of age[129]. Intramuscular and intrahepatic fat are particularly increased in older persons, and this increase has been linked to insulin resistance[130]. Partially on the basis of these changes, ageing has been proposed to be an independent determinant of glucose tolerance, which progressively worsens with age[131,132].

Ageing likewise affects metabolic parameters in rodents. Analogous to what occurs in humans, the body weight of the C57BL/6J mouse, the most commonly used mouse strain for metabolic studies, increases with age, peaking at ~9 months[133], and older C57BL/6J mice (22 months) have reduced lean mass and increased fat mass compared with young 3-month-old mice[134]. In both rats and mice, fasting glucose levels are mostly stable throughout life, but whereas glucose tolerance generally worsens with age in rats, mice are less affected[135–140]. In fact, 2-year-old male C57BL/6J mice were significantly more glucose tolerant than their 5-month-old counterparts[138]. Consistent with these findings, glucose-stimulated insulin release from the pancreas decreases with age in rats, but not in mice[137,138].

Another important consideration is age at the beginning of a dietary intervention study. If, for example, mice are put on a HFD at too young of an age (<8 weeks, in our experience), the subsequent development of obesity and adiposity is not particularly pronounced[141].

Diet. Minor dietary differences can greatly affect metabolic parameters and experimental outcomes. For example, in rats, dietary fats from an animal source, such as lard, have a more pronounced effect on adiposity and insulin resistance than vegetable fats[142]. Even subtle modifications, such as changes in the ratio of unsaturated to saturated fatty acids[143] and the physical form of the diet (liquid versus solid)[144,145], lead to different DIO outcomes. For example, the fat source used for the preparation of HFDs is the critical element that determines whether germ-free mice are resistant or susceptible to DIO[146–148]. Lastly, even the texture and hardness of the food pellets must be considered. One study showed that mice fed on a powdered form of a low-fat diet spontaneously developed excess fat[149]. Therefore, taking into account these data, part of the DIO response might depend on altered texture rather than modification of macronutrient composition, as the pellets used in HFDs are usually softer than those used in the respective control diets.

The aforementioned factors highlight the importance of standardizing diets and accurately detailing diet composition and administration. Experimental diets are composed of defined purified raw materials and should guarantee that nutrient composition is reproducible in each production lot. Researchers must also take care to select the proper control diets if the primary goal is to assess the effects of specific dietary components. For example, grain-based chow control diets are all too frequently compared with purified experimental HFDs, although the adjoining caveats are known[150]. These chow diets are likely to vary in composition by batch, season and vendor. Variability in non-nutritive dietary components, such as soluble fibre content and plant-derived phytoestrogens, affects the progression of DIO and metabolic disease, even affecting behavioural traits[151,152].

Another consideration is that humans consume ~30% of their daily calories from fat. This fat intake is remarkably consistent across age and BMI[153] and lower than the 40% to 60% calories from fat used in many preclinical rodent studies of DIO. In our opinion, the translational value of results obtained with exceptionally HFDs, with nearly 80% calories from fat, is questionable. The metabolic endotoxaemia hypothesis, which states that high dietary fat intake impairs the gut barrier, is largely based on data from dietary interventions studies in mice that received 78% of their daily calorie intake as fat in an essentially carbohydrate-free diet formulation[154]. Follow-up studies have shown that the proposed effect of HFD consumption on gut barrier integrity largely depends on housing conditions and bacterial colonization of the gut[155,156].

A rise in sugar consumption is linked to the human obesity crisis[157,158]. With its high energy density and palatability, sugar easily facilitates a positive energy balance. Further, common sugar is composed of large parts of fructose, which exacerbates obesity complications such as liver disease[159,160]. Mice with access to fructose-supplemented water have increased adiposity compared with mice with access to glucose water, despite a comparable calorie intake[161]. Compared with what occurs on a HFD, body weight gain and adiposity observed in rodents on high-sugar diets are often less pronounced, but glycaemic control is equally negatively affected[162,163]. Sugar can also elicit neurochemical changes reminiscent of addictive drugs[164].

Most experimental obesity-inducing diets lack the complexity and variety of the human diet. Moreover, rodent diets are typically pelleted for easy handling and weighing. The absence of dietary variety might explain why rodents often do not exhibit pronounced or persistent hyperphagia when fed such diets[165]. This finding is supported by the observation that mice largely overeat during the first days upon transition from the standard low-fat diet to a more palatable HFD but then

Four core genotypes
A mouse model system that dissociates the effects of the gonadal sex (testes or ovaries) from the effects of the sex chromosomes (XX or XY)[121].

progressively reduce their energy intake approaching the levels of animals fed the control diet[166]. The cafeteria diet, in which rodents have free access to several different palatable high-energy 'junk' foods (in addition to regular chow and water)[167], is an alternative approach to better mimic the hedonic hyperphagia[168] that is observed in humans who are obese[169]. Consequently, compared with a HFD, the cafeteria diet promotes more pronounced weight gain and more severe diabetic symptoms[167].

Epigenetic inheritance of diet-induced obesity. The accurate description and standardization of experimental manipulation of the envirotype pose one major challenge for improving the mouse as a model system for meaningful obesity research[170,171]. Whether traits acquired during lifetime are passed on to the progeny via sperm and oocyte has been discussed but remains controversial[172]. Studies in rodents conducted under conditions of natural fecundation found that parental HFD feeding propagates obesity and glucose intolerance in their offspring[173–179]. With natural fecundation, however, factors such as the *in utero* environment, lactation and parental microbiomes, as well as behavioural differences, confound the interpretation. By using healthy surrogate mothers to carry zygotes from *in vitro* fertilizations with sperm and oocytes from parental mice that were fed either a low-fat diet or a HFD, one group elegantly circumvented the confounding factors and clearly demonstrated epigenetic germline inheritance of DIO and insulin resistance[180]. This finding is consistent with epidemiological data in humans demonstrating that an offspring's BMI is associated with the degree of parental obesity[181–183], whereas diminished glucose tolerance is more prominently associated with maternal rather than paternal impaired glycaemic control[184].

Changes in the gametes' transcriptomes, proteomes and metabolomes as well as characteristic marks in their genomic methylomes are potential candidates for the epigenomic information that transmits the acquired metabolic phenotype. Studies indicate that microinjection of specific sperm-derived small RNAs into zygotes can elicit persistent gene transcriptional effects and contribute to the metabolic phenotype in the offspring generation[185–187]. In contrast to genetic changes in the sequence of the DNA, such epigenetic changes can be reversed by environmental factors or nutritional supplements[188]. For example, the transgenerational effect of *in utero* exposure to a HFD can be reversed by a normal diet for three generations[189]. Interestingly, the authors of the study noted that histone modifications in the respective gene promoters accompanied changes in the expression levels of leptin and adiponectin in this *in utero* exposure model. The molecular mechanisms that increase, maintain or diminish epigenetic signatures throughout multiple generations following different dietary exposures remain unclear. Researchers, however, should take care to be aware of the age and health (and dietary) status of the parent generation, as these factors could confound findings and/or results, such as the development of obesity in the animals of interest — this point emphasizes why littermate comparisons are best for most experiments.

Envirotype
Factors that are exogenous to an organism.

Genetic rodent models

Spontaneous and targeted monogenic models. In the 1960s, Coleman and colleagues at the Jackson Laboratory discovered and isolated two stocks of mutant mice — the mildly diabetic but severely obese (ob) stock and the more moderately obese but severely diabetic (db) stock[190–192]. In now iconic parabiosis studies, researchers surgically joined mice from the ob stock with mice from the db stock, which resulted in ob mice rapidly losing weight, while the parabiosis had no effect on the db mice[192]. These findings suggested the presence of a hitherto unknown circulating factor (and a corresponding sensor of that factor) that is essential in the regulation of energy metabolism and food intake. More than 2 decades later, through positional cloning of a gene that at the time was referred to as *Ob* but now is known as *Lep*, leptin was identified as the circulating factor[6], igniting an explosion of research into the genetic causes of obesity. Subsequent work identified numerous genes that function in the hypothalamic leptin–melanocortin feeding pathway, including *Lepr*, *Mc4r*, *Pomc* and *Pcsk1*. Each of these genes has been functionally studied in gene-knockout mice and found to affect energy intake and expenditure and thus control body weight[193]. In addition, several of these genes are affected in human monogenic obesity syndromes[194].

Investigators have intensely studied ob and db mice for decades, and to this day, they are used as preclinical models. The leptin-null *ob/ob* mouse has a spontaneous mutation in *Lep* that precludes the secretion of bioactive leptin. The *db/db* mouse has a defect in leptin signal reception, which is caused by a spontaneous mutation in *Lepr*. Remarkably, and often forgotten, when these mice are on the same genetic background, a lack of leptin production (*ob/ob*) or lack of leptin sensing (*db/db*) elicits nearly identical phenotypes[195]. Traditionally, however, *ob/ob* mice are maintained on a C57BL/6J genetic background, and the *db/db* mice are maintained on a C57BLKS/J genetic background. This difference in background is what imparts the phenotypic differences of severe obesity (*ob/ob*) versus severe diabetes (*db/db*)[195] that were the inspiration for the nomenclature.

On the C57BL/6J genetic background, leptin-null *ob/ob* mice exhibit early-onset obesity that is promoted by hyperphagia and reduced energy expenditure[196], as non-shivering thermogenesis of the brown adipose tissue is reduced[197,198]. Additionally, *ob/ob* mice develop hyperinsulinaemia, mild hyperglycaemia and insulin resistance. These manifestations are likely secondary to the obesity. In addition, *ob/ob* mice are infertile[199], have increased circulating corticosterone levels, suffer from hypothyroidism[200] and have insufficient growth hormone (GH) levels, which results in stunted linear growth[201]. On the C57BLKS/J background, *ob/ob* mice suffer from pronounced diabetes mellitus marked by severe hyperglycaemia and atrophy of pancreatic islets[202], leading to premature death[202]. Treatment with recombinant leptin normalizes all apparent complications in *ob/ob* mice[203], as well as in humans with leptin deficiency[204].

Leptin-receptor-deficient *db/db* mice on the C57BLKS/J background largely recapitulate the obesity phenotype of the *ob/ob* mouse. The nomenclature of db (that is, diabetic) stems from the original observation of marked hyperglycaemia in these mice. *db/db* mice are hyperphagic and have reduced energy expenditure, leading to early-onset obesity[195]. They are also hypothermic, have decreased linear growth owing to GH deficiency and are infertile[195], and leptin levels in *db/db* mice are markedly elevated[205]. Hyperinsulinaemia can be detected as early as 10 days of age, and insulin levels continue to increase until 3 months of age. The hyperinsulinaemia is accompanied by hyperplasia and hypertrophy of the pancreatic β-cells. After 3 months, levels of insulin in *db/db* mice drop profoundly, which is concomitant with the atrophy of β-cells. Consequently, marked and sustained hyperglycaemia with blood glucose values >400 mg/dl promotes premature death around 5–8 months of age. However, the *db/db* model does not capture all the diabetic complications observed in the human disease. Vascular and retinal complications, for example, are rarely documented in *db/db* mice, likely because of the dramatically shortened lifespan. Notably, *db/db* mice on a C57BL/6J background exhibit only mild diabetic symptoms and a normal lifespan, despite marked obesity[78,79,195].

Analogous to the *db/db* mouse model, there are rat models with spontaneous defects in leptin signal reception. The obese Zucker rat has a defective leptin receptor; a missense mutation in *Lepr* results in the receptor becoming trapped intracellularly, leading to blunted leptin signal transduction[206,207]. The Koletsky rat, also known as the spontaneously hypertensive obese (SHROB) rat, lacks a functional leptin receptor because of a nonsense point mutation in *Lepr*[208–210]. These rats are hyperphagic and morbidly obese and have reduced energy expenditure, impaired glucose tolerance, impaired insulin sensitivity and stunted linear growth owing to lower activity of the GH–IGF1 axis and to hypothyroidism. Female and male rats that are homozygous for the mutation are infertile. In addition, and in contrast to the Zucker rat, the Koletsky rat develops hypertension starting at an age of 30 days[211]. Neither the obese Zucker rat nor the Koletsky rat is prone to developing diabetes mellitus.

The ZDF rat was derived through selective breeding of hyperglycaemic obese Zucker rats. ZDF rats carry an autosomal recessive defect in the β-cell transcription machinery that is inherited independently from the mutation in *Lepr*. The resulting animal model is one of obesity with a severe diabetic syndrome, with sustained and early-onset hyperglycaemia and progression to β-cell death, hypoinsulinaemia and premature death[212].

Following adult-onset chronic hyperglycaemia and insulin resistance, the Otsuka Long–Evans Tokushima Fatty (OLETF) rat develops diabetes mellitus, with characteristic symptoms such as polyuria and polydipsia, owing to β-cell exhaustion. These rats also exhibit a mild hyperphagia-induced obesity[213,214]. The OLETF rats also lack the cholecystokinin (CCK) receptor type A, contributing to their phenotype. CCK is a gut-derived peptide hormone that functions as a peripheral satiation signal[215], and experiments on the OLETF rat have been pivotal in unravelling the function of CCK in metabolism and have progressed our understanding of gut–brain crosstalk. These rats also exhibit a behavioural phenotype of self-correcting energy balance; when provided with concomitant access to a HFD and running wheels, the OLETF rats greatly increase their energy expenditure and are consequently indistinguishable from non-mutant control rats with respect to food intake and adiposity[216]. These findings underline the complex role of gut–brain signals in behavioural aspects of energy homeostasis[217] as well as the importance of using appropriate transgenic models to research the underlying mechanisms of obesity and diabetes mellitus.

Overall, monogenic animal models of metabolic diseases are valuable for understanding human-specific gene functions and monogenic forms of obesity, and they have emerged as central research tools in modern drug discovery research. Researchers frequently use the *ob/ob* mouse model on the C57BL/6J background to assess the potency of novel anti-obesity medications to overcome a strong hyperphagia-driven obese phenotype (for example, REF. 218). Furthermore, studies investigating whether leptin action is required as a cofactor for metabolic benefits of specific therapeutics have used this model[219]. The *db/db* mouse on the C57BLKS/J background is often used in studies investigating the efficacy of anti-diabetic drugs[218]. Frequently, however, researchers fail to sufficiently consider that these mouse models are based on a very specific receptor mutation, while the patient populations for which these drug candidates are being designed cannot be reduced to a single locus.

The use of gene-editing tools to create targeted monogenic animal models to explore the physiological role of specific genes has burgeoned in the past 20 years. Although this endeavour has taught us a great deal about the cellular and molecular underpinnings of energy homeostasis, it is becoming increasingly clear that metabolic characterization of transgenic animal models might be less predictive of the physiological function of the gene of interest than is often assumed. For example, genetic manipulation could impose compensatory biological changes during development that in turn take over the function of an otherwise key gene. Exemplifying the predictive limitations of germline gene knockouts, mice deficient in glucagon-like peptide 1 receptor (GLP-1R) are protected from DIO and exhibit only mild defects in glucose tolerance. Taken at face value, these data imply that GLP-1R agonism would have little to no metabolic benefits[220,221]. Yet, GLP-1R agonists belong to the currently best-in-class therapeutics for treating obesity and T2DM, exemplifying the hazards of determining gene function on the basis of germline transgenic models and also illustrating the possibility of overlooking relevant therapeutic utility[222].

Despite this well-known phenomenon of genetic redundancy, we have still not described the majority of mammalian gene functions. Instead, genetic research has continued to focus on those molecular pathways that were already discovered before the sequencing

of the first mammalian genomes (human and mouse) at the beginning of the 21st century[223]. International consortia are currently undertaking unbiased systemic phenotyping screens of gene-targeted mutant mice to specifically explore new and pleiotropic gene functions of the ignored genes[97]. Studies have identified more than 50 genetic loci associated with phenotypic parameters of glucose homeostasis, metabolism or obesity in a systemic phenotyping screen of 27,000 mice from 449 mutant lines[224]. These data suggest that we can expect that the number of monogenetic animal models for obesity and diabetes will dramatically increase over the next decade.

Polygenic models. Human obesity has a strong hereditable component. GWAS linking common genetic variability to specific traits (such as obesity) identified ~100 obesity candidate-genes, including, for example, *FTO*[225]. Intriguingly, most of these obesity-associated genes are associated with neuronal processes, consistent with the hypothesis that obesity is a disease of the central nervous system[226]. In addition, the sheer (and increasing) number of genetic loci that have been associated with obesity emphasizes that common human obesity is a polygenic disease with a large degree of inter-individual heterogeneity. Therefore, polygenic obesity-prone rodent models are important to better capture the human condition.

As discussed above, the C57BL6/J is a polygenic obesity-prone mouse strain that is widely used in experiments, as these mice develop hyperphagia-induced obesity when put into an obesogenic environment; however, several groups have observed heterogeneity in the response of C57BL6/J mice to a HFD. Approximately 60% of C57BL6/J mice in an obesogenic environment will have an increase in body weight, whereas the others have a comparable body weight to control mice fed a standard diet. This finding, which is possibly a result of epigenetic differences, is reminiscent of the diverse range of obesity susceptibility among humans. We are able to distinguish between DIO responder and non-responder C57BL6/J mice at 6 weeks of age, when the two groups differ with regards to levels of plasma insulin and leptin as well as in insulin sensitivity[227,228].

Analogous to C57BL6/J mice, only ~50% of outbred Sprague Dawley rats develop obesity on a HFD; the remainder are resistant to DIO[229]. When fed a low-fat chow diet, DIO-prone (DIO-P) rats eat and weigh about the same as DIO-resistant (DIO-R) rats, but when fed a HFD, DIO-P rats present increased feeding efficiency, hyperinsulinaemia and hyperleptinaemia as well as a rapid onset of obesity. Notably, compared with DIO-R rats, DIO-P rats have reduced central leptin and insulin sensitivity before the inception of obesity[230–232]. The DIO-P and DIO-R phenotypes are inheritable, making it possible to generate stable DIO-P and DIO-R substrains that reproduce many of the features of polygenic human obesity[94].

The sand rat, which is actually a diurnal gerbil (*Psammomys obesus*), is an outbred polygenic model of nutrition-dependent early-onset obesity and associated diabetic sequelae. In its native semi-desert habitat, with access to a low-calorie plant-based diet, the sand rat is lean and normoglycaemic. In the laboratory setting, however, with *ad libitum* access to an energy dense laboratory rodent diet, the sand rat becomes obese and rapidly develops hyperglycaemia followed by hallmark manifestation of T2DM such as severe hypoinsulinaemia, hyperlipidaemia and ketosis that are often observed within 3–4 weeks of birth[233]. Similar to the end-stage T2DM progression in humans, the diabetic sand rat suffers from β-cell degradation, nephropathy, body weight loss and premature death[234,235]. On a cholesterol-rich diet, the sand rat also develops non-alcoholic steatohepatitis with morphological and functional consequences that are similar to the human pathology[236]. Through selective breeding, researchers have also created diabetes-prone and diabetes-resistant sand rat cohorts to study the interaction between diet and genes in the development of diabetes and obesity[234].

The New Zealand Obese (NZO) mouse is another inbred polygenic strain that develops obesity and T2DM[79]. Adiposity in the NZO mouse is driven by a moderate hyperphagia, reduced energy expenditure and reduced voluntary activity. These effects are accompanied by dysregulated leptin signalling and glycaemic disturbances related to both pancreatic and hepatic defects[237,238]. By the age of 4–5 weeks, NZO mice exhibit insulin resistance in brown adipose tissue and skeletal muscle[239]. Notably, the macronutrient composition of the diet is important for the diabetogenic phenotype[240]. NZO mice fed a carbohydrate-free diet remain normoglycaemic despite marked insulin resistance and obesity[241]. However, when switched to a carbohydrate-containing diet, there is a dramatic effect on the pancreatic islets including a loss of RAC-α serine/threonine-protein kinase (AKT) activation, decreased expression of glucose transporter GLUT2 and reduction in several transcription factors that are essential for insulin synthesis and β-cell integrity[242]. This makes the model suitable for assessing the ability of novel therapeutics to prevent carbohydrate-mediated β-cell failure[243]. Similar to what occurs in humans, the onset of T2DM in NZO mice decidedly depends on the degree of hepatosteatosis early in life. When the liver fat content is <10% at the age of 10 weeks, NZO mice are protected from ensuing hyperglycaemia and β-cell loss[244]. Dietary interventions such as a moderate calorie restriction or intermittent fasting (fasting every other day) can protect NZO mice from hyperglycaemia. This effect is accompanied by reduced hepatic triglyceride and diacylglycerol concentrations, a decrease in protein kinase C-ε type (PKCε) activation and improved insulin sensitivity[245].

The TALLYHO/Jng (TH) mouse is an inbred polygenic model of T2DM that exhibits moderate obesity. TH mice have reduced insulin-stimulated glucose uptake in adipose tissue and skeletal muscle and abnormal pancreatic morphology and function, symptoms that, in many aspects, resemble polygenic human T2DM[246]. Despite hyperinsulinaemia and obesity, female TH mice remain euglycaemic[247]. The molecular underpinnings of the diabetic phenotype might relate to lower levels of insulin receptor substrate 1 (IRS-1) and impaired GLUT4

mobilization in the adipose tissue[248]. Moreover, male TH mice present drastically increased plasma triglyceride levels as part of the diabetic phenotype[247]. Finally, the TH mouse displays reduced bone mineral density[249], making it an interesting model for studying the interplay between T2DM and the skeleton.

The University of California (UC) Davis-T2DM (UCD-T2DM) rat model was created by crossing Sprague Dawley rats with adult-onset obesity and insulin resistance with ZDF-lean rats that are wild-type for the leptin receptor but have a pancreatic β-cell and/or islet defect and that together result in the development of overt T2DM with marked hyperglycaemia[250]. UCD-T2DM rats have impaired glucose-stimulated insulin secretion, and their fasting and fed glucose concentrations exceed 250 mg/dl and 450 mg/dl, respectively, and their HbA_{1c} is >7% within 3–4 months following diabetes onset. The rats develop T2DM on a low-fat, low-sugar diet, and both sexes are affected; however, the onset of diabetes is earlier in males (~4–8 months of age) than in females (~8–12 months of age). UCD-T2DM rats develop a number of complications of T2DM, including albuminuria[250], defects in vertebral disc and bone structure and function[251] and defects in brain and/or neuronal plasticity and metabolism[252]. Because they are leptin-responsive, UCD-T2DM rats are fertile, unlike leptin-deficient ZDF rats. Chronic leptin administration normalizes fasting hyperglycaemia and lowers levels of HbA_{1c} in UCD-T2DM rats[253]. Exogenous leptin administration also results in a reduction in agouti-related protein (AgRP) mRNA, an increase in pro-opiomelanocortin (POMC) mRNA in the hypothalamus, a decrease in hepatic gluconeogenic enzymes (glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK)) and a reduction of endoplasmic reticulum stress in the liver, muscle, adipose tissue and pancreas[253]. Treatment with a GLP-1R agonist[254], a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist[255] or two types of bariatric surgery, ileal interposition[256] and vertical sleeve gastrectomy[257], delays the onset of T2DM in UCD-T2DM rats by 4 months or more (equivalent to >10 years in a human lifespan). Overall, the UCD-T2DM rat is a versatile rodent model that can be used in the development and evaluation of novel strategies for the therapeutic management and prevention of T2DM and its sequelae.

In sum, it is without question that the greatest challenge when selecting an appropriate genetic model for metabolic studies is in ensuring that the model is appropriately tailored to the specific research question under investigation. For preclinical drug development programmes, employing susceptible polygenic models can be a good starting point to test compound efficacy and safety. For subsequent follow-up target validation of novel therapeutics, genetic loss-of-function models are the most relevant.

Rodent models for pancreatic β-cell dysfunction. The defining feature in the progression of T2DM is the decrease in β-cell function and β-cell number that ultimately leads to insufficient insulin production and secretion[258,259]. The mechanisms underpinning this β-cell failure are multifactorial (for example, environment and nutrition) and include a complex genetic architecture[260]. Several polygenic rodent models of T2DM exist, but for most, the progression from insulin resistance to T2DM with β-cell failure can be secondary to obesity (such as with the TH mouse, sand rat and NZO mouse discussed above). By contrast, the Goto–Kakizaki (GK) rat is a non-overweight polygenic T2DM model[261]. GK rats have a neonatal β-cell mass deficit, and in adult animals, total β-cell mass is depleted by 50%[262]. Several distinct genetic lesions occur in GK rats including defects in β-cell metabolism and function. When combined with chronic hyperglycaemia, inflammation and oxidative stress, the consequence of defects in β-cell metabolism and function is a perturbed islet architecture and loss of β-cell mass, which makes GK rats an established model to investigate the interconnection between β-cell failure and T2DM[263].

To understand specific molecular pathways that underpin β-cell dysfunction, the use of monogenic rodent models is invaluable. These models usually have mutations in genes that encode transcription factors important for β-cell identity or protein components of machineries that regulate glucose sensing or insulin secretion. The Akita mouse, for example, carries a spontaneous mutation in *Ins2* that causes the accumulation of misfolded proinsulin, which leads to endoplasmic reticulum stress and ultimately loss of β-cells[264–266].

Other models of β-cell dysfunction target the maturity onset diabetes of the young (MODY) — an umbrella term for several hereditary forms of diabetes mellitus that develop from spontaneous mutations in essential β-cell-specific genes and that account for ~1% of all diabetic cases in humans[267]. MODYs exemplify the utility of personalized therapy. Mutations in *KCNJ11*, for example, lead to the transcription of a defective potassium channel that can cause neonatal diabetes in humans. If detected, this particular MODY can be effectively treated with sulfonylurea[267]. Similarly, transgenic mice overexpressing the same defective potassium channel exhibit hyperglycaemia and β-cell de-differentiation, which can also be reversed by treatment with sulfonylurea or insulin therapy[268–271]. Mutations in *Gck* (which result in MODY2 in humans) and *Hnf1a* (which result in MODY3 in humans) induce impaired glucose tolerance and deficient insulin secretion in mice, respectively[272–275]. In humans, MODY4 is caused by a heterozygous mutation in *PDX1*, which is essential for β-cell development and function[276]. *Pdx1* heterozygous mice develop hyperglycaemia in adulthood, and β-cell-specific *Pdx1* deletion induces loss of β-cell identity[277,278].

A similar phenotype to that of MODYs occurs in Foxa2–Venus Fusion (FVF), Pdx1–BFP Fusion (PBF) double homozygous ($FVFPBF^{DHom}$) reporter mice, in which *Foxa2* and *Pdx1* are genetically fused with fluorescent proteins. In these mice, impaired β-cell maturation and loss of identity trigger transdifferentiation of β-cells into other endocrine cell types that consequently decreases β-cell number and causes diabetes[279].

Interestingly, female FVFPBF^{DHom} mice are protected from diabetes mellitus; however, during pregnancy, they develop gestational diabetes. Therefore, FVFPBF^{DHom} mice provide a sexually dimorphic diabetic model with the ability of β-cell tracking due to the fused fluorescent proteins.

Mechanical or chemical induction

Obesity. Early research on the role of the brain in obesity examined the effects of lesions targeting different regions of the hypothalamus. These efforts uncovered the ventromedial hypothalamus as a pivotal satiety centre and the lateral hypothalamus as an eating centre[280]. The effects of such lesions were profound and led to the recognition of the hypothalamus as a key brain area with regards to influencing metabolism. For example, lesions in the ventromedial hypothalamus lead to hyperphagia, weight gain and adiposity, despite increases in circulating leptin. A similar phenotype can be induced by physically cutting — and therefore severing — ventromedial hypothalamus axonal connections, by stimulating the ventromedial hypothalamus via locally implanted electrodes or by locally injecting procaine or other neuronal blockers. Each of these procedures results in an immediate phenotype of ravenous overeating and weight gain.

Although these interventions might appear somewhat crude, in some studies, the physiological consequences observed were rather sophisticated. In rats, lesions in the lateral hypothalamic area reduced food intake but did not disrupt the ability of the animal to regulate food intake in a fasting and/or feeding paradigm. Rather, the lesioning altered the settling point for body mass, or adiposity[281]. Today, targeted genetic disruption of certain brain regions or specific cells in the brain is often preferred over the lesion approach, which influences not only all neurons in the impacted area but also neuronal connectivity between brain regions[78,280].

Diabetes mellitus. Type 1 diabetes mellitus (T1DM) and T2DM have different causes, but both ultimately lead to pancreatic β-cell dysfunction. Damaging the pancreas chemically or mechanically can induce experimental diabetes mellitus. Pancreatic damage can be achieved by surgically removing parts of or all of the pancreatic tissue (pancreatectomy) to reduce or fully ablate endogenous insulin production[282]. The benefit of this method is the lack of toxic adverse effects (compared with diabetogenic drugs) on other organs. However, a prerequisite for specialized training and surgical equipment, as well as the confounding effects of eradicating exocrine pancreatic digestive enzymes and other islet hormones, limits the widespread use of pancreatectomy.

Chemical approaches, such as the use of the diabetogenic drugs streptozotocin or alloxan, to target the insulin-secreting β-cells are also available. Both drugs are cytotoxic glucose analogues that, owing to their high affinity for the GLUT2 transporter, primarily target β-cells. Streptozotocin is favoured over alloxan because it is more stable and less toxic. Researchers initially used these drugs to elicit a stable model of T1DM (that is, they were used to destroy all β-cells), but low doses elicit only partial β-cell loss, more reminiscent of T2DM. A popular approach is to combine HFD feeding with a subsequent injection of a low dose (~30–40 mg/kg intraperitoneally) of streptozotocin to model the transition from the pre-diabetic insulin-resistant state to overt T2DM[283]. The key advantage of this nongenetic model is that researchers can customize it to resemble the slow pathogenesis of T2DM that occurs in most humans, encompassing the slow development from adult-onset DIO to glucose intolerance, insulin resistance, the resulting compensatory insulin release and finally streptozotocin-induced partial β-cell death.

To achieve a slow pathogenesis of T2DM, young adult mice[284] or rats[285] are fed a high-fat or Western diet to elicit DIO and insulin resistance. Single or multiple injections with low-dose streptozotocin (~30–40 mg/kg intraperitoneally) then elicit partial loss of β-cells, which results in hypoinsulinaemia and hyperglycaemia. Protocols are being continuously refined and likely differ between species and even strains[283]. The HFD streptozotocin rat is sensitive to metformin, further demonstrating the utility of this model[285]. Downsides of streptozotocin treatment include liver and kidney toxicity and mild carcinogenic adverse effects (TABLE 1).

Energy balance measurements in rodents

Obesity is the consequence of a positive energy imbalance of energy intake over energy expenditure. To accurately calculate energy intake, the ingestion of a standardized diet, as well as the energy resorption efficiency, is measured. For the latter, the energy content of the faeces and food are quantified over time, and the difference between the calorie intake and the energy that is lost with faeces is then considered the metabolized energy. Of note, this process neglects the energy that is lost with urine and combustible gases. In humans, measurements of energy intake over sufficiently long periods are very involved and costly. Techniques to measure energy expenditure in humans vary[286], but they are also very elaborate and are usually performed in laboratory settings.

The small size of the laboratory mouse has enabled researchers to develop several commercial systems that can monitor food and drink consumption as well as energy expenditure over long periods, with high throughput, resolution and accuracy and in conditions that closely approximate regular mouse housing. These systems use indirect calorimetry to determine energy expenditure on the basis of the amount of oxygen consumed and carbon dioxide produced[287].

To correctly interpret mouse energy expenditure data, one must conduct a careful and appropriate analysis. Researchers have been intensely discussing the best methods to normalize the metabolic rate of laboratory mice for more than 50 years. The various approaches, some of which have been used more than others, include the direct comparison of uncorrected values (kcal per hour), correction by lean body mass, correction by body weight, correction by the body weight raised to the power of 0.75 or, as more recently suggested, by analysis of covariance (ANCOVA)[287–289]. The current consensus

is that analysis of energy expenditure data by use of ANCOVA, with body weight and body composition as covariants, is the most appropriate way to analyse energy expenditure[287,289,290].

In line with this notion, the correction of energy expenditure data by body weight should be avoided, as the metabolic rate does not increase in direct linear proportion to body weight (for example, the basal metabolic rate of a mouse weighing 50 g is not twice as great as the basal metabolic rate of a mouse weighing 25 g). Correction of energy expenditure by the body weight raised to the power of 0.75 seems more appropriate given that the basal metabolic rate of different species can quite accurately be predicted using this formula; however, the correction of energy expenditure by use of the so-called metabolic factor ($BW^{0.75}$) is suitable for the comparison of only different species and not for the comparison of two cohorts of mice. In addition, the regression line of such a normalization typically does not go through the zero intercept, a clear indication that this correction is inappropriate. Correction by lean body mass is also not advisable because such a correction assumes that the amount of fat mass is not contributing to metabolic rate. Brown adipose tissue in mice, however, can under certain circumstances account for up to 60% of the basal metabolic rate[287], and the correction of energy expenditure by lean body mass leads to spurious results by overcompensating for the lean mass effect[287,291]. The preferred method to best express changes in energy expenditure, in a way that takes differences in body weight and body composition into account, is thus the ANCOVA[287,289], and there are step-by-step guides available to help scientists with such an analysis[290].

Other considerations and limitations

A myriad of factors affect animal experiments. Men elicit a greater stress response in mice than women[292], likely confounding feeding behaviour. Rodents from different production facilities (for example, Jackson Laboratory and Taconic) have unique gut microbiotas[293], perhaps contributing to differences in their susceptibility to DIO and related diabetic complications[293]. Similarly, cage position within a rack of cages, single versus group housing, the skill level of the researcher, ambient room temperature or the type of cage bedding can all affect experimental outcomes.

Researchers must carefully consider the primary end point of each experiment. For example, if body weight is the key metric, researchers should limit or abstain from performing noninvasive procedures such as a glucose tolerance test. The stress and the fasting period inherent to these procedures confound weight gain. Researchers should also be aware of strain differences in rats and mice with regards to the susceptibility to stress[294,295]. Even within a strain, such as the widely used C57BL/6J mouse, there are responders and non-responders to stress[296]. When characterizing novel rodent models that are hypothesized to have a metabolic phenotype, a good idea is to conduct an initial experiment in which only food intake and body weight progression are measured weekly until at least middle age. Obtaining high-quality data for body weight and food intake is a pivotal foundation for subsequent experiments in metabolic research.

We believe there are several factors that researchers should consider when conducting obesity and diabetes mellitus research in rodents (FIG. 2). Although our list is by no means an exhaustive, it demonstrates the complexity and interconnectedness of the myriad of factors that can confound experimental outcomes. Although it is impossible to control for everything, researchers should accurately detail all experimental conditions and methods to allow for better interpretation of the results and, importantly, for better reproducibility.

Another concern pertains to control mice. Compared with free-living mice in the wild, laboratory control mice with *ad libitum* access to food are sedentary, overweight, glucose intolerant and tend to die at a younger age[297]. Comparisons between mice with DIO and control mice might be analogous to investigating the genetic cause of obesity-resistance by comparing humans who are overweight or obese. This potential problem with control mice could explain why the use of DIO diets that have 40% to 60% of total energy from fat is so prevalent, as this might be necessary to achieve divergent weight gains. With free access to running wheels, C57BL/6J mice voluntarily run 5–10 km per day[298,299]. As is the case with humans[300], mice get health benefits from regular physical activity including weight loss, decreased adiposity and improved insulin sensitivity[301,302]. Physical activity might also affect the epigenome over several generations[303]. An enriched physical and social cage environment alone improves leptin sensitivity and energy expenditure in mice, independent of physical activity[304,305]. Overall, these data suggest that with standard mouse husbandry, chow-fed laboratory mice are not the ideal healthy and lean control group for meaningful obesity research.

Lastly, while rodents recapitulate many aspects of human metabolism, researchers should be aware of key species differences in basal metabolic rate, feeding behaviour, fecundity, immune system[306] and gut microbiota[293]. Another important difference is the primary site of glucose disposal: in humans, this is skeletal muscle, while in rodents, the primary site is the liver, which is reflected by the relative liver weight being ~2.5 times greater in rats and mice than in humans[307].

Large animal models

The need for a large model that allows chronic cannulation of vessels and access to anatomic regions not possible in humans (for example, the hepatic portal vein, hepatic and renal veins or cerebroventricles) has greatly increased as new hypotheses developed in non-mammalian organisms and rodents require examination under conditions closer to human biology. Many metabolic studies on dogs historically used non-selected animals available from dealers, such as studies of insulin entry into[308] and action within the brain[309,310]. Since these early studies, however, researchers have developed mongrel hound models that consistently develop overweight and/or obesity, prediabetes or low-dose-streptozotocin-induced diabetes mellitus within a reasonable period of time[311–313].

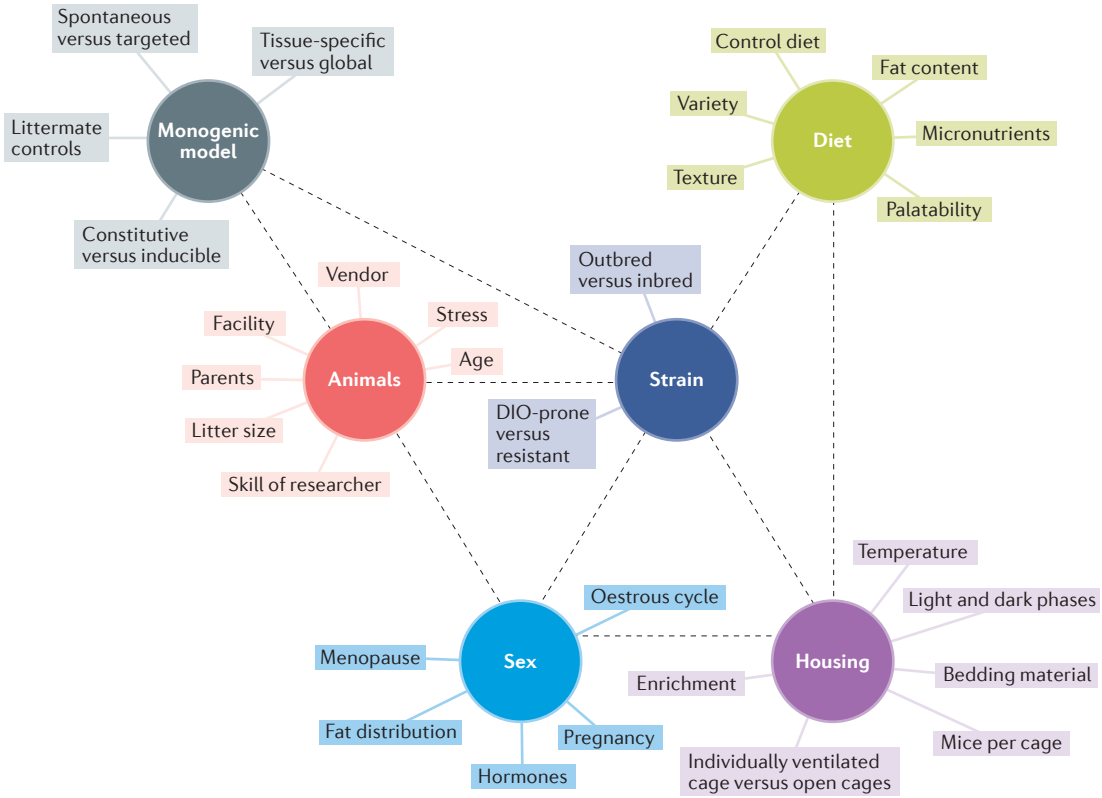

Figure 2 | **Important experimental parameters and potential confounders of experimental outcomes in obesity and diabetes research and their interrelatedness.** Countless factors influence experimental outcomes when using animal models, and what is enumerated here is by no means a complete list. This figure is one depiction of the multifactorial and interconnected genetic and environmental matrix that makes it virtually impossible to design the perfect experiment. For example, single-housing mice to obtain more accurate food intake data introduces a stress that in turn affects food intake. The severity of this stress response is both strain-specific and sex-dependent. What is important is to be aware of these challenges and to control for them in the most optimal manner. It is equally, if not more, important to accurately and comprehensively detail all experimental conditions in research papers, as these have bearing on the interpretation and reproducibility of the published results. DIO, diet-induced obesity.

Overweight can be induced in dogs within a 4–12-week period by providing excess amounts of dietary energy in various forms: a standard diet of meat and chow[314]; a meat and chow diet supplemented with fat[315,316]; or commercially prepared diets with either high-fat or high-fructose content or both[317]. An increase in energy intake is most evident during the first 1–2 weeks on these supplemented diets, but hyperphagia is maintained throughout the time the animals have free access to the diets[314,315,317]. Elevated fasting glucose and insulin concentrations are not observed in these models, but metabolic defects are clearly evident in all of them, despite modest increases in body weight. MRI studies identified increased visceral, subcutaneous and total adipose tissue mass on the high-fat, high-fructose diet (HFFD)[318] and on a HFD[315]. The increase of body fat in mongrel dogs fed a HFD is inversely correlated with insulin transport into the brain[316], an indication of increased insulin resistance at the blood–brain barrier[319]. Dogs fed the HFFD have elevated glycaemic excursions during oral glucose tolerance tests[320], and there is no change in the areas under the curve of insulin, indicating a β-cell defect. Moreover, after 10 weeks on the HFFD, dogs have a decrease in the glucose infusion rate required to maintain euglycaemia under hyperinsulinaemic–euglycaemic clamp conditions, indicating a decrease in insulin sensitivity[320].

Older and more obese dogs are prone to exhibit insulin resistance, accompanied by postprandial hyperglycaemia and hyperlipidaemia, but fasting hyperglycaemia is rare[321,322]. Hyperinsulinaemia in both the fasting and fed state is common in these dogs, and this is apparently sufficient to maintain normoglycaemia in the fasting state, although not after feeding. A small percentage of dogs (~1%) spontaneously develop T1DM, but spontaneous T2DM is uncommon[323], perhaps because of the capacity of the β-cell to compensate for insulin resistance[268]. As with rodents, researchers can induce diabetes mellitus in dogs with pancreatectomy or with the use of alloxan and/or streptozotocin[313,324–326]. The advantage of the use of diabetogenic drugs is that the dose can be carefully titrated, which results in the maintenance of some insulin secretion. By combining a diet that includes excessive amounts of energy with

diabetogenic drugs, researchers have developed a canine model of pre-diabetes and can create a model of T2DM. This dog model allows invasive measures and assessments not possible in humans or rodent models. Moreover, in times of metabolic stress, such as pregnancy, researchers can induce pre-diabetes and diabetes mellitus by feeding dogs solely on a HFFD[327,328].

The dog provides a useful model for examining the metabolic effects of overweight, obesity and diabetes mellitus, as well as for testing interventions for the management of these conditions. The canine model is especially powerful in allowing quantification of liver glucose uptake, which is impossible to assess directly in humans or small animal models. Being able to assess liver glucose uptake is particularly relevant because during normal meal feeding, the liver exhibits net glucose uptake for at least two-thirds of the day[329]. Because the dog absorbs a meal more slowly than a human[330], examination of repeated meal responses within a day is not possible, but all other aspects of absorption and glucose disposition appear to reflect those in the human.

The pig is another large animal model used for translational studies in research on obesity and diabetes mellitus. Obesity in pigs is routinely induced by high-energy high-fat and/or high-carbohydrate diets[331,332]. Minipig lines, such as Ossabaw, Yucatan or Göttingen minipigs, are most widely used because they can be reared to adulthood at reasonable costs. Dietary supplementation of cholesterol provokes pronounced dyslipidaemia (elevated cholesterol, LDL cholesterol and HDL cholesterol). Although impaired glucose tolerance is inconsistently observed, DIO in domestic or minipigs does not lead to an overt diabetic phenotype[332]. Rather, this requires additional manipulations, such as damage of β-cells by streptozotocin or alloxan or impairment of β-cell function by specific genetic modifications.

Genetic engineering of pigs has been remarkably refined[333] and represents an approach of generating tailored large animal models for diabetes mellitus research (TABLE 2). Currently available pre-diabetic and diabetic models include transgenic pigs expressing a dominant-negative glucose-dependent insulinotropic polypeptide receptor (GIPR)[334] and transgenic pigs expressing mutant insulin[335] or hepatocyte nuclear factor 1α (HNF1A)[336]. In addition, researchers have generated a number of genetically engineered pig models with dyslipidaemia and atherosclerosis[337], facilitating studies of potential interactions between these metabolic disturbances.

On the basis of their anatomical and physiological similarities to humans, their high fertility and easy maintenance, the possibility of dietary and surgical interventions and the efficient and specific genetic modifications, pigs are promising models to overcome gaps between proof-of-concept models and clinical studies in obesity and diabetes mellitus research. In addition, pigs might serve as tissue donors for β-cell replacement therapies of

Table 2 | **Examples of genetically engineered pig models for diabetes and dyslipidaemia research**

Genetic modification and/or mechanism	Phenotypic consequences	Potential applications
Expression of a dominant-negative GIPR in β-cells[334]	Impaired incretin effect; reduced glucose tolerance and insulin secretion; progressive reduction of β-cell mass[334]	Screening for biomarkers of prediabetes[382]; testing of incretin-based therapies[383]
Expression of mutant insulin-C94Y in β-cells[335]	Permanent neonatal diabetes mellitus; impaired insulin secretion; β-cell apoptosis; reduced growth; cataract[335]; reduced vascularization and pericyte investment in the myocardium[384]; retinal changes similar to diabetic retinopathy[385]	Testing of insulin or β-cell replacement therapies; treatments to prevent endoplasmic reticulum stress; studying effects of chronic hyperglycaemia on different organ systems and organ crosstalk[332,386,387]
Ubiquitous expression of a dominant-negative human HNF1A[336]	Persistent diabetes mellitus; abnormal pancreatic islet morphogenesis; immature renal development; pathological alterations of the kidneys and liver[336]	Studying diabetic complications*[388]
Liver-specific expression of the PCSK9-D374Y gain-of-function mutant[389]	Reduced hepatic LDL cholesterol receptor levels; impaired LDL cholesterol clearance; severe hypercholesterolaemia; spontaneous development of atherosclerotic lesions[389]	Testing of therapeutic compounds and imaging and intravascular devices[389]; evaluating the role of hyperglycaemia in atherogenesis[390]
Expression of human ApoC-III (REF. 391)	Increased plasma triglyceride levels; delayed clearance of plasma triglyceride; reduced lipoprotein lipase activity[391]	Understanding the roles of ApoC-III in lipid metabolism and of triglycerides in atherosclerosis; evaluating drugs for hypertriglyceridemia[391]
Expression of human ApoA[392,393]	High plasma levels of human ApoA[393]	Evaluating the pharmacology and efficacy of new drugs for atherosclerosis[393]
Knockout of the gene encoding LDL receptor[394,395]	Moderate (*LDLR*$^{+/-}$) or severe (*LDLR*$^{-/-}$) increase in total and LDL cholesterol on a standard diet; more severe on a high-fat, high-cholesterol diet[21]; atherosclerotic lesions in the coronary arteries and abdominal aorta (*LDLR*$^{-/-}$)[394,395]	Developing and testing novel detection and treatment strategies for coronary and aortic atherosclerosis and its complications[394–396]
Overexpression of LDL-PLA(2)[397]	Increased postprandial plasma triglyceride levels; increased expression of pro-inflammatory genes in peripheral blood mononuclear cells[397]	Studying the consequences of elevated circulating LDL-PLA(2) levels; testing LDL-PLA(2) inhibitors[397]

ApoA, apolipoprotein(a); ApoC-III, apolipoprotein C-III; GIPR, glucose-dependent insulinotropic polypeptide receptor; HNF1A, hepatocyte nuclear factor 1α; LDL-PLA(2), LDL-associated phospholipase A2; PCSK9, proprotein convertase subtilisin/kexin type 9. *The pathological changes in this model may be caused by expression of mutant HNF1A and not be a consequence of diabetes mellitus.

insulin-dependent diabetes mellitus[338,339] or as a source of reporter islets for *ex vivo* studies of β-cell maturation, proliferation and heterogeneity[340]. While pigs are increasingly used as models for obesity and diabetes mellitus research, dogs remain an important model in this field, particularly for studies involving oral administration of compounds, which is much easier in dogs than in pigs (TABLE 3).

Non-human primates

A long history of studying non-human primates for translational research in metabolic diseases, including hyperlipidaemia, atherosclerosis, T2DM, hypertension and fatty liver disease, exists. The most commonly used species in metabolic disease research include rhesus macaques (*Macaca mulatta*), cynomolgus monkeys (*Macaca fascicularis*), baboons (*Papio* species), African Green Monkeys (*Chlorocebus* species) and common marmosets (*Callithrix jacchus*), although a number of other species have been used[341]. With respect to metabolic physiology, non-human primates differ from rodents and are more similar to humans in the major site of *de novo* lipogenesis (adipose tissue versus liver), in the major circulating lipoprotein subclasses and in the physiology of thermogenesis and utilization of insulin-meditated glucose utilization[341]. Rhesus macaques have been useful in the translation of studies on the regulation of ingestive behaviour by gastrointestinal peptides such as glucagon-like peptide 1 (REF. 342) and peptide YY[343] as well as on the physiology and pharmacokinetics of leptin administration, in models ranging from rodents to primates[344,345]. Rhesus macaques have also proved valuable for investigations into the role of the autonomic nervous system in postprandial insulin secretion[346] and in the regulation of glucagon secretion during insulin-induced hypoglycaemia[347], as well in studies of compensatory insulin secretion in glucocorticoid-induced insulin resistance[348]. Studies in non-human primates have led directly to translational studies confirming the same mechanisms in humans[349,350]. Studies of long-term energy restriction in aged rhesus macaques have also been important for translating the effects of limiting calorie intake, including extending lifespan and improving glucose and lipid metabolism, originally reported in rodents to primates[351–355].

Diabetes mellitus secondary to insulin resistance and to inadequate β-cell and/or islet compensation has been reported to spontaneously develop in a number of captive non-human primate species[341]. Data show a high prevalence of T2DM in captive *Macaca nigra*[356] and a substantial prevalence of T2DM in captive rhesus macaques[357]. The progression of obesity accompanied by the progression from insulin resistance to β-cell failure and overt diabetes mellitus with ageing in captive rhesus macaques has been well characterized in longitudinal studies and is similar to the progression observed in cross-sectional studies in humans[358]. The presence of islet amyloidosis characteristic of T2DM in humans is also observed in diabetic monkeys, implicating a similar aetiology of islet lesions in monkeys and humans[359]. Diabetes mellitus associated with insulin resistance can be induced by administering nicotinic acid to baboons with reduced β-cell mass produced with a low dose (40 mg/kg) of intravenous streptozotocin[360]; however, this model is less representative of T2DM in humans owing to the chemically induced islet lesion.

Diet-induced models of non-human primate metabolic disease are commonly used to accelerate the development of the metabolic disease phenotype observed in captive non-human primate species that are fed a standard low-fat, low-sugar laboratory primate diet. Examples of diet-induced models include a baboon (*Papio hamadryas*) model that is exposed to a high-fat, high-sugar diet for 8 weeks, which increases fat mass and plasma

Table 3 | **Major advantages of dog and pig models for obesity and diabetes mellitus research**

Parameter	Dog	Pig
Monogastric omnivore, approaching the size and weight of humans over a wide range of developmental periods	Yes	Yes
High fecundity (8–14 offspring per litter)	Yes	Yes
Similar pancreas and islet structure, total β-cell mass, ratio of β-cell mass to body mass and β-cell replication capacity as in humans	Yes	Yes
Suitable for testing medical devices (for example, bioartificial pancreas) and surgical techniques (for example, bariatric surgery)	Yes	Yes
Pharmacokinetics of orally or subcutaneously administered compounds similar to humans	Yes (oral, subcutaneous)	Yes (subcutaneous)
Placement of permanent catheters allows physiological tests and repeated blood sampling without anaesthesia or stress	Yes	Yes
Existing protocols for diet-induced obesity and diet-induced atherosclerosis	Yes (diet-induced obesity)	Yes (diet-induced obesity, diet-induced atherosclerosis)
Diet-induced glucose intolerance	Yes	Yes
Genetic engineering is well established	–	Yes
Well-established model for assessment of hepatic glucose metabolism, including hepatic glucose uptake	Yes	–

triglycerides and reduces circulating adiponectin concentrations[361]. Marmosets (*C. jacchus*) fed a HFD or a high-sugar diet have also been studied as a non-human primate model of metabolic disease[362]. A Japanese macaque model has been used to investigate the effects of diet-induced maternal obesity and of metabolic perturbations during pregnancy on offspring[341,363,364]. In rhesus macaques, additional consumption of 300 kcal per day from flavoured fructose-sweetened beverages for up to 1 year results in the induction of many of the features of the metabolic syndrome including the following: increased body weight and fat mass; insulin resistance assessed by intravenous glucose tolerance tests; dyslipidaemia with hypertriglyceridaemia; decreased HDL cholesterol; increased apolipoprotein B, apolipoprotein C-III and apolipoprotein E; and decreased adiponectin[365]. A subset of these animals develops overt diabetes mellitus over the course of a year on the dietary regimen[365]. Interestingly, supplementation with a high dose (4 g per day) of omega-3 fatty acids from fish oil largely prevents the dyslipidaemia (as defined by an increase in triglycerides and apolipoprotein C-III) and the development of insulin resistance over a period of 6 months in the fructose-fed rhesus macaque model[366].

Dietary fructose also results in hepatic steatosis and inflammation in Old World monkeys[367] and increases liver triglyceride and cholesterol content in rhesus macaques. Studies investigating the pathogenesis of fatty liver disease have also been performed in baboons and marmosets[368–370]. Importantly, DIO non-human primate models have proved valuable in evaluating the therapeutic potential and/or safety of a number of interventions, including tyrosine-protein phosphatase non-receptor type 1 antisense oligonucleotides[371], fibroblast growth factor 21[372,373], glucagon-like peptide 1 agonists[374], omega-3 fatty acids[366], melanocortin receptor 4 agonists[375], tropomyosin receptor kinase B agonism[376] and oxytocin administration[377], in the treatment of obesity, insulin resistance and obesity-related metabolic diseases.

Diet-induced non-human primate models might soon be used to investigate the relationship between metabolic dysfunction, cognitive decline and dementia (including Alzheimer disease). Researchers are becoming increasingly aware that both insulin resistance and diabetes mellitus are risk factors for cognitive impairment and an age-associated decline in cognition. The decline of cognitive function in aged non-human primates has been well described[378–380], and there is evidence that feeding a high-fat, high-sugar diet activates pathways involved in oxidative stress, apoptosis and inflammation in the cortex of middle-aged male rhesus macaques[381]. Research performed in non-human primates enables the study of both cognitive function and markers of synaptic morphology and plasticity in the same animal. Performing these types of studies in non-human primates with diet-induced metabolic dysfunction, including dyslipidaemia, insulin resistance and T2DM, will be important in understanding the effect of dietary components (both harmful and protective) and their metabolic consequences in the aetiology, prevention and treatment of cognitive decline and dementia in humans.

Important advantages of non-human primate models include their close genetic relationship to humans and physiological similarity to humans. The baboon and rhesus macaque genomes have been sequenced, and this information can be used in target identification and validation studies. Approaches used in the metabolic evaluation of non-human primates include hyperinsulinaemic and hypoglycaemic clamps[347], modelled intravenous glucose tolerance tests and indirect calorimetry[365]. In addition, their relatively larger size compared with the earlier mentioned models, and their anatomical similarity to humans, make non-human primates particularly suitable for studies using advanced imaging techniques, including DXA, ultrasonography, PET and functional MRI. Another advantage of studies in non-human primates compared with humans is that adherence to diet and pharmacological interventions can be closely controlled, whereas in free-living human studies, compliance with dietary and treatment regimens is generally quite poor. In addition, researchers can conduct sequential biopsies of tissues including liver and intra-abdominal adipose tissue in studies of non-human primates. By contrast, in humans, these samples can be obtained in cross-sectional studies at only a single time point during elective abdominal or bariatric surgery, which seriously limits the selection of study participants as well as the ability to assess the effects of different interventions. Terminal collection of tissues such as brain (hypothalamus), kidney and pancreas for islet studies is also possible in non-human primate studies. The disadvantages of using non-human primates versus other models include the limited number of animals available for study, the expense of maintaining non-human primate colonies and the limited number of facilities that provide support for non-human primate research (for example, the seven National Primate Research Centers supported by the NIH).

In summary, non-human primates represent a valuable and physiologically relevant model and serve as a critical translational bridge between basic studies performed in rodent models and clinical studies in humans. Given the substantial cost of conducting clinical trials in humans, studies in non-human primate models represent a cost-effective avenue for evaluating the efficacy and safety of novel therapeutic strategies targeting metabolic diseases, including dyslipidaemia, atherosclerosis, T2DM, hepatic steatosis and potentially cognitive dysfunction and dementia, before taking these therapies into clinical trials.

Conclusions

The existing therapeutic approaches to treat diabetes mellitus and obesity, which are saving millions of patient lives every day, were discovered, validated and optimized in animal models. More sophisticated organoids are becoming available and offer useful complementary models for selected scientific questions, but so far, the models cannot replace mammalian models to study food intake, nutrient portioning, body fat distribution, systemic glucose metabolism, brain control over metabolic fluxes, exercise metabolism or many other key aspects of metabolic health and disease.

Studies in animal models of targeted diseases are also essential in identifying potential undesired adverse effects and toxicities, thereby protecting healthy volunteers and patients enrolled in consecutive clinical studies. Of course, there is a constant need to improve, adjust and refine preclinical models to meaningfully mirror clinical observations and processes. Here, we have summarized the key information on currently available animal models of obesity and diabetes mellitus, offering guidance as to the usefulness, advantages and limitations of these models depending on species, altered pathway, environmental conditions and genetic background. Choosing the right models for any planned translational studies in the right order and combination, and a consideration of shortcomings when generating data and offering conclusions, will continue to be of critical importance to any study. Failing to select and use appropriate animal models impedes successful discovery and development of safer and more potent therapeutics with the potential to stop the obesity and T2DM pandemics.

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Author contributions

M.Kle. and M.H.T conceptualized the review. All authors wrote parts of the review article, contributed to discussion of content and reviewed and/or edited the manuscript before submission.

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