1 | INTRODUCTION

Diabetes has a complex pathophysiology involving several organs and systems. For this reason, animal models are used extensively in preclinical diabetes research. Mouse models specifically are excellent in vivo tools owing to their similarity to humans in their glucose homeostasis. Indeed, numerous mouse models of diabetes are available which simulate different components of diabetes pathogenesis. In addition, genetically altered mice can be used to dissect mechanistic pathways involved in normal and disease states.

When planning an in vivo experiment, many factors inherent to the mouse model exist. These must be considered alongside experimental planning for the hypothesis to be appropriately tested and to ensure robustness and reproducibility of results. This review discusses commonly used mouse models, when they are appropriate to use and considerations for their use. Additionally, the impact of physiological model characteristics and husbandry practices on common experimental readouts will be explored.

2 | MODELS OF DIABETES

It is vital that an appropriate model is selected during experimental planning that corresponds with the aspect of...
the disease being studied. A plethora of mouse models of diabetes are available, including not only models of Type 1 (T1D) and Type 2 diabetes (T2D) but also less common types including neonatal diabetes.

2.1 Models of T1D and overt hyperglycaemia

T1D is characterised by an absolute deficit in insulin resulting from the autoimmune destruction of pancreatic β-cells. Accordingly, mass β-cell destruction is used to model T1D. Diabetogenic chemicals are frequently used to ablate β-cells in mice. However, this strategy often does not capture the autoimmune aspect of T1D and only models the end stage of disease, characterised by β-cell mass depletion and overt hyperglycaemia. Other models are available that recapitulate the β-cell-directed autoimmune reaction, and these include models where insulitis develops spontaneously and those where insulitis is induced chemically. The choice of model will depend on the experimental question and requires careful consideration.

2.2 Chemical induction of hyperglycaemia

Alloxan and streptozotocin (STZ) mediate β-cell loss via several mechanisms including through reactive oxygen species production after uptake via the GLUT2 transporter. Alloxan can also induce β-cell loss via mechanisms independent of GLUT2. Although both are widely used to induce hyperglycaemia, STZ is preferable owing to its enhanced specificity and reduced toxicity. STZ can be administered as a single high dose (100–200 mg/kg) via intraperitoneal or intravenous injection, or as multiple low doses over 5 days (20–40 mg/kg). Mice typically develop hyperglycaemia within ~48 h of a high dose and thus this is often the model of choice for rapid diabetes induction. However, stable diabetes should be confirmed before starting experiments, and many researchers wait 5–7 days after STZ injection. It is important to note that a one-time high STZ dose does not induce insulitis making it a poor model for studying this aspect of T1D. In contrast, multiple low-dose STZ administration has been found to drive T-cell-mediated β-cell destruction, and this, therefore, represents a model of insulitis. However, other studies have disputed this based on several differences compared with spontaneous autoimmune insulitis. High-dose STZ, although a more reliable inducer of diabetes compared with lower-dose regimens, is associated with higher mortality rates and so mice should be frequently monitored for ill health and/or severe weight loss (>15%). Of note, residual β-cell functional mass can be detected in 68% of humans with long-standing T1D. A varying degree of residual β-cell mass can be seen in animal models depending on model used. Therefore, the extent of residual β-cell mass should be confirmed in the control group to allow more accurate interpretation of the action of the treatment.

What is already known?
- Many mouse models of diabetes are available for studying different aspects of normal glucose homeostasis and diabetes.
- Physiological characteristics (including sex, age and genetic background) may influence experimental readouts.
- Husbandry practices often differ between laboratories and across studies.

What this study has found?
- Experimental outcomes in preclinical diabetes research can be influenced by choice of model, physiological characteristics of that model, as well as husbandry procedures.

What are the implications of this study?
- This study emphasises the importance of fully researching the model of choice during experimental planning. It also highlights how model-inherent and husbandry factors can impact experimental outcome and stresses the need to fully disclose and standardise these to ensure data reproducibility.
2.3 Models of spontaneous T1D

A more representative model of human insulinitis and T1D is the non-obese diabetic (NOD) mouse. This model develops spontaneous insulitis at 3–4 weeks resulting in β-cell destruction and overt hyperglycaemia from ~12 to 14 weeks. However, age at hyperglycaemic onset varies dramatically within and between laboratories. A benefit of this model is that it shares many similarities to the autoimmune response seen in human T1D, including islet-specific autoantibody generation and autoreactive CD4+ and CD8+ T-lymphocytes. Moreover, although susceptibility to diabetes in these mice is polygenic and influenced by environmental factors, the most important genetic factor is the harbouring of a unique major histocompatibility complex haplotype (called H-2k7), which is also an important genetic factor predisposing human T1D. Despite these similarities, many drugs tested in this model have been found to be ineffective in humans, suggesting that translatability is limited. This may be due to difficulties in translating drug dose or that drugs are typically administered at an early stage when diabetes can be prevented in the NOD mouse. Considerations when using this model include the need to house NOD mice in specific pathogen-free (SPF) environments since microbial exposure in standard housing facilities reduces diabetes incidence. Another difficulty when using this model is the unpredictable disease incidence and onset, as well as a female predominance in incidence (60%–90% vs. 10%–30%). These considerations represent logistical and financial barriers to using this model over STZ. It is important to note that hyperglycaemia and weight loss is severe in these mice; therefore, regular insulin administration is required.

2.4 Models of T2D and impaired glucose tolerance

In T2D, insulin resistance combined with the inability of β-cells to compensate for this, leads to β-cell dysfunction and eventual failure in the later stages of disease. This is driven by a combination of genetic and environmental factors. In humans, obesity and resultant insulin resistance often leads to impaired glucose tolerance which is regarded as a pre-diabetic stage. Therefore, models of T2D mimic insulin resistance and/or β-cell failure with severity ranging from glucose intolerance (pre-diabetes) to overt diabetes (established disease). Some of the most used models include monogenic obese mice defective in leptin signalling and diet-induced obesity.

Models mimicking human obesity include those defective in leptin signalling. Leptin signalling is important in controlling satiety; hence, both Lep^ob/ob (leptin deficient) and Lep^db/db (leptin-receptor deficient) mice are hyperphagic and become severely obese. This results in insulin resistance and a progressive worsening of glycaemic control from 4-weeks. In mice with the Lep^ob/ob and Lep^db/db mutations on a C57BL6/J background, hyperglycaemia peaks between 3–5 months and falls thereafter as mice begin to compensate through increasing β-cell mass. However, when these mutations exist on a C57BLKS/J background β-cell failure occurs resulting in ketoadosis and thus a shortened lifespan (8–10 months). Lep^ob/ob mice are typically available on a C57BL6/J background whilst Lep^db/db are typically available on a C57BLKS/J background. Increased diabetic susceptibility in C57BLKS/J mice is thought to be under polygenic control. These obese models also display impaired immune function, hyperlipidaemia, dysfunctional thermoregulation and inactivity. Importantly, leptin signalling defective mice are infertile, and so breeding programmes require the use of breeders heterozygous for these mutations. Despite these models being well characterised and used extensively, their monogenic nature is not translatable to human T2D, where the cause is polygenic and involves environmental factors.

Consequently, diet-induced models of obesity and insulin resistance (DIO) are often used as these model the progression towards human T2D more accurately. Young mice are fed a high-fat diet (HFD) or high-fat-high-sugar (HFHS) diet for ~12–16 weeks although exact protocols vary. DIO mice exhibit glucose intolerance and insulin resistance from as early as 1 week of diet. However, unlike the leptin signalling deficient mice, they do not develop overt hyperglycaemia. Supporting this, we have used continuous glucose monitoring (CGM) to show that, despite impaired glucose tolerance, median blood glucose concentrations in HFD male mice is only 0.4 mM higher than normal chow controls (7.6 ± 0.2 mM vs. 7.2 ± 0.2 mM) and, therefore, cannot be regarded as a model of overt diabetes. There are several factors to consider when using DIO models. We have found that although both male and female mice become glucose intolerant from 1 to 2 weeks into a HFD or HFHSD, this is more pronounced in male mice. This is concurrent with other studies reporting a pronounced sex difference in sensitivity to HFD or HFHS, with male mice becoming more obese, insulin resistant and glucose intolerant.

The choice of HFD is also an important consideration since commercial diets vary in fat content, source of fat and sucrose content, and these have implications on phenotype outcome. Diets providing 60% or 45% calories from fat are commonly used. A 60% HFD induces more severe obesity at a quicker rate compared with 45% HFD and so represents the more inexpensive option as animals...
require housing and feeding for a shorter period. However, it should be mentioned that the 45% HFD is more representative of human fat consumption and, therefore, may serve as a better model. The fat content also impacts sucrose content; a 60% HFD typically contains less sucrose than a 45% diet.\(^{25}\) Owing to the role that sucrose plays in metabolic health, this should be considered. Other diets include those high in fat and sucrose (HFHS), although the precise composition varies. It has been suggested that a HFHS is more representative of a human western diet and thus may be more translatable to human physiology. Studies have also reported increasing dietary sucrose by dissolving it in the animal drinking water.\(^{26}\) Although liquid sucrose ingestion has been found to result in increased body fat and enhanced glucose intolerance compared with solid ingestion, the amount of sucrose ingested in this way cannot be controlled, although it can be measured by monitoring water intake.\(^{26}\) In addition, this raises welfare concerns when using mice with diabetes displaying polydipsia, and furthermore sucrose solution may stick to the animals’ fur resulting in dermatitis. Owing to the different compositions of research diets, the diet manufacturer and product number should be reported to avoid reproducibility issues.

Dietary modification can also be used in combination with STZ to induce T2D. HFD/STZ mice are initially high-fat fed to induce insulin resistance, glucose intolerance and hyperinsulinemia, and are subsequently administered low-dose STZ (30–40 mg/kg) to reduce \(\beta\)-cell mass.\(^{15,27}\) An advantage of this model is that HFD and STZ protocols can be customised to better mimic the progressive changes in metabolic profile which take place during T2D development.\(^{15}\)

### 2.5 Models of monogenic diabetes

Although T1D and T2D are the most common types of diabetes, rarer monogenic forms exist with single mutations causing maturity-onset diabetes of the young (MODY) or neonatal diabetes, and these have been instrumental in understanding the role of key genes in blood glucose homeostasis. Although many monogenic models have been generated through genetic manipulation, others occur spontaneously. An example of this is the KINGS mouse, which was recently characterised in our laboratory after a spontaneous mutation led to overt diabetes in male mice within a C57BL/6J colony.\(^{28}\) Further investigation revealed that these mice harbour an \(Ins2\) mutation which causes a glycine to serine substitution in proinsulin2 at position 32. This also causes a form of neonatal diabetes in humans. Mice heterozygous for this mutation exhibit impaired glucose tolerance but only males develop overt diabetes by 5 weeks, whereas females remain normoglycaemic.

Despite overt diabetes in males, insulin treatment is not required to maintain weight. Impaired glycaemia in these mice results from \(\beta\)-cell endoplasmic reticulum (ER) stress, which is driven by misfolding of the mutated proinsulin2. \(\beta\)-cell ER stress impairs glycaemic control in other models harbouring \(Ins2\) mutations including the Akita mouse.\(^{29-31}\) There is growing recognition of the importance of ER stress in other forms of diabetes, including T1D and T2D, and these models will prove useful in understanding how ER stress mediates \(\beta\)-cell failure and offer a platform to test ER stress-directed therapeutics.\(^{32}\)

Spontaneous mutations leading to a clear diabetic phenotype are rare. A more common route to evaluating a gene’s role in metabolic function is through genetic manipulation.

### 2.6 Genetic manipulation of mice

Transgenic mice are a key tool in diabetes research and the use of global knockout or knock-in mice can be used to measure dramatic effects in metabolism, fertility, morbidity and mortality across multiple organs. However, interpretations of gene function within specific tissues can be confounded by indirect phenotypic effects from other tissues, such as the indirect effects within fat or liver on \(\beta\)-cell function. Site-specific recombinase technologies, such as Cre\(-\text{loxP}\), allow for tissue-specific gene manipulation to study the direct effects of a gene within a particular cell-type or tissue. A successful tissue-specific transgenic mouse, the generation of which is discussed elsewhere,\(^{33,34}\) has recombinase expression driven by a promoter from a gene, which is tissue-specific and is highly and consistently expressed. Availability of such promoters is largely tissue dependent; however, there are now large databases of tissue-specific Cre recombinase mouse lines available\(^{35}\) driven by different promoters, different promoter fragments and often containing other expression drivers in these sequences.

Despite this ever-growing choice in transgenic tools, compromises are often necessary, and lines should be properly investigated and controlled for prior to experimental use. A key starting point is establishing whether the insertion of the Cre-transgene alone alters tissue function or mass,\(^{36-38}\) by studying the tissue and metabolic phenotype of these mice versus wild types in the absence of any target gene knock-in or knockout by the presence of \(loxP\). Another common issue with transgenic lines involves ectopic expression of Cre in a non-target organ, such as regions of the brain,\(^{39}\) leading to indirect effects. The amount of ectopic expression should be measured and the functional significance of ectopic gene manipulation in a specific tissue or cell population, as well as its influence on the target tissue should be investigated and controlled.
for through Cre-positive/loxP-negative controls. Attempts to improve tissue specificity can have a negative impact on recombination efficiency or decrease the population of target cells able to maintain transgenic expression. Levels of gene knock-in or knockout should, therefore, be measured, preferably in a way that can measure cell heterogeneity of expression prior to interpretation of results.

Inducible transgenic models allow researchers to induce knock-in or knockout of a gene at a specific timepoint or life stage. This can be advantageous, allowing a tissue to develop naturally until the point of study, potentially bypassing early knockout lethality and preventing any long-term compensatory adaptations within the tissue. Furthermore, in some cases it can increase tissue specificity, if, for example, the Cre-driving promoter plays a role in fetal development, where it is expressed in a wide variety of progenitor cells, the transgene can be induced following differentiation. Conversely, however, gene knockout within the target tissue is often of a lower efficiency or with basal leakage of expression prior to induction.

Transgene induction relies on administration of a chemical, commonly tamoxifen or tetracycline, which results in potential side effects from both the inducing chemical and the method of administration. As a selective oestrogen receptor modulator, tamoxifen has side effects on adipogenesis, obesity and β-cells, as well as fertility and bone turnover. Tetracycline has cytotoxic effects on mitochondrial function, lipid metabolism, the liver, and gut flora, altering food absorption. The use of these chemicals in Cre-negative and/or wild-type controls is, therefore, essential.

3 | THE IMPACT OF PHYSIOLOGICAL CHARACTERISTICS AND HUSBANDRY ON EXPERIMENTAL READOUTS

Alongside choice of model, other factors inherent to the model need to be considered as these can have profound impacts on results, data reproducibility and animal welfare. Factors include the models genetic background (strain/stock and breeding), sex and age. Furthermore, husbandry practices, which include housing densities and animal enrichment, can also influence results.

3.1 | Strain/stock

Inbred (strains include C57BL/6, BALB/c and DBA/2) or outbred (stocks include CD1 and NMR1) mice can be used for in vivo research. Inbred strains are genetically defined and homogeneous, whereas outbred stocks are genetically undefined and variable. Each strain or stock is genetically distinct and therefore experimental readouts are likely to vary between them. Indeed, not only have different strains/stocks been found to differ in their glucose homeostasis in a physiologically normal state, but differences in genetic background can also impact the phenotype of models with diabetes.

Several studies have shown strain/stock variations in glucose homeostasis under normoglycaemic conditions. For example, BALB/c mice are more insulin resistant when directly compared with C57BL/6, NMR1 and CD-1 mice. Additionally, differences in insulin action and secretion, and counter-hypoglycaemic responses have been reported between commonly used inbred mice (C57BL/6, DBA/2, 129X1/Sv, FVB/N). Interestingly, it has also been found that variability in common preclinical diabetes readouts is unchanged between inbred strains and outbred stocks, challenging the perception that inbred strains are less variable.

Genetic background also influences diabetes onset and severity. BALB/c mice are more resistant to HFD-induced glucose intolerance compared with other strains including C57BL/6. Moreover, susceptibility to STZ-induced diabetes varies widely depending on the mouse strain; whilst C57BL/6 mice are more susceptible, BALB/c mice are more resistant, and this has been attributed to increased STZ metabolite accumulation in C57BL/6 β-cells. A different susceptibility between strains is also true of genetic models of diabetes. As mentioned previously, the Lepob/ob genotype on a C57BL/KS background results in more severe and ultimately lethal diabetes compared with when it is on a C57BL/6J background.

Finally, distinctions in glucose homeostasis also exist between sub-strains. C57BL/6J mice harbour a mutation in nicotinamide nucleotide transhydrogenase (Nnt), which is involved in NADPH production. This has been associated with impaired β-cell function compared with C57BL/6N mice, with C57BL/6J displaying impaired glucose stimulated insulin secretion when glucose is administered intravenously.

Owing to differences between strain/stocks, caution is encouraged when making direct comparisons between studies. Furthermore, the strain/stock should be fully researched during experimental planning and fully disclosed to avoid reproducibility concerns.

3.2 | Sex

Biological sex is an important consideration in preclinical diabetes research as many studies have demonstrated sex
differences in glucose homeostasis and diabetic incidence and induction. The latter, combined with the perception that female blood glucose concentrations are more variable due to the oestrus cycle, have been reasons for the historic exclusion of female mice from diabetes research. However, the importance of using females should not be overlooked with evidence that anti-diabetic drugs tested preclinically primarily in males are less effective and/or have more side effects in women. 65

Male and female normoglycaemic mice have been found to differ in glucose and insulin tolerance with females exhibiting improvement in both over males. 66,67 We have also found that non-fasted blood glucose concentrations are higher in male mice compared with age-matched females (Kennard, M.R., et al, unpublished). In addition, CGM carried out in our laboratory has found that non-fasted blood glucose concentrations in females are significantly less variable compared with male mice. This is despite the perception of enhanced variability in female mice due to oestrogen fluctuations during the oestrus cycle. Indeed, oestrogen has been shown to increase insulin secretion and sensitivity and help maintain β-cell mass. 68 Reduced glycaemic variation is important in detecting drug effects and so the use of females should be considered.

Profound sex differences also exist in diabetes incidence and severity in numerous mouse models, and this is important to consider when using these. Typically, female mice have reduced incidences of diabetes and severity, although the NOD mouse represents an exception. Models of ER stress-induced diabetes, including the Akita, KINGS and Munich mice, show a male predominance in diabetes despite both sexes showing glucose intolerance. 28,29,31 Nevertheless, incorporation of females may improve translatability of diabetes treatments as they offer an opportunity to study varying degrees of disease severity. Moreover, human diabetes is more predominant in men (specifically when analysing the middle-aged population). The International Diabetes Federation have found that the global prevalence of diabetes is 0.7% higher in men and the US National Health and Nutrition Examination Survey found a diabetic incidence of 13% in men and 11% in women between 2013 and 2016. 69,70 This further emphasises the need to use female mouse models as sex differences reflect the situation in humans. 70

Female mice are also more resistant to diabetes and obesity induction using diabetogenic drugs and dietary modification. It is widely accepted that female mice are resistant to STZ-induced T1D, and although reasons for this remain elusive, it has been hypothesised that 17-β-estradiol protects against STZ-mediated β-cell oxidative stress and apoptosis. However, increasing STZ dose abrogates these sex differences, reliably inducing diabetes in females. 11 Numerous studies have also reported that female mice are more resistant to HFD-induced obesity and metabolic impairment. 24,71 Again, 17-β-estradiol has been implicated, although progesterone may also play a role by mitigating dietary induced inflammatory responses. 71-74 However prolonging dietary modification has been shown to induce obesity and metabolic syndrome in female mice. 75 Therefore, in models of induced diabetes, protocol augmentation should be considered to incorporate female mice rather than excluding them completely.

In summary, sex differences exist in glucose homeostasis and diabetes incidence and so whilst female mice should not be excluded from preclinical diabetes research, it is important that an equal number of male and female mice are recruited to any given study so that any sex differences in outcome can be observed and if so, comparisons can be made between sex-matched groups.

3.3 | Age

Age is an important intrinsic variable that should be considered carefully when conducting preclinical diabetes research and will determine experimental design. This is not only because metabolic profile changes with age in normoglycaemic mice but that age of diabetes onset in mouse models critically depends on the model.

Although research into the impact of age on glucose homeostasis in normoglycaemic mice is limited, it is generally accepted that age influences body composition, glucose tolerance and insulin resistance. 67 Reynolds et al. found that an age-related impairment in glucose homeostasis was more pronounced in male mice compared with female mice. Males became significantly more glucose intolerant between 6 and 18 months, whereas this was not seen in females, and this was attributed to an age-related increase in percent body fat in males but not female mice. 67 Interestingly, an improvement in glucose tolerance was observed between 20 and 28 months in males. Supporting this, a separate study found that older males (21–25 months) display improved non-fasted blood glucose concentrations and glucose tolerance compared with 4–5-month mice, a result of increased islet size and pancreatic insulin content. 76 Although further research is required, it is clear from existing studies that age could impact experimental end points in diabetes research. For this reason, mice should always be age-matched to controls, age should be accurately reported and consistency in mouse ages should not be overlooked.

Diabetes development is age dependent in many models of diabetes, and it is important to account for this during experimental planning. Some models have predictable ages of disease onset; for example, the KINGS
mouse reliably develops hyperglycaemia between 5 and 6 weeks. On the other hand, some models have less predictable onset ages. Disease onset in the NOD mouse varies considerably, even within the same colony, making experiments logistically difficult and meaning staggered recruitment is often required. Disease progression is also model-specific. In many models, hyperglycaemia increases progressively with age, but this is not always the case. Hyperglycaemia in the Lep\textsuperscript{ob/ob} mouse peaks at 3–5 months and decreases to below the diabetes threshold thereafter. Therefore, to ensure that the model of choice displays the appropriate phenotype for the research question, it is vital to fully research phenotype onset and variation with age.

### 3.4 | Husbandry

Mouse husbandry encompasses their care, housing and breeding and despite often being overlooked, it represents an important consideration when planning and running an in vivo experiment. This is because certain husbandry practices can have direct impacts on experimental outcome. Examples of this include the need to house NOD mice in SPF environments. In addition, certain husbandry practices increase psychological stress which, through the effects of glucocorticoids and adrenaline, can increase blood glucose concentration—a primary end point in diabetes research.

A major factor influencing stress is housing density. Mice are typically housed in single-sex groups or individually, depending on experimental requirements. Individual housing leads to social isolation and so is usually avoided owing to welfare concerns. However, grouped housing can lead to aggressive behaviour especially in male mice. Both social isolation and aggressive environments heighten stress and thus can impact glucose homeostasis. Indeed, we found that individual housing compared with pair housing resulted in increased non-fasted blood glucose concentration and glycaemic variability in male and female mice, although this was only significant in males (male median: 8.37 ± 0.40 mM vs 6.93 ± 0.14 mM, female median: 6.84 ± 0.47 mM vs. 6.07 ± 0.07 mM). Despite this, corticosteroid levels measured in single versus grouped housed mice varies between studies and it is not clear what variable to be investigated between a control and experimental variation and allows for the effect of a single housing density is optimal to achieve minimal stress.

Housing density also impacts weight and food intake, which is important for metabolic studies. Therefore, cage densities should be standardised across the study to minimise the effect of this on end points. However, this may not always be practical or possible, especially when mice are derived from an in-house small colony. Additionally, mice should be housed with littermates, whose dominance hierarchy has already been established prior to weaning, leading to a reduced risk of in-fighting. Since odour cues are important in maintaining the social hierarchy, during regular cage changes, bedding should be retained as this has been found to reduce fighting. Appropriate enrichment such as nesting materials are also important as these reduce stress and aggressive behaviour. However, it should be noted that several studies have found that enrichment increases aggression. Regular (once a week) handling prior to experimentation is also a widely used practice as it reduces stress associated with novel handling.

There are some husbandry considerations pertaining specifically to the use of obese and diabetic models. The maximum cage density is determined by the size of the cage and animal weight, and it is recommended that 60–100 cm\textsuperscript{2} floor area is provided per animal. This requires review when using obese models which are substantially larger than lean mice and require more space. However, because thermogenesis is impaired, individual housing should be avoided. Obesity can also restrict mobility impairing the mouse’s ability to reach the feeder and water supply. Therefore, some food pellets should also be placed at the bottom of the cage and a longer nozzle should be fitted to the water supply. Because obese mice are hyperphagic, food pellet levels should be checked regularly and refilled.

Diabetic mice are polyureic and polydipsic and so to reduce excessive soiling of bedding, lower housing densities should be used. Additionally, mice should be supplied with more bedding, and cages should be changed more frequently. Furthermore, water supply should be regularly checked and re-filled.

### 3.5 | Breeding considerations for inbred mice

Inbred mouse strains are a valuable tool in research. They are achieved through sibling mating for a minimum of 20 generations, which induces homogeneity at gene loci and results in phenotypic uniformity across the colony. Reduced genetic variation in inbred lines reduces experimental variation and allows for the effect of a single variable to be investigated between a control and experimental group since any differences between these genetically identical groups can be ascribed to that variable. However, the use of inbred mice has its drawbacks, the most important being genetic drift.
they can be propagated and become fixed in the population. Any introduction, loss, or fixation of a mutation in a population is defined as genetic drift. Most spontaneous mutations will appear silent and thus go unnoticed, but these have the potential to change the phenotype of the strain and thus experimental results and reproducibility. Whilst the risk of breeding a mouse with a germline mutation is higher in smaller colonies, which are commonly maintained in research laboratories, genetic drift should also be considered by researchers using inbred mice purchased from established breeding suppliers who maintain larger colonies.

Genetic drift ultimately cannot be prevented when maintaining a mouse colony. However, steps can be taken to minimise this or reverse it if indicators of drift are noted. Indicators include unexpected phenotype changes and a 5–10 generation colony which has not been refreshed. Genetic drift can be minimised by keeping accurate mating records so that phenotype changes can be traced back to the affected mating pair/trio and descendants, and by keeping two or more pedigrees so a pedigree exhibiting a changed phenotype can be removed completely. Additionally, accurate record keeping of generation numbers, backcrossing and sibling mating should be kept. Genetic drift can also be reversed. For inbred strains which are also maintained by reputable suppliers (who employ procedures to reduce drift), the colony should be restarted with newly purchased mating pairs. For novel genetically modified strains, these should be backcrossed to a newly purchased inbred wildtype mouse with the same genetic background in a way that ensures both male and female sex chromosomes, and the mitochondrial genome, is refreshed (Figure 1, an example of an appropriate mating scheme). Finally, for novel mouse strains or those used infrequently, sperm and embryos can be cryopreserved and can be used to recover a colony subjected to breeding errors or genetic drift.

Even when the above precautions are taken, genetic drift will still occur over time resulting in changes to the colony’s genetic makeup. As such, an inbred colony is considered a sub-strain of the founder colony when it has been maintained separately for 20 generations. This has resulted in a series of sub-strains in commonly used inbred mice being described. To reduce reproducibility issues, it is critical for the researcher to accurately state the inbred strain, sub-strain and supplier, as different sub-strains could have profoundly different responses in any given experiment. Additionally, the use of control mice from the same colony as the experimental group (achieved through heterozygous mating) is important, as is the use of current rather than historical control mice.

3.6 Alternatives to animal models of diabetes

In line with the three R’s it is important to consider how we can reduce, refine and replace animal use in diabetes.
research. Consideration of animal inherent characteristics, as discussed in this review, can lead to a reduction in the number of mice used through enhancing data reproducibility and robustness. We have also detailed how animal welfare can be enhanced through husbandry refinements, but refinements can also be made to common experimental procedures used in preclinical diabetes research.\(^1\) The replacement of animals is harder to achieve.

One alternative is the use of human islets, which are considered the ‘gold standard’ owing to their direct translatability to humans.\(^91\) However, human islets are scarce and therefore use of primary mouse islets is more common. The latter represents a partial replacement as animals are not used throughout the experiment and so suffering is minimised. Absolute replacements include the use of β-cell lines such as MIN6 which display β-cell characteristics including glucose stimulated insulin secretion, especially when three dimensional pseudo-islets are formed.\(^91\) In the last decade protocols for differentiating stem cells (embryonic and pluripotent) into beta-like cells have also been developed and offer another alternative to animal-derived primary β-cells.\(^92\) Unlike animal models, these alternatives do not recapitulate the multisystem control of blood glucose. However, islet-on-chip technology goes some way towards overcoming this limitation. Recent efforts to model the multisystem control of blood glucose have combined islets with liver hepatocytes on microfluidic chips.\(^93\) Combining components of the glucose-regulatory system on chips (multi-organ-on-a-chip) may go some way to replacing animal use but this technology is in its infancy and a source of β-cells is still required.\(^94\) Indeed, whereas the use of primary tissue, cell lines and organ-on-chip technologies can reduce animal numbers and certainly play an important role in determining which in vivo experiments should be prioritised, currently a full replacement of animals is not possible due to the multisystem pathology of diabetes.

This includes background strain, sex and age. (3) It is critical that animal characteristics are well documented and reported. This includes the animal model, background strain, breeding history, age and sex. (4) When using inbred mice, it is important that the researcher uses mice from reputable suppliers that have in place procedures to limit genetic drift, and when maintaining a colony that an appropriate breeding programme is set up to limit genetic drift. Moreover, appropriate controls with the same genetic background should be used.

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**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

LFDG, MRK, LIFS, AJFK participated in writing and reviewing the manuscript.

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