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Peripheral mouse models of metabolic disease

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1   **Abstract**

2   Metabolic disease risk is driven by defects in the function of cells that regulate energy

3   homeostasis, as well as altered communication between the different tissues or organs that

4   these cells occupy. Thus, it is desirable to use model organisms to understand the contribution

5   of different cells, tissues and organs to metabolism. Mice are widely used for metabolic

6   research, since well-characterised mouse strains (in terms of their genotype and phenotype)

7   allow comparative studies and human disease modelling. Such research involves strains

8   containing spontaneous mutations that lead to obesity and diabetes, surgically- and

9   chemically-induced models, those that are secondary to caloric excess, genetic mutants

10 created by transgenesis and gene knockout technologies, and peripheral models generated by

11 Cre-Lox or CRISPR/Cas9 approaches. Focusing on obesity and type 2 diabetes as relevant

12 metabolic diseases, we systematically review each of these models, discussing their use,

13 limitations, and future potential.

14

15 **Key words:** mouse; metabolism; obesity; diabetes; insulin resistance; insulin; glucagon;

16 somatostatin; beta-cell; alpha-cell; beta-cell; delta-cell; liver; adipose; muscle.

17

Introduction

Metabolic disease constitutes one of the foremost non-communicable disease states affecting global health. Almost 35% of adults over 20 years of age are obese, defined as BMI $\geq$ 25 kg/m^2, and these individuals are more likely to suffer type 2 diabetes mellitus (T2DM), as well as subfertility, hypertension and orthopaedic issues [1]. T2DM itself afflicts almost 10% of adults worldwide (30% in some genetically-predisposed populations), accounting for 5% of deaths secondary to significant co-morbidities including cardiovascular disease, neuropathy, nephropathy, vasculopathy and cancer [1]. The majority of metabolic disease states such as T2DM and obesity have complex polygenic aetiology, which increases disease risk when combined with a permissive environment. Many different cell types, as well as interactions between disparate tissues and organs (*e.g.* muscle, liver, adipose, pancreas) are involved in metabolic regulation, and may be subtly affected by these changes to produce a clinical phenotype. Dissecting the mechanisms underlying metabolic disease is thus challenging, requiring appreciation of how genotype and environment interact to influence cell/tissue/organ function. Since genes cannot (yet) be manipulated in humans, key to understanding metabolic disease is the development of animal models that allow genes in specific cells/tissues/organs to be linked with particular phenotypic traits. Focusing on obesity and T2DM as examples of metabolic disease partly driven by peripheral factors, the present review will discuss the animal models currently available, highlighting any limitations, before discussing future possibilities.

Spontaneous mutation mouse models of obesity and diabetes

Before the widespread adoption of technologies for conditional gene manipulation, a vast body of research relied on the use of animal models with perturbed peripheral metabolism due to spontaneous mutations resulting from selective breeding strategies. Many of these

animal models are still used today, since they are well characterised, tend to develop profound and reproducible metabolic deficits and arguably reflect the complex nature of human disease better.

Obese or ob/ob mouse: The *obese* or *ob/ob* mouse was discovered in 1949 by Jackson Laboratories due to a chance mutation in the House Mouse (*Mus Musculus*) that was eventually transferred onto an inbred C57BL/6J background. Mice homozygous for the mutation rapidly gain weight from 2-14 weeks, with body mass almost 3-fold higher than non-obese wild-type (C57BL/6J) controls (reviewed in [2]). Seminal studies cloned and sequenced the *ob* gene, which encodes a 4.5 kb mRNA with a 167-amino acid ORF 84% conserved between mice and humans [3]. It was found that the *ob/ob* strain arose from a nonsense mutation in codon 105 of the *ob* (leptin) gene located on chromosome 6 (7 in humans), replacing an Arg with a STOP codon and leading to translation of a truncated non-functional protein [3]. Thus, mutant mice are hypoleptinemic and present with hyperphagia, obesity, insulin resistance, hyperinsulinemia, transient hyperglycemia subsiding at 14-16 wks, elevated HDL cholesterol fraction, sub/infertility and delayed wound healing [2]. Implying normal leptin receptor signalling, but a loss of leptin secretion, injection of recombinant leptin reduces body weight and food intake in ob/ob mice [4]. Moreover, hyperglycemia is only transient- probably owing to increased insulin levels and maintained beta-cell mass expansion- subsiding at 14-16 wks unless mice are bred onto the C57BLKS background , where severe glucose intolerance is associated with profound beta-cell loss, ketosis and early death [5].

Whilst the *ob/ob* mouse has been pivotal in the discovery of leptin as a key regulator of food intake, obesity and energy homeostasis, its relevance for human obesity is less clear. Indeed, obesity in humans is a complex genetic trait influenced by environment [6]. Moreover, obese humans do no tend to present with weight gain from an early age, hyperglycemia progresses

with time, marked dyslipidemia is accompanied by increased LDL and not HDL and leptin increases rather than decreased [2]. Conversely, *ob/ob* mice may be a better model of exceedingly rare monogenic obesity (*e.g.* congenital leptin deficiency [7]), or where the general molecular and cellular effects of obesity and insulin resistance need to be studied on a candidate tissue (*e.g.* brain, islets, liver).

Diabetes db/db mouse: Similarly to *ob/ob* mice, the *db/db* mouse results from a single autosomal recessive Gly -> Thr mutation in the leptin receptor (*Lepr*) gene located on chromosome 4 [8]. This results in an inactive Lepr and hyperleptinemia [8], with a phenotype closely matching the *ob/ob* mouse. Use of *db/db* mice is however complicated by profound infertility, interactions between steroid sulfotransferase enzymes and the Lepr resulting in gender differences in diabetes severity [9], and maintained metabolic performance in heterozygotes due to compensation [10].

TALLYHO JngJ mouse: This strain was derived from inbreeding male Theiler Original mice that spontaneously developed hyperglycemia and hyperinsulinemia without hypoleptinemia or defective Lepr signalling [11, 12]. This is due to inheritance of a single recessive *Tannidd1* (TallyHo-associated non-insulin-dependent diabetes) gene on chromosome 19, which interacts with a range of other TallyHo-associated loci including Tanidd2, Tanidd3, Tabw2 and Tafat located on different chromosomes [11]. TALLYHO JngJ mice represent a model of polygenic moderate obesity combined with gradual onset insulin resistance, hyperglycemia, hyperinsulinemia, hyperlipidemia and beta-cell mass expansion from 10-14 weeks [11, 12]. As such, they share many characteristics of T2DM secondary to obesity in humans, although only males display overt hyperglycemia and impaired glucose intolerance ruling out study of the disease in female animals. Moreover, due to the polygenic nature of TALLYHO JngJ mice, experiments are complicated by lack of control animal, with most studies using either standard chow-fed C57BL6 or SWR/J [12].

1   See Table 1 for a concise summary of spontaneous mutation mouse models

2   **Caloric excess mouse models of obesity and diabetes**

3   The high fat diet (HFD) mouse is perhaps one of the most-cited animal models of obesity due

4   to its relevance for T2DM and ease of use in both wild-type and transgenic animals. In this

5   model, maintenance diet is replaced with a HFD containing ~ 60% calories from fat (lard)

6   and maintained for 1-20 wks, depending on the study and severity required [13]. After 1

7   week of HFD, animals display impaired glucose tolerance and insulin secretion using IV

8   glucose tolerance testing [14]. Weight gain is noticeable from 2-4 wks, with maximal body

9   weight achieved at 16-20 wks, being almost 20-30% higher than wild-types [13, 14]. Mice

10   generally display obesity (including adipocyte hyperplasia and mesenteric fat deposition),

11   hypertension, hyperglycemia, hyperinsulinemia due to increased fasting insulin levels and

12   insulin resistance. Impaired oral/intravenous/intraperitoneal glucose tolerance and glucose-

13   stimulated insulin release are usually apparent by 8-12 wks [13, 14]. Thus, the HFD mouse

14   more closely mimics the progression of obesity and T2DM in humans, with insulin resistance

15   and obesity occurring in parallel to compensatory beta-cell mass expansion and eventual

16   beta-cell failure (> 18 wks) depending on strain, lab and colony [15].

17   The mechanisms underlying beta-cell failure are controversial, but may reflect defective

18   stimulus-secretion coupling [16], rather than a reduction in beta-cell mass as may be present

19   in humans [15], although the extent to which this occurs is debated due to limitations in the

20   assessment of beta-cell mass [15]. It should be noted that responses to HFD vary with strain-

21   C57BL/6J, 129X1, DBA/2 and FVB/N animals being susceptible, whilst BALB/c are

22   relatively protected [17]. Moreover, even among C57BL/6J, there tends to be some

23   heterogeneity in HFD-responsiveness ('low' and 'high' responders), with commensurate

24   differences in phenotype [18], meaning that food intake and weight should be closely

monitored. Sex differences also exist, with C57BL/6 female mice displaying milder glucose intolerance and systemic inflammation, normal insulin levels and less islet hypertrophy [19]. Lastly, HFD provides a useful way of revealing environment-genotype interactions, which may increase or decrease susceptibility to obesity/insulin resistance/insulin secretion in transgenic mouse models (see later).

See Table 1 for a concise summary of caloric excess mouse models

Surgical or chemically-induced models of obesity or diabetes

Surgical or chemical ablation of brain centres involved in the regulation of energy homeostasis has led to the generation of rodent models of obesity. Since the use of these techniques are discussed elsewhere in this volume, we will focus here on the physical and/or chemical manipulation of adipose tissue, pancreas, and the ovaries.

Adipose tissue: Humans possess functional brown adipose tissue (BAT) in the cervical–supraclavicular depot, and BAT activity was shown to be negatively associated with adiposity and indices of metabolic syndrome (reviewed in [20]). Thus, modelling BAT deficiencies is relevant to the study of obesity and metabolic syndrome in humans. Lowell and colleagues demonstrated that removal of BAT by adipocyte-specific expression of diphtheria toxin in mice leads to obesity and hyperphagia, ostensibly due to the lack of UCP-1 driven energy dissipation of heat [21]. BAT-ablated mice developed insulin resistance and a T2DM-like phenotype [21]. The lowered energy expenditure for BAT thermogenesis contributes to the development of obesity: BAT-ablated mice did not display obesity or hyperphagia when maintained under conditions of thermoneutrality [22], making this mouse model different from *ob/ob* and *db/db* mouse models where a combination of hyperphagia, hyperinsulinemia and energy expenditure appear to be more important [2].

Endocrine pancreas: The endocrine pancreas is critical for the regulation of energy homeostasis *via* secretion of the hormones insulin, glucagon and somatostatin from the beta-, alpha-, and delta-cells, respectively. The beta-cell in particular has been the subject of intense research- loss of beta-cell function is associated with the development and progression of T2DM [15] (discussed further under "Conditional mouse lines for diabetes research")- and many methods to study the function of this cell type have been developed over the decades following the discovery of insulin by Banting and Best in 1921. Alloxan and streptozotocin (STZ) are widely used to induce diabetes since their pro-beta-cell necrosis and thus diabetogenic properties were first described almost a half a century ago (reviewed in [23]). The specificity of both alloxan and STZ for beta-cells is due to their uptake by the low-affinity glucose transporter, GLUT2, in rodent beta-cells [23]. GLUT2 is also expressed in the kidney and liver, leading to cytotoxic effects in these tissues [24]. Alloxan is an inhibitor of glucokinase (GK) and induces production of reactive oxygen species, thereby leading to beta-cell dysfunction and death [23]. The methylnitrosurea moiety of STZ is responsible for the toxicity effects of STZ, leading to modification of proteins, fragmentation of genomic and mitochondrial DNA, and ultimately beta-cell dysfunction and death [23]. The loss of functional beta-cell mass following exposure to alloxan and STZ leads to profound glucose dyshomeostasis. STZ is more commonly used than alloxan for the induction of diabetes in rodents as it is a more stable chemical, therefore leading to more reproducible results [23].

As described earlier in the section on BAT ablation, pancreatic endocrine-cells can be eliminated by cell type-specific expression of diphtheria toxin. Studies using such approaches have been instrumental in lineage tracing analysis of pancreatic endocrine cells (for example, see [25]).

Surgical methods have previously been used to deliver adeno-associated viruses (AAVs) in the vicinity of islets to facilitate gene delivery to these micro-organs [26, 27]. This technique

1 is technically demanding and involves injecting AAVs into the mesenteric artery [26] or the

2 pancreatic duct [27] following laparotomy. AAV serotypes 6, 8, and 9 were shown to mediate

3 long-term transduction of beta-cells [27], with more efficient transduction of the islets than

4 systemic administration of AAVs [26, 27].

5 *Ovariectomy:* Menopause, or reproductive senescence, is part of the aging process in women

6 and is commonly associated with weight gain and increased adiposity. Rodent models share

7 multiple features of menopause with humans (reviewed in [28]). Although rodents do

8 undergo natural menopause, only a small proportion of individuals within a colony would

9 transition to a state that is akin to human menopause [28]. Ovariectomy (OVX) is a widely

10 used method to increase the number of mice within a colony transitioning to the menopausal

11 state. OVX induces a phenotype- increased body weight, adiposity (although the fat depots

12 affected differ) and feeding- which closely mimics that seen in human females [28].

13 However, OVX is a model of immediate loss of function rather than a gradual transition of

14 loss of the oestrus cycle, as in menopause, and does not accurately reflect the adaptations

15 enacted to compensate for the gradual drop of ovarian hormones [29]. This temporal

16 difference in effect may account for some of the phenotypic differences seen between the

17 OVX model and human menopausal females, *e.g.* in total lean mass [30].

18 **Genetically-modified mouse lines for diabetes research**

19 A key event in the development of T2DM in humans is expansion of the beta-cell mass to

20 functionally compensate insulin resistance, mainly due to defective insulin signalling in

21 muscle, liver and adipose tissue [15, 31]. This is followed by beta-cell failure and increased

22 blood glucose levels after an poorly defined period of time [15]. Alpha-cells further aggravate

23 the situation, by hypersecreting the glycogenolytic/gluconeogenic hormone glucagon [32],

24 although this may be balanced by their conversion into beta-cells following extreme loss (in

mouse models at least) [33]. Much less is known about somatostatin-secreting delta-cells, although they likely act to suppress insulin and glucagon release in a paracrine manner [34], and recent studies have shown that they may in fact provide a source of beta-cells in pre-pubertal animals, allowing diabetes recovery [35]. Obesity is a major risk factor for development of T2DM, since excess circulating fatty acids, glycerol, cytokines conspire to drive insulin resistance in muscle, liver and adipose depots, in addition to directly influencing beta and other cell function [31]. The mechanisms underlying insulin resistance, alpha-beta-delta-cell interactions and conversion during diabetes, as well as the chronology of these events, are poorly understood. This is a major roadblock to treating the disease, since it is difficult to preserve insulin secretion or regenerate beta-cells *in vivo* without this knowledge. Key to this are mouse models that allow conditional and inducible gene manipulation specifically in muscle, liver and adipose tissue, as well as beta, alpha and delta-cells, allowing cellular/molecular interrogation of the processes involved in T2DM.

*Beta-cell-specific driver lines***:** Conditional gene recombination (expression or inactivation) can be achieved in pancreatic beta-cells using site-specific Cre-Lox technology. Early Cre driver lines consisted of Cre expressed under the control of the R̲at i̲nsulin p̲romoter (Rip-Cre), otherwise termed the ancestral *Ins2* gene, but were plagued with extra-pancreatic recombination, including in the hypothalamus (reviewed in [36, 37]), which is involved in glucose homeostasis. Depending on the exact Rip-Cre transgenic line used, recombination efficiency in beta-cells is ~80-98%, with varying degrees of expression in the brain depending on construct length [36, 37]. Such leakiness has however allowed these mice to reveal many brain-periphery phenomena, including a role for arcuate nucleus GABAergic RIP-Cre neurons in energy expenditure [38]

The Pdx1-Cre deleter line also displays some leakiness, although this is mainly restricted to the hypothalamus, as shown using GFP reporters [37]. Unlike Rip (*Ins2*), which is restricted

to beta-cells within the pancreas, pancreatic and duodenal homeobox 1 (Pdx1) is expressed in the posterior gut endoderm during development and as such throughout the exocrine and endocrine pancreas in the adult. Pancreas-wide deletion can however be avoided by using an tamoxifen-inducible Cre (Pdx1-CreER) to switch on expression in the postnatal period when Pdx1 is largely confined to beta-cells within the pancreas [39]. This model also has the added advantage of being less leaky, with virtually no recombination detected in the brain [39].

Both Rip-Cre and Pdx1-Cre strains are now superseded by animals in which Cre is expressed under the *Ins1* promoter, this gene being more beta-cell-specific than *Ins2,* the expression of which is sufficient for normal insulin production following loss of both *Ins1* alleles [40]. Ins1-CreERT (termed MIP-CreERT) animals lack Cre expression in the brain [37], but were developed using a transgenic construct containing the human growth hormone (GH) minigene, now shown to cause pregnancy-like proliferative changes in beta-cell mass through autocrine actions on the prolactin receptor (part of the cytokine receptor superfamily together with the growth hormone receptor) [41]. By contrast, Ins1-Cre knock-in animals rely on Cre expression from the translation initiation codon of the endogenous *Ins1* gene [40], with the added advantage of driving more moderate Cre expression, the enzyme itself shown to induce beta-cell hypoplasia when present at high levels [42]. Nonetheless, this is sufficient for excellent (> 90%) recombination efficiency in beta-cells [40]. However, these animals are relatively new, and in light of issues with previous beta-cell-specific driver lines, more in-depth metabolic phenotyping is required.

Alpha-cell-specific driver lines: Glu-Cre mice were first developed over a decade ago using a short (1.6. kb) promoter fragment containing the rat *Gcg* promoter. Whilst initial recombination efficiency was high, this appears to have diminished over time from 100% -> 40% depending on the colony studied [25, 43]. Similarly, the Glu-CreERT deleter strain displays expression in 50-75% of alpha-cells, but functional analyses in these animals are

complicated by haploinsufficiency due to loss of a preproglucagon gene [43], as well as expression in enteroendocrine L-cells and potentially in brainstem/hypothalamic neurons, which cleave preproglucagon into glucagon-like peptide 1 [36]. By contrast, animals engineered with an iCre upstream of the proglucagon gene (Glu-iCre) show Cre expression in a higher proportion of alpha-cells, as well as 70% of enteroendocrine L-cells, although recombination is also detected in ~ 20% beta-cells and the hindbrain, both sites involved in glucose homeostasis [44, 45]. Recent studies used CRISPR-Cas9 genome editing to insert CreER into the 3' UTR of the endogenous *Gcg* locus, without disrupting preproglucagon expression [43]. Whilst the resulting Glucagon-CreER mice display a normal phenotype, even as homozygotes, and display good recombination efficiency in adult alpha-cells (~ 95%), Cre expression was detected in only 33% of L-cells [43], limiting their use for enteroendocrine deletion compared to Glu-iCre animals [44].

Delta-cell-specific driver lines: The widespread expression of the neuropeptide somatostatin (Sst) in the brain [46], makes assessment of any conditional gene recombination in delta-cells particularly challenging *in vivo*. Nonetheless, Sst-iCre mice possessing mCherry-2A-iCre after the Sst start codon show recombination in ~ 80% of delta-cells and have proven useful for delta-cell lineage tracing experiments [35]. However, it has been recently reported that another Sst-Cre mouse line in which Cre is inserted into the 3'UTR of the *Sst* gene possess depleted (~20-50%) Sst levels throughout the brain, leading to altered behavioural responses [46]. Thus, results should be interpreted carefully pending further phenotypic validation of delta-cell-specific driver lines for metabolic research, especially given the reported reduction in Sst expression in the hypothalamus of Sst-Cre animals [46].

See Table 2 for a concise summary of pancreatic Cre-deleter lines.

Muscle-specific driver lines: Muscle is a major site for regulation of glucose and fatty acid metabolism, and hence energy expenditure. Peripheral insulin resistance is the first detectable defect in T2DM, with insulin resistance greatest in skeletal muscle at the initiating phase of the disease (reviewed in [47]). Manipulation of gene expression in skeletal muscle is thus a useful means with which to investigate the involvement of energy homeostasis *via* this tissue. Muscle-specific Cre-deleter strains have been produced using the muscle creatine kinase (MCK) [48], human alpha-skeletal actin (HSA) [49], myogenic factor 5 (Myf5) [50], myosin light chain 1/3 fast (MLC1/3f) [51], myogenic differentiation 1 (Myod1) [52], myogenin (Myog) [53] and paired box gene 7 (Pax7) [54] promoters. Additionally, tamoxifen-inducible Cre-deleter strains are also available for muscle-specific deletion using HSA and αMHC promoters. The MCK Cre-deleter strain has Cre expression driven by a 6.5 kb genomic DNA fragment from the *Mck* gene, containing the MCK promoter, enhancer 1, the untranslated exon 1, and intron 2/enhancer 2, conferring specificity for expression in skeletal muscle and heart. MCK-driven Cre expression is restrained during development [48], thus making this a useful deleter strain for the study of adult muscle phenotype.

Liver-specific driver lines: The albumin promoter driven Cre-deleter strain (Alb-Cre) [55] has been widely used in the study of energy homeostasis by the liver. The Alb-Cre transgene consists of 2335-bp of the rat albumin enhancer/promoter fragment located 8.5-10.4 kb upstream of the albumin promoter [55]. The first study using this line demonstrated that hepatic and beta-cell glucokinase are involved in the pathogenesis of hyperglycaemia in MODY-2 [55]. A tamoxifen-inducible albumin promoter driven Cre-deleter strain, SA-Cre-ERT2 is also available for targeted somatic mutagenesis in hepatocytes [56]. Whilst the transthyretin promoter driven Cre-deleter strain (TTR-Cre) leads to deletion in the foetal liver, visceral endoderm and yolk sac [57], a tamoxifen inducible version of this deleter strain allows specific recombination in the foetal and adult liver [58]. The Alfp-Cre-deleter strain

contains the mouse albumin enhancer and albumin promoter and alpha-fetoprotein enhancers, and was reported to lead to liver-specific Cre recombinase expression [59]. However, it was also reported that the transgene used for the generation of the deleter strain contains the human growth hormone gene and could, therefore, lead to the expression of human growth factor, which may confound data similarly to affected beta-cell-specific driver lines [41, 60].

An alternative approach to deliver Cre recombinase expression vectors to the liver in mouse models is the use of a technique termed hydrodynamic delivery [61]. This technique has been used successfully for hepatic gene delivery and involves the injection of plasmids, encoding for Cre-recombinase under the control of a tissue-specific promoter *e.g.* the albumin promoter, by hydrodynamic gene transfer [61]. Hydrodynamic gene transfer avoids problems such as positional insertion artefacts, variations caused by differences in genetic background, leaky expression, and toxicity associated with elevated Cre expression, which are confounding factors commonly encountered in the use of Cre-deleter strains.

Adipose-specific driver lines: Adipose tissue- consisting of WAT and BAT- is an important site of energy homeostasis. We have already described how BAT can be targeted by chemical means. The Cre/lox system has also been used effectively to target adipocytes using adipocyte-specific promoters to drive Cre expression. Amongst these are aP2-Cre mice [62], where Cre expression is driven by the promoter of the fatty acid binding protein 4 (Fabp4) gene, and the Adipoq-Cre strains [63], where Cre expression is driven by the adiponectin promoter. The Adipoq-Cre strains have been shown to exhibit highly-selective expression of Cre in adipose tissue whilst the aP2-Cre strains have been shown to possess leaky expression in the brain, endothelial cells, macrophages, adipocyte precursors and embryonic tissues [64, 65], and differing recombination efficiencies in WAT *vs.* BAT [65]. Platelet derived growth factor receptor alpha (Pdgfrα) promoter driven Cre-deleter strains (Pdgfrα-Cre) have also been generated based upon reports that Pdgfrα is expressed on adipocyte precursor cells in

WAT and can be used to trace adipocyte cell lineage [66]. Tamoxifen-inducible versions of the Adipoq-Cre (Adipoq-CreERT) and Pdgfrα-Cre (Pdgfrα-CreERT) deleter strains are also available for temporal regulation of Cre expression, all of which exhibit good cell type specificity [65]. There are currently no deleter strains that allow Cre expression specifically in WAT or BAT.

See Table 3 for a concise summary of muscle, liver and adipose Cre-deleter lines

See Schematic 1 for a diabetes mouse model phenotyping workflow

Genetically-modified mouse lines for obesity research

Most obesity models are centrally-mediated (discussed elsewhere in this volume) due to effects on neurons that regulate appetite and energy homeostasis *etc*. Some peripheral models of obesity are however listed below.

11beta-hydroxysteroid dehydrogenase type 1 (HSD11B1): Increased 11 beta-hydroxysteroid dehydrogenase type 1 (HSD11B1), the key glucocorticoid regenerating enzyme, in the adipose tissue is characteristic of obesity in humans and rodents, and associated with metabolic syndrome [67]. Tissue-specific over-expression of 11BHSD1 also correlates with weight gain (or lack of, *e.g.* where HSD11B1 expression is high in liver) in menopausal women [68]. Thus, transgenic mice over-expressing HSD11B1 in adipose tissue *via* the aP2 promoter display elevated intra-adipose corticosterone levels (the more active glucocorticoid in mice *versus* 11-dehydrocorticosterone), albeit with the caveat that there are specificity issues surrounding the use of the aP2 promoter for adipocyte-specific expression, including possible expression in the brain, as discussed earlier in this chapter [69]. Transgenic mice with apoE driven over-expression of HSD11B1 largely in the liver displayed normal corticosterone levels, glucose tolerance, body weight and fat depots, with concomitant

hyperinsulinaemia, suggesting that the obese phenotype is likely due to altered HSD11B1 activity in other tissues, *e.g.* adipose tissue, rather than liver [70].

*MafB deletion in macrophage: M*afB is expressed in macrophages (and rodent alpha-cells), where it is important for lineage commitment [71]. Transgenic mice with haematopoietic cell and macrophage-specific loss of MafB expression exhibited increased body weight and adipose tissue weight when exposed to HFD, with increased fat storage and serum cholesterol [72], linking the immune system with regulation of metabolism.

Androgen receptor knockout mice: Androgen deprivation is linked to metabolic syndrome in men, whilst androgen replacement therapy has beneficial effects on body mass [73]. Androgens bind to the androgen receptor (AR) to exert their effect and various AR null (ARKO) mouse models exist. Global AR-KO mice display, late-onset visceral obesity, altered lipid metabolism, glucose homeostasis, and insulin and leptin resistance [74]. Liver-specific AR-KO mice, generated using the Alb-Cre-deleter strain, exhibited obesity and hepatic steatosis when challenged with HFD, with concomitant liver insulin resistance resulting in hyperglycaemia, and altered fatty-acid oxidation [75]. Loss of AR in the brain, effected through synapsin-Cre deletion of floxed AR alleles, led to hypothalamic insulin resistance and reduced suppression of hepatic gluconeogenic gene expression [76]. Muscle-specific deletion of AR using the MCK- or HSA-promoter deleter strains, led to altered fibre type composition, reduction in intra-abdominal fat mass, and contractility of fast- and intermediary-twitch muscles [77, 78]. Over-expression of AR in myocytes increased oxidative metabolism in skeletal muscle, and led to improved glucose tolerance [79, 80]. Deletion of AR using the aP2-Cre-deleter strain led to impaired insulin sensitivity and age-related glucose intolerance, with increased susceptibility to HFD-induced visceral obesity [81]. Conversely, over-expression of AR in female mice protected against HFD-induced

atherosclerosis, obesity and dyslipidemia [82]. These data indicate that AR signalling may be protective against metabolic syndrome.

GLUT4 mouse models: The low affinity insulin-sensitive glucose transporter isoform 4 (GLUT4) is expressed in adipocytes, skeletal muscle and cardiac muscle, where it mediates glucose disposal [83]. In line with this, ablation of GLUT4 from adipocyte (aP2-Cre) [62] and muscle (MCK-Cre) [84] compartments led to reduced glucose uptake, hyperglycaemia, hyperinsulinaemia and secondary insulin resistance in other insulin sensitive tissues. By contrast, over-expression of GLUT4 prevented HFD-induced hyperglycaemia and insulin resistance [85].

Beta 3 adrenergic receptor knockout mouse: Beta 3 adrenergic receptor (beta3-AR) is expressed in human BAT, with decreased expression levels in genetically-obese mouse models (reviewed in [86]). Mutation in the beta3-AR is associated with obesity, decreased energy expenditure, insulin resistance and T2DM [86]. Beta3-AR knockout mice (beta3-ARKO) have increased fat mass indicating an impact on energy homeostasis [87].

See Schematic 2 for an obesity mouse model workflow

Mouse models for metabolic phenotyping of GWAS hits

Linkage and candidate gene analyses have identified genes with high penetrance for T2DM and obesity (*e.g. HNF1A*, *GCK* and *MC4R*) [88], although these are rare causal variants seen only in a small proportion of individuals worldwide. It was not until the human genome was mapped that understanding of the genetics behind complex polygenic disease such as obesity and diabetes accelerated. To date, genome-wide association studies (GWAS) have identified > 100 single nucleotide polymorphisms (SNPs) or gene variants associated with an increased odds ratio of developing obesity or T2DM [6]. Whilst this only explains a small proportion

(~10%) of disease heritability [6], it is likely that many more low-effect size common variants exist whose discovery requires increased power through larger sample sizes. Pertinently, the majority of the SNPs associated with T2DM affect intravenous glucose tolerance rather than insulin resistance in humans, pointing to defects in beta-cell function [89], whereas for obesity BMI-associated loci tend to overlap with feeding and fasting circuits or molecules involved in neurodevelopment [90]. Despite this, few of the GWAS hits identified to date have been functionally validated for their effects on metabolism in animal models. This may reflect: 1) the time and investment taken to generate and phenotype animal models relative to the pace at which international consortia are identifying new gene variants; and 2) the difficulty assigning GWAS signals to specific genes, since they generally map to non-coding regions of the genome. Whilst functional validation could be speeded up by using non-mammalian species such as drosophila and zebrafish, or even cells lines, these do not allow assessment of gene effects on multi-systemic traits such as glucose homeostasis and adiposity.

Most studies to date have relied on global knockout or Cre-Lox models to delete the gene of interest. Whilst this allows functional interrogation of gene variants that have restricted expression patterns (*e.g. SLC30A8*- largely expressed in the islet and retina [91]), it is less useful for those that are ubiquitously expressed. In such cases, conditional approaches may allow the identification of the tissue(s) where the variant may act to influence phenotype (with the important caveat that many SNPs may also affect neighboring loci). Whether the gene should be deleted (*e.g.* with Cre-Lox) or overexpressed (*e.g.* with Tet-ON) can be determined from expression quantitative trait loci (eQTL) analyses, which link possession of the variant with up- or down-regulation of gene expression. It should also be noted that SNPs may not necessarily occur in isolation, and the contributions of gene interactions to phenotype may require more than one gene to be manipulated. Another caveat is that over-

expression or deletion may not recapitulate the subtle changes in expression levels that occur

in humans with possession of gene variants, and variants may affect neighboring gene loci

[92].

Nonetheless, animal models have already provided unique insight into GWAS-identified

genes, often revealing new facets of cell function along the way, even if this is not necessarily

how gene variants may influence disease in humans (due to the caveats listed above). FTO

encodes 2-Oxoglutarate–Dependent Nucleic Acid Demethylase, and SNPs in this gene are

associated with obesity [93]. *Fto-/-* mice have been shown to possess reduced fat mass and

increased energy expenditure, pointing to a protective role of *FTO* in obesity (reviewed in

[90]). Although hypothalamic FTO expression levels are high, regional Cre-mediated FTO

deletion led to decreased food intake without effects on energy expenditure and body

composition, pointing to effects if FTO deficiency in multiple body compartments, including

the brain and adipose tissue [90]. Gene variants in TCF7L2, encoding for a transcription

factor downstream of Wnt signalling, are strongly associated with T2DM. As predicted from

RNA-silencing studies in isolated tissue [94], Tcf7l2 deletion specifically in beta-cells using

Ins1Cre$^{+/-}$:Tcf7l2$^{-/-}$ mice impairs glucose tolerance [95]. Another gene strongly associated

with T2DM is *SLC30A8*, which encodes zinc transporter 8 (ZnT8), involved in importing

Zn^{2+} into the beta-cell cytoplasm where it is required for proper insulin crystallisation [91].

Global ZnT8 results in glucose intolerance, although depending on the colony studied this

was sex-specific, disappeared with age, or did not occur at all (reviewed in [92]). The reasons

for this are unknown, but may involve metabolic adaptation, genetic background (mixed

versus C57BL6) or housing conditions [92]. By contrast beta-cell-specific knockout of ZnT8

consistently reveals impaired glucose tolerance [96, 97], whilst beta-cell-specific over-

expression mildly improves the same parameter [97]. Together, these data show the utility of

1   animal models for functionally validating GWAS, allowing conditional gene KO or over-

2   expression in the *in vivo* setting.

3   See Schematic 1 for a diabetes mouse model phenotyping workflow

4   **Mouse models for understanding gene by environment interactions in metabolic disease**

5   Complex metabolic diseases such as obesity and T2DM typically result from gene-by-

6   environment (GXE) interactions. Whilst the genetic risk factors for development of metabolic

7   disease can be identified using GWAS and genetically-engineered mouse models, GXE are

8   more difficult to dissect due to the inability to control environment (in human studies at

9   least), and the single genetic background of inbred mice. The latter can be partly

10 circumvented using recombinant inbred strain families, genetic reference populations (GRP)

11 where an isogenic line represents each genotype due to brother-sister mating, thus resembling

12 rare human populations [98]. This allows studies to be replicated in multiple cohorts from

13 lines spanning differing levels of genetic complexity [98]. Combined with eQTL analyses,

14 GRPs provide a powerful platform to understand how certain regions of the genome affect

15 phenotype, and can even be used to define new gene candidates for human metabolic disease

16 by comparing against parental sequence data ("reverse" GWAS) [99]. For example, using

17 140 clinical phenotypes from the BXD GRP showed metabolic traits (glucose tolerance, body

18 composition, blood pressure *etc*.) were shown to be highly variable and hereditary, and could

19 be associated with known gene variants [99].

20 **CRISPR-Cas9: the future for models of metabolic disease?**

21 CRISPR-Cas9 technology allows single nucleotides to be edited with high precision in the

22 endogenous genome, opening up the possibility to produce mouse models harboring identical

23 gene variants to those identified by GWAS in humans. This is showcased by studies of FTO

where the rs1421085 T-to-C single nucleotide polymorphism was found to switch BAT to WAT, reducing mitochondrial thermogenesis and increasing lipid storage [100]. CRISPR-Cas9 replication of the T-to-C in the equivalent primary adipocytes from a non-risk allele carrier mimicked this switch [100]. Whilst similar studies can be envisaged in rodents, especially where genotyped human tissue is difficult to access (*e.g.* pancreas), it is worth noting that the sequences housing gene variants identified by GWAS in man may not be present in mice [92]. Lastly, whilst CRISPR-Cas9 can be used to create Cre deleter lines, as alluded to above [43], homologous recombination still remains a rare event conferring minimal advantages over conventional knock-in approaches at present.

Summary

Mouse models have been incredibly important for furthering our understanding of metabolic disease. An extensive genetic toolbox of mice now exists, allowing the precise spatial and temporal manipulation of gene expression in cells and tissues involved in metabolic homeostasis. From this review, it should be clear that no perfect animal model exists, and lines of enquiry should always be complemented and confirmed by other experimental models (*e.g.* cell lines, human tissue, clinical data), and preferably a second mouse model, where a well-validated option exists. Interpretation of data from mouse experiments also requires careful consideration of the controls. For example, in Cre-Lox models, the floxed allele should be maintained on a varying Cre background and *vice versa* to determine any effects of the Cre *per se* or interactions between loss/gain of a Cre on a floxed background or *vice versa*. Similarly, animals fed HFD should always be assessed in parallel to littermates fed standard chow to avoid the dataset being confounded by heterogeneity in responsiveness. Lastly, there is an assumption that all animal facilities are the same. However, small variations in housing conditions (light, temperature, noise) have large effects on metabolic

parameters. We should therefore work toward better enforced consensus guidelines that regulate the housing conditions for animal models of metabolic disease.

Practice points

- Metabolic disorders stem from a complex interplay between different cells and tissues.

- Metabolic disease such as obesity and T2DM are polygenic and strongly influenced by environment.

- Mouse models with single genetic backgrounds allow the contributions of specific cells and tissues to metabolic homeostasis to be dissected out.

- Genetic reference populations allow the effect of environment on phenotypic traits to be better understood.

- No mouse model is perfect and careful attention should be paid to controls and housing conditions.

- Future studies may rely more and more on CRISPR-Cas9, which allows precise gene editing.

Research Agenda

- Mouse models that better mimic human obesity and diabetes progression are required, whether spontaneous mutation, caloric excess or genetic mutants.

- Consensus guidelines are required for mouse metabolic phenotyping, including standardised driver/deleter-line validation and housing conditions.

- Use of "open-source" genetically-modified mouse depositories should be encouraged, as this will accelerate phenotyping, as well as help uncover any potential issues more quickly.

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1   Schematics and Tables
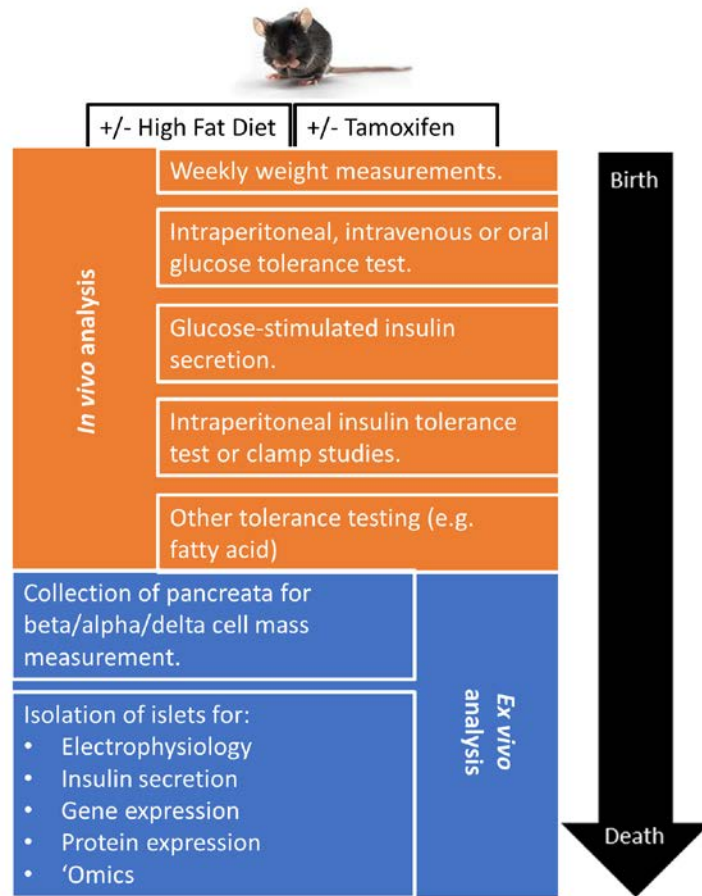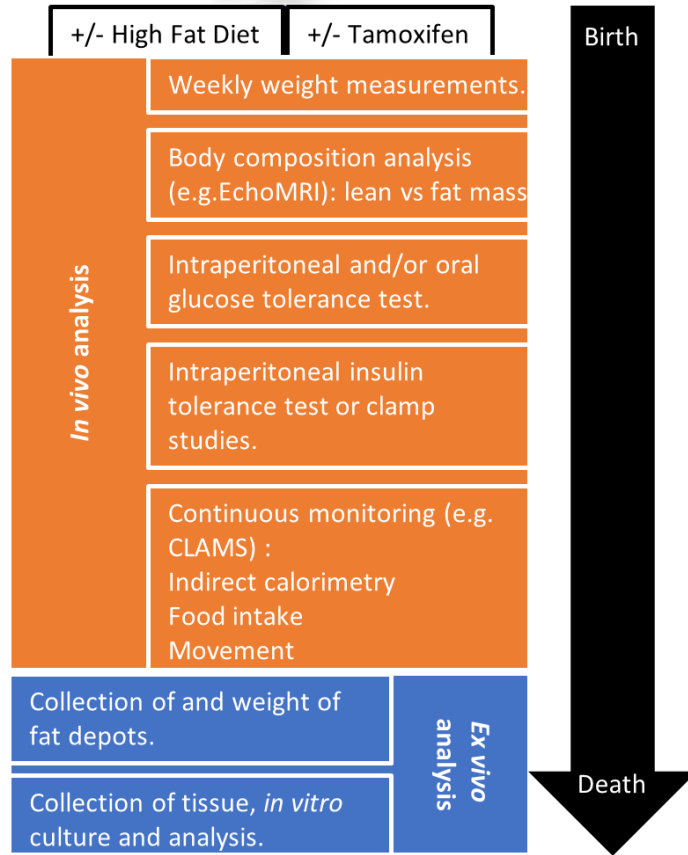

2

3   **Schematic 1:** diabetes mouse model phenotyping workflow

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2   **Schematic 2** obesity mouse model phenotyping workflow

3

1   **Table 1:** Spontaneous mutation and caloric excess mouse models

Cause	Strain	Comments	References
Spontaneous mutation in leptin (*Lep^{ob}*) gene	Obese or *ob/ob*	- Rapid weight gain. - Hyperphagia. - Insulin resistance. - Hyperinsulinemia. - Altered energy expenditure. - Dyslipidemia (although HDL and not LDL like humans). - Transient hyperglycemia (abates from 14-16 wks). - Model of rare monogenic obesity.	[2, 3].
Spontaneous mutation in leptin receptor (*Lepr*) gene	Diabetes or *db/db*	- Similar to *ob/ob* strain. - Infertility. - Sex-specific metabolic phenotype. - Metabolism may be improved in heterozygotes. - Model of rare monogenic obesity.	[8-10]
Inheritance of single recessive *Tannidd1* gene	TALLYHO JngJ mouse	- Hyperglycemia and hyperinsulinemia but without hypoleptinermia. - Gradual onset phenotype. - Preserved energy balance. - Polygenic T2D secondary to obesity model. - Controls complicated by mixed genetic background.	[11, 12]
60% calories by fat	High fat diet (HFD) mouse	- Moderate weight gain. - Hyperglycemia. - Hyperinsulinemia. - Insulin resistance. - Impaired insulin release. - Hypertension. - Adiposity. - Heterogeneity in responsiveness to HFD (some animals are low responders). - Strain and sex-dependent.	[13, 14, 17, 18]

2

3

1   **Table 2:** Pancreatic Cre-deleter lines for diabetes research

Cell type	Promoter	Comments	References
Beta-cell	Rat insulin promoter (RIP) (also termed *Ins2*)	• Good recombination efficiency in beta-cells. • Expression in various brain regions depending upon strain used. • GH minigene may be present.	[36, 37, 41]
Endocrine and exocrine pancreas	Pancreatic and duodenal homeobox 1 (*Pdx1*) (non-inducible)	• Good recombination efficiency in beta-cells. • Expression in the hypothalamus. • Pancreas-wide deletion. • GH minigene may be present.	[36, 37, 41]
Beta-cell Or Endocrine and exocrine pancreas	Pancreatic and duodenal homeobox 1 (*Pdx1*) (tamoxifen-inducible)	• Moderate recombination efficiency in beta-cells. • Expression in the hypothalamus depending on strain. • Some strains show no recombination in brain. • Beta-cell-specific if activated in adult. • GH minigene may be present.	[37, 39].
Beta-cell	Insulin 1 (*Ins1*) (transgenic)	• Good recombination efficiency in beta-cells. • Some strains show no recombination in brain. • GH minigene may be present.	[37, 41]
Beta-cell	Insulin 1 (*Ins1*) (knock-in)	• Excellent recombination efficiency in beta-cells. • Virtually no leakiness. • More natural gene expression due to use of endogenous locus. • No GH minigene present. • Recently developed strain- further phenotypic validation required.	[40, 45]
Alpha-cell L-cell	Glucagon (*Gcg*) (Preproglucagon, Cre)	• Recombination efficiency in alpha-cells has declined since line creation. • Poor recombination efficiency (~30%) in enteroendocrine L-cells. • Haploinsufficency due to loss of a preproglucagon (*Ppg*) gene.	[25, 36, 43-45].
Alpha-cell L-cell	Glucagon (*Gcg*) (Proglucagon, iCre)	• Good recombination efficiency in alpha-cells. • Good recombination efficiency in enteroendocrine L-cells. • Some expression in the beta-cell complement (~20%). • Hindbrain expression.	[44, 45]
Alpha-cell	Glucagon (*Gcg*) (Glucagon, Cre-ER,	• Excellent recombination efficiency in alpha-cells. • Poor recombination efficiency in	[43].

		CRISPR)	• enteroendocrine L-cells. • No haploinsufficiency.	
Delta-cell	Somatostain (*Sst*)		• Good recombination efficiency in delta-cells. • Sst gene depletion in brain? • General lack of phenotypic validation.	[35, 46].

1

2

1 **Table 3:** Muscle, liver and adipose Cre-deleter lines for obesity and diabetes research

Cell type	Promoter	Comments	References
Muscle	Muscle Creatine Kinase (MCK)	- Good recombination efficiency in skeletal and cardiac muscle. - Expression restrained during development.	[48]
Muscle	Human Alpha-Skeletal Actin (HSA) (HSA-Cre and HSA-Cre-ERT2)	- Adult striated muscle fibres and embryonic striated muscle cells of the somites and heart - Tamoxifen inducible version with good temporal regulation.	[49]
Muscle	Myogenic Factor 5 (Myf5)	- Cre expression in skeletal muscle, dermis. - Cre expression also in non-muscle.	[50]
Muscle	Myosin Light Chain 1/3 fast (MLC1/3f)	- Cre recombinase expression in skeletal muscle tissue, including gastrocnemius, tibialis anterior and biceps femoris muscles. - Cre expression not detected in brain, liver, heart or stomach.	[51]
Muscle	Myogenin Differentiation 1 (MyoD1)	Cre expression in committed myogenic cells and skeletal muscle.	[52]
Muscle	Myogenin (MyoG)	Recombination good in all skeletal muscle.	[53]
Muscle	Paired Box 7 (Pax7) (Pax7-Cre, Pax7-CreER, Pax7-CreERT2)	- Cre expression in progenitor cells of skeletal muscle lineage and satellite cells. - Tamoxifen-inducible expression available, leading to ablation of satellite cells.	[54]
Liver	Albumin (Alb-Cre, SA-Cre-ERT2)	- Efficient recombination in hepatocytes - Good temporal resolution with tamoxifen in inducible Cre-line	[55, 56]
Liver	Transthyretin (TTR-Cre, TTR-CreER)	- Deletion in foetal liver, visceral endoderm and yolk sac - Tamoxifen inducible version has specific recombination in foetal and adult liver.	[57, 58]
Liver	Albumin+Alpha fetoprotein (Alfp-Cre)	- Liver specific Cre-recombinase expression. - Possible expression of human growth hormone from transgene.	[59]
Adipose	Fatty Acid binding protein 4 (aP2)	- Recombination in adipocytes, adipocyte precursor, brain, embryonic tissues, endothelial cells, macrophages. - Differing recombination efficiencies in WAT vs BAT.	[62]
Adipose	Adiponectin (Adipoq-Cre, Adipoq-	- High specificity for adipocytes. - Tamoxifen-inducible version with	[63]

	CreERT)	good temporal resolution and cell specificity.	
Adipose	Platelet derived growth factor alpha (Pdgfrα-Cre, Pdgfrα-CreERT)	• Cre expresson in adipocyte precursor cells in WAT. • Tamoxifen-inducible version with good temporal resolution and cell specificity.	[66]

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