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Mouse models of peripheral 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 these cells occupy. Thus, it is desirable to use model organisms to understand the contribution 4 5 of different cells, tissues and organs to metabolism. Mice are widely used for metabolic research, since well-characterised mouse strains (in terms of their genotype and phenotype) 6 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 created by transgenesis and gene knockout technologies, and peripheral models generated by 10 Cre-Lox or CRISPR/Cas9 approaches. Focusing on obesity and type 2 diabetes as relevant 11 metabolic diseases, we systematically review each of these models, discussing their use, 12 13 limitations, and future potential.

14

Key words: mouse; metabolism; obesity; diabetes; insulin resistance; insulin; glucagon;
somatostatin; beta-cell; alpha-cell; beta-cell; delta-cell; liver; adipose; muscle.

1 Introduction

Metabolic disease constitutes one of the foremost non-communicable disease states affecting 2 global health. Almost 35% of adults over 20 years of age are obese, defined as BMI > 253 kg/m², and these individuals are more likely to suffer type 2 diabetes mellitus (T2DM), as 4 well as subfertility, hypertension and orthopaedic issues [1]. T2DM itself afflicts almost 10% 5 of adults worldwide (30% in some genetically-predisposed populations), accounting for 5% 6 7 of deaths secondary to significant co-morbidities including cardiovascular disease, neuropathy, nephropathy, vasculopathy and cancer [1]. The majority of metabolic disease 8 states such as T2DM and obesity have complex polygenic aetiology, which increases disease 9 risk when combined with a permissive environment. Many different cell types, as well as 10 interactions between disparate tissues and organs (e.g. muscle, liver, adipose, pancreas) are 11 involved in metabolic regulation, and may be subtly affected by these changes to produce a 12 clinical phenotype. Dissecting the mechanisms underlying metabolic disease is thus 13 challenging, requiring appreciation of how genotype and environment interact to influence 14 cell/tissue/organ function. Since genes cannot (yet) be manipulated in humans, key to 15 understanding metabolic disease is the development of animal models that allow genes in 16 specific cells/tissues/organs to be linked with particular phenotypic traits. Focusing on 17 18 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 19 20 limitations, before discussing future possibilities.

21

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 4 Laboratories due to a chance mutation in the House Mouse (Mus Musculus) that was 5 eventually transferred onto an inbred C57BL/6J background. Mice homozygous for the 6 mutation rapidly gain weight from 2-14 weeks, with body mass almost 3-fold higher than 7 non-obese wild-type (C57BL/6J) controls (reviewed in [2]). Seminal studies cloned and 8 sequenced the ob gene, which encodes a 4.5 kb mRNA with a 167-amino acid ORF 84% 9 10 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 11 humans), replacing an Arg with a STOP codon and leading to translation of a truncated non-12 13 functional protein [3]. Thus, mutant mice are hypoleptinemic and present with hyperphagia, obesity, insulin resistance, hyperinsulinemia, transient hyperglycemia subsiding at 14-16 14 wks, elevated HDL cholesterol fraction, sub/infertility and delayed wound healing [2]. 15 Implying normal leptin receptor signalling, but a loss of leptin secretion, injection of 16 recombinant leptin reduces body weight and food intake in ob/ob mice [4]. Moreover, 17 18 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 19 background, where severe glucose intolerance is associated with profound beta-cell loss, 20 21 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).

6 <u>Diabetes db/db mouse</u>: Similarly to *ob/ob* mice, the *db/db* mouse results from a single 7 autosomal recessive Gly -> Thr mutation in the leptin receptor (*Lepr*) gene located on 8 chromosome 4 [8]. This results in an inactive Lepr and hyperleptinemia [8], with a phenotype 9 closely matching the *ob/ob* mouse. Use of *db/db* mice is however complicated by profound 10 infertility, interactions between steroid sulfotransferase enzymes and the Lepr resulting in 11 gender differences in diabetes severity [9], and maintained metabolic performance in 12 heterozygotes due to compensation [10].

TALLYHO JngJ mouse: This strain was derived from inbreeding male Theiler Original mice 13 14 that spontaneously developed hyperglycemia and hyperinsulinemia without hypoleptinemia 15 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 16 interacts with a range of other TallyHo-associated loci including Tanidd2, Tanidd3, Tabw2 17 and Tafat located on different chromosomes [11]. TALLYHO JngJ mice represent a model of 18 polygenic moderate obesity combined with gradual onset insulin resistance, hyperglycemia, 19 20 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 21 22 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 23 mice, experiments are complicated by lack of control animal, with most studies using either 24 25 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

The high fat diet (HFD) mouse is perhaps one of the most-cited animal models of obesity due 3 to its relevance for T2DM and ease of use in both wild-type and transgenic animals. In this 4 model, maintenance diet is replaced with a HFD containing ~ 60% calories from fat (lard) 5 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 glucose tolerance testing [14]. Weight gain is noticeable from 2-4 wks, with maximal body 8 9 weight achieved at 16-20 wks, being almost 20-30% higher than wild-types [13, 14]. Mice generally display obesity (including adipocyte hyperplasia and mesenteric fat deposition), 10 hypertension, hyperglycemia, hyperinsulinemia due to increased fasting insulin levels and 11 12 insulin resistance. Impaired oral/intravenous/intraperitoneal glucose tolerance and glucosestimulated insulin release are usually apparent by 8-12 wks [13, 14]. Thus, the HFD mouse 13 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 beta-cell failure (> 18 wks) depending on strain, lab and colony [15]. 16

17 The mechanisms underlying beta-cell failure are controversial, but may reflect defective stimulus-secretion coupling [16], rather than a reduction in beta-cell mass as may be present 18 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 relatively protected [17]. Moreover, even among C57BL/6J, there tends to be some 22 23 heterogeneity in HFD-responsiveness ('low' and 'high' responders), with commensurate differences in phenotype [18], meaning that food intake and weight should be closely 24

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).

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

7 Surgical or chemically-induced models of obesity or diabetes

8 Surgical or chemical ablation of brain centres involved in the regulation of energy 9 homeostasis has led to the generation of rodent models of obesity. Since the use of these 10 techniques are discussed elsewhere in this volume, we will focus here on the physical and/or 11 chemical manipulation of adipose tissue, pancreas, and the ovaries.

Adipose tissue: Humans possess functional brown adipose tissue (BAT) in the cervical-12 supraclavicular depot, and BAT activity was shown to be negatively associated with 13 14 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 15 and colleagues demonstrated that removal of BAT by adipocyte-specific expression of 16 diphtheria toxin in mice leads to obesity and hyperphagia, ostensibly due to the lack of UCP-17 1 driven energy dissipation of heat [21]. BAT-ablated mice developed insulin resistance and a 18 T2DM-like phenotype [21]. The lowered energy expenditure for BAT thermogenesis 19 contributes to the development of obesity: BAT-ablated mice did not display obesity or 20 21 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, 22 23 hyperinsulinemia and energy expenditure appear to be more important [2].

Endocrine pancreas: The endocrine pancreas is critical for the regulation of energy 1 2 homeostasis via secretion of the hormones insulin, glucagon and somatostatin from the beta-, 3 alpha-, and delta-cells, respectively. The beta-cell in particular has been the subject of intense 4 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 5 many methods to study the function of this cell type have been developed over the decades 6 7 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 8 9 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-10 affinity glucose transporter, GLUT2, in rodent beta-cells [23]. GLUT2 is also expressed in 11 12 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 13 beta-cell dysfunction and death [23]. The methylnitrosurea moiety of STZ is responsible for 14 15 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 16 functional beta-cell mass following exposure to alloxan and STZ leads to profound glucose 17 dyshomeostasis. STZ is more commonly used than alloxan for the induction of diabetes in 18 rodents as it is a more stable chemical, therefore leading to more reproducible results [23]. 19

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

is technically demanding and involves injecting AAVs into the mesenteric artery [26] or the
pancreatic duct [27] following laparotomy. AAV serotypes 6, 8, and 9 were shown to mediate
long-term transduction of beta-cells [27], with more efficient transduction of the islets than
systemic administration of AAVs [26, 27].

Ovariectomy: Menopause, or reproductive senescence, is part of the aging process in women 5 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 transition to a state that is akin to human menopause [28]. Ovariectomy (OVX) is a widely 9 10 used method to increase the number of mice within a colony transitioning to the menopausal state. OVX induces a phenotype- increased body weight, adiposity (although the fat depots 11 affected differ) and feeding- which closely mimics that seen in human females [28]. 12 13 However, OVX is a model of immediate loss of function rather than a gradual transition of loss of the oestrus cycle, as in menopause, and does not accurately reflect the adaptations 14 15 enacted to compensate for the gradual drop of ovarian hormones [29]. This temporal difference in effect may account for some of the phenotypic differences seen between the 16 OVX model and human menopausal females, *e.g.* in total lean mass [30]. 17

18 Genetically-modified mouse lines for diabetes research

A key event in the development of T2DM in humans is expansion of the beta-cell mass to functionally compensate insulin resistance, mainly due to defective insulin signalling in muscle, liver and adipose tissue [15, 31]. This is followed by beta-cell failure and increased blood glucose levels after an poorly defined period of time [15]. Alpha-cells further aggravate the situation, by hypersecreting the glycogenolytic/gluconeogenic hormone glucagon [32], 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, 1 2 although they likely act to suppress insulin and glucagon release in a paracrine manner [34], 3 and recent studies have shown that they may in fact provide a source of beta-cells in prepubertal animals, allowing diabetes recovery [35]. Obesity is a major risk factor for 4 development of T2DM, since excess circulating fatty acids, glycerol, cytokines conspire to 5 drive insulin resistance in muscle, liver and adipose depots, in addition to directly influencing 6 7 beta and other cell function [31]. The mechanisms underlying insulin resistance, alpha-betadelta-cell interactions and conversion during diabetes, as well as the chronology of these 8 9 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. 10 Key to this are mouse models that allow conditional and inducible gene manipulation 11 12 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. 13

Beta-cell-specific driver lines: Conditional gene recombination (expression or inactivation) 14 can be achieved in pancreatic beta-cells using site-specific Cre-Lox technology. Early Cre 15 driver lines consisted of Cre expressed under the control of the Rat insulin promoter (Rip-16 Cre), otherwise termed the ancestral Ins2 gene, but were plagued with extra-pancreatic 17 18 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 19 efficiency in beta-cells is ~80-98%, with varying degrees of expression in the brain 20 21 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 22 23 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].

7 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 8 which is sufficient for normal insulin production following loss of both *Ins1* alleles [40]. 9 10 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) 11 minigene, now shown to cause pregnancy-like proliferative changes in beta-cell mass through 12 13 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 14 15 expression from the translation initiation codon of the endogenous Insl gene [40], with the added advantage of driving more moderate Cre expression, the enzyme itself shown to induce 16 beta-cell hypoplasia when present at high levels [42]. Nonetheless, this is sufficient for 17 18 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-19 depth metabolic phenotyping is required. 20

21 <u>Alpha-cell-specific driver lines:</u> Glu-Cre mice were first developed over a decade ago using a
22 short (1.6. kb) promoter fragment containing the rat *Gcg* promoter. Whilst initial
23 recombination efficiency was high, this appears to have diminished over time from 100% ->
24 40% depending on the colony studied [25, 43]. Similarly, the Glu-CreERT deleter strain
25 displays expression in 50-75% of alpha-cells, but functional analyses in these animals are

1 complicated by haploinsufficiency due to loss of a preproglucagon gene [43], as well as 2 expression in enteroendocrine L-cells and potentially in brainstem/hypothalamic neurons, 3 which cleave preproglucagon into glucagon-like peptide 1 [36]. By contrast, animals 4 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 5 recombination is also detected in ~ 20% beta-cells and the hindbrain, both sites involved in 6 glucose homeostasis [44, 45]. Recent studies used CRISPR-Cas9 genome editing to insert 7 CreER into the 3' UTR of the endogenous Gcg locus, without disrupting preproglucagon 8 9 expression [43]. Whilst the resulting Glucagon-CreER mice display a normal phenotype, even as homozygotes, and display good recombination efficiency in adult alpha-cells (~ 10 95%), Cre expression was detected in only 33% of L-cells [43], limiting their use for 11 12 enteroendocrine deletion compared to Glu-iCre animals [44].

13 <u>Delta-cell-specific driver lines:</u> The widespread expression of the neuropeptide somatostatin (Sst) in the brain [46], makes assessment of any conditional gene recombination in delta-cells 14 15 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 16 for delta-cell lineage tracing experiments [35]. However, it has been recently reported that 17 18 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 19 [46]. Thus, results should be interpreted carefully pending further phenotypic validation of 20 delta-cell-specific driver lines for metabolic research, especially given the reported reduction 21 in Sst expression in the hypothalamus of Sst-Cre animals [46]. 22

23 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 1 2 metabolism, and hence energy expenditure. Peripheral insulin resistance is the first detectable 3 defect in T2DM, with insulin resistance greatest in skeletal muscle at the initiating phase of 4 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. 5 Muscle-specific Cre-deleter strains have been produced using the muscle creatine kinase 6 7 (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 8 9 (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 aMHC 10 promoters. The MCK Cre-deleter strain has Cre expression driven by a 6.5 kb genomic DNA 11 12 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 13 heart. MCK-driven Cre expression is restrained during development [48], thus making this a 14 useful deleter strain for the study of adult muscle phenotype. 15

Liver-specific driver lines: The albumin promoter driven Cre-deleter strain (Alb-Cre) [55] 16 has been widely used in the study of energy homeostasis by the liver. The Alb-Cre transgene 17 18 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 19 hepatic and beta-cell glucokinase are involved in the pathogenesis of hyperglycaemia in 20 21 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 22 23 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 24 allows specific recombination in the foetal and adult liver [58]. The Alfp-Cre-deleter strain 25

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].

6 An alternative approach to deliver Cre recombinase expression vectors to the liver in mouse 7 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 8 for Cre-recombinase under the control of a tissue-specific promoter e.g. the albumin 9 10 promoter, by hydrodynamic gene transfer [61]. Hydrodynamic gene transfer avoids problems such as positional insertion artefacts, variations caused by differences in genetic background, 11 leaky expression, and toxicity associated with elevated Cre expression, which are 12 confounding factors commonly encountered in the use of Cre-deleter strains. 13

14 Adipose-specific driver lines: Adipose tissue- consisting of WAT and BAT- is an important 15 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 16 adipocyte-specific promoters to drive Cre expression. Amongst these are aP2-Cre mice [62], 17 where Cre expression is driven by the promoter of the fatty acid binding protein 4 (Fabp4) 18 gene, and the Adipoq-Cre strains [63], where Cre expression is driven by the adiponectin 19 20 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 21 22 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 23 factor receptor alpha (Pdgfra) promoter driven Cre-deleter strains (Pdgfra-Cre) have also 24 25 been generated based upon reports that Pdgfra 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.

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

7 See Schematic 1 for a diabetes mouse model phenotyping workflow

8 Genetically-modified mouse lines for obesity research

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

11beta-hydroxysteroid dehydrogenase type 1 (HSD11B1): Increased 11 beta-hydroxysteroid 12 dehydrogenase type 1 (HSD11B1), the key glucocorticoid regenerating enzyme, in the 13 adipose tissue is characteristic of obesity in humans and rodents, and associated with 14 metabolic syndrome [67]. Tissue-specific over-expression of 11BHSD1 also correlates with 15 weight gain (or lack of, e.g. where HSD11B1 expression is high in liver) in menopausal 16 women [68]. Thus, transgenic mice over-expressing HSD11B1 in adipose tissue via the aP2 17 promoter display elevated intra-adipose corticosterone levels (the more active glucocorticoid 18 19 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 20 possible expression in the brain, as discussed earlier in this chapter [69]. Transgenic mice 21 22 with apoE driven over-expression of HSD11B1 largely in the liver displayed normal corticosterone levels, glucose tolerance, body weight and fat depots, with concomitant 23

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: MafB 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.

8 Androgen receptor knockout mice: Androgen deprivation is linked to metabolic syndrome in 9 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 10 11 (ARKO) mouse models exist. Global AR-KO mice display, late-onset visceral obesity, 12 altered lipid metabolism, glucose homeostasis, and insulin and leptin resistance [74]. Liverspecific AR-KO mice, generated using the Alb-Cre-deleter strain, exhibited obesity and 13 14 hepatic steatosis when challenged with HFD, with concomitant liver insulin resistance 15 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 16 resistance and reduced suppression of hepatic gluconeogenic gene expression [76]. Muscle-17 specific deletion of AR using the MCK- or HSA-promoter deleter strains, led to altered fibre 18 type composition, reduction in intra-abdominal fat mass, and contractility of fast- and 19 20 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]. 21 22 Deletion of AR using the aP2-Cre-deleter strain led to impaired insulin sensitivity and agerelated glucose intolerance, with increased susceptibility to HFD-induced visceral obesity 23 [81]. Conversely, over-expression of AR in female mice protected against HFD-induced 24

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

3 <u>*GLUT4 mouse models:*</u> The low affinity insulin-sensitive <u>glucose transporter isoform 4</u> 4 (GLUT4) is expressed in adipocytes, skeletal muscle and cardiac muscle, where it mediates 5 glucose disposal [83]. In line with this, ablation of GLUT4 from adipocyte (aP2-Cre) [62] 6 and muscle (MCK-Cre) [84] compartments led to reduced glucose uptake, hyperglycaemia, 7 hyperinsulinaemia and secondary insulin resistance in other insulin sensitive tissues. By 8 contrast, over-expression of GLUT4 prevented HFD-induced hyperglycaemia and insulin 9 resistance [85].

10 <u>Beta 3 adrenergic receptor knockout mouse:</u> Beta 3 adrenergic receptor (beta3-AR) is
 11 expressed in human BAT, with decreased expression levels in genetically-obese mouse
 12 models (reviewed in [86]). Mutation in the beta3-AR is associated with obesity, decreased
 13 energy expenditure, insulin resistance and T2DM [86]. Beta3-AR knockout mice (beta3 14 ARKO) have increased fat mass indicating an impact on energy homeostasis [87].

15 See Schematic 2 for an obesity mouse model workflow

16 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 1 2 variants exist whose discovery requires increased power through larger sample sizes. 3 Pertinently, the majority of the SNPs associated with T2DM affect intravenous glucose 4 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 5 circuits or molecules involved in neurodevelopment [90]. Despite this, few of the GWAS hits 6 7 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 8 9 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 10 non-coding regions of the genome. Whilst functional validation could be speeded up by using 11 12 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 13 adiposity. 14

Most studies to date have relied on global knockout or Cre-Lox models to delete the gene of 15 interest. Whilst this allows functional interrogation of gene variants that have restricted 16 expression patterns (e.g. SLC30A8- largely expressed in the islet and retina [91]), it is less 17 18 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 19 (with the important caveat that many SNPs may also affect neighboring loci). Whether the 20 21 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 22 23 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 24 phenotype may require more than one gene to be manipulated. Another caveat is that over-25

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].

4 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 5 how gene variants may influence disease in humans (due to the caveats listed above). FTO 6 7 encodes 2-Oxoglutarate-Dependent Nucleic Acid Demethylase, and SNPs in this gene are 8 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 9 10 [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 11 12 composition, pointing to effects if FTO deficiency in multiple body compartments, including 13 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 14 RNA-silencing studies in isolated tissue [94], Tcf712 deletion specifically in beta-cells using 15 Ins1Cre^{+/-}:Tcf7l2^{-/-} mice impairs glucose tolerance [95]. Another gene strongly associated 16 with T2DM is SLC30A8, which encodes zinc transporter 8 (ZnT8), involved in importing 17 Zn^{2+} into the beta-cell cytoplasm where it is required for proper insulin crystallisation [91]. 18 Global ZnT8 results in glucose intolerance, although depending on the colony studied this 19 was sex-specific, disappeared with age, or did not occur at all (reviewed in [92]). The reasons 20 for this are unknown, but may involve metabolic adaptation, genetic background (mixed 21 versus C57BL6) or housing conditions [92]. By contrast beta-cell-specific knockout of ZnT8 22 consistently reveals impaired glucose tolerance [96, 97], whilst beta-cell-specific over-23 expression mildly improves the same parameter [97]. Together, these data show the utility of 24

animal models for functionally validating GWAS, allowing conditional gene KO or over 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-byenvironment (GXE) interactions. Whilst the genetic risk factors for development of metabolic 6 7 disease can be identified using GWAS and genetically-engineered mouse models, GXE are more difficult to dissect due to the inability to control environment (in human studies at 8 9 least), and the single genetic background of inbred mice. The latter can be partly circumvented using recombinant inbred strain families, genetic reference populations (GRP) 10 where an isogenic line represents each genotype due to brother-sister mating, thus resembling 11 12 rare human populations [98]. This allows studies to be replicated in multiple cohorts from lines spanning differing levels of genetic complexity [98]. Combined with eQTL analyses, 13 GRPs provide a powerful platform to understand how certain regions of the genome affect 14 phenotype, and can even be used to define new gene candidates for human metabolic disease 15 by comparing against parental sequence data ("reverse" GWAS) [99]. For example, using 16 17 140 clinical phenotypes from the BXD GRP showed metabolic traits (glucose tolerance, body composition, blood pressure etc.) were shown to be highly variable and hereditary, and could 18 19 be associated with known gene variants [99].

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

CRISPR-Cas9 technology allows single nucleotides to be edited with high precision in the endogenous genome, opening up the possibility to produce mouse models harboring identical 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 1 2 WAT, reducing mitochondrial thermogenesis and increasing lipid storage [100]. CRISPR-3 Cas9 replication of the T-to-C in the equivalent primary adipocytes from a non-risk allele 4 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 5 noting that the sequences housing gene variants identified by GWAS in man may not be 6 7 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 8 9 minimal advantages over conventional knock-in approaches at present.

10 Summary

Mouse models have been incredibly important for furthering our understanding of metabolic 11 12 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 13 homeostasis. From this review, it should be clear that no perfect animal model exists, and 14 15 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, 16 where a well-validated option exists. Interpretation of data from mouse experiments also 17 requires careful consideration of the controls. For example, in Cre-Lox models, the floxed 18 allele should be maintained on a varying Cre background and vice versa to determine any 19 20 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 21 22 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 23 variations in housing conditions (light, temperature, noise) have large effects on metabolic 24

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

3 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.
- 16 **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 **REFERENCES**

2	1.	Ezzati, M. and O. World Health, Comparative quantification of health risks : global
3		and regional burden of disease attributable to selected major risk factors. 2004.

- 4 2. Wang, B., P. Chandrasekera, and J. Pippin, Leptin- and Leptin Receptor-Deficient
- *Rodent Models: Relevance for Human Type 2 Diabetes.* Current Diabetes Reviews,
 2014. 10(2): p. 131-145.
- 7 3. Zhang, Y., et al., *Positional cloning of the mouse obese gene and its human*
- 8 *homologue*. Nature, 1994. **372**(6505): p. 425-432.
- 9 *4. Halaas, J.L., et al., Weight-reducing effects of the plasma protein encoded by the
 10 obese gene. Science, 1995. 269(5223): p. 543-6.
- 11 5. King, A.J., *The use of animal models in diabetes research*. Br J Pharmacol, 2012.
 12 166(3): p. 877-94.
- Grarup, N., et al., *Genetic susceptibility to type 2 diabetes and obesity: from genome- wide association studies to rare variants and beyond.* Diabetologia, 2014. 57(8): p.
- 15 1528-41.
- 16 7. Montague, C.T., et al., *Congenital leptin deficiency is associated with severe early-*17 *onset obesity in humans.* Nature, 1997. **387**(6636): p. 903-8.
- *8. Chen, H., et al., Evidence That the Diabetes Gene Encodes the Leptin Receptor: *Identification of a Mutation in the Leptin Receptor Gene in db/db Mice.* Cell, 1996.
 84(3): p. 491-495.
- Khor, V.K., et al., Gender-Specific Expression and Mechanism of Regulation of Estrogen Sulfotransferase in Adipose Tissues of the Mouse. Endocrinology, 2008.
 149(11): p. 5440-5448.

- Kanda, Y., et al., Molecular analysis of db gene-related pancreatic beta cell
 dysfunction; evidence for a compensatory mechanism inhibiting development of
 diabetes in the db gene heterozygote. Endocr J, 2009. 56(8): p. 997-1008.
- 4 11. Kim, J.H., et al., *Genetic analysis of a new mouse model for non-insulin-dependent*5 *diabetes.* Genomics, 2001. 74(3): p. 273-86.
- Kim, J.H., et al., *Phenotypic characterization of polygenic type 2 diabetes in TALLYHO/JngJ mice*. J Endocrinol, 2006. **191**(2): p. 437-46.
- 8 13. Wang, C.-Y. and J.K. Liao, A Mouse Model of Diet-Induced Obesity and Insulin
 9 Resistance. Methods Mol Biol, 2012. 821: p. 421-433.
- 10 14. Winzell, M.S. and B. Ahren, *The high-fat diet-fed mouse: a model for studying*11 *mechanisms and treatment of impaired glucose tolerance and type 2 diabetes.*12 Diabetes, 2004. 53 Suppl 3: p. S215-9.
- *15. Halban, P.A., et al., β-Cell Failure in Type 2 Diabetes: Postulated Mechanisms and
 Prospects for Prevention and Treatment. Diabetes Care, 2014. 37(6): p. 1751-1758.
- 15 16. Rorsman, P., M. Braun, and Q. Zhang, *Regulation of calcium in pancreatic alpha- and beta-cells in health and disease*. Cell Calcium, 2012. **51**(3-4): p. 300-8.
- Montgomery, M.K., et al., *Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding*. Diabetologia, 2013. 56(5): p. 1129-1139.
- 19 18. Peyot, M.L., et al., -*Cell Failure in Diet-Induced Obese Mice Stratified According to*
- Body Weight Gain: Secretory Dysfunction and Altered Islet Lipid Metabolism Without
 Steatosis or Reduced -Cell Mass. Diabetes, 2010. 59(9): p. 2178-2187.
- Pettersson, U.S., et al., *Female Mice are Protected against High-Fat Diet Induced Metabolic Syndrome and Increase the Regulatory T Cell Population in Adipose Tissue.* PLoS ONE, 2012. 7(9): p. e46057.

- Cypess, A.M. and C.R. Kahn, *Brown fat as a therapy for obesity and diabetes.* Current Opinion in Endocrinology, Diabetes and Obesity, 2010. 17(2): p. 143-149.
- 3 *21. Lowell, B.B., et al., *Development of obesity in transgenic mice after genetic ablation*4 of brown adipose tissue. Nature, 1993. 366(6457): p. 740-2.
- 5 22. Melnyk, A., M.E. Harper, and J. Himms-Hagen, *Raising at thermoneutrality prevents obesity and hyperphagia in BAT-ablated transgenic mice*. Am J Physiol, 1997. 272(4
 7 Pt 2): p. R1088-93.
- 8 23. Lenzen, S., *The mechanisms of alloxan- and streptozotocin-induced diabetes.*9 Diabetologia, 2008. 51(2): p. 216-26.
- Weiss, R.B., *Streptozocin: a review of its pharmacology, efficacy, and toxicity.*Cancer Treat Rep, 1982. 66(3): p. 427-38.
- Herrera, P.L., Adult insulin- and glucagon-producing cells differentiate from two
 independent cell lineages. Development, 2000. **127**(11): p. 2317-22.
- 14 26. Maione, F., et al., Semaphorin 3A is an endogenous angiogenesis inhibitor that blocks
 15 tumor growth and normalizes tumor vasculature in transgenic mouse models. J Clin
- 16 Invest, 2009. **119**(11): p. 3356-72.
- 17 27. Jimenez, V., et al., In vivo genetic engineering of murine pancreatic beta cells
 18 mediated by single-stranded adeno-associated viral vectors of serotypes 6, 8 and 9.
- 19 Diabetologia, 2011. **54**(5): p. 1075-86.
- 20 28. Diaz Brinton, R., *Minireview: translational animal models of human menopause:*21 *challenges and emerging opportunities.* Endocrinology, 2012. **153**(8): p. 3571-8.
- 22 29. Zhao, L., et al., *Estrogen receptor beta-selective phytoestrogenic formulation prevents*
- 23 physical and neurological changes in a preclinical model of human menopause.
- 24 Menopause, 2011. **18**(10): p. 1131-42.

1	30.	Gloy, V., et al., Ovariectomy and overeating palatable, energy-dense food increase
2		subcutaneous adipose tissue more than intra-abdominal adipose tissue in rats. Biol
3		Sex Differ, 2011. 2 : p. 6.
4	31.	Samuel, Varman T. and Gerald I. Shulman, Mechanisms for Insulin Resistance:
5		Common Threads and Missing Links. Cell, 2012. 148(5): p. 852-871.
6	32.	Unger, R.H. and A.D. Cherrington, Glucagonocentric restructuring of diabetes: a
7		pathophysiologic and therapeutic makeover. J Clin Invest, 2012. 122(1): p. 4-12.
8	33.	Thorel, F., et al., Conversion of adult pancreatic alpha-cells to beta-cells after
9		extreme beta-cell loss. Nature, 2010. 464(7292): p. 1149-54.
10	34.	Hauge-Evans, A.C., et al., Somatostatin secreted by islet delta-cells fulfills multiple
11		roles as a paracrine regulator of islet function. Diabetes, 2009. 58(2): p. 403-11.
12	35.	Chera, S., et al., Diabetes recovery by age-dependent conversion of pancreatic δ -cells
13		into insulin producers. Nature, 2014. 514(7523): p. 503-507.
14	*36.	Magnuson, Mark A. and Anna B. Osipovich, Pancreas-Specific Cre Driver Lines and
15		Considerations for Their Prudent Use. Cell Metabolism, 2013. 18(1): p. 9-20.
16	37.	Wicksteed, B., et al., Conditional gene targeting in mouse pancreatic ss-Cells:
17		analysis of ectopic Cre transgene expression in the brain. Diabetes, 2010. 59(12): p.
18		3090-8.
19	38.	Kong, D., et al., GABAergic RIP-Cre Neurons in the Arcuate Nucleus Selectively
20		Regulate Energy Expenditure. Cell, 2012. 151(3): p. 645-657.
21	39.	Estall, J.L. and R.A. Screaton, To Be(ta Cell) or Not to Be(ta cell): New Mouse
22		Models for Studying Gene Function in the Pancreatic beta-Cell. Endocrinology,
23		2015. 156 (7): p. 2365-7.
24	40.	Thorens, B., et al., Ins1 knock-in mice for beta cell-specific gene recombination.
25		Diabetologia, 2014. 58(3): p. 558-565.

- *41. Brouwers, B., et al., *Impaired islet function in commonly used transgenic mouse lines due to human growth hormone minigene expression*. Cell Metabolism, 2014. 20(6): p.
 979-90.
- 4 42. Pomplun, D., et al., Alterations of pancreatic beta-cell mass and islet number due to
 5 Ins2-controlled expression of Cre recombinase: RIP-Cre revisited; part 2. Horm
 6 Metab Res, 2007. 39(5): p. 336-40.
- Ackermann, A.M., et al., *High-fidelity Glucagon-CreER mouse line generated by CRISPR-Cas9 assisted gene targeting*. Mol Metab, 2017. 6(3): p. 236-244.
- 9 44. Parker, H.E., et al., *Predominant role of active versus facilitative glucose transport*10 for glucagon-like peptide-1 secretion. Diabetologia, 2012. 55(9): p. 2445-55.
- Soedling, H., et al., *Limited impact on glucose homeostasis of leptin receptor deletion from insulin- or proglucagon-expressing cells.* Mol Metab, 2015. 4(9): p. 619-30.
- 46. Viollet, C., et al., *Somatostatin-IRES-Cre Mice: Between Knockout and Wild-Type?*Front Endocrinol (Lausanne), 2017. 8: p. 131.
- 15 47. DeFronzo, R.A. and D. Tripathy, *Skeletal Muscle Insulin Resistance Is the Primary* 16 *Defect in Type 2 Diabetes*. Diabetes Care, 2009. **32**(suppl_2): p. S157-S163.
- Bruning, J.C., et al., A muscle-specific insulin receptor knockout exhibits features of
 the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell,
 19 1998. 2(5): p. 559-69.
- 49. Miniou, P., et al., *Gene targeting restricted to mouse striated muscle lineage*. Nucleic
 Acids Res, 1999. 27(19): p. e27.
- 50. Haldar, M., et al., A conditional mouse model of synovial sarcoma: insights into a
 myogenic origin. Cancer Cell, 2007. 11(4): p. 375-88.

- Mourkioti, F., et al., Analysis of CRE-mediated recombination driven by myosin light
 chain 1/3 regulatory elements in embryonic and adult skeletal muscle: a tool to study
 fiber specification. Genesis, 2008. 46(8): p. 424-30.
- 4 52. Chen, J.C., et al., *MyoD-cre transgenic mice: a model for conditional mutagenesis*5 and lineage tracing of skeletal muscle. Genesis, 2005. 41(3): p. 116-21.
- 53. Li, S., et al., Requirement for serum response factor for skeletal muscle growth and *maturation revealed by tissue-specific gene deletion in mice*. Proc Natl Acad Sci U S
 A, 2005. 102(4): p. 1082-7.
- 9 54. Nishijo, K., et al., *Biomarker system for studying muscle, stem cells, and cancer in*10 *vivo.* FASEB J, 2009. 23(8): p. 2681-90.
- *55. Postic, C., et al., *Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase.* J Biol
 Chem, 1999. 274(1): p. 305-15.
- Schuler, M., et al., *Efficient temporally controlled targeted somatic mutagenesis in hepatocytes of the mouse.* Genesis, 2004. **39**(3): p. 167-72.
- 16 57. Kwon, G.S. and A.K. Hadjantonakis, *Transthyretin mouse transgenes direct RFP*17 *expression or Cre-mediated recombination throughout the visceral endoderm.*18 Genesis, 2009. 47(7): p. 447-55.
- 19 58. Tannour-Louet, M., et al., A tamoxifen-inducible chimeric Cre recombinase
 20 specifically effective in the fetal and adult mouse liver. Hepatology, 2002. 35(5): p.
 21 1072-81.
- 59. Kellendonk, C., et al., *Hepatocyte-specific expression of Cre recombinase*. Genesis,
 2000. 26(2): p. 151-3.
- Pruniau, V.P., et al., *The AlfpCre mouse revisited: evidence for liver steatosis related to growth hormone deficiency.* Hepatology, 2013. 58(6): p. 2209-10.

1	61.	Zhu, H.Z., et al., Conditional gene modification in mouse liver using hydrodynamic
2		delivery of plasmid DNA encoding Cre recombinase. FEBS Lett, 2006. 580(18): p.
3		4346-52.
4	62.	Abel, E.D., et al., Adipose-selective targeting of the GLUT4 gene impairs insulin
5		action in muscle and liver. Nature, 2001. 409(6821): p. 729-33.
6	63.	Wang, Z.V., et al., Identification and characterization of a promoter cassette
7		conferring adipocyte-specific gene expression. Endocrinology, 2010. 151(6): p. 2933-
8		9.
9	*64.	Lee, K.Y., et al., Lessons on conditional gene targeting in mouse adipose tissue.
10		Diabetes, 2013. 62 (3): p. 864-74.
11	65.	Jeffery, E., et al., Characterization of Cre recombinase models for the study of
12		adipose tissue. Adipocyte, 2014. 3(3): p. 206-11.

- Berry, R. and M.S. Rodeheffer, *Characterization of the adipocyte cellular lineage in vivo.* Nat Cell Biol, 2013. 15(3): p. 302-8.
- 15 67. Seckl, J.R., et al., *Glucocorticoids and 11beta-hydroxysteroid dehydrogenase in adipose tissue*. Recent Prog Horm Res, 2004. **59**: p. 359-93.
- 17 68. Andersson, T., et al., *Tissue-specific increases in 11beta-hydroxysteroid*18 *dehydrogenase type 1 in normal weight postmenopausal women.* PLoS One, 2009.
 19 4(12): p. e8475.
- 20 69. Masuzaki, H., et al., A transgenic model of visceral obesity and the metabolic
 21 syndrome. Science, 2001. 294(5549): p. 2166-70.
- 22 70. Paterson, J.M., et al., *Metabolic syndrome without obesity: Hepatic overexpression of*
- 23 *11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice*. Proc Natl Acad Sci
- 24 U S A, 2004. **101**(18): p. 7088-93.

- Sarrazin, S., et al., *MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells.* Cell, 2009. 138(2): p. 300-13.
- 3 72. Tran, M.T., et al., *MafB deficiency accelerates the development of obesity in mice*.
 4 FEBS Open Bio, 2016. 6(6): p. 540-7.
- 5 73. Allan, C.A. and R.I. McLachlan, *Androgens and obesity*. Curr Opin Endocrinol
 6 Diabetes Obes, 2010. 17(3): p. 224-32.
- 7 74. Lin, H.Y., et al., *Insulin and leptin resistance with hyperleptinemia in mice lacking androgen receptor*. Diabetes, 2005. 54(6): p. 1717-25.
- 9 75. Lin, H.Y., et al., *Increased hepatic steatosis and insulin resistance in mice lacking hepatic androgen receptor.* Hepatology, 2008. 47(6): p. 1924-35.
- 11 76. Yu, I.C., et al., Neuronal androgen receptor regulates insulin sensitivity via
 12 suppression of hypothalamic NF-kappaB-mediated PTP1B expression. Diabetes,
 13 2013. 62(2): p. 411-23.
- 14 77. Ophoff, J., et al., Androgen signaling in myocytes contributes to the maintenance of
 15 muscle mass and fiber type regulation but not to muscle strength or fatigue.
 16 Endocrinology, 2009. 150(8): p. 3558-66.
- 17 78. Chambon, C., et al., *Myocytic androgen receptor controls the strength but not the*18 *mass of limb muscles.* Proc Natl Acad Sci U S A, 2010. 107(32): p. 14327-32.
- 19 79. Fernando, S.M., et al., Myocyte androgen receptors increase metabolic rate and
 20 improve body composition by reducing fat mass. Endocrinology, 2010. 151(7): p.
 21 3125-32.
- 80. Semirale, A.A., X.W. Zhang, and K.M. Wiren, *Body composition changes and inhibition of fat development in vivo implicates androgen in regulation of stem cell lineage allocation.* J Cell Biochem, 2011. **112**(7): p. 1773-86.

1	81.	McInnes, K.J., et al., Deletion of the androgen receptor in adipose tissue in male mice
2		elevates retinol binding protein 4 and reveals independent effects on visceral fat mass
3		and on glucose homeostasis. Diabetes, 2012. 61(5): p. 1072-81.
4	82.	Fagman, J.B., et al., The androgen receptor confers protection against diet-induced
5		atherosclerosis, obesity, and dyslipidemia in female mice. FASEB J, 2015. 29(4): p.
6		1540-50.
7	83.	Shepherd, P.R. and B.B. Kahn, Glucose transporters and insulin actionimplications
8		for insulin resistance and diabetes mellitus. N Engl J Med, 1999. 341(4): p. 248-57.
9	84.	Zisman, A., et al., Targeted disruption of the glucose transporter 4 selectively in
10		muscle causes insulin resistance and glucose intolerance. Nat Med, 2000. 6(8): p.
11		924-8.
12	85.	Atkinson, B.J., et al., Moderate GLUT4 overexpression improves insulin sensitivity
13		and fasting triglyceridemia in high-fat diet-fed transgenic mice. Diabetes, 2013.
14		62 (7): p. 2249-58.
15	86.	Lowell, B.B. and J.S. Flier, Brown adipose tissue, beta 3-adrenergic receptors, and
16		obesity. Annu Rev Med, 1997. 48: p. 307-16.
17	87.	Susulic, V.S., et al., Targeted disruption of the beta 3-adrenergic receptor gene. J
18		Biol Chem, 1995. 270(49): p. 29483-92.
19	88.	Jafar-Mohammadi, B. and M.I. McCarthy, Genetics of type 2 diabetes mellitus and
20		obesitya review. Ann Med, 2008. 40(1): p. 2-10.
21	89.	Florez, J.C., Newly identified loci highlight beta cell dysfunction as a key cause of
22		type 2 diabetes: where are the insulin resistance genes? Diabetologia, 2008. 51(7): p.
23		1100-10.
24	90.	Loos, R.J. and G.S. Yeo, The bigger picture of FTO: the first GWAS-identified obesity

gene. Nat Rev Endocrinol, 2014. **10**(1): p. 51-61.

1	91.	Rutter, G.A., et al., Intracellular zinc in insulin secretion and action: a determinant of
2		diabetes risk? Proc Nutr Soc, 2016. 75(1): p. 61-72.
3	*92.	da Silva Xavier, G., et al., Animal models of GWAS-identified type 2 diabetes genes. J
4		Diabetes Res, 2013. 2013: p. 906590.
5	93.	Frayling, T.M., et al., A common variant in the FTO gene is associated with body
6		mass index and predisposes to childhood and adult obesity. Science, 2007. 316(5826):
7		p. 889-94.
8	94.	da Silva Xavier, G., et al., TCF7L2 regulates late events in insulin secretion from
9		pancreatic islet beta-cells. Diabetes, 2009. 58(4): p. 894-905.
10	95.	Mitchell, R.K., et al., Selective disruption of Tcf7l2 in the pancreatic beta cell impairs
11		secretory function and lowers beta cell mass. Human Molecular Genetics, 2014.
12	96.	Wijesekara, N., et al., Beta cell-specific Znt8 deletion in mice causes marked defects
13		in insulin processing, crystallisation and secretion. Diabetologia, 2010. 53(8): p.
14		1656-68.
15	97.	Mitchell, R.K., et al., Molecular genetic regulation of Slc30a8/ZnT8 reveals a positive
16		association with glucose tolerance. Mol Endocrinol, 2015: p. me20151227.
17	98.	Singer, J.B., et al., Genetic dissection of complex traits with chromosome substitution
18		strains of mice. Science, 2004. 304(5669): p. 445-8.
19	*99.	Andreux, P.A., et al., Systems genetics of metabolism: the use of the BXD murine
20		reference panel for multiscalar integration of traits. Cell, 2012. 150 (6): p. 1287-99.
21	100.	Claussnitzer, M., et al., FTO Obesity Variant Circuitry and Adipocyte Browning in
22		Humans. New England Journal of Medicine, 2015. 373(10): p. 895-907.
23		

Schematics and Tables



3 Schematic 1: diabetes mouse model phenotyping workflow



2 Schematic 2 obesity mouse model phenotyping workflow

Table 1: Spontaneous mutation and caloric excess mouse models

Cause	Strain	Comments	References
Spontaneous mutation in leptin (<i>Lep^{ob}</i>) gene	Obese or <i>ob/ob</i>	 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 (<i>Lepr</i>) gene	Diabetes or <i>db/db</i>	 Similar to <i>ob/ob</i> strain. Infertility. Sex-specific metabolic phenotype. Metabolism may be improved in heterozygotes. Model of rare monogenic obesity. 	[8-10]
Inheritance of single recessive <i>Tannidd1</i> 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]

Table 2: Pancreatic Cre-deleter lines for diabetes research

Cell type	Promoter	Comments	Poforoncos
Endocrine and exocrine pancreas	Promoter Rat insulin promoter (RIP) (also termed Ins2) Pancreatic and duodenal homeobox	 Good recombination efficiency in beta-cells. Expression in various brain regions depending upon strain used. GH minigene may be present. Good recombination efficiency in beta-cells. 	[36, 37, 41]
	1 (<i>Pdx1</i>) (non-inducible)	 Expression in the hypothalamus. Pancreas-wide deletion. GH minigene may be present. 	
Beta-cell Or Endocrine and exocrine pancreas	Pancreatic and duodenal homeobox 1 (<i>Pdx1</i>) (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 (<i>Ins1</i>) (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 (<i>Ins1</i>) (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 (<i>Gcg</i>) (Preproglucagon, Cre)	 Recombination efficiency in alpha-cells has declined since line creation. Poor recombination efficiency (~30%) in enteroendocrine L-cells. Haploinsuffiency due to loss of a preproglucagon (<i>Ppg</i>) gene. 	[25, 36, 43-45].
Alpha-cell L-cell	Glucagon (<i>Gcg</i>) (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 (<i>Gcg</i>) (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].

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]