# Of Rodents and Men: Species-Specific Glucose Regulation and Type 2 Diabetes Research

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**Summary**

*Type 2 diabetes mellitus (T2DM) has reached epidemic proportions worldwide and animal models mimicking human T2DM are widely used to study mechanisms of disease and to develop pharmacotherapeutics. Over the last three decades, rodent models of T2DM have yielded more than 50 publications per month; however, many details of human T2DM pathogenesis remain unclear, and means of preventing disease progression remain elusive. This review investigates the reasons for this translational discrepancy by analyzing the experimental evidence from rodent models of T2DM. The analysis reveals significant species-specific differences at every level of glucose regulation, from gene/protein expression, cellular signaling, tissue and organ to whole organism level, when compared with data acquired using human cells, tissues, organs, and populations. Given the extensive species-specific barrier that creates an immutable translational gap, there is an urgent need to further employ and develop human-based research strategies to make significant strides against the current T2DM epidemic.*

*Keywords: Type 2 diabetes, glucose homeostasis, rodent models, species specificity*

## 1 Introduction

Dysregulation of glucose homeostasis can result in serious health consequences, most prominently type 2 diabetes mellitus (T2DM). T2DM has reached epidemic proportions over the last three decades, and it is predicted that by 2030, more than 366 million people worldwide will have T2DM (Wild et al., 2004). In the United States, the prevalence of T2DM is 26 million with another 79 million considered pre-diabetic, and it is estimated that one in three Americans will have diabetes by 2050 (CDC, 2011). Thus it is imperative to develop strategies to understand, prevent, treat, and, hopefully, cure T2DM. In order to achieve these goals, it is necessary to further our understanding of the pathophysiology of this multifactorial disease.

An abundance of animal models displaying various characteristics of T2DM have been generated to study the underlying pathology and to develop potential treatments. These efforts have created a considerable knowledge base regarding rodent glucose biology, from gene regulation to the maintenance of whole-animal glucose homeostasis. However, the precise molecular and biochemical mechanisms leading to disease pathogenesis in humans remain unclear and treatment methods for humans remain unsatisfactory. Erroneous extrapolations from animal research have resulted in poor translational efficacy at every level from molecular mechanistic findings to phenotypic and natural history findings to the development of effective pharmaceuticals. Despite the wealth of knowledge acquired from rodent studies, only a limited number of anti-diabetic drugs are in clinical use for humans, most of which have adverse health effects, but little impact on disease progression, and none of which cures T2DM or clearly prolongs life.

The purpose of this review is to examine the underlying molecular, biological, and physiological differences – from gene regulation to whole-animal and population levels – that help explain why rodents do not serve as reliable models for studying human T2DM. This review will also address how researchers may overcome this translational barrier by employing a wide range of human-based investigational methods that will promote human-relevant discoveries while reducing – and eventually replacing – the use of animals in T2DM research.

## 2 T2DM: The characteristic pathophysiology in humans

The natural history of human T2DM involves a progressive transition from normal glucose regulation to a pre-diabetic stage characterized by impaired fasting glucose and impaired glucose tolerance to T2DM characterized by overt hyperglycemia resulting from a combination of peripheral insulin resistance and β-cell dysfunction (ADA, 2012; Kahn et al., 2006). Risk factors associated with T2DM in humans include obesity, dyslipidemia, hypertension, lifestyle factors such as physical inactivity and excessive dietary intake, and genetic predisposition (Zimmet et

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al., 2001). T2DM may be foreshadowed by a specific metabolic syndrome that includes abdominal obesity, hypertension, dyslipidemia, impaired fasting glucose, and hypercoagulability. The morbidity and mortality associated with human T2DM – retinopathy, nephropathy, neuropathy, coronary heart disease, peripheral arterial disease, and stroke – derive from dysregulation of glucose homeostasis, in combination with hypertension and dyslipidemia, and the resulting vascular pathologies (Forbes and Cooper, 2013). Elucidating the biochemical mechanistic links among T2DM risk factors, insulin resistance, pancreatic β-cell dysfunction and T2DM sequelae – as these occur in humans – is therefore key to understanding and treating this disease.

## 3 T2DM: Rodent models of human T2DM

A large number of animal models have been generated to study various aspects of T2DM. A PubMed database search focused on rodent models of T2DM revealed that more than 50 articles per month have been published over the last three decades, allowing for possible duplication among studies (Fig. 1). Rodent species have been preferred since they facilitate progression from hypothesis generation to data acquisition with relative ease in a manageable period of time due to factors such as short breeding periods, short lifespan, ease of use for genetic manipulation, low maintenance cost, and ease of handling.

Obese and non-obese rodent models of T2DM have been generated by several methods, including surgical, chemical, dietary and genetic manipulations, and combinations thereof. The standard surgical approach for producing non-obese models of T2DM is partial or complete pancreatectomy, in which the extent of pancreatectomy appears to determine the severity of the resulting condition (Islam and Loots du, 2009). T2DM is chemically induced in rodents using drugs that destroy pancreatic β-cells by mechanisms involving irreversible cytotoxicity (Lenzen, 2008). Nutritional modification has been used to model the metabolic disorders characteristic of human obesity, primarily by increasing the fat content in rodent feed (Lutz and Woods, 2012). Genetic models of rodent T2DM can be broadly categorized into two major groups: spontaneous genetically derived models (naturally occurring mutations such as leptin and leptin receptor mutations) and genetically modified models (transgenic, knock-out, and knock-in models). Transgenic models are designed primarily to address the pathophysiological consequences resulting from global or tissue-specific targeted disruption or overexpression of a defined single gene (or multiple genes) on numerous signal transduction pathways involved in glucose homeostasis (Neubauer and Kulkarni, 2006).

These animal models display various phenotypic manifestations of human T2DM, albeit to varying degrees of disease penetrance, severity, and duration. These features include fasting and non-fasting hyperglycemia, reduced pancreatic mass, reduced glucose-stimulated insulin secretion, decreased serum insulin levels, glucose intolerance, dyslipidemias, and obesity (Chatzigeorgiou et al., 2009). However, most rodent models do not replicate the natural history and pathophysiological mecha-

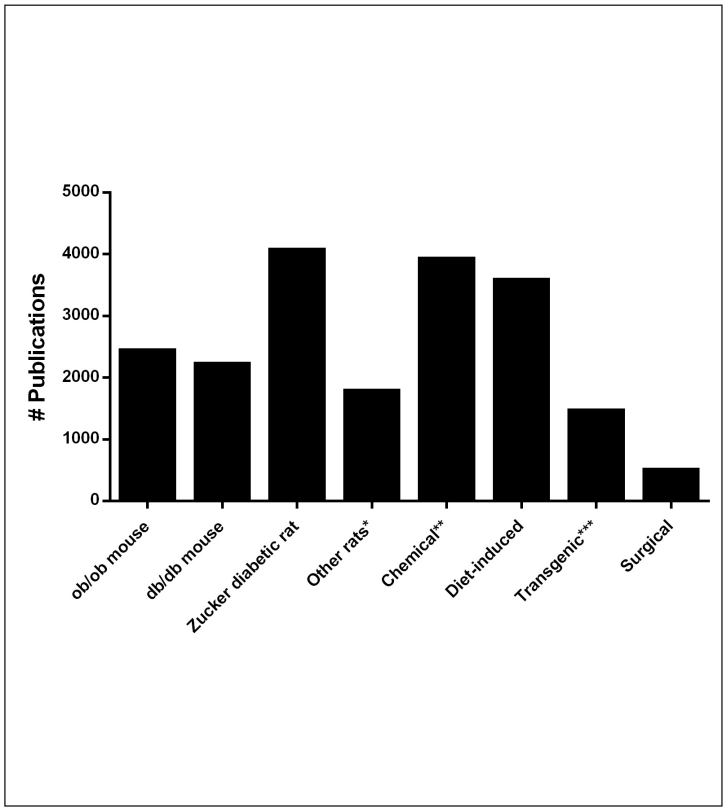


**Fig. 1: Scientific publications based on rodent models of type 2 diabetes**

The PubMed database was searched to determine the number of papers published with rodent models of T2DM with filters "other animals" and "publication dates" (1982-2012). For the data shown, relevant papers were obtained using combinations of specific search terms: "model name", "rodents", and "type 2 diabetes." The mean number of rodent studies published monthly was 56. Please note that this number may vary slightly due to the difficulty in teasing out potential duplication from one category to another. *Other rat models included in this search were GK (Goto-Kakizaki), OLETF (Otsuka Long Evans Tokushima Fatty), and JCR: LA-cp. **Searching for "rodent" diabetes chemically induced with streptozotocin (STZ) and alloxan (ALX) yielded 17,367 and 2,437, respectively, for a total of 19,804 publications. However, STZ and ALX have been used widely to generate models of type 1 diabetes as well. Therefore, random sampling of references was taken in order to estimate the number of papers that utilized STZ to specifically study T2DM. The results indicate that at least 20% of the published work can be assigned to the T2DM category. Thus, the value reported here reflects 20% of 19,804, but the actual number may be higher. ***Searching PubMed with the terms "transgenic" and "type 2 diabetes" yields 1466 papers, but is likely higher since many "knock-out" and "knock-in" models may not have been included.

nisms that lead to the human diabetogenic phenotype, and no individual model or combination of models replicates the complex human T2DM disease state.

## 4 From rodents to humans: Species-specificity of glucose biology

The coordinated function of multiple organs as well as genetic and environmental factors affect β-cell function and tissue insulin sensitivity, all of which contribute to the etiopathology of this polygenic multifactorial disease in humans. Increased use of human-based methodologies over the last decade has significantly broadened our understanding of the species-specificity of glucose biology. It is now apparent that crucial differences exist at every level of glucose regulation, from gene and protein expression and intracellular signaling to tissue, organ, and whole organism manifestations, and extending to population and environment levels (Fig. 2). Given the breadth of this topic and the extensive literature (Fig. 1), the following sections will address interspecies differences only in tissues and organs that play a direct dominant role in the control of glucose homeostasis – endocrine pancreas, skeletal muscle, liver, and adipose tissue.

### 4.1 Glucose biology: Nucleic acid level

Interspecies differences at the nucleic acid level exist at various stages, including but not limited to chromosomal regulation, orthologous and lineage-specific paralogous gene regulation, tissue-specific transcriptional regulation, total gene (mRNA) expression, and post-transcriptional regulation. Humans have only a single copy of the β-cell-specific and glucose-regulated insulin gene, located on chromosome 11 (Owerbach et al., 1980). In contrast, rodents are unique compared to all other mammals in that they express two functional forms of insulin from two non-allelic insulin genes. In mice, they are located on chromosomes 19 (gene 1) and 7 (gene 2), and in rats, both insulin genes are located >100 Mb apart on chromosome 1 (Lomedico et al., 1979; Soares et al., 1985; Wentworth et al., 1986; Davies et al., 1994). In general, rodent insulin gene 2 is thought to be the ortholog of the human insulin gene based on gene structure and sequence homology, but both insulin genes are functionally expressed and regulated in rodents under basal

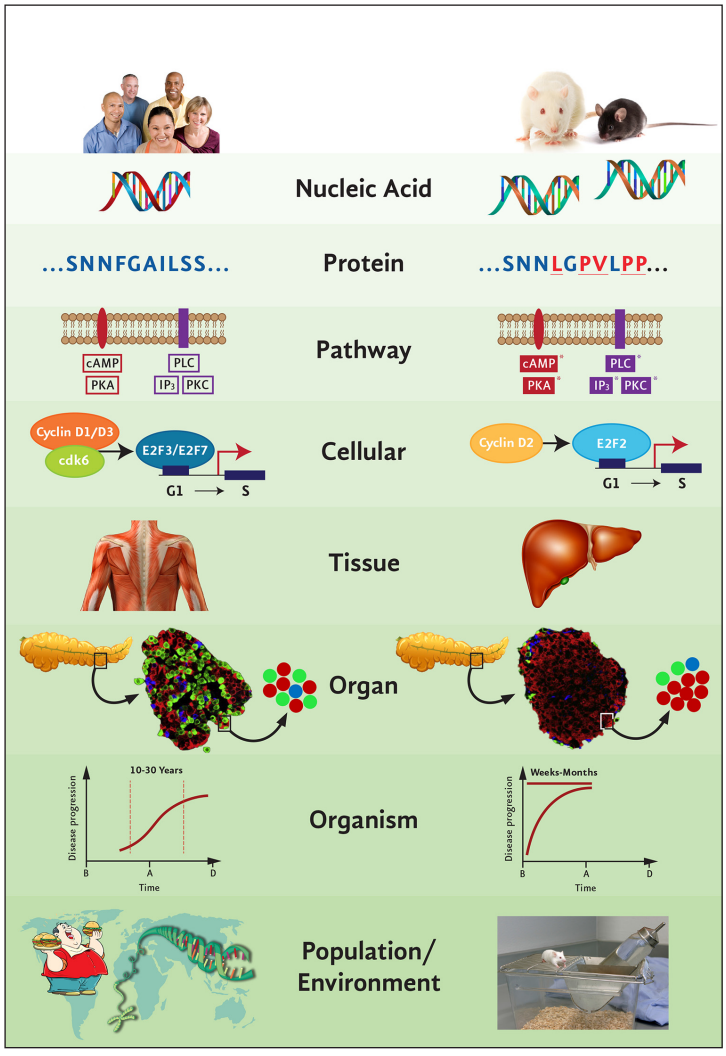


**Fig. 2: Species-specificity of glucose regulation**
This figure illustrates examples of rodent-human species-specific differences that exist at every level of glucose regulation, from nucleic acid to environment level: (1) Nucleic acid level – one insulin gene in humans vs two non-allelic insulin genes in rodents; (2) Protein level – sequence divergence in rodents in the region that confers amyloidogenicity to human islet amyloid polypeptide; (3) Pathway level – species disparity in $K_{ATP}$-independent second phase insulin secretion due to altered signaling via cAMP/PKA and PLC/$IP_3$/PKC pathways; (4) Cellular level – control of β-cell proliferation (G1/S transition) differs in humans; (5) Tissue level – primary site of glucose clearance is skeletal muscle tissue in humans and liver in rodents; (6) Organ level – pancreatic cytoarchitecture is strikingly different and this has functional consequences, as cell-to-cell interactions within the islet markedly vary between humans and rodents; red cells=insulin-secreting beta cells, green cells=glucagon-secreting alpha cells, blue cells=somatostatin-releasing delta cells; (7) Organism level – progressive transition from insulin resistance to overt hyperglycemia over a long period of time in humans versus rapid disease progression and T2DM from birth often observed in rodents (B=birth, A=adult, D=death); (8) Environment level – uncontrolled life style, epigenetic factors in humans versus controlled laboratory environments for rodents. Refer to text for further details. cAMP-cyclic adenosine monophosphate; PKA-protein kinase A; PLC-phospholipase C, $IP_3$-inositol triphosphate; PKC-protein kinase C, $K_{ATP}$-ATP-sensitive potassium channel. Tissue staining images of human and rodent pancreata are reprinted from "*Seminars in Cellular and Developmental Biology*, Vol 24, Caicedo A, Paracrine and autocrine interactions in the human islet: more than meets the eye, pages 11-21 (2012)", with permission from Elsevier.

and high glucose concentrations that stimulate insulin biosynthesis (Cordell et al., 1982; Kakita et al., 1982; Wentworth et al., 1986, 1992; Babaya et al., 2006). In addition to primary rodent β-cells, two functional, glucose-sensitive insulin genes are expressed in the heterologous rodent β-cell line MIN6, which is widely used to model human β-cell function *in vitro* (Roderigo-Milne et al., 2002). Comparative sequence analysis has revealed that the overall homology of the insulin promoter (-600 to +1 region) between humans and rodents is only ~45-48%. Many features of the *cis*-regulatory elements, such as the cyclic AMP response element, negative regulatory element, and CCAAT box, also display marked species specificity (Hay and Docherty, 2006; Boam et al., 1990). The exact and relative contributions of the two insulin genes to rodent glucose regulation remain unclear and often overlooked, thereby limiting data extrapolation to the single insulin gene system in humans. These effects may be further amplified since insulin transcriptionally regulates the expression of more than 150 genes in various tissues (Desvergne et al., 2006).

Species-specific differences also occur at proximal *trans*-acting factor expression and localization, post-translational modifications, DNA binding, and autoregulation. For example, the V-maf musculoaponeurotic fibrosarcoma oncogene homolog (Maf), hepatocyte nuclear factor (HNF), and pancreatic duodenal homeobox 1 (Pdx-1) transcription families play key roles in human and rodent islet and liver transcriptional regulation. However, their expression is differentially regulated in human and mouse islets with respect to spatio-temporal cell type-specific isoform expression and glucose-mediated regulation (Artner et al., 2010; Dai et al., 2012; Hang and Stein, 2011; Dorrell et al., 2011; Harries et al., 2009). Interestingly, mice lacking HNF1a, 1b, and 4a isoforms – genes directly linked to maturity onset diabetes of the young (MODY) in humans – do not emulate human MODY-like phenotypes (Harries et al., 2009).

There is marked divergence in DNA binding between mouse and human glucose regulatory transcription factors. For example, of 4000 orthologous gene pairs tested in human and mouse liver, "*41%-89% of the orthologous promoters bound by a protein in one species were not bound by the same protein in the second species*" (Odom et al., 2007). The location of binding events also varied to an extent that could not be predicted from rodent-human sequence alignments, and transcriptional programs in homologous tissues (at least in the liver) appear to be directed by species-specific gene sequences (Odom et al., 2007; Wilson et al., 2008). Autoregulation can also be species-specific: despite significant sequence homology between mouse and human promoter, the CCAAT/enhancer binding protein alpha (C/EBPα), a transcription factor critical for regulating liver-specific gene expression (including those of gluconeogenic, cell cycle control, and apoptotic genes), employs a different autoregulatory mechanism in mice (direct binding to a *cis* element) as compared to humans (indirect *trans*-acting autoregulation via upstream stimulatory factor) (Schrem et al., 2004; Timchenko et al., 1995).

The above examples provide only a glimpse into the species specificity of the complex gene regulatory networks involved in glucose homeostasis. Although the same families of genes and transcription factors may be involved, differential expression and regulation manifest divergent phenotypes between rodents and humans. With additional regulation by many other factors such as micro RNA (Klein et al., 2013; Lin et al., 2012), chromatin packing and *cis*-regulatory element-specific chromatin signatures (Mikkelsen et al., 2010), multiple long range interactions (Maston et al., 2006; Sanyal et al., 2012), dimerization (Guo et al., 2010; Mendel et al., 1991), post-translational modifications such as phosphorylation (Hang and Stein, 2011; Guo et al., 2010), and cross-talk among signaling systems (Velloso et al., 2006) – all of which can be species-specific – even subtle differences would have dramatic effects *in vivo*. Thus, it is important to consider how the cumulative effects of such fundamental differences across the entire gene regulatory network may profoundly limit interspecies translatability.

### 4.2 Glucose biology: Protein level

Rodent and human β-cells are equipped with glucose transporters and enzymes that play a key role in β-cell glucose metabolism. The principal glucose transporter present in rodent β-cells is glucose transporter 2 (GLUT2), and greatly reduced GLUT2 expression levels (both mRNA and protein) have been shown to correlate with elements of T2DM in various diabetic rodent models such as db/db mouse, Goto-Kakizaki rat, Zucker diabetic fatty rat, and streptozotocin mouse (Thorens et al., 1992; Orci et al., 1990; Johnson et al., 1990; De Vos et al., 1995). Based on such evidence, GLUT2 had been presumed to play the same role in the human islet; however, human islets predominantly express glucose transporters 1 (GLUT1) and 3 (GLUT3) (De Vos et al., 1995; McCulloch et al., 2011), and GLUT2 expression levels do not correlate with human T2DM (Ferrer et al., 1995). In fact, T2DM due to decreased β-cell GLUT2 or defects in GLUT2 may represent only a small subset of humans with T2DM – the well-known inactivating mutations of human GLUT2 are associated with a rare disease of carbohydrate metabolism (Fanconi-Bickel syndrome), which results in significant impairment of hepatic and renal glucose metabolism, but not defective β-cell insulin secretion (Santer et al., 1998, 1997), with a subset of patients displaying transient neonatal diabetes before the clinical manifestations of Fanconi-Bickel syndrome appear (Sansbury et al., 2012). In marked contrast, mice lacking GLUT2 (GLUT2-null mice and mice expressing GLUT2 antisense RNA in β-cells) display altered glucose tolerance, loss of first-phase insulin secretion, inverse α-to-β cell ratio, hyperglycemia, hypoinsulinemia, and increased plasma glucagon, non-esterified fatty acids, and β-hydroxybutyrate levels. GLUT2 null mice die within the first 2-3 weeks of life following severe growth retardation and impaired postnatal pancreatic development (Valera et al., 1994; Guillam et al., 1997). Biochemically, GLUT2 is a low affinity transporter (*Km* ~25 mM) while GLUT1 and GLUT3 are high affinity transporters (*Km* ~1-5 mM) (Bell et al., 1993; Bouche et al., 2004). Functionally, this translates into altered glucose sensing and secretion such that human islets secrete more insulin at lower glucose concentrations than mouse islets (Dai et al., 2012) despite the fact that expression levels of glucokinase (the enzyme that catalyzes the rate-limiting step in β-cell glycolysis) is the same

in rodents and humans (De Vos et al., 1995). These studies indicate that GLUT2 does not play the same key role in the human pancreas as it does in rodents.

The rate-limiting step in human glucose metabolism is insulin-dependent glucose uptake into skeletal muscle, which is facilitated by glucose transporter 4 (GLUT4) being recruited to the plasma membrane in clathrin-coated vesicles. In humans, the heavy chains of the clathrin triskelion are encoded by two distinct clathrin heavy chain (CHC) genes, CHC17 and CHC22 (Kedra et al., 1996). CHC22 is highly expressed in human skeletal muscle, and it is associated with expanded GLUT4 compartments in T2DM patients (Vassilopoulos et al., 2009; Wakeham et al., 2005). Unlike in humans, only a pseudogene exists in mice (Wakeham et al., 2005; Vassilopoulos et al., 2009). In addition, CHC22 appears to be biochemically and functionally distinct with unique protein-protein interactions and transport properties (Wakeham et al., 2005; Brodsky, 2012). For example, CHC22 does not bind the classic adapter proteins or Golgi-localized, γ-ear-containing Arf-binding proteins that CHC17 interacts with, but directly binds to the membrane cargo recognition family member sorting nexin 5 abundant in skeletal muscle through a coiled-coil domain absent in CHC17 (Towler et al., 2004a,b). GLUT4 translocation has been studied extensively in mice with the goal of understanding the role of insulin-mediated glucose uptake in T2DM, but translatability is limited due to species differences in the GLUT4 trafficking pathway.

In terms of pathophysiological features of human T2DM at the protein level, one of the most striking differences in rodents is the absence of islet amyloid deposits. Islet amyloid polypeptide (IAPP) is a 37-amino acid peptide that in humans is co-expressed and co-secreted in a 1:1 ratio with insulin. IAPP and insulin genes have similar promoter elements, and IAPP transcription is regulated by the same transcription factor utilized by insulin promoter in a glucose-dependent manner (Lukinius et al., 1989; Watada et al., 1996; Macfarlane et al., 2000). One of the primary reasons for β-cell apoptosis and decreased β-cell mass in human T2DM is the accumulation of extracellular oligomeric fibrils of IAPP, which disrupt membrane interactions (cell-to-cell adherence and coupling), induce apoptosis, impair insulin secretion, and lead to progressive β-cell failure (Haataja et al., 2008; Ritzel et al., 2007). In contrast, rodent IAPP exists in monomeric form and does not form oligomers or amyloid deposits (Westermark et al., 2011); rodents, therefore, do not fully recapitulate this important aspect of human T2DM islet pathology. The amino- and carboxy-terminals of IAPP display high homology between humans and rodents, but due to the presence of proline residues in the serine-rich region that confers human IAPP its amyloidogenic properties (residues 20-29, Fig. 2), rodent IAPP is not amyloidogenic (Westermark et al., 1990). Many attempts have been made to create amyloidogenic islets in rodents by transgenic expression of human IAPP; however, the results were highly variable with limited similarity to human IAPP pathophysiology (Matveyenko and Butler, 2006). From pancreatic glucose sensing to skeletal muscle glucose transport to loss of β-cell mass, human and rodent proteins (and their biological effects) have diverged significantly to an extent that does not permit reliable interspecies extrapolation.

### 4.3 Glucose biology: Pathway level

Rodent-human species differences are also seen in cellular signal transduction and glucose metabolic pathways. Glucose metabolism via anaplerosis and cataplerosis plays an important role in insulin secretion by rodent pancreatic β-cells, and metabolic enzymes pyruvate carboxylase and ATP citrate lyase play important roles in rodents. However, the levels and activities of the key anaplerotic enzymes pyruvate carboxylase and ATP citrate lyase are reported to be 80-90% and 60-75% lower in human islets compared to rodent islets, respectively (MacDonald et al., 2011). The role of pyruvate carboxylase in insulin secretion in human islets is also different from rodents: human islets are less dependent on pyruvate carboxylation, with only 15% pyruvate carboxylation activity compared to that in rodents. Furthermore, glucose-stimulated human islets form more acetoacetate than rodent islets, and human islets also contain higher levels of other metabolic enzymes such as succinyl-CoA-3-ketoacid-CoA transferase and acetoacetyl-CoA synthetase (MacDonald, 2002). Fatty acid synthase levels in human islets are also higher than in rodent islets (MacDonald, 2002). Glucose-6-phosphatase (G6P) plays an indispensable role in rodent and human liver by catalyzing the final step in gluconeogenesis and glycogenolysis; however, unlike murine G6P, human G6P catalytic subunit gene expression is not subject to modulation by peroxisome proliferator activated receptor gamma coactivator-1 via HNF4a due to a mere 3-base-pair sequence variation (Schilling et al., 2008). Such differences in critical glucose metabolic pathways between rodents and humans have fundamental implications for understanding the regulation of human glucose homeostasis under normal and disease conditions.

Insulin secretion is the end result of a coordinated effort by a host of signaling molecules from various intracellular signaling cascades. The secretory responsiveness characteristic of human perfused pancreas and perifused islets includes biphasic insulin secretion (a rapid robust first phase and a sustained rising second phase) as well as time-dependent potentiation and inhibition of insulin secretion (Nesher and Cerasi, 2002; Grodsky, 1989; Eizirik et al., 1992). In marked contrast, perfused and perifused mouse islets lack the sustained second phase and the time-dependent potentiation and inhibition responses to priming with high glucose. Moreover, mice also differ from rats in this regard, as rat islets readily display biphasic insulin release and time-dependent potentiation/inhibition (Berglund, 1980, 1987; Zawalich and Zawalich, 1996a; Zawalich et al., 1998). This species disparity in ATP-sensitive potassium (KATP) channel-independent second phase insulin secretion can be attributed – at least in part – to altered intracellular signaling pathways involving cAMP (Ma et al., 1995), ATP and protein kinase A (Takahashi et al., 1999), glyceraldehyde (Lenzen, 1979), amino acids (Liu et al., 2003), and phospholipase C/protein kinase C (Zawalich et al., 2000, 2001, 1995; Zawalich and Zawalich, 1996b). Studies with humans have shown that in T2DM, the first-phase insulin secretion is almost abolished and the second-phase insulin secretion is significantly reduced (Del Prato, 2003). Notably, insulin secretion in the conscious mouse is biphasic and pulsatile, and thus it has been argued that the mechanism(s) generating the murine second phase response *in vivo* is lost during *in vitro* islet dis-

sociation (Nunemaker et al., 2006). This would complicate interspecies extrapolation by raising the possibility that mice may contain a labile signaling factor absent in humans and rats.

Pronounced species differences in islet glucose sensitivity can substantially affect drug testing. This may be particularly true for drugs that are targeted to intracellular signaling cascades to improve glucose-dependent insulin secretion. For example, drugs targeted to G protein-coupled receptor 119 and incretins that increase insulin secretion via cAMP/PKA-mediated pathways may exert variable results in rodents. In addition, even subtle changes in the signal transduction network can generate differential effects. For example, the activation of adenosine monophosphate-activated protein kinase by the most widely prescribed anti-diabetic drug, metformin, is more potent in primary human hepatocytes compared to primary rodent hepatocytes despite similar intracellular ratios of adenosine monophosphate and adenosine triphosphate (Stephenne et al., 2011). Taken together, the cumulative effects of alterations in key signaling molecules and their intrinsic properties within the human cellular milieu (further affected by post-translational modifications, subcellular localization, kinetics, feedback mechanisms, and cross-talk, etc.) can result in distinct signaling events in diverse cellular contexts that cannot be reliably extrapolated across the species barrier.

### 4.4 Glucose biology: Cellular level

Pancreatic dysfunction concomitant with a decrease in β-cell mass is a key feature of human T2DM, and therefore much effort has been dedicated to studying β-cell survival and replication for β-cell replacement therapy. There is ample evidence depicting the ability to robustly induce adult murine β-cell replication *in vitro*, but such replication rarely occurs in adult human β-cells (Butler et al., 2003; Parnaud et al., 2008). In the murine β-cell $G_1$/S proteome, the E2F2 protein is abundantly expressed, and loss of E2F2 expression by gene knockout leads to severe pancreatic dysfunction (Iglesias et al., 2004). However, human islets lack E2F2, but contain E2F3 and E2F7 that are absent in murine islets (Fiaschi-Taesch et al., 2009). The cyclin family members involved in cell cycle progression also differ: humans primarily express cyclin D3 with variable amounts of cyclin D1, and little or no cyclin D2, but rodents express and utilize cyclin D2 to an extent that rodents lacking cyclin D2 develop islet hypoplasia, hypoinsulinemia, and diabetes (Fiaschi-Taesch et al., 2010). Similarly, expression of cdk-6 (a protein kinase of the CDK family that is critical for $G_1$ progression and $G_1$/S transition) markedly varies between human and murine islets. Human β-cells express cdk-4 and cdk-6, the latter capable of promoting robust human β-cell proliferation, which is in marked contrast to murine islets that do not express cdk-6 (Fiaschi-Taesch et al., 2010). In terms of cell survival, there are species-specific differences in the role calcineurin plays for β-cell survival: inhibition of calcineurin induces marked human β-cell apoptosis, but only moderates murine β-cell proliferation (Soleimanpour et al., 2010). The translation of β-cell survival and replication in mouse models to β-cell survival and islet transplantation in humans, with a different complex of cell cycle proteins and regulatory pathways, is poor.

The trademark biological function that defines a pancreatic β-cell is its ability to secrete insulin in response to glucose. Recent evidence obtained using human islets suggests that human β-cell stimulus-secretion coupling differs greatly with respect to ion channel composition and function. Both rodent and human β-cells express the voltage-gated $K_{ATP}$ channels, the primary glucose sensors that initiate β-cell stimulus-secretion coupling. Polymorphisms in the $K_{ATP}$ channel subunit genes have been associated with increased risk of T2DM in humans. In addition, loss-of-function mutations in either of the $K_{ATP}$ channel subunits, sulfonylurea receptor type 1 (SUR1) or inwardly rectifying $K^+$ channel ($K_{ir}$ 6.2), cause severe dysregulation of insulin secretion leading to persistent hyperinsulinemic hypoglycemia of infancy (Denton and Jacobson, 2012). Unlike humans, SUR1-ablated mice remain euglycemic with normal insulin secretion in response to feeding (Shiota et al., 2002). This is due, in part, to the activation of a cholinergic response that compensates for the severe defects in glucose-induced insulin secretion mediated by SUR1 (Shiota et al., 2002), but such compensation does not occur in humans with SUR1 inactivation. Similarly, $K_{ir}$ 6.2 null mice have no $K_{ATP}$ channel activity in β-cells, but show only mild impairment in glucose tolerance (Miki et al., 1998). It is instructive that genetic deletion of such genes critical for insulin secretion in mice does not always result in deleterious consequences nor manifest the same way as in humans.

The repertoire of other voltage-gated ion channels involved in stimulus-secretion coupling also differs between mice and humans. Human β-cells express functionally important $Ca^{2+}$-channels that are not thought to be as critical in the mouse β-cell (Braun et al., 2008). For example, R-type $Ca^{2+}$ channels, which play a critical role in mouse β-cells by modulating the kinetics of insulin release and glucose-mediated suppression of glucagon secretion, are not expressed at detectable levels in human β-cells (Jing et al., 2005; Rorsman and Braun, 2013). The expression of voltage-gated delayed rectifier potassium channels that regulate exocytosis and delayed phase ionic current in humans also differs in rodents. Voltage-gated $Na^+$ channels ($Na_v$) are expressed in mouse β-cells, but there is no functional $Na^+$ current at physiological membrane potential (-80 mV) due to voltage-dependent inactivation. In marked contrast, human β-cells carry voltage-gated $Na^+$ current (via $Na_v1.6$ isoform and Nav1.7) important for action potential generation and glucose-mediated insulin secretion (Braun et al., 2008). Functional implications of such discoveries cannot be understated given the diverse roles of ion channels like $Na_v1.7$. For example, $Na_v1.7$ is involved in nociception (Dib-Hajj et al., 2013), and any $Na_v1.7$ antagonists developed for use as analgesics could have serious implications for human patients who face potential impairment of insulin secretion as a side effect, an effect that would not be readily evident in mice since they lack functional β-cell $Na_v1.7$.

The expression and function of plasma membrane receptor and intracellular ion channel complements facilitating voltage-independent insulin release also differ between rodents and humans. Extracellular ATP provides an important autocrine regulatory mechanism that enhances the sensitivity and responsiveness of the human β-cell to glucose (Jacques-Silva et al.,

2010). Human β-cells evoke this response predominantly via ion channel-gated, ATP-selective P2X purinergic receptors, particularly $P2X_3$ subtype (Jacques-Silva et al., 2010). In contrast, rodent β-cells primarily act via biochemically, pharmacologically, and functionally distinct, G-protein coupled P2Y purinergic receptors (Petit et al., 1998; Farret et al., 2004; Leon et al., 2005; Poulsen et al., 1999). Intracellular signaling via the ubiquitous second messenger $Ca^{2+}$ is an important aspect of β-cell stimulus-secretion coupling. Receptor-mediated $Ca^{2+}$-signaling involves the release of $Ca^{2+}$ from intracellular stores through inositol triphosphate ($IP_3$) and ryanodine receptors (RyR), and there is interspecies variation in expression levels and isoforms (Zawalich et al., 2001; Rorsman and Braun, 2013; Zhang et al., 2007; Johnson et al., 2004). In rodents, β-cell intracellular $Ca^{2+}$ generates synchronized oscillations that spread throughout the islet (Valdeolmillos et al., 1989; Santos et al., 1991; Jonas et al., 1998; Poulsen et al., 1999). In contrast, islet-wide synchronized $Ca^{2+}$ oscillations do not occur in humans (Cabrera et al., 2006). Examination of fundamental cellular activities – from human β-cell division to stimulus-secretion coupling to β-cell survival – reveals major species differences in the β-cell machinery and mechanisms.

### 4.5 Glucose biology: Tissue level

Skeletal muscle comprises the primary site of glucose clearance in humans, accounting for 50%-90% of glucose uptake, making it the primary insulin-sensitive tissue and the primary site of dysregulation in human peripheral insulin resistance (Shulman et al., 1990; DeFronzo and Tripathy, 2009; Koistinen and Zierath, 2002; Abdul-Ghani and DeFronzo, 2010). By contrast, liver is the primary site of glucose clearance in rodents, with 5 to 10-fold higher glycogen storage in the liver in rodents versus ~10-fold more glycogen storage in muscle than in liver in humans (Ivy, 1999; Kasuga et al., 2003). This has functional implications since various aspects of glucose regulation differ between human skeletal muscle and rodent liver. For example, human skeletal muscle glucose transport is primarily facilitated by high affinity GLUT4 while rodent liver glucose transport is primarily facilitated by low affinity GLUT2. Glucose transport is the rate-limiting step in human skeletal muscle glucose metabolism whereas the rate-limiting step in rodent liver is glucose phosphorylation (Petersen and Shulman, 2002; Ploug and Vinten, 2006). Moreover, exercise can greatly increase glucose uptake and glycogen synthesis in skeletal muscle in a manner similar to that mediated by insulin, but liver does not display such exercise-mediated glucose transport (Jensen and Richter, 2012).

Impaired glucose trafficking in skeletal muscle is the primary cause of insulin resistance and an important element for development of T2DM in humans. Skeletal muscle tissue from human subjects with T2DM has significantly reduced GLUT4 expression, and real-time evaluation of molecular defects using magnetic resonance spectroscopy has also revealed that insulin resistance in humans can result from decreased insulin-stimulated GLUT4 activity and subsequent glycogen synthesis (Cline et al., 1999; DeFronzo and Tripathy, 2009; Del Prato et al., 1994). In contrast, rodents lacking GLUT4 (global GLUT4-null) do not develop hyperglycemia (Katz et al., 1995). Furthermore, muscle-specific GLUT4 knockouts also show that lack of muscle GLUT4 does not impair glycemic control in mice (Fam et al., 2012). Additionally, unlike in humans, reduced glucose uptake in rodents appears to result not from decreased GLUT4 levels, but rather from impaired GLUT4 translocation and reduced GLUT4 availability at the cell surface (King et al., 1992; Hansen et al., 1998). Interestingly, compared to wild type controls, knockouts lacking muscle-specific glycogen synthase disposed glucose more effectively, and had normal glucose levels and better glucose tolerance (Pederson et al., 2005), further implicating the reliance on other organs for rodent glucose disposal. Perturbation of insulin signaling through tissue-specific deletion of the insulin receptor in muscle does not alter glucose homeostasis in mice – these mice display normal blood glucose levels, serum insulin levels, and glucose tolerance (Bruning et al., 1998). However, liver-specific deletion of insulin receptor causes severe insulin resistance with marked hyperinsulinemia, hyperglycemia, and glucose intolerance in mice (Michael et al., 2000). In addition, liver-specific deletion of insulin receptor substrate 1 and 2 also causes insulin resistance (Kubota et al., 2008).

Obesity is a major risk factor for human insulin resistance and type 2 diabetes. Adipose tissue plays a central role in the regulation of obesity and metabolism by modulating the release of non-esterified fatty acids (increased lipolysis), glycerol, hormones such as adiponectin and leptin, and pro-inflammatory cytokines, and other factors contributing to human insulin resistance (Kahn et al., 2006). Human and rodent adipose tissue regulation has diverged on various fronts from adipogenesis to adipokine secretion. For example, a recent study has shown that major species differences exist in the signaling pathways which promote adipogenesis by modulating the master regulator of adipogenesis, peroxisome proliferator-activated receptor γ (PPARγ). Human adipogenesis appears to be critically dependent on the modulation of the PPARγ axis by glucocorticoid-dependent gene LIM domain only 3 (LMO3). However, mouse LMO3 does not have the same functional consequences due to mutations in the critical glucocorticoid induction site GRE1 in the mouse genome (Lindroos et al., 2013).

Adipocyte PPARγ binding sites also vary between humans and rodents – there is low retention of binding sites between species, and the binding site specificity is also dependent on the context. In humans, binding retention appears to depend on "actual sequence conservation and chromatin context" whereas retention in mice is dependent more on "*vicinity to highly expressed genes, co-binding with C/EBPα, and binding strength*" (Schmidt et al., 2012). Also at variance in adipose tissue signaling is the regulation of adipocyte glucose sensing and systemic glucose metabolism via the adipokine retinol binding protein-4 (RBP4). Expression and regulation of adipose and circulating RBP4 markedly varies between human and mouse obesity (Janke et al., 2006). Taken together, species specificity – from adipose tissue gene expression to adipokine regulation – can affect translatability from mechanistic findings to drug development. PPAR isoforms have been the target of the thiazolidinedione class of anti-diabetic drugs, and the combined effect of these species differences may contribute to the lack of efficacy and the various drug toxicities seen in humans.

### 4.6 Glucose biology: Organ level

For decades it had been assumed that human pancreatic islet cytoarchitecture is similar to that in rodents. While human and rodent islets contain the same cell types, in rodent islets, there is clear segregation of different cell types characterized by predominant β-cells (70-80%) clustering in the center with a characteristic "mantle-core" pattern, in which the core of β-cells is surrounded by a mantle of α-cells (along with other islet cell types, δ and γ) localized to the periphery of the islet (Baetens et al., 1979; Bennett et al., 1996; Ku et al., 2002). Comprehensive analysis of human pancreatic islet composition by confocal laser scanning microscopy and multiple immunofluorescence labeling has shown that human islet cytoarchitecture is strikingly different. Human α-cells are not discretely localized to the periphery, and the vast majority of human β-cells intermingle with all other cell types compared to the homotypic associations prominent in mouse β-cells (Brissova et al., 2005; Cabrera et al., 2006; Kim et al., 2009), other than in one study that identified some small human islets displaying mouse-like segregation (Bosco et al., 2010). The relative islet cell composition also varies between rodents and humans. Image analysis of human islet optical sections and morphometric analysis of discrete histological sections have shown that human islets contain (with slight variations among studies) approximately 50-60% β cells, 30-40% α cells, and 10% δ cells in contrast to >70% β cells, <20% α cells, and <10% δ cells in rodents (Brissova et al., 2005; Cabrera et al., 2006).

Islet architecture also differs with respect to vasculature and innervation. Human islets have a more prominent intra-islet vasculature with higher smooth muscle content. In contrast, rodent islet vasculature is primarily composed of endothelial tubes, with only a few arterioles containing smooth muscle cells (Bonner-Weir and Orci, 1982; Rodriguez-Diaz et al., 2011a). Islet β-cells in mice directly interact with vascular endothelial cells, which contain a single basement membrane. In contrast, human capillary endothelia have two separate sheaths of basement membranes, generating altered molecular interactions between islet and endothelial cells (Otonkoski et al., 2008).

It has long been known that rodent islets are densely innervated with sympathetic, parasympathetic, and sensory nerves with less innervation in the exocrine pancreas (Ahren, 2000). In contrast, human islets display sparse innervation with only a few neuronal axons penetrating the islet (and only in discrete regions within the islet), while much denser innervation is present in the exocrine pancreas (Rodriguez-Diaz et al., 2011a; Caicedo, 2012). In mouse islets, sympathetic fibers preferentially innervate α-cells while parasympathetic fibers innervate α- and β-cells equally. In contrast, the sympathetic axons do not innervate or contact human islets directly – they innervate contractile "*vascular smooth muscle cells deep inside human islets*" (Rodriguez-Diaz et al., 2011a). Thus, in humans, sympathetic nerves regulate islet function indirectly by controlling blood flow or by acting on islet regions located downstream, whereas mouse islets are directly innervated by the autonomic nervous system (Rodriguez-Diaz et al., 2011a), introducing a different set of mechanisms to regulate islet function and subsequent glucose metabolism.

Morphological differences translate into functional differences between human and rodent islets at many levels. For example, in human islets, α-cells appear to exert stronger influence on β-cells compared to rodent islets due to the higher proportion of α-cells and their unstructured association with β-cells. In the human islet, α-cells store, exocytose, and secrete acetylcholine via the vesicular acetylcholine transporter under lower glucose concentrations, and this endogenous acetylcholine in turn "primes" the juxtaposed β-cells for fluctuations in glucose levels by amplifying β-cell-specific glucose-induced insulin secretion (Rodriguez-Diaz et al., 2011b). With only a small number of α-cells juxtaposed to β-cells, this paracrine non-neural cholinergic activity of human islets cannot be modeled in rodent islets. Instead, mouse islets are densely innervated by cholinergic axons, and acetylcholine exerts direct neural effects on insulin secretion via M3 muscarinic receptors expressed on β-cells (Gilon and Henquin, 2001; Gautam et al., 2007).

The cumulative effects of islet architecture and signaling appear to identify human islets as unique. Human islets have a different set-point for glucose sensing, with a lower threshold for insulin secretion (Rorsman and Braun, 2013). The magnitude of glucose-induced insulin secretion is much lower in human islets (3-fold) compared to rodent islets (30-fold) (Dai et al., 2012). Of note, the insulin secretory capacity differs not only between humans and rodents, but also among rodent species and strains (Lenzen, 1979). In addition to insulin secretion, glucagon secretion and subsequent effects also differ among species, and postulations regarding "glucagonocentric" diabetes should be based solely on human studies, as "*[important] species differences in the α-cell stimulus secretion coupling as well as in the relative importance of the different components of the signaling networks have significantly hampered our ability to propose a unifying hypothesis for regulation of glucagon secretion*" (Gromada et al., 2007). With 70-80% of β-cells juxtaposed to α-cells in humans, intra-islet insulin secretion prevents hyperglucagonemia, and unregulated α-cells hypersecrete glucagon, thereby generating a diabetic phenotype (Unger and Cherrington, 2012), whereas these interactions are different in rodents with different pancreatic cytoarchitecture (cell-to-cell interactions and innervation, as described above). Taken together, these findings indicating marked differences in islet architecture and function raise heretofore under-appreciated concerns regarding the species specificity of glucose biology and subsequent translatability, especially with respect to treatment modalities for human T2DM. These differences may partly explain why many treatments shown to reverse diabetes in rodents have not translated to humans.

### 4.7 Glucose biology: Organism level

One hallmark feature of human T2DM is the progressive transition from pre-diabetic metabolic dysfunction to insulin resistance and reduced β-cell compensation and failure, eventually leading to T2DM with overt hyperglycemia (Kahn et al., 2006). Due to the nature of rapid experimental induction of dysglycemia, insulin resistance does not precede hyperglycemia in many rodent models, and overt hyperglycemia can be present without elevated plasma insulin or insulin resistance (Srinivas-

an and Ramarao, 2007). Most rodent models do not allow the researcher to control the onset or the severity of insulin resistance, and the inability to accurately model human disease progression becomes problematic, especially when developing therapies for the early phase of human T2DM. Systemic glucose regulation is also subject to further modulation by cross-talk among parallel systems. For example, estrogen exerts various effects on skeletal muscle, liver, adipose tissue, and cells of the immune system; and estrogen action on the pancreas can regulate insulin secretion (Mauvais-Jarvis et al., 2013). Such interactions introduce another level of species-specific differences, hindering rodent-to-human extrapolation.

Human T2DM is associated with vascular complications, which start developing long before the clinical diagnosis of overt hyperglycemia. One of the primary limitations of rodent models of T2DM is that they either lack these complications altogether or do not mirror the human etiopathology accurately. Cardiovascular complications remain the leading cause of morbidity and mortality in human T2DM, with accelerated atherosclerosis being the dominant underlying mechanism. It is well-known that an altered lipid profile, characterized by elevated total cholesterol and low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL), and other factors such as inflammation, oxidative stress, and insulin resistance contribute to the chronic complex process of atherosclerosis in humans (Van Gaal et al., 2006). However, rodents are resistant to atherosclerosis in general – atherosclerosis is typically absent or very mild unless specific atherogenic manipulations are enforced with diet and/or genetic modifications (Pellizzon, 2008; Ishibashi et al., 1994; Coleman et al., 2006; Daniels et al., 2012). Rodents have highly effective lipid clearance and a notably different anti-atherogenic lipid profile where HDL is the major lipoprotein in circulation rather than LDL. Mouse strains differ considerably in their susceptibility to atherosclerosis, and some strains resist atherosclerosis even when fed atherogenic diets (Pellizzon, 2008; Galman et al., 2007; Ishida et al., 1991; Nishina et al., 1994; Jiao et al., 1990; Mu et al., 1999).

Furthermore, platelet aggregation, a major component of the human atherosclerotic process, is not a critical factor in rodent models. Notably, one of the most widely used mouse models of T2DM, the db/db model, which has generated more than 2,300 publications over the last three decades (Fig. 1), does not readily develop atherosclerotic lesions despite obesity, hyperlipidemia, and cardiomyopathy (Belke et al., 2004). The db/db mouse develops atherosclerosis only on a highly atherogenic diet or when crossed into a vulnerable genetic background such as apolipoprotein E-deficient mice (Wendt et al., 2006). However, such manipulations do not follow the same etiology as in humans. In addition, the method of T2DM induction in rodents can often induce irrelevant cardiovascular abnormalities such as decreased blood pressure and resting bradycardia observed in streptozotocin-induced rodent T2DM models (Hicks et al., 1998). In terms of other diabetic complications, most rodent models lack overt degenerative pathological alterations seen in human diabetic neuropathy, which include segmental demyelination and axon loss (Sharma and Thomas, 1974). Similarly, rodents may develop early stages of diabetic retinopathy, but intravitreal neovascularization leading to blindness in humans is not seen in any rodent model of T2DM (Robinson et al., 2012). Instead of diabetic complications inherent in humans, rodents tend to develop other secondary – often lethal – conditions such as ketoacidosis, growth retardation, and neonatal mortality, which are absent in human T2DM (Srinivasan and Ramarao, 2007).

Rodents also develop unrelated pathophysiological complications that arise due to the technique of induction. For example, induction of T2DM using the toxic glucose analogues streptozotocin and alloxan also results in extrapancreatic genotoxic and cytotoxic effects including disruption of the hypothalamic-pituitary-gonadal axis (Szkudelski et al., 1998; Thliveris et al., 1984), and therefore it is often not possible to segregate the effects caused by pancreatic cytotoxicity versus those effects stemming from extrapancreatic sites. Moreover, with many rodent models, the presence of phenotypic features from birth makes it difficult to distinguish between developmental effects versus experimentally induced effects. This is particularly true for gene deletion studies, since it is often not possible to differentiate the effects of gene deletion on development from the effects attributable to redundant compensatory mechanisms.

Rodent data also vary markedly due to factors such as species, strain, age, gender, and mode of T2DM induction. The common inbred mouse strains used in T2DM research display considerable strain-dependent variability in whole-body glucose metabolism, such as insulin secretion and action, and counter-regulatory responses. The impact of genetic background on glycemic control has been reported in many studies (Kulkarni et al., 2003; Almind and Kahn, 2004; Berglund et al., 2008), and the susceptibility to diabetes varies among even the most closely related strains such as C57BL/6J and C57BLKS/J (Mu et al., 1999). Gender differences also exist at the species and strain levels to the extent that in some models female disease penetrance is nil. These rodent models also vary in the age of disease onset, disease penetrance, disease severity, and duration of phenotypic manifestations – from the absence of the main T2DM metabolic features such as hyperglycemia and insulin resistance to the presence of only one such feature to reversion of metabolic abnormalities. The loss of phenotype over time also has been reported, for example, in the β-cell specific insulin receptor knockout models (Kulkarni et al., 2003). Such high variability in the most basic parameters of glucose regulatory mechanisms consequently yields data that are unreliable for humans and also for the *Mus* and *Rattus* lineages.

Rodent data extrapolation is further complicated by experimental challenges associated with techniques such as the hyperinsulinemic-euglycemic clamp used for measuring various parameters of glucose regulation in rodents (Ayala et al., 2006). In addition, administration of glucose via the intraperitoneal route averts the incretin response known to potentiate glucose-mediated insulin secretion in humans. Even the techniques utilized for pancreatic imaging cannot be compared directly between rodents and humans. For example, radiolabeled tetrabenazine analogues are used for imaging human

β-cell mass, as these ligands selectively bind to the vesicular monoamine transporter 2 (VMAT2) abundantly expressed in human pancreas, but absent in rodent endocrine pancreas (Schafer et al., 2013).

### 4.8 Glucose biology: Population and environment level

It is axiomatic that various environmental factors influence human T2DM development and predisposition. In general, lifestyle choices such as diet and physical activity are superimposed on genetic predisposition and other risk factors. The effect of high-fat diet has been studied extensively in various rodent models of T2DM, but the data have been highly variable. Most rodents tend to become obese on a high fat diet, but there has been considerable variability in weight gain, glucose tolerance, insulin resistance, serum triglycerides, and various other parameters, even among strains (Buettner et al., 2007; Rossmeisl et al., 2003). In addition, regardless of dietary content, some strains have higher fasting plasma glucose levels and lower fasting plasma insulin levels (Andrikopoulos et al., 2005). Changes in dietary composition (fatty acids, carbohydrates, proteins) has also led to considerable variability in rodent studies with some strains being more susceptible to weight gain on a high-fat diet and others on a high-carbohydrate diet (Smith et al., 1997; Warden and Fisler, 2008; Buettner et al., 2006). Some mouse strains are altogether resistant to high-fat diet-induced pathological changes (West et al., 1992). Dietary modifications are often combined with other manipulations, such as gene modifications and chemical inductions, further exacerbating rodent-model variability and correlation with human T2DM pathophysiology. It is clear that for rodents it is not possible to define either an "ideal" high-fat diet or the metabolic perturbations based on dietary composition. Conversely, acute and long-term human studies yield species-specific data indicating that improper diet and sedentary lifestyle contribute to the human metabolic syndrome and T2DM (Wang et al., 2013; Pan et al., 1997; Bienso et al., 2012).

Genetic predisposition is also a risk factor contributing to human T2DM. Human gene linkage analysis, candidate gene approaches, and genome-wide association studies have yielded several single nucleotide polymorphisms (SNP) possibly related to T2DM risk, but they vary greatly among different populations and may not be reproducible or individually significant (Amato et al., 2009). Using information obtained from human linkage analysis to generate transgenic animals has typically not yielded results relevant to humans (Harries et al., 2009). It is evident that no single gene is responsible for a particular phenotype. Rather, the collective actions of multiple genes contribute to the overall pathogenesis of human T2DM. It is interesting that the two most commonly studied genetic-derived rodent models of T2DM (*ob*/*ob* and *db*/*db*), with more than 4,500 publications during the last three decades (Fig. 1), are the models with leptin and leptin receptor mutations. However, leptin or leptin receptor deficiency is rarely associated with human T2DM (Gibson et al., 2004), and it is difficult to derive genetic information relevant to human disease etiopathogenesis from such models.

Epigenetic factors that mediate gene-environment interactions are also important. There are links among diet/nutrients, obesity, energy metabolism, physical activity, and gene expression and regulation such as DNA methylation and histone modifications. For example, genetic background (family history of T2DM) and exercise can alter expression and DNA methylation of several genes involved in skeletal muscle glucose regulation (Nitert et al., 2012). Adding another layer of complication is the recent identification of the effects of regulatory small RNAs and micro RNAs on gene regulation and T2DM complications (Kantharidis et al., 2011). Though humans and rodents have approximately the same number of genes, it is in large part the differences in gene structure and regulation that give rise to two completely different organisms (Dunham et al., 2012). It is also noteworthy that lab animals fed *ad libitum* are inbred for many generations and thus many genes and phenotypes are enriched for certain traits that may not be relevant to T2DM pathophysiology even for that species or strain. Therefore, data obtained from rodents cannot be extrapolated directly to humans with respect to genetic and epigenetic influences.

Also, rodents housed in controlled environments without exposure to toxins, environmental hazards, and other factors causing cellular stress in humans do not recapitulate the human condition (Murea et al., 2012). The standard laboratory environment also has negative impacts on rodents. Mice are generally housed at 18-22°C, which is well below their preferred and critical thermoneutrality temperature of ~30°C (Gaskill et al., 2012). These suboptimal temperatures exert chronic thermal stress, and rodents try to maintain thermoneutrality by increasing metabolism via increased food intake by as much as 50% (Lodhi and Semenkovich, 2009; Cannon and Nedergaard, 2011). Even standard control rodents used in research do not serve as appropriate controls since they are "*metabolically morbid…sedentary, obese, glucose intolerant, and on a trajectory to premature death*" (Martin et al., 2010). These factors have a tremendous potential to skew interspecies extrapolation, particularly in obesity, metabolism, and T2DM fields.

Taken together, data obtained from rodents are affected by many different factors ranging from gene structure to gene expression, organ function to environment. Despite the evolutionarily conserved genes and biochemical pathways humans may have in common with rodents, the cumulative differences and overall integrative physiological processes which account for glucose regulation are markedly different.

## 5 T2DM: Bridging the translational gap by "humanizing" research

Despite the wealth of knowledge regarding mechanisms of glucose regulation in rodents, only a limited number of anti-diabetic drugs are currently available for humans, most of which have little impact on disease progression and outcomes. Erroneous extrapolations from animal models have resulted in poor translational efficacy in the development of effective pharmaceuticals. Perhaps more concerning are serious adverse effects associated with widely used T2DM drugs. Thiazolidinedione

drugs such as troglitazone, pioglitazone, and rosiglitazone have caused liver disease, myocardial infarctions, and heart failure (Scheen, 2001; Smith, 2003; Taylor and Hobbs, 2009; Nissen and Wolski, 2010). The dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin and glucagon-like peptide-1 (GLP-1) mimetics exenatide and liraglutide have been suggested to increase risks for pancreatitis and pancreatic cancer (Elashoff et al., 2011; Butler et al., 2013). These drugs tested safe and effective in rodents, but deleterious consequences resulted in humans. Identifying drug targets and disease mechanisms in rodent models has often prompted researchers to develop treatments based on those optimistic findings, but this may produce effective drugs for humans only if the same targets, mechanisms, and effects are at work in the human disease. Regardless of the method used to induce T2DM-like phenotypes in rodents, it is evident that immutable differences at the molecular and physiological levels of glucose regulation in rodents and humans severely restrict reliable translation. Therefore, future research efforts should focus on increasing the utility of already available methods and the development of new technologies that are more human-based and directly human-relevant. A detailed discussion of this topic is beyond the scope of this review, but a brief discussion follows.

Human-based data can be acquired at various levels ranging from *in vitro* and *in vivo* technologies to population studies. *In vitro* technologies utilizing human heterologous cell lines (e.g., transformed cell lines and human mesenchymal stem cells), human primary cells and tissues (e.g., from biopsies, surgeries, cadavers), and explanted or donated human organs can be used to study various aspects of T2DM ranging from gene expression to organ function. Functional heterologous human β-cell lines have not been available to researchers to date, but a robust novel technology involving targeted oncogenesis in human fetal tissue holds promise for making functional β-cell lines as well as other human cell lines from cell types for which cell-specific promoters are readily available (Ravassard et al., 2011). With respect to primary cells, it is important to utilize human primary cells since physiological differences between rodent and human primary cells can interfere with signal transduction and drug responses, as has been reported for primary hepatocytes (Kotokorpi et al., 2007). Using these human cells and tissues, molecular factors involved in signal transduction can be identified with high-throughput microarray techniques (Mei et al., 2010) and signaling pathway protein arrays. Human islets cultured for up to 7 days have been used successfully for high-throughput drug screening (Walpita et al., 2012). Integrating data from multiple sources such as high-density SNP genotyping, microarray data, proteomic and metabolomic data will prove especially useful.

Organotypic 3D cell culture techniques can be adapted to assess biological processes in an environment more consistent with the *in vivo* environment. For example, upcyte® Hepatocyte 3D Culture System[1] offers an environment that mimics the *in vivo* cellular architecture of the liver (Barrila et al., 2010). Isolated primary cells can be used immediately for acute assays or cultured as appropriate for chronic studies. Many aspects of human β-cell function have already been studied in this manner, as discussed in preceding sections. Using co-culture systems with media-exchange perfusions, interactions among multiple cell types as well as paracrine interactions can be assessed using techniques similar to the "Quasi-Vivo® system" [2]. Events such as paracrine signaling can also be detected in real time using biosensor cells in the vicinity of isolated human islets (Rodriguez-Diaz et al., 2012). Minimally invasive techniques involving phlebotomy and muscle biopsies can be utilized for various biochemical and systemic regulation analyses (Chavez et al., 2009; Zierath et al., 2000), and gene-silencing studies can be extended to human skeletal muscle samples (Austin et al., 2008; Bouzakri and Zierath, 2007).

The use of human organs *ex vivo* facilitates the integration of cellular data to the organ level. For example, explanted hearts from human patients have been utilized by the Langendorff method to examine electrical activity and contractility, and these data have shown divergence from data obtained in mice (Nanthakumar et al., 2007; Fedorov et al., 2011). Sophisticated organ culture systems like the IdMOC (integrated discrete multiple organ co-culture) allow the researcher to mimic the whole-body systemic interactions affecting various organs as well as paracrine interactions (Li, 2009). Emerging tools such as the "organ-on-a-chip" technology can be utilized for further organ-based analyses (disease modeling and drug testing), and will greatly reduce the reliance on animal-based research in the future (Huh et al., 2012; Mowatt, 2012b). Future improvements on such technologies to include "multiple organs-on-a-chip" and "human-on-a-chip" (Mowatt, 2012a) will further facilitate human-based data acquisition.

Whole-animal studies can be done with human subjects using noninvasive or minimally invasive techniques. Positron emission tomography (PET), magnetic resonance imaging (MRI), functional MRI, and advanced ultrasound techniques have emerged as useful noninvasive research methods in recent years. For example, $^{18}$F-FDG (fluorodeoxyglucose) has been used successfully with PET to study how insulin stimulates glucose uptake in the human liver (Bertoldo et al., 2006; Iozzo et al., 2003; Roden et al., 2001). The ability to monitor glucose and insulin real-time with advanced β-cell imaging techniques offers valuable insights into human glucose biology (Kilimnik et al., 2011; Ahlgren and Kostromina, 2011). Human epidemiological studies have shown that it is possible to obtain clues to pathological complications in diabetes, for example by measuring retinal vascular caliber from photographs of human retinas (Robinson et al., 2012). Human population studies will likely prove invaluable for identifying T2DM susceptibility genes, for drug testing, and for characterization of other aspects of T2DM such as environmental and lifestyle factors. Evaluation of epigenetic interactions in glucose regulation can also be studied in T2DM patients (Yang et al., 2012; Volkmar et al., 2012).

[1] http://www.medicyte.com/productsservices/human-upcyte-hepatocytes/3d-cell-culture-kit.html

[2] http://kirkstall.org/index.php/quasi-vivo-system/

Integration of information derived from human-based methods can also be facilitated by computational modeling. Systems biology approaches to studying interactions among various biological molecules (and drugs) can be predicted through advanced computer simulations and modeling. For example, Dalla Man et al. (2007) developed an *in silico* model of the glucose-insulin system to monitor postprandial glucose regulation. This physiologically based model was generated using quantitative information such as plasma glucose concentration, glucose and insulin fluxes previously obtained from normal and T2DM human subjects, and this guided the development of closed-loop glucose control via implantable insulin pumps. Based on the predictive value of the "artificial pancreas control algorithms" computational model, the FDA approved closed-loop insulin pumps as a substitute for animal testing (Dalla Man et al., 2007; Kovatchev et al., 2009; Patek et al., 2009). This is merely one example that "*realistic computer simulation is capable of providing invaluable information about the safety and the limitations of closed-loop control algorithms, guiding clinical studies, and out-ruling ineffective control scenarios in a cost-effective manner*" (Kovatchev et al., 2009). Therefore, much effort should be dedicated to developing and using such alternative technologies guided by human-based data acquisition methods.

## 6 Human T2DM: What the future holds

This analysis of rodent model translation in T2DM indicates that cumulative differences from gene expression to cellular signaling to phenotypic expression to environmental impact involved in glucose homeostatic and regulatory characteristics render rodent research results unreliable for human T2DM characterization, prevention, and treatment. While decades of T2DM research efforts have elucidated the details of rodent glucose regulation, the critical knowledge base that is lacking is a detailed understanding of the mechanisms underlying *human* glucose homeostasis, obesity, insulin resistance, and β-cell dysfunction as well as their sequelae and responses to interventions in human T2DM.

Incompatible findings from rodent models have raised concerns in other medical fields, including type 1 diabetes, regarding the usefulness of rodent models to study human diseases (Roep et al., 2004; Seok et al., 2012; Mestas and Hughes, 2004; Demetrius, 2005). Researchers are now questioning the validity of rodent models to study human glucose biology, with many lamenting that "*clinical investigations have fallen out of favor with funding agencies and with reviewers and scientists*" and that "*[w]e need to refocus and adapt new methodologies for use in humans to understand disease biology in humans*"(Caicedo, 2012; Bell, 2013; McManus, 2013). However, "*[the] rodent islet model is so ingrained that there is a reluctance to view discrepant findings as real differences*" (Caicedo, 2012). With the T2DM epidemic at hand, it is vital to consider whether rodent models provide reliable, reproducible, and translatable insights into human T2DM mechanisms and therapeutics.

Research and clinical advances in the field of T2DM require a paradigm shift, focusing on the use of human-based methods that can greatly improve bench-to-bedside success. Decades of research have made it clear why *a priori* application of rodent data to humans is inappropriate and why human-based data must go from being anecdotal to systematic frontline evidence. Therefore, scientists and funding agencies should prioritize human-based strategies to study human T2DM characteristics and therapeutic options. This is the clear path to deal with the global epidemic of T2DM.

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## Declarations/conflict of interest

None

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