

REVIEW

Current views on type 2 diabetes

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Abstract

Type 2 diabetes mellitus (T2DM) affects a large population worldwide. T2DM is a complex heterogeneous group of metabolic disorders including hyperglycemia and impaired insulin action and/or insulin secretion. T2DM causes dysfunctions in multiple organs or tissues. Current theories of T2DM include a defect in insulin-mediated glucose uptake in muscle, a dysfunction of the pancreatic β -cells, a disruption of secretory function of adipocytes, and an impaired insulin action in liver. The etiology of human T2DM is multifactorial, with genetic background and physical inactivity as two critical components.

The pathogenesis of T2DM is not fully understood. Animal models of T2DM have been proved to be useful to study the pathogenesis of, and to find a new therapy for, the disease. Although different animal models share similar characteristics, each mimics a specific aspect of genetic, endocrine, metabolic, and morphologic changes that occur in human T2DM. The purpose of this review is to provide the recent progress and current theories in T2DM and to summarize animal models for studying the pathogenesis of the disease.

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Introduction

It is estimated that diabetes affects about 150 million people worldwide, and this figure is expected to be doubled in the next 20 years (Zimmet *et al.* 2001). About 90–95% of all North American cases of diabetes are type 2 diabetes mellitus (T2DM), and about 20% of the population over the age of 65 has T2DM (Zimmet *et al.* 2001). About 5–10% of the total health care budget has been used for T2DM in many countries. T2DM may result in severe complications, including renal failure, blindness, slow healing wounds, and arterial diseases. Here, we review the pathophysiology of T2DM and the major animal models of T2DM.

Diabetes mellitus is often simply considered as diabetes, a syndrome of disordered metabolism with abnormally high blood glucose levels (hyperglycemia) (Tierney 2002). The two most common forms of diabetes are type 1 diabetes (diminished production of insulin) and type 2 diabetes (impaired response to insulin and β -cell dysfunction). Both lead to hyperglycemia, excessive urine production, compensatory thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism.

T2DM is a complex heterogeneous group of metabolic conditions characterized by increased levels of blood glucose

due to impairment in insulin action and/or insulin secretion (Das & Elbein 2006). Physiologically, the pancreatic β -cells constantly synthesize insulin, regardless of blood glucose levels. Insulin is stored within vacuoles and released once triggered by an elevation of the blood glucose level. Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells, including skeletal muscle cells and adipocytes. Insulin is also the major signal for conversion of glucose to glycogen for internal storage in liver and skeletal muscle cells. A drop in the blood glucose level results in a decrease in release of insulin from the β -cells and an increase in release of glucagon from the α -cells, which stimulates the conversion of glycogen to glucose. Following an overnight fast, glucose is largely produced by glycogenolysis and gluconeogenesis.

There are three key defects in the onset of hyperglycemia in T2DM: increased hepatic glucose production, diminished insulin secretion, and impaired insulin action (DeFronzo *et al.* 1992, Stumvoll *et al.* 2005). Insulin resistance refers to suppressed or delayed responses to insulin. Insulin resistance is generally 'post-receptor', which refers to a problem with the cells that respond to insulin rather than a problem with insulin production. Some rare causes of diabetes include pregnancy, certain medications, or diseases such as maturity onset diabetes in the young (MODY).

Recent advances in insulin signaling and glucose transport

Insulin signaling

Human insulin receptor, a heterodimer, is composed of two extracellular α -subunits and two transmembrane β -subunits (White & Kahn 1994). The α -subunit contains the extracellular ligand-binding domain that regulates intracellular tyrosine kinase activity of β -subunits (Sciaccia *et al.* 2003, McKern *et al.* 2006, Ward *et al.* 2007). Mammalian insulin receptor gene has 22 exons that generate two isoforms by alternative splicing of exon-11, the a-isoform (IRa) retains exon-11 and the b-isoform (IRb) omits exon-11. IRb binds to insulin strongly and predominates in classical insulin-sensitive target tissues such as adult liver, muscle, and adipose tissues. By comparison, IRa binds both insulin-like growth factor 2 (IGF2) and insulin with moderate affinity and predominates in fetal tissues, the adult central nervous system, and hematopoietic cells (Louvi *et al.* 1997). Insulin and IGF receptors reside in the plasma membrane as inactive covalent dimers (Schlessinger 2000). The ligand binding increases flexibility of the activation loop to allow ATP to enter the catalytic site and stabilize the activation loop in the active

conformation by autophosphorylation (Hubbard *et al.* 1994). In higher animals, activated insulin receptor phosphorylates the tyrosine residues in cellular substrates, including insulin receptor substrate (IRS)1, IRS2, IRS3, or IRS4, or other scaffold proteins. Data from transgenic mice suggests that many insulin responses are mediated through IRS -1 or -2 (White 2003).

IRS proteins have a pleckstrin homology (PH) at the NH₂-terminal and phosphotyrosine-binding (PTB) domains, followed by a tail of tyrosine and Ser/Thr phosphorylation sites. The PTB domain binds directly to the phosphorylated NPXY motif in the activated receptors for insulin, IGF1, or interleukin-4 (IL4); the PH domain also facilitates coupling between the IRS proteins and the activated receptors. The tyrosine phosphorylation sites coordinate downstream signaling cascades by binding to the SH2 domains in common effector proteins – including enzymes (phosphoinositide 3-kinase, the phosphatase SHP2, or the tyrosine kinase fyn) or adapters (GRB2, NCK, CRK, SHB, and others). The classic insulin-like signaling cascades involve the production of PI(3,4,5)P₃ by the phosphatidylinositol 3-kinase (PI3K; Fig. 1). PI(3,4,5)P₃ recruits the Ser/Thr kinases phosphatidylinositol-dependent protein kinase 1 (PDK1) and AKT

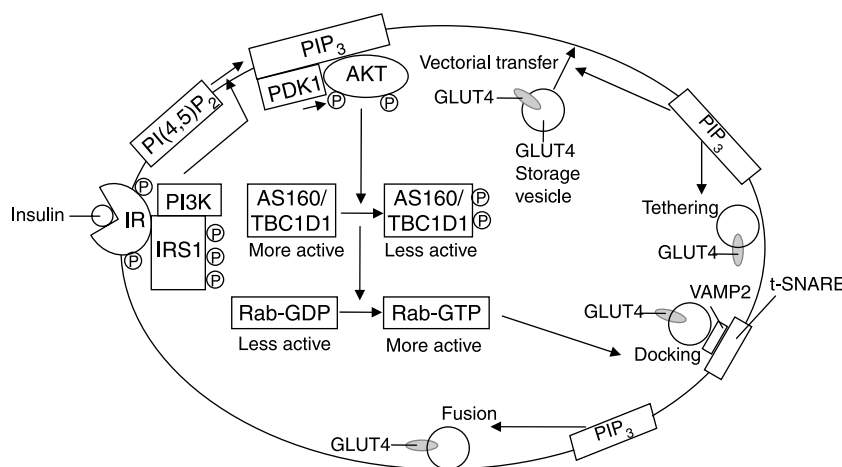


Figure 1 Insulin signaling pathways regulating GLUT4 translocation in mammalian skeletal muscle. Insulin activates tyrosine kinase activity of insulin receptor (IR) by binding with α -subunit of IR. Activated IR phosphorylates itself and insulin receptor substrate-1 (IRS1). Phosphorylated IRS1 binds to phosphatidylinositol 3-kinase (PI3-kinase), which is recruited to plasma membrane and converts phosphatidylinositols-4,5-bisphosphate (PIP₂) to phosphatidylinositols-3,4,5-trisphosphate (PIP₃). Increased PIP₃ recruits phosphatidylinositol-dependent protein kinase-1 (PDK1) and AKT to plasma membrane where AKT is activated by PDK1-mediated phosphorylation. Activated AKT phosphorylates AS160/TBC1D1, which inhibits its Rab GTPase-activating protein (GAP) activity towards particular Rab isoform(s). Inhibition of GAP increases conversion of less active GDP-loaded Rab to more active GTP-loaded Rab. Increased active GTP-loaded Rab then allows GLUT4 storage vesicles to move to, dock, and fuse with plasma membrane. Four stages of GLUT4 translocation have been proposed. Vectorial transfer: GLUT4 vesicles are transported to the cell periphery, possibly along microtubules. Tethering: GLUT4 vesicles are retained near the cell periphery through remodeling actin cytoskeleton. Docking: GLUT4 vesicles bind to plasma membrane via interaction of VAMP2 with target-SNARE complexes. Fusion: irreversible incorporation of GLUT4 vesicles on to plasma membrane is enhanced through action of Munc18c on SNARE proteins.

to the plasma membrane, where AKT is activated by PDK1-mediated phosphorylation. AKT phosphorylates many proteins, including glycogen synthase kinase 3 β (GSK3B) in liver, AS160 (GLUT4 translocation), the BAD/BCL2 heterodimer (antiapoptosis), and forkhead box O transcription factors (regulation of gene expression) (Fig. 1; Taguchi & White 2008).

Protein phosphatases and phospholipid phosphatases modulate the strength and duration of insulin signals. Depending upon the tissue site, dysregulation of these heterologous signaling mechanisms can progress to glucose intolerance, hyperinsulinemia, dysregulated lipid metabolism, and insulin resistance (White 2003).

Translocation of glucose transporter to plasma membrane

The transport of glucose into the skeletal muscle cells is the rate-limiting step in whole body glucose metabolism under normoglycemic conditions (Ren *et al.* 1993). Glucose enters the cell by facilitated diffusion mediated via a group of structurally related glucose transporter proteins (GLUTs). At least 12 GLUTs have been described (Joost *et al.* 2002). In skeletal muscle and adipose tissue, GLUT1 mediates basal glucose transport, whereas GLUT4 is responsible for insulin-mediated glucose uptake (Tordjman *et al.* 1989).

The stimulation of GLUT4 translocation and glucose uptake by insulin is a complex process. The necessity of the IRS–PI3K–AKT–AS160 axis in insulin-stimulated glucose uptake is well documented (Bjornholm & Zierath 2005). Insulin regulates translocation of GLUT4 in vectorial transfer, vesicle tethering, vesicle docking, and vesicle fusion (Fig. 1; Zaid *et al.* 2008).

Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) mediate both docking and fusion events (Foster & Klip 2000, Kawanishi *et al.* 2000). Vesicle-associated membrane protein 2 (VAMP2), syntaxin4, and soluble NSF attachment protein 23 are three elements of the SNARE complex involved in docking/fusion of insulin-sensitive GLUT4 vesicles with the plasma membrane. The SNARE complex consists of four parallel α -helices formed from the coiled-coil segments of SNARE proteins in two opposite membranes (McNew *et al.* 2000). Prior to docking/fusion, *cis*-SNARE complexes exist on vesicles and must be disassembled to allow *trans*-SNARE complex formation. The disassembly of *cis*-SNARE complex is driven by the ATPase activity of *N*-ethylmaleimide-sensitive factor (NSF). NSF recruitment to the *cis*-SNARE complex requires SNAP (Jahn & Scheller 2006). NSF is believed to associate with GLUT4 vesicles and cell membranes, but this interaction itself does not impact on the formation of fusion complexes (Mastick & Falick 1997). The regulation of SNARE complex formation is believed to be exerted via SM (Sec1p/Munc18) (Latham *et al.* 2006). Among the three mammalian SM genes, Munc18c is involved in GLUT4 trafficking and stabilizing syntaxin in the closed inactive conformation in the basal state. Data have shown that Munc18c dissociates from syntaxin4

upon insulin stimulation (Thurmond *et al.* 1998, Kanda *et al.* 2005) and Munc18c may switch to a different binding site on the target-SNARE, thereby allowing VAMP2 and GLUT4 vesicle docking at the plasma membrane (Smithers *et al.* 2008).

Current understanding of T2DM

The pathophysiology of T2DM varies with tissues or organs as detailed below. The contribution of each tissue or organ to T2DM is summarized in Fig. 2.

Skeletal muscle

Since skeletal muscle accounts for ~75% of whole body insulin-stimulated glucose uptake, defects in this tissue play a major role in the glucose homeostasis in patients with T2DM (Bjornholm & Zierath 2005). Insulin receptor tyrosine phosphorylation appears to be normal or reduced in nonobese T2DM (Zierath *et al.* 2000). IRS1 knockout mice demonstrate peripheral insulin resistance and reduced growth; however, these defects are partly compensated by the existence of an IRS1-independent pathway for insulin signal transduction (Araki *et al.* 1994). This compensatory pathway was shown to be mediated by IRS2 (Sun *et al.* 1995). IRS2 knockout mice have a progressive development of T2DM, with insulin resistance in skeletal muscle (Lin *et al.* 2004). Type 2 diabetic subjects have impaired insulin-stimulated tyrosine phosphorylation of IRS1 in skeletal muscle. This is not related to decreased protein expression of IRS1. A similar impairment is observed at the level of PI3K in type 2 diabetic muscle (Zierath *et al.* 2000). Impaired PI3K activation has been observed in Zucker fatty obese rat (Asano *et al.* 2007). Whereas IRS1 and PI3K phosphorylation/activation is impaired under *in vivo* and *in vitro* insulin stimulation in the skeletal muscle from type 2 diabetic subjects, AKT phosphorylation is impaired only under *in vitro* conditions (Zierath *et al.* 2000).

The dysregulation of the insulin receptor or IRS constitutes a common feature of insulin resistance. Mechanisms for this dysregulation might include tumor necrosis factor α (TNF α)-mediated downregulation of mRNA transcription (Hotamisligil *et al.* 1993, Frittitta *et al.* 1997, Moller 2000), kinase-mediated serine/threonine phosphorylation (Bandyopadhyay *et al.* 1997b, De Fea & Roth 1997, Aguirre *et al.* 2000, Yu *et al.* 2002), proteasome-mediated degradation (Zhande *et al.* 2002), and phosphatase-mediated dephosphorylation (Worm *et al.* 1996, Ahmad *et al.* 1997, Bandyopadhyay *et al.* 1997a, Elchebly *et al.* 1999). These signaling abnormalities could result in an impaired glucose transport. Indeed, the insulin-stimulated glucose transport is reduced in the skeletal muscle of type 2 diabetic subjects (Zierath *et al.* 2000).

Patients with T2DM are characterized by a decreased fat oxidative capacity and high levels of circulating free fatty acids (FFAs; Kelley & Simoneau 1994, Blaak *et al.* 2000a,b).

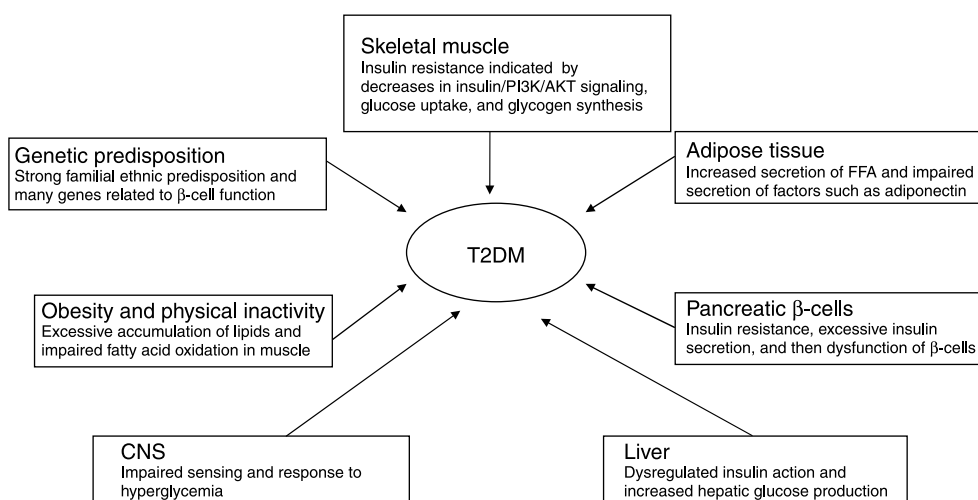


Figure 2 Pathogenesis of T2DM. Current theories on T2DM include a defect in insulin-mediated glucose uptake in skeletal muscle, a disruption of secretory function of adipocytes, a dysfunction of pancreatic β -cells, impaired sensing and response to hyperglycemia in the CNS, an excessive accumulation of lipids, and impaired fatty acid oxidation due to obesity, physical inactivity, and genetic predisposition.

The latter is known to cause insulin resistance, particularly in skeletal muscle, by reducing insulin-stimulated glucose uptake, most likely via accumulation of lipid inside the muscle cell (Boden 1999, Santomauro *et al.* 1999). T2DM is associated with impaired metabolic flexibility, i.e. an impaired switching from fatty acid to glucose oxidation in response to insulin (Kelley & Mandarino 2000). Thus, a reduced fat oxidative capacity and metabolic inflexibility are important components of skeletal muscle insulin resistance. The cause of these derangements in skeletal muscle of type 2 diabetic patients remains to be elucidated. An impaired mitochondrial function is a likely candidate.

Adipose tissue

GLUT4 expression is down-regulated in adipose tissue in patients with T2DM (Shepherd & Kahn 1999). Given that skeletal muscle is the major site for glucose disposal, the hyperglycemia associated with T2DM cannot be explained by the decreased uptake of glucose into adipose tissue due to downregulation of GLUT4 in adipocytes. Furthermore, adipocyte-selective knockout of GLUT4 (adipose-*Glut4*^{−/−}) in mice resulted in systemic insulin resistance similar to that induced by muscle-selective *Glut4* knockout mice (Zisman *et al.* 2000, Abel *et al.* 2001). These studies indicate that adipocyte GLUT4 deficiency may result in generation of circulating factors that are responsible for cross-organ communication. Retinol-binding protein-4 (RBP4) was recently identified as one candidate for that crosstalk in the adipose tissue of adipose-specific *Glut4* knockout mice (Yang *et al.* 2005). Furthermore, serum RBP4 protein levels were elevated in insulin-resistant mice and obese and diabetic humans. Transgenic overexpression of the human *RBP4* gene

or injection of recombinant RBP4 protein in normal mice caused insulin resistance, whereas RBP4 knockout mice had enhanced insulin sensitivity (Yang *et al.* 2005). Increased serum RBP4 protein levels induced hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and impaired insulin signaling in muscle. Adipocytes, both visceral and peripheral, secrete a plethora of factors, which may alter systemic insulin action and hepatic glucose production, including adiponectin, resistin, leptin, cytokines IL6 and TNF α , visfatin, RBP4, as well as FFA (Gimeno & Klamann 2005, Lazar 2005, Wellen & Hotamisligil 2005). The inhibition of signaling downstream of the insulin receptor may be a primary mechanism through which this inflammatory state causes insulin resistance. Exposure of cells to TNF α or elevated levels of FFAs stimulates phosphorylation of serine residues of IRS1. This phosphorylation reduces tyrosine phosphorylation of IRS1 in response to insulin (Hotamisligil *et al.* 1996, Paz *et al.* 1997, Aguirre *et al.* 2000, 2002). Both adipose tissue and the macrophages within adipose tissue serve in both endocrine and paracrine fashions to promote inflammation and decrease insulin sensitivity (Wellen & Hotamisligil 2005, de Luca & Olefsky 2006).

Pancreatic β -cells

Many mechanisms contributing to T2DM may also trigger β -cell apoptosis and reduce β -cell mass or ability to compensate for insulin resistance (Rhodes 2005). Mechanisms include endoplasmic reticulum stress (Harding & Ron 2002), chronic hyperglycemia (Donath & Halban 2004), chronic hyperlipidemia (Poitout & Robertson 2002), oxidative stress (Kaneto *et al.* 2006), and inflammatory cytokines (Donath *et al.* 2003). Decreased IRS2 expression

may lead to spontaneous β -cell apoptosis (Hennige *et al.* 2003). Because several mechanisms relevant to pathogenesis of T2DM may increase IRS2 serine/threonine phosphorylation (Werner *et al.* 2004) with resultant IRS2 ubiquitination and proteosomal degradation, defects in insulin signaling and insulin secretion may be coupled. When β -cell function is viewed in the context of reduced insulin sensitivity, considerable data support the early failure of insulin secretion in T2DM pathogenesis (Ferrannini 1998, Kahn 2003). Animal studies also support this concept. However, the signals that cause normal β -cell compensation and hyperinsulinemia, the mechanisms of this compensation, the point in the pathogenesis of T2DM where this compensatory mechanism fails, and the etiology of this failure all remain unclear. Insulin sensitivity and insulin secretion are deteriorated in human T2DM.

Liver

Liver is the major organ with the ability to consume, store, and produce glucose and lipids. Hepatic glucose metabolism includes the formation of glycogen (short-term energy storage), generation of glucose from nonsugar carbon substrates and intracellular energy supply via glycolysis (Klover & Mooney 2004). Fatty acid oxidation, *de novo* synthesis of fatty acids, cholesterol and bile acid synthesis, as well as lipoprotein assembly are the essential processes in lipid metabolism. These metabolic pathways are coordinately regulated to maintain glucose and lipid homeostasis under physiological conditions (Raddatz & Ramadori 2007). Consequently, the liver is a key target for the anabolic hormone insulin and its catabolic counterpart glucagon. Insulin is released from the pancreatic β -cells in response to increased blood glucose concentrations, and this is amplified in the presence of FFAs. Impaired insulin sensitivity and dysregulated insulin action in the liver contributes significantly to the pathogenesis of T2DM (Fritsche *et al.* 2008). The pancreatic α -cells release glucagon in response to decreasing blood glucose concentrations as it occurs during fasting. Glucagon affects mainly the liver and adipose tissue. It induces the breakdown of glycogen and the mobilization of fatty acids. Most importantly, it promotes gluconeogenesis, i.e. the formation of glucose from lactate, glycerol and glucogenic amino acids.

IRS -1 and -2 are complementary key players in the regulation of hepatic insulin signaling and expression of genes involved in gluconeogenesis, glycogen synthesis, and lipid metabolism (Fritsche *et al.* 2008). The function of IRS proteins is regulated by their expression levels and post-translational modifications. Dysfunction of IRS proteins initially leads to postprandial hyperglycemia, increased hepatic glucose production, and dysregulated lipid synthesis, and is considered as a major pathophysiological mechanism for the development of insulin resistance and T2DM (Taniguchi *et al.* 2005, Dong *et al.* 2006, Simmgen *et al.* 2006). *Irs2* knockout mice have a progressive development of

T2DM, increased adiposity and female infertility (Withers 2001), with insulin resistance in the liver and skeletal muscle, together with a lack of β -cell compensation for the peripheral insulin resistance (Lin *et al.* 2004).

Multiorgan disease

Previously, resistance to insulin-mediated nonoxidative glucose metabolism was viewed as the early and primary defect leading to T2DM, and thereafter the progressive failure of the β -cell to compensate for insulin resistance has received considerable attention (Kahn 2003). Most recently, data from animal models have challenged this paradigm. Muscle-specific insulin receptor (IR or INSR as given in the HUGO Database) inactivation did not lead to impaired glucose tolerance (IGT) despite insulin resistance, whereas muscle-specific inactivation of glucose transporter GLUT4 caused both profound insulin resistance and T2DM (Minokoshi *et al.* 2003). GLUT4 inactivation limited to white adipose tissue resulted in insulin resistance, glucose intolerance, and even T2DM with impairment in whole body glucose uptake markedly out of proportion to the expected contribution of white adipose tissue (Minokoshi *et al.* 2003). This isolated defect in white adipose tissue resulted in defective insulin action in both muscle and liver. These studies suggest a central and previously under-appreciated role of adipocytes in the pathogenesis of T2DM. However, insulin receptor inactivation in the liver also resulted in hyperinsulinemia, hepatic insulin resistance, and peripheral insulin resistance (Michael *et al.* 2000, Baudry *et al.* 2002, Mauvais-Jarvis *et al.* 2002, Fisher & Kahn 2003). These studies suggest considerable crosstalk among organ systems, as well as an important role for insulin signaling pathways in the β -cell (Accili 2004).

The central nerve system (CNS) has been shown to have an essential role in regulating glucose metabolism by sensing and integrating information from neural, hormonal, and nutrient signals, and then modulating glucose output in the liver and glucose uptake in peripheral tissues (Sandoval *et al.* 2009). Data from animal experiments have been documented to show that overeating and obesity dampen the ability of the CNS to sense and respond to the information, whereas selective CNS interventions decrease insulin resistance and blood glucose levels (Sandoval *et al.* 2009).

Thus, T2DM is the final outcome of a multiorgan disease, which is characterized by early direct or indirect defects in muscle, adipocytes, hepatocytes, β -cells, and the CNS.

Mitochondria and reactive oxygen species

Reactive oxygen species (ROS) may regulate insulin resistance. Several lines of evidence support the association of oxidative stress markers with diabetes (Urakawa *et al.* 2003, Furukawa *et al.* 2004, Lin *et al.* 2005). Recent data showed that treatment of 3T3-L1 adipocyte with either TNF α or dexamethasone increased the ROS level and resulted in decreased insulin action (Houstis *et al.* 2006).

Antioxidant molecules or transgenes encoding ROS-scavenging enzymes both ameliorated the insulin resistance of TNF α - or dexamethasone-treated 3T3-L1 adipocytes to varying degrees. Furthermore, antioxidant molecules improved insulin sensitivity and glucose homeostasis in ob/ob mice (Houstis *et al.* 2006). Reduced mitochondrial oxidative phosphorylation has recently been demonstrated in human diabetes, using both microarrays to identify down-regulated genes involved in oxidative phosphorylation (OXPHOS genes). PGC1 (PPARGC1A as given in the HUGO Database) appears to be at least partially responsible for this defect (Mootha *et al.* 2003, Patti *et al.* 2003). Supportive studies in humans have suggested a role for defective mitochondrial fatty acid oxidation, mitochondrial dysfunction, and reduced numbers of skeletal muscle mitochondria in T2DM pathogenesis, and have suggested that increased intramyocellular lipid content was associated with defects in mitochondrial activity (Maechler & Wollheim 2001, Petersen *et al.* 2004, Lowell & Shulman 2005, Morino *et al.* 2005). There is 38% less mitochondrial density in muscle of insulin resistant individuals compared with controls (Morino *et al.* 2005). The decreased mitochondrial fatty acid oxidation, caused by either mitochondrial dysfunction and/or reduced mitochondrial numbers, produces increased levels of intracellular fatty acyl CoA and diacylglycerol. These molecules activate a novel protein kinase C, which in turn activates a serine kinase cascade, leading to increased serine phosphorylation of IRS1. As described above, serine phosphorylation blocks IRS1 tyrosine phosphorylation and inhibits downstream signaling, including recruitment of GLUT4 to the plasma membrane and insulin-mediated glucose uptake in skeletal muscle (Lowell & Shulman 2005).

Obesity and physical inactivity

Only about one-third of adults in America are considered to have 'normal' weight, and similar trends are being observed worldwide (Kahn *et al.* 2006). About 80% of T2DM is associated with obesity and sedentary life styles (Venables & Jeukendrup 2009). It is well accepted that obesity and physical inactivity are risk factors for the development of T2DM (Weinstein *et al.* 2004).

In obesity, the disparity between uptake of fatty acids into skeletal muscle and oxidation results in excessive accumulation of triacylglycerol and fatty acid metabolites such as long-chain acyl-CoAs, diacylglycerols, and ceramides in the sarcoplasm of skeletal muscle (Venables & Jeukendrup 2009). It has been reported that an elevation in circulating fatty acids was associated with a decrease in insulin signaling and glucose disposal rates (Belfort *et al.* 2005). An increase in skeletal muscle diacylglycerol content in human and animal models of insulin resistance activates specific isoforms of protein kinase C, leading to inhibition of the insulin signal through serine phosphorylation of IRS1 (Itani *et al.* 2002, Yu *et al.* 2002). For obesity and insulin resistance to be

associated with type 2 diabetes, β -cells must be unable to compensate fully for decreased insulin sensitivity (Kahn *et al.* 2006). Dysfunction of β -cells exists in individuals who are at high risk of developing T2DM even when their glucose levels are still normal, suggesting that β -cell dysfunction could be crucial (Kahn *et al.* 2006).

Many prospective studies have shown strong association between daily physical activity and a reduced risk of developing T2DM (Venables & Jeukendrup 2009). Clinical trials have also shown that addition of physical activity and dietary modification can reduce the incidence of T2DM (Venables & Jeukendrup 2009). Recent data have indicated that elevations in mitochondrial oxidative capacity following an acute bout of exercise can increase insulin-stimulated glucose uptake (Thyfault *et al.* 2007).

Genetic analysis

The genetic information in the family history has been used in clinical assessment of T2DM (Majithia & Florez 2009). There is ample evidence that T2DM has a strong genetic component, which includes monogenic disease such as MODY1–6 (described to date) under 25 years of age and polygenic disease such as common T2DM (Majithia & Florez 2009). The lifetime risk of T2DM is about 7% in a general population, about 40% in offspring of one parent with T2DM, and about 70% if both parents have diabetes (Majithia & Florez 2009). The first-degree family with a history of T2DM is associated with twofold increased risk of future T2DM (Lyssenko *et al.* 2008).

Genetic studies including linkage analysis, candidate gene approaches, and most recent genome-wide association studies have identified 20 common genetic variants associated with T2DM (Ridderstrale & Groop 2009). Many loci appear to regulate the capacity of β -cells to increase insulin secretion in response to an increase in insulin resistance or obesity, which includes eight genes such as *TCF7L2*, *KCNJ11*, *HHEX*, *SLC30A8*, *CDKAL1*, *CDKN2A/2B*, *IGF2BP2*, and *KCNQ*; *PPARG* gene is related to insulin sensitivity; *CAPN10* gene is related to glucose transport; *MC4R* and *FTO* genes are related to obesity; eight other loci have unknown roles in T2DM (Ridderstrale & Groop 2009).

Animal models of T2DM

Animal models of T2DM are excellent tools to study the pathogenesis of human disease. Owing to the uncertain etiology of T2DM and its causes are multifarious, the use of various animal models, each of which induces the disease by a different mechanism yet with the same end result, is advantageous. Animal models of T2DM include a wide range of species with genetic, experimental, or nutritional causation (Table 1; Srinivasan & Ramarao 2007).

Table 1 Phenotypes, advantages, and disadvantages of major animal models of type 2 diabetes mellitus (T2DM)

Animal model	Phenotype	Advantages	Disadvantages
ob/ob mouse (Shafir 2003)	Mild hyperglycemia, mild glucose intolerance, severe hyperinsulinemia, insulin resistance, and impaired wound healing	Develop spontaneous T2DM and animals display features resembling human T2DM; require small sample size	Homogenous and monogenic inheritance unlike heterogeneity seen in human T2DM
db/db mouse (Shafir 2003)	Hyperphagic, obese, hyperglycemic, hyperinsulinemic. Animals then display diabetic nephropathy and ketosis	Develop spontaneous T2DM and animals show features resembling human T2DM; require small sample size	Homogenous and monogenic inheritance unlike heterogeneity seen in human T2DM
Zucker diabetic fatty (ZDF) rat (Shafir 1992)	Hyperglycemia and insulin resistance. Male ZDF rat exhibits impaired insulin secretory β -cell response to glucose, while it remains intact to nonglucose secretagogues like arginine	Develop spontaneous T2DM and animals show features resembling human T2DM; require small sample size	Homogenous and monogenic inheritance unlike heterogeneity seen in human T2DM
Obese rhesus monkey (Kim <i>et al.</i> 2005)	Develops obesity, hyperinsulinemia, insulin resistance, and necrosis of β -cells; deposition of amylin/amyloid in β -cells	Develop spontaneous obesity and T2DM and animals show features resembling human T2DM. Polygenic inheritance resembling to human T2DM	Expensive
Sand rat (Shafir <i>et al.</i> 1999)	Develop hyperphagia, obesity, hyperinsulinemia, glucose intolerance with pancreatic islet cells remain intact followed by β -cell degeneration and necrosis resulting in profound insulin deficiency and overt diabetes and ketosis, ultimately leading to death of animal	Develop diabetes associated with obesity as a result of overnutrition, which is similar to human population wit T2DM. Polygenic model	Require long period of dietary treatments. No frank hyperglycemia
Streptozotocin-induced adult diabetic animals (Rakieten <i>et al.</i> 1963)	Develops hyperglycemia due to cytotoxic action on pancreatic β -cells	Selective loss of pancreatic β -cells. Residual insulin secretion makes animal live long without insulin treatments. Comparatively cheaper; ketosis and mortality are relatively less	Develop hyperglycemia primarily by insulin deficiency rather than insulin resistance; long-term treatments. May be toxic to other organs. Variability may be high
Complete or partial pancreatectomy in animals (Sasaki <i>et al.</i> 2000)	Develop hyperglycemia	Resemble human T1DM due to reduced islet β -cell mass	May also remove other cells, e.g. α -cells, which are also important in regulation of glucose homeostasis. Mortality is high
Insulin receptor (IR) knockout (Accili <i>et al.</i> 1996)	Severe hyperglycemia and hyperketonemia; die within days after birth due to ketoacidosis	Study single gene in diabetes	Expensive for production
Insulin receptor (IR) in β -cell knockout (Kulkarni <i>et al.</i> 1999)	Develops hyperglycemia due to decreased insulin secretion. 20% of animals develop diabetes	Study single gene in specific organ in diabetes	Expensive for production

Commonly used animal models of T2DM

ob/ob mouse ob/ob mouse is inherited as autosomal recessive mutation on chromosome 6 in C57BL/6J mouse strain (Shafir 2003). The mutation is in *leptin* gene, which encodes leptin protein. Ob/ob mice are hyperphagic and obese. Ob/ob mice display diabetes like syndrome of mild hyperglycemia, mild IGT, severe hyperinsulinemia, insulin resistance, and impaired wound healing. However, when ob gene is expressed on C57BL/KS background, mice become severely diabetic with regression of islets (Bell & Hye 1983).

db/db mouse The db/db (diabetic) mouse is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KSJ strain (Shafir 2003). The mutation is in db gene, which encodes for leptin receptors. Db/db mice mimic several features of human T2DM. These mice are spontaneously hyperphagic and obese. These mice show insulin resistance within the first month of age and develop hyperinsulinemia and hyperglycemia later with a peak between 3 and 4 months of age. Animals then display diabetic nephropathy and ketosis, which are similar to advanced human T2DM (Shafir 2003, Sharma *et al.* 2003).

Zucker fatty rat The spontaneous mutation obese (fatty) model was found in the rat stock of Sherman and Merck by Zucker in 1961. The Zucker (fa/fa) fatty or obese rat results from the simple autosomal recessive (fa) gene on chromosome 5, which encodes leptin receptors. It has hyperphagia and early onset of obesity, which appear at 4 weeks of age along with increased growth of subcutaneous fat depot, which mimics the early stage of human T2DM. It shows mild hyperglycemia, insulin resistance, mild glucose intolerance, hyperlipidemia, hyperinsulinemia, and moderate hypertension (Durham & Truett 2006).

Zucker diabetic fatty rat It is a substrain of Zucker fatty rat selectively inbred for hyperglycemia. Male Zucker diabetic fatty rat progresses to diabetes due to failure to compensate adequately for insulin resistance (Shafir 1992). In contrast to fa/fa rats, the ability to over secrete insulin to compensate peripheral insulin resistance is limited, and the β -cells are brittle and easily succumb to over secretion pressure. It exhibits impaired insulin secretory β -cell response to glucose, while it remains intact to nonglucose secretagogues like arginine, a phenomenon similar to human T2DM.

Obese rhesus monkey (*Macaca mullata*) Obese rhesus monkey develops obesity, hyperinsulinemia, and insulin resistance when maintained on *ad libitum* laboratory diet, which gradually progresses to necrosis of β -cells, severe fall in insulin levels and overt hyperglycemia over a period of several years (Kim *et al.* 2005). The most remarkable similarity may be the prediabetic phase with insulin resistance and compensatory hyperinsulinemia, which is well documented in the human condition. The final loss of insulin secretion is interestingly associated with the deposition of

amylin/amyloid in β -cells (Ramarao & Kaul 1999, Kim *et al.* 2005). Islet amyloid is found in about 90% of human T2DM (Cefalu 2006).

These obese animal models have provided unique tools for studying how obesity causes T2DM. For example, db/db mice were generated by mutation of the leptin receptor gene. Owing to genetic deficiency of leptin receptors, db/db mice are hyperphagic and therefore obese. These animals develop severe hyperglycemia, insulin resistance, and β -cell dysfunction. These characteristics mimic those of human T2DM. Indeed, each animal model was developed to mimic certain aspects of human T2DM. In addition, animal models of T2DM are essential tools for testing new anti-diabetic drugs.

The characteristics, advantages, and disadvantages of the major T2DM models are provided in Table 1.

Summary

T2DM is a complex heterogeneous group of metabolic disorders characterized by hyperglycemia and impaired insulin action and/or insulin secretion. The complex nature of T2DM reflects the multifaceted genetic background and the varied genetic-environmental interaction. The current theories of T2DM include a defect in insulin-mediated glucose uptake, a dysfunction of the pancreatic β -cell, a dysregulation of the adipocyte as a secretory organ, and a dysfunction of the liver. Dysregulation of metabolism in diabetes also causes extensive complications in nearly all organs or tissues. Recent advances in animal models of T2DM make it possible to explore the previously unidentified pathogenesis of the disease.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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