Editor: Patricia Joseph-Bravo

5

Molecular basis of type-2 diabetes

Cristina Fernandez-Mejia
Nutritional Genetics Unit, Biomedical Research Institute
National University of Mexico

Abstract

Diabetes mellitus is taking its place as one of the most important diseases in the world. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion, insulin action, or both. There are two main types of diabetes: type-1 and type-2, the worldwide diabetes epidemic relates particularly to type-2 diabetes. Type-2 diabetes is characterized by impaired insulin action and/or abnormal insulin secretion. An early abnormality in the disease is insulin resistance; a defective state in which insulin is unable to exert its biological effects at circulation concentrations that are effective in normal subjects. Insulin resistance leads to profound decreases in glucose uptake and glycogen synthesis in
Insulin resistance yields to defective suppression of hepatic glucose output. Resistance to the antilipolytic action of insulin also favors triglyceride breakdown in adipose tissue and the generation of free fatty acids, which interfere with insulin receptor signals. Changes in serum adipokine concentrations are also part of the insulin resistant state. At the pre-onset of type-2 diabetes, resistance to the glucose-lowering action of insulin tends to lead a slight increase of blood glucose concentration, which stimulates insulin secretion and causes hyperinsulinemia. Initially hyperinsulinemia is able to overcome insulin resistance. The diabetic state develops when insulin secretion cannot longer be sustained to compensate insulin resistance, and it is at this stage that fasting and post-prandial hyperglycemia is apparent. Plentiful efforts have been made to understand the molecular basis of type 2-diabetes. It is now well accepted that defective postreceptor insulin signaling is the main feature involved in insulin resistance of type-2 diabetes. As a result the metabolic insulin actions are affected. Several mechanisms such as insulin receptor tyrosine dephosphorylation, imbalance of serine/threonine phosphorylation, or insulin receptor internalization impair insulin signaling. A number of molecules associated to insulin resistance such as free fatty acids, interleukin-6, TNF-alpha, affect insulin receptor signaling, interestingly most of them are related to adipose tissue. Transcriptional factors such as peroxisome proliferator activated receptor gamma (PPAR gamma) and peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1 alpha) have also been found to be associated with insulin resistance. Beta-cell function plays a pivotal role in determining progress to type-2 diabetes. Beta-cell gene expression defects, as seen in the monogenic diabetes forms (MODY) or secondary beta-cell defects, caused by glucotoxicity, increased free fatty acids, cytokines and/or mitochondrial disfunction may be implicated in the physiopathology of type-2 diabetes. Classical physiology studies, biochemical methods, tissue culture technology, the use of gene targeting approaches in mice, and naturally occurring mutations in patients have shed some light on the molecular causes of type-2 diabetes and have contributed to a deeper understanding of the molecular mechanisms involved in the disease.

Molecular basis of type-2 diabetes
Diabetes mellitus is taking its place as one of the most important diseases in the world. In the past two decades the disease has experienced an explosive increase, now reaching epidemic proportions. Global estimates for the year 2010 predict a further growth of approximately 50%, with the greatest increase in developing countries [1]. Pronounced changes in environment, food
availability and lifestyle have resulted in escalating rates of obesity and diabetes.

1. History

The first document known to mention descriptions of a polyuric state resembling diabetes is an Egyptian papyrus dating from approximately 1500 BC. The term “diabetes” was first used by Aretaeus of Cappadocia in the 2nd century AD and described a condition showing increased urine production. During the same period, Galen, the Roman physician, spoke of two cases of a rare disease characterized by polyuria and polydipsia. In the 5th and 6th centuries, two notable Indian physicians, Susruta and Sharuka, described for the first time the association of polyuria with a sweet-tasting substance in the urine. In the 17th century, Thomas Willis made several clever observations about the disease, which still hold true today. He wrote that diabetes had been rare in classical times (Galen) “but in our age, given to good fellowship and gusling down chiefly of unallayed wine, we meet with examples and instances enough, I may say daily, of this disease..... As to what belongs the cure, it seems a most hard thing of this disease to draw propositions for curing, for this cause lies so deeply hid, and hath its origin so deep and remote” [2]. Since then, plentiful efforts have been made to unravel the deep causes of this frequent disease.

2. Definition

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion, insulin action, or both [3].

3. Diabetes classification

There are two main types of diabetes: type-1 and type-2. Type-1 diabetes is due to the autoimmune-mediated destruction of pancreatic beta cells, resulting in insulin deficiency. Patients with type-1 diabetes require exogenous insulin for survival. Its frequency amounts to nearly 10% of all diabetes cases. There is marked geographical variation in its prevalence, Scandinavian countries showing the highest rate of this illness [4].

Type-2 diabetes accounts for approximately 90% of diabetes cases, and is characterized by impaired insulin action and/or abnormal insulin secretion. The worldwide diabetes epidemic relates particularly to type-2 diabetes. Besides type-1 and type-2 diabetes, there are other specific types of diabetes as described on Table 1.

To understand the metabolic and molecular mechanisms responsible for type-2 diabetes, it is necessary to understand the regulation of fuel metabolism in the human body.
Table 1. Etiologic classification of diabetes mellitus [3].

| I TYPE-1 DIABETES (beta-cell destruction, usually leading to absolute insulin deficiency) |
|---------------------------------|-----------------------------------------------------------------------------------|
| A) Immune mediated               |
| B) Idiopathic                   |

<table>
<thead>
<tr>
<th>II TYPE-2 DIABETES (may range from predominantly impaired insulin action with relative insulin secretion deficiency, to a predominantly secretory defect with impaired insulin action)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>III OTHER SPECIFIC TYPES:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Genetic defects of the beta cell, usually referred to as maturity-onset diabetes of the young (MODY). Inherited in an autosomal dominant pattern.</td>
</tr>
<tr>
<td>1. Chromosome 12, HNF-1 alpha (MODY3)</td>
</tr>
<tr>
<td>2. Chromosome 7, glucokinase (MODY2)</td>
</tr>
<tr>
<td>3. Chromosome 20, HNF-4 alpha (MODY1)</td>
</tr>
<tr>
<td>4. Chromosome 13, insulin promoter factor-1 (IPF-1; MODY4)</td>
</tr>
<tr>
<td>5. Chromosome 17, HNF-1Beta (MODY5)</td>
</tr>
<tr>
<td>6. Chromosome 2, NeuroD1 (MODY6)</td>
</tr>
<tr>
<td>7. Mitochondrial DNA</td>
</tr>
<tr>
<td>8. Others</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Genetic defects of insulin action. Certain infrequent causes of diabetes are due to genetic abnormalities of insulin action caused by mutations of the insulin receptor. Some individuals with these mutations may present acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. Leprechaunism and the Rabson-Mendenhall syndrome are two pediatric syndromes that show mutations in the insulin receptor gene with subsequent alterations in insulin receptor function. Other abnormalities are related to insulin postreceptor signal transduction pathways, such as insulin-resistant lipoatrophy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Type-A insulin resistance</td>
</tr>
<tr>
<td>2. Leprechaunism</td>
</tr>
<tr>
<td>3. Rabson-Mendenhall syndrome</td>
</tr>
<tr>
<td>4. Lipoatrophic diabetes</td>
</tr>
<tr>
<td>5. Others</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Diseases of the exocrine pancreas. Processes that injure the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Endocrinopathies. Pathologies with excess amounts of hormones that antagonize insulin secretion (e.g., growth hormone, cortisol, glucagon, epinephrine) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is controlled.</td>
</tr>
<tr>
<td>E. Drug- or chemical-induced diabetes. Many drugs, such as vacor, glucocorticoids, diazoxide and thiazides, among others, can impair glucose homeostasis. These drugs may not cause diabetes by themselves, but they may precipitate diabetes.</td>
</tr>
<tr>
<td>F. Infections. Congenital rubella, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing the disease.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G. Uncommon forms of immune-mediated diabetes. Examples are the stiff-man syndrome, and conditions with anti-insulin receptor antibodies.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>H. Other genetic syndromes sometimes associated with diabetes. Several genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down’s syndrome, Klinefelter’s syndrome, Prader-Willi syndrome and Turner’s syndrome. Wolfram’s syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of beta-cells at autopsy.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>IV. GESTATIONAL DIABETES MELLITUS (GDM). GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy.</th>
</tr>
</thead>
</table>
4. Basic principles of metabolism

In the fed state, fuels in excess are stored as glycogen and triglycerides. During the fasting state these reservoirs are broken down to provide fuels. Energy reservoirs are built up and broken down in response of hormonal messages.

In the fed state, coordination of insulin secretion by the pancreatic beta cells along with the responsiveness to insulin of major glucose metabolic tissues such as muscle, liver and fat, control plasma glucose. Insulin promotes glucose uptake, glycogen synthesis in the liver and muscle, lipid formation to be stored in the adipose tissue, and protein synthesis in most cells. The rate limit step in whole body glucose uptake is the transport of glucose into skeletal muscle cells, this accounts for more than 75% of glucose uptake [5]. Alongside the insulin stimulatory effect on fuel reservoir synthesis, in the fed state, the hormone has restrained functions on glucose output and lipolysis [6, 7].

In the fasting state, decreased plasma insulin concentration and increased counterinsulin hormones, such as glucagon, glucocorticoids, and catecholamines, contribute to glucose output via glycogen breakdown and gluconeogenesis, and via lipolysis as well as decreased synthesis and increased protein degradation.

Beside the classical regulating hormones, a considerable piece of evidence indicates that adipose tissue hormones, adipokines, as well as free fatty acids, influence metabolism and fuel expenditure.

Below, we describe the basic knowledge of the molecular mechanisms involved in insulin secretion and of responsiveness to insulin in normal conditions. We also describe the role of adipose tissue in fuel metabolism.

5. Molecular mechanisms involved in the regulation of metabolism under normal conditions

Molecular mechanisms of insulin secretion

Insulin secretion in response to glucose is a complex, multistep process that requires transport and oxidation of glucose, electrophysiological changes and fusion of insulin-containing secretory granules with the beta-cell plasma membrane (Fig. 1). Glucose enters the cell by facilitated diffusion mediated by a group of structurally related glucose transport proteins (GLUT), characterized by 12 hydrophobic helical domains. To date, at least 12 GLUTs have been described [8]. In the pancreatic beta cell, glucose is transported by the glucose transporter 2 isoform (GLUT2). Glucose is phosphorylated to form glucose-6-phosphate by glucokinase. This enzyme plays a critical role in glucose-induced insulin secretion and is considered the glucosensor of the pancreatic beta cell. Due to its kinetic characteristics, glucokinase is a determining factor for glucose phosphorylation [9] and hence for its metabolism through glycolysis and oxidation.
Figure 1. Insulin secretion in response to glucose. Glucose enters the cell by facilitated diffusion mediated the glucose transporter 2 isoform (GLUT2) and it is phosphorylated to form glucose-6-phosphate by glucokinase. The generation of ATP by glucose oxidation leads to closure of the ATP-sensitive K+ channel, a hetero-octamer comprised of four subunits of the sulphonylurea 1 receptor (SUR1) and four subunits of the inwardly rectifying K+ channel Kir6.2. The closing of the ATP-sensitive K+ channel leads to depolarization of the plasma membrane and influx of extracellular calcium. This leads to fusion of insulin-containing secretory granules with the plasma membrane and the release of insulin into the circulation.

The generation of ATP by glycolysis and the Krebs cycle leads to closure of the ATP-sensitive K+ channel — a hetero-octamer comprised of four subunits of the sulphonylurea 1 receptor (SUR1) and four subunits of the inwardly rectifying K+ channel Kir6.2 [10]. The closing of the ATP-sensitive K+ channel leads to depolarization of the plasma membrane and influx of extracellular calcium [11]. Together with calcium mobilized from intracellular stores, this leads to fusion of insulin-containing secretory granules with the plasma membrane and the release of insulin into the circulation [12]. These pathways are stimulated to varying degrees by neurotransmitters and hormones via activation of receptors on the pancreatic beta-cell. For instance, acetylcholine, acting via the muscarinic receptor type 3 activates protein kinase C: Similarly, glucagon-like peptide-1 (GLP-1) promotes a rise in cyclic AMP and therefore activation of protein kinase A [13].
Molecular mechanisms of insulin signaling

Insulin starts its action by binding to the insulin receptor; this leads to a cascade of events that involves protein and membrane phospholipid phosphorylation, scaffold and docking proteins, and cytoskeleton activity (Fig. 2).

Figure 2. Molecular mechanisms of insulin signaling. The insulin interaction with its receptor promotes insulin receptor autophosphorylation, and catalyses the phosphorylation of cellular proteins such as members of the IRS family and Cbl. Upon tyrosine phosphorylation, these proteins interact with signalling molecules, resulting in a diverse series of signalling pathways, including activation of PI3K and the activation of TC10. These pathways act in a concerted fashion to coordinate the regulation of glucose transporter 4 (GLUT4) vesicle trafficking, protein synthesis, enzyme activation and inactivation, and gene expression, which results in the regulation of glucose, lipid and protein metabolism.

Insulin receptor. The insulin receptor belongs to a subfamily of receptors, all with protein tyrosine kinase activity. The insulin receptor is a heterotetrameric membrane glycoprotein consisting of two alfa-subunits and two beta-subunits. Insulin binds to the extracellular alpha-subunit of the receptor and induces a conformational change that brings the alpha-subunits closer together. This leads to a rapid auto-phosphorylation of the receptor, and catalyzes the phosphorylation of intracellular proteins such as: a) members of the insulin receptor substrate family, b) Shc and c) Cbl. Upon tyrosine phosphorylation, these proteins act as docking sites for proteins that contain SH2 (Src-homology-2) domains, such as phosphoinositide 3-kinase, Grb-2 and SHP-2, resulting in a diverse series of signaling pathways. These pathways act concertedly to transmit the signal from the insulin receptor to biological
endpoints, such as glucose transport, protein, lipid and glycogen synthesis, mitogenesis and gene expression [14].

**Insulin receptor substrate family (IRS).** At least 12 substrates of the insulin receptor have been identified: IRS-1, IRS-2, IRS-3, IRS-4, IRS-5, IRS-6, Gab-1, three isoforms of She, p62dok and APS (adapter protein containing a PH and SH2 domain) [15]. Studies in knockout mice suggest that IRS proteins serve complementary, rather than redundant, roles in insulin signaling. IRS-1 knockout mice demonstrate defective action of insulin in peripheral tissues, and reduced growth [16]. IRS-2 knockout mice have a progressive development of type-2 diabetes, increased adiposity and female infertility [17], with abnormal insulin action in the liver and skeletal muscle, together with a lack of beta-cell mass compensation for the impaired actions of insulin in peripheral tissues [18]. These animal models have provided clues for the pathogenesis of type-2 diabetes.

**Phosphoinositide 3-kinase (PI3K).** PI3K is a heterodimeric lipid kinase that plays a critical role in the actions of insulin and other growth factors [reviewed in [19]. PI3K consists of a regulatory subunit (p85) that associates with a catalytic subunit (p110). The regulatory subunit binds the IRSs, whereas the catalytic subunit phosphorylates phosphatidylinositols in the membrane to generate the lipid second messenger: phosphatidylinositol 3,4,5-trisphosphate, which binds to the plecstrin homology (PH) domains of a variety of signaling molecules, thereby altering their activity or subcellular localization. [20].

**Insulin signaling downstream of PI3K**

Downstream PI3K intracellular serine/threonine kinases, Akt and atypical PKC (aPKC), are activated by the formation of lipid products of PI3K. These kinases require the involvement of IRS to transmit the insulin signal to downstream biological events [21].

**Akt.** The generation of phosphatidylinositol 3,4,5-trisphosphate, by PI3K activates Akt (a serine/threonine kinase also called PKB). Increasing levels of phosphatidylinositol 3,4,5-trisphosphate: a) recruit Akt to the plasma membrane through specific interactions with its amino-terminal PH domain, and b) promote the activation of PI-dependent kinases (PDK1 y PDK2). In turn, PDK activate Akt by phosphorylation on threonine 308 and serine 473 [14].

Three ubiquitously expressed isoforms of Akt have been identified: Akt1, Akt2 and Akt3. Both Akt1 and Akt2 are involved in insulin signal transduction in skeletal muscle and adipose tissue. In contrast, Akt3 is not activated by insulin in liver, muscle or adipose tissue [22].

**Atypical protein kinase C (aPKC).** In addition to Akt, the aPKC ζ and λ have also been implicated in insulin signaling. Unlike the conventional PKCs, aPKCs are not activated by diacylglycerol. However, although aPKCs lack PH domains, phosphatidylinositol 3,4,5-trisphosphate bind to the regulatory domains
of these kinases and apparently induce conformational changes that lead to aPKC activation. [23]. Activation of aPKC activity has also been reported to occur through autophosphorylation, phosphorylation by PDK1, and relief from pseudosubstrate inhibition [23].

**Insulin signaling downstream Grb2**

Insulin stimulates the mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK). This pathway involves the tyrosine phosphorylation of IRS proteins and/or Shc, which in turn interact with the adapter protein Grb2, recruiting the Son-of-sevenless (SOS) exchange protein to the plasma membrane for activation of Ras. The activation of Ras also requires stimulation of the tyrosine phosphatase SHP2, through its interaction with receptor substrates such as Gab-1 or IRS1/2. Once activated, Ras operates as a switch, stimulating a serine kinase cascade through the activation of Raf, MEK and ERK. Activated ERK can translocate into the nucleus, where it catalyzes the phosphorylation of transcription factors, that leads to cellular proliferation or differentiation [21].

**Insulin signaling downstream Cbl**

This insulin signaling pathway involves tyrosine phosphorylation of the Cbl protooncogene. Cbl is associated with the adapter protein CAP. Upon phosphorylation, the Cbl–CAP complex translocates to lipid raft domains in the plasma membrane. Translocation of phosphorylated Cbl recruits the adapter protein CrkII to the lipid raft via interaction of the SH2 domain of CrkII with phospho-Cbl. CrkII also forms a constitutive complex with the guanyl nucleotide-exchange protein C3G. Once translocated into lipid rafts, C3G comes into proximity with the G protein TC10, and catalyzes the exchange of GTP for GDP, resulting in the activation of the protein. Once activated, TC10 seems to provide a second signal to the GLUT4 protein that functions in parallel with the activation of the PI3K pathway. This may involve the stabilization of cortical actin, which seems to be important in GLUT4 vesicle translocation to the plasma membrane [21].

**Inhibition of insulin signaling**

Termination of the insulin signal is critical for the maintenance of metabolic control. Signaling of the insulin receptor cascade is terminated by specific phosphatases. They act to terminate insulin’s effects through activation of lipid or protein phosphatases and through the induction of serine/threonine kinases that phosphorylate and uncouple various elements along the insulin signaling pathways [24]. Because Ser/Thr phosphorylation of IRS proteins is stimulated by insulin treatment and by inducers of insulin resistance, it suggests that the same Ser kinases might be utilized to
phosphorylate the IRS proteins under both physiological and pathological conditions [24]. Protein-tyrosine phosphatase IB (PTP1B), phosphatase and tensin homologue protein (PTEN) and SHIP2, which inactivate the lipid products of PK3I, are involved in the termination of insulin signaling. Other negative feedback control mechanisms operate on a longer time scale, and involve a reduction in the cellular content of insulin receptor and its substrates. Inhibition of insulin signal represents a new area of research that might shed light to the molecular mechanisms involved in the physiopathology of type-2 diabetes [24].

6. Molecular mechanisms of metabolic insulin actions

Insulin-mediated glucose transport

In fed normoglycemic conditions the transport of glucose into the skeletal muscle cells represents the main pathway for glucose uptake [5]. In skeletal muscle and adipose tissue, GLUT1 mediates basal glucose transport, whereas GLUT4 is responsible for insulin-stimulated glucose uptake. In the basal state, GLUT4 is primarily intracellular. Insulin stimulates the translocation of GLUT4-vesicles to the plasma membrane, where they facilitate glucose uptake. [25]. The molecular mechanism by which insulin causes redistribution of GLUT4 has been extensively studied. Two pathways appear to be involved: The first, and more established, pathway is PI3K/Akt. However, activation of PI3K is insufficient for insulin-stimulated glucose transport. A second pathway indicates that the CAP (Cbl-associated protein)/Cbl complex, may play a role in glucose uptake [26].

Recent studies have revealed the importance of the actin cytoskeleton in insulin-induced GLUT4 translocation [27]. Actin is an exceedingly complex molecule in terms of its dynamics and interactions with other proteins. The insulin-induced dynamic actin rearrangements necessary for GLUT4 translocation apparently result from a combination of the PI3K signal and spatially compartmentalized TC10 signal since both are activated by insulin stimulation [28].

Insulin-mediated glycogen synthesis

Insulin stimulates glycogen accumulation through a coordinated increase in glucose transport and glycogen synthesis. The hormone activates glycogen synthase by promoting its dephosphorylation, through the inhibition of kinases such as PKA or GSK-3 [29], and activation of protein phosphatase 1 (PP1). [30]. Insulin, via PI3K/Akt, phosphorylates and inactivates GSK-3, decreasing the rate of phosphorylation of glycogen synthase, thus increasing its activity state. On the other hand, the mechanism by which insulin activates glycogen-associated PP1 remains unknown, inhibitors of PI3K block
this effect, suggesting that PtdIns(3,4,5)P3-dependent protein kinases are involved.

**Insulin-mediated lipid synthesis**

Insulin induces the transcription of critical enzymes of lipid synthesis. The transcriptional factor, sterol regulatory element binding protein 1c (SREBP-1c), has a pivotal role on lipogenic gene expression, and has been proposed as a key mediator of insulin transcriptional effects [31]. Insulin stimulates the transcription of SREBP-1c [32], this effect of insulin on SREBP-1c appears to involve PI3K/Akt pathway [33]. Dominant negative forms of SREBP-1c can block expression of lipogenic genes, whereas overexpression can increase their transcription effects [31].

Alongside the anabolic actions of insulin (i.e. glycogen synthesis, lipogenesis) in the fed state, the hormone has restricted functions on glucose output and lipolysis [6,7].

**Insulin repression of glucose output**

Insulin inhibits the production and release of glucose by the liver by blocking gluconeogenesis and glycogenolysis. This occurs through a direct effect of insulin on the liver, as well as by indirect effects of insulin on substrate availability, such as free fatty acids, lactate and aminoacids [21]. Insulin inhibits the transcription of the gene encoding phosphoenolpyruvate carboxylase, the rate-limiting step in gluconeogenesis [34]. The hormone also decreases transcription of the genes encoding fructose-1, 6-bisphosphatase and glucose-6-phosphatase. The mechanism is produced via phosphatidylinositol 3-kinase (PI3K) and Akt through phosphorylation of the transcription factor Foxo 1 [6]. In the fasting state, Foxo1 is largely dephosphorylated and is localized in the nucleus, where it binds the peroxisome proliferator–activated receptor-coactivator (PGC)-1 [35] and Cbp/p300 [36] to promote phosphoenolpyruvate carboxylase and glucose-6-phosphatase transcription. Insulin-dependent phosphorylation via AKT dissociates the Foxo1/PGC-1 complex and causes Foxo1 nuclear export and consequently inhibition of target gene expression [6].

**Insulin repression of lipolysis**

The hormone strongly inhibits lipolysis in adipocytes, through the inhibition of the hormone-sensitive lipase [37]. Insulin inhibits the activity of the lipase primarily through reductions in cAMP levels, owing to the activation of a cAMP-specific phosphodiesterase 3B by the serine-threonine kinase Akt [38]. The low concentration of insulin required to inhibit the hormone-sensitive lipase may explain why patients with mild type-2 diabetes and glucose
intolerance are hyperglycemic in the absence of significant elevations of plasma free fatty acids or ketone bodies.

7. Adipose tissue

Adipose tissue is a unique tissue in fuel metabolism in several ways: a) it stores and releases excess energy in the form of lipids, modifying its size and number in accordance with changing metabolic needs; b) it releases free fatty acids that inhibit glucose uptake, glucose oxidation and glycogen synthesis and stimulate hepatic glucose output [39-42]; c) adipose metabolism, specifically glucose transport mediated by GLUT4, is capable of directing insulin action in peripheral tissues [43]; d) moreover, adipose tissue is capable of integrating signals from other organs and responds by regulating secretion of multiple proteins, termed adipokines [44] that interfere with insulin action. Cytokines such as, TNF-alpha, IL-6, and resistin decrease insulin action. On the contrary, adiponectin and visfatin possess a positive effect on insulin action [For review see 44].

8. Metabolism in type-2 diabetes

Type-2 diabetes is characterized by impaired insulin action and/or abnormal insulin secretion. An early abnormality in the disease is insulin resistance; a defective state in which insulin is unable to exert its biological effects at circulation concentrations that are effective in normal subjects. Insulin resistance has been proposed as the key linking factor for the metabolic syndrome disease cluster of glucose intolerance, hypertension and dyslipidemia.

Insulin resistance leads to profound decreases in glucose uptake and glycogen synthesis in peripheral tissues [45, 46]. Impaired hepatic glycogen stores and glycogen synthase activity are also observed in insulin resistance [42, 47]. Insulin resistance yields to defective suppression of hepatic glucose output, under the fasting as well as the fed state [6]. Resistance to the antilipolytic action of insulin also favors triglyceride breakdown in adipose tissue and the generation of free fatty acids, which inhibit insulin-stimulated glucose uptake and metabolism in skeletal muscle, stimulate hepatic gluconeogenesis [41,42] and interfere with insulin receptor signals [21]. Changes in serum adipokine concentrations are also part of the insulin resistant state [44].

At the pre-onset of type-2 diabetes, resistance to the glucose-lowering action of insulin, tends to lead a slight increase of blood glucose concentration, which stimulates insulin secretion and causes hyperinsulinemia. Initially hyperinsulinemia is able to overcome insulin resistance. The diabetic state develops when insulin secretion cannot longer be sustained to compensate insulin resistance, and it is at this stage that fasting and post-prandrial hyperglycemia is apparent.


9. Molecular mechanisms affected in type-2 diabetes

**Insulin resistance**

It is now well accepted that defective postreceptor insulin signaling is the main feature involved in insulin resistance of type-2 diabetes. As a result the metabolic insulin actions are affected. Several mechanisms such as tyrosine dephosphorylation, imbalance of serine/threonine phosphorylation, or insulin receptor internalization impair insulin signaling [24]. A number of molecules associated to insulin resistance affect these mechanisms, and most of them are related to adipose tissue. Transcriptional factors have also been found to be associated with insulin resistance.

**Adipose tissue**

Dysfunction of adipose tissue plays a crucial role in the development of insulin resistance in type-2 diabetes. Both obesity and lipodystrophy lead to insulin resistance in muscle. Impaired glucose transport by GLUT4 in adipose tissue results in insulin resistance in the muscle and liver [43]. Emerging data suggest that molecules released from adipocytes, such as free fatty acids, TNF alpha, interleukin 6, inhibit insulin signaling and induce insulin resistance, and activate serine/threonine kinases that phosphorylate the IRS proteins and inhibit their function [48, 44].

**Free fatty acids.** At molecular level, elevated FFAs are associated with a reduction in insulin-stimulated IRS-1 phosphorylation and IRS-1-associated PI3K activity [21]. Free fatty acids activate cellular kinases, including atypical protein kinase C isoforms by increasing cellular diacylglycerol concentrations, which can activate the inflammatory kinase inhibitor kB (IKK) and c.jun N-terminal kinases, increasing serine/threonine phosphorylation of IRS-1 and reducing downstream IRS-1 signalling [49].

**Interleukin 6 (IL-6).** In insulin resistance states, a two- to threefold elevation of circulating IL-6 has been observed. IL-6 decreases tyrosine phosphorylation of the IR substrate (IRS)1, and decreases association of the p85 subunit of phosphatidylinositol 3-kinase with IRS-1 in response to physiologic insulin levels. In addition, insulin-dependent activation of Akt, is markedly inhibited by IL-6 treatment [50]. These events are mediated through increases in the expression of the suppressor of cytokine signaling-3 (SOCS-3) protein. [51].

**Tumor necrosis alpha (TNF-alpha).** TNF-alpha is a cytokine produced by adipocytes that has been widely implicated as a causative factor in obesity-associated insulin resistance and the pathogenesis of type-2 diabetes [revised in 52]. Multiple mechanisms have been suggested to account for the metabolic effects of TNF-alpha. These include the induction of elevated free fatty acids
Cristina Fernandez-Mejia

via stimulation of lipolysis, downregulation of genes that are required for normal insulin action, such as GLUT4, direct effects on insulin signaling, and negative regulation of PPAR gamma [revised in 52].

**Peroxisome proliferator activated receptor gamma (PPAR gamma).** PPAR’s are nuclear receptors that heterodimerize with the retinoic X receptor and regulate transcription of a number of genes. PPAR-gamma, in particular, has been shown to be involved in regulating genes involved in insulin action, such as, SREBP-1c and PEPCK [53,54]. In addition, PPAR activation inhibits leptin gene expression, as well as the expression of TNF-alpha, which, in turn, is an inhibitor of PPAR gene expression [55]. The notion that PPAR’s play an important role of type-2 diabetes is supported by the recent description of families whose mutations in PPAR caused severe insulin resistance and diabetes [56].

**Fuel oxidation**

Several investigations have found that mitochondrial function might be involved in the pathogenesis of insulin resistance and type-2 diabetes [revised in 57]. Microarray studies in obese and type-2 diabetic subjects found decreased gene expression of genes regulated by the transcriptional factor peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1 alpha) [58], a transcriptional factor that plays an important role in mitochondria biogenesis. Furthermore, the activity of mitochondrial oxidative enzymes was found to be lower in type-2 diabetic patients [59]. These data support the concept that insulin resistance arises from defects in mitochondrial fatty acid oxidation, which, in turn, lead to increases in fatty acid metabolites, such as fatty acyl CoA and diacylglycerol) that disrupt insulin signaling [57]. Additional evidence of the importance of PGC-1 in metabolic regulation is its role in the regulation of hepatic gluconeogenesis genes such as PEPCK and glucose-6-phosphate phosphatase, and its molecular interaction with diabetogenic agents such as glucocorticoids [6, 60]. Defects in fuel oxidation are also involved in impaired insulin secretion and in diabetic genetic defects (see below).

**Insulin secretion**

Type-2 diabetes arises when pancreatic beta cells fail to secrete sufficient insulin to handle the insulin resistance demand, because of acquired beta-cell secretory dysfunction and/or decreased beta-cell mass. Recent studies have presented evidence that the beta-cell mass plays a pivotal role in determining whether an individual will progress to type-2 diabetes [61]. These defects may be caused by primary beta-cell defects, such as seen in the monogenic diabetes forms of MODY (see below in Genetic Factors in Diabetes Section), or by secondary beta-cell defects, caused by glucotoxicity, increased free fatty acids, cytokines, mitochondrial disfunction and/or metabolic stress.
Beta-cell mass

Pancreatic beta mass is regulated by: a) beta-cell replication, b) beta-cell size, c) beta-cell neogenesis, and d) beta-cell apoptosis. The beta-cell mass adapts to an increased metabolic load caused by insulin resistance. The onset of type-2 diabetes is accompanied by a progressive decrease in the beta-cell mass that arises from a marked increase of beta-cell apoptosis which prevails over beta-cell replication and neogenesis [61]. Studies in IRS-2 knockout mice have provided clues for the important role of IRS-2 on beta-cell mass regulation [18]. Since then, several lines of evidence support a role of IRS-2 in the pathogenesis of type diabetes [revised in 61]. Similar mechanisms to those produced by cytokines and free fatty acids on insulin signaling in the peripheral tissues (see above, Insulin Resistance) affect IRS-2 activity in beta cells and could account for the acquired beta-cell secretory dysfunction.

Glucotoxicity

Deterioration of insulin secretion over time is the usual course in most type-2 diabetic patients, and many patients will end with more or less severe insulin deficiency after about 10 years of diabetes [62]. Hyperglycemia itself decreases insulin secretion and is implicated in the damage of beta cells [63]. Chronic hyperglycemia impairs insulin gene expression of two major beta-cell transcription factors, pancreatic-duodenum homeobox-1 (PDX-1) and the activator of the rat insulin promoter element 3b1 [64]. The molecular mechanisms of glucotoxicity have been proposed to involve generation of chronic oxidative stress [63]. Furthermore, a new study demonstrates that hyperglycemia-induced mitochondrial superoxide production activates uncoupling protein 2, which decreases the ATP/ADP ratio and thus reduces the insulin-secretory response [65]. Additionally, reactive oxygen species are known to enhance NFk-Beta activity, which potentially induces beta-cell apoptosis, and might account for glucotoxicity [61].

10. Genetic factors

After the elucidation of Mendelian disorders with diabetes as a major phenotypic feature it has become clear that type-2 diabetes is heterogeneous and may result from defects in one or more molecular pathways. Genetic defects of the beta cell, usually referred to as maturity-onset diabetes of the young (MODY), can result from mutations in any of at least six different genes (see Table 1). Most of the MODY subtypes are caused by mutations in transcription factors, which are involved in the tissue-specific regulation of gene expression in the liver and in pancreatic beta-cells. Other related genetic factors are due to insulin receptor mutations [66].
Genetic defects of the beta cell

**HNFs.** HNF-1 alpha and HNF-1 Beta are members of the homeodomain-containing family of transcription factors and HNF-4 alpha is an orphan nuclear receptor. They involve part of a network of transcription factors that controls gene expression during embryonic development and in the adult tissues in which they are co-expressed. In the pancreatic beta cell, they regulate the expression of insulin as well as proteins involved in glucose transport, glycolysis and mitochondrial metabolism, all of which are important in the regulation of insulin secretion [67].

**IPF-1.** is a homeodomain-containing transcription factor involved in pancreatic development [68] transcriptional regulation of a number of critical beta-cell genes including insulin, glucokinase and glucose transporter 2 (GLUT2), all involved in glucose-induced insulin secretion.

**NeuroD1/BETA2.** The basic helix–loop–helix transcription factor NeuroD1/BETA2 participates in the regulation of transcription of the insulin gene and is required for normal pancreatic islet development [69]. Mutations in NeuroD1 are a rare cause of MODY and result in reduced serum insulin concentrations [70].

**Glucokinase.** MODY-2 is due to mutations in the glucokinase gene. Glucokinase is a tissue-specific enzyme expressed in liver, in pancreatic beta cells and in certain neuroendocrine cells of the brain and gut [71] and it plays a key-role in glucose homeostasis. In the liver, its activity is critical for glucose uptake and glycogen synthesis in the post-prandial state [72]. In beta cells, glucokinase plays a key-role regulating insulin secretion in response to glucose [9]. The combination of reduced glucose-induced insulin secretion from the pancreatic beta cell and reduced glycogen storage in the liver leads to an increase in plasma glucose concentrations.

**Mitochondrial DNA.** Abnormal mitochondrial function resulting from mutations in the mitochondrial genome can lead to diabetes [73]. The most common diabetes-associated mutation is an A-to-G transition in the mitochondrial tRNALeu(UUR) gene at base pair 3,243. This results in defects in insulin secretion including failure of glucose to prime the insulin secretory response and abnormal insulin secretory oscillations [74].

Genetic factors in polygenic type-2 diabetes

Considerable efforts aiming to identify genes responsible for more common, polygenic forms of type-2 diabetes have been underway for the last two decades, however, the result of these investigations has been largely disappointing. Sequence differences in a few genes have been associated, so far, with complex, polygenic forms of type-2 diabetes [49]. Among the variants identified are: PPAR gamma, glycogen synthase, IRS-1, proinsulin, sulfonylurea receptor1, potassium inward rectifier 6.2, PGC1 alpha, and
calpain 10 a ubiquitously expressed protein protease which plays roles in membrane fusion [75] and hydrolyzes various proteins that participate in cellular signaling, such as kinases, receptors, and transcription factors. Further, calpain is also important for differentiation of preadipocytes into adipocytes. These facts raise the possibility that calpain modulates both insulin secretion and action. Because many steps could be modulated by calpain, further precise experiments are essential to clarify the molecular and physiological mechanism explaining the association of calpain with type-2 diabetes. [75].

Conclusions

Classical physiology studies, biochemical methods, tissue culture technology, the use of gene targeting approaches in mice, and naturally occurring mutations in patients have shed some light on the molecular causes of type-2 diabetes and have contributed to a deeper understanding of the underlying mechanisms involved in the disease. With some variations, the observations of Thomas Willis [2] remain true until today. Now we can write that diabetes is rare in rural life, but in our age, given sedentary life style and gussling down chiefly of soda and junk food, we meet with examples and instances enough, I may say daily, of this disease. As to what belongs the cure, it seems a most hard thing of this disease to draw propositions for curing, for this cause lies hid inside the molecular mechanisms of insulin signaling and insulin secretion.

Acknowledgments

We would like to thank Isabel Pérez Montfort for correcting the English version of the manuscript and to Dr. Silvestre Frenk for critical reading of the manuscript. Supported by research grants from the Consejo Nacional de Ciencia y Tecnologia 44266M and the Direccion General de Asuntos del Personal Academico IN208605.

References

2. Willis, T. Pharmaceutica rationalis sive diatriba de medicamentorum operationitus in humano corpore. 2 vols. London 1674-1675
Molecular basis of type-2 diabetes


