3. REVIEW OF LITERATURE
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3.1. DIABETES MELLITUS: DEFINITION, HISTORY, EPIDEMIOLOGY AND CLASSIFICATION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2008).

Diabetes develops due to a diminished production of insulin (in type 1) or resistance to its effects (in type 2 and gestational). Both lead to hyperglycemia, which largely causes the acute signs of diabetes: excessive urine production, resulting compensatory thirst and increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism. All forms of diabetes have been treatable since insulin became medically available in 1921, but there is no cure. Type 2 diabetes mellitus is managed with a combination of dietary treatment, tablets and insulin supplementation. Diabetes and its treatments can cause many complications. Acute complications (hypoglycemia, ketoacidosis, or nonketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long-term complications include cardiovascular disease (doubled risk), chronic renal failure, retinal damage (which can lead to blindness), nerve damage (of several kinds), and microvascular damage, which may cause impotence and poor wound healing. Adequate treatment of diabetes, as well as increased emphasis on blood pressure control and lifestyle factors (such as not smoking and maintaining a healthy body weight), may improve the risk profile of most of the chronic complications (WHO, 1999).
History

Diabetes mellitus often referred to simply as diabetes (Ancient Greek: διαβήτης "to pass through [urine]") is often referred to simply as diabetes (Ancient Greek: διαβήτης, διαβήτης) was coined by Aretaeus of Cappadocia. It was derived from the Greek verb διαβάλειν, διαβαίνειν, itself formed from the prefix δια-, "across, apart," and the verb βαίνειν, "to walk, stand." The verb διαβαίνειν meant "to stride, walk, or stand with legs asunder"; hence, its derivative διαβήτης meant "one that straddles," or specifically "a compass, siphon." The sense "siphon" gave rise to the use of διαβήτης as the name for a disease involving the discharge of excessive amounts of urine. Diabetes is first recorded in English, in the form diabete, in a medical text written around 1425. In 1675, Thomas Willis added the word mellitus, from the Latin meaning "honey," a reference to the sweet taste of the urine. This sweet taste had been noticed in urine by the ancient Greeks, Chinese, Egyptians, Indians, and Persians. In 1776, Matthew Dobson confirmed that the sweet taste was because of an excess of a kind of sugar in the urine and blood of people with diabetes (Dobson, 1776). Diabetes mellitus appears to have been a death sentence in the ancient era. Hippocrates makes no mention of it, which may indicate that he felt the disease was incurable. Aretaeus did attempt to treat it but could not give a good prognosis; he commented that "life (with diabetes) is short, disgusting and painful (Medvei, 1993). Sushruta (6th century BCE) identified diabetes and classified it as Madhumeha. He further identified it with obesity and sedentary lifestyle, advising exercises to help cure it (Dwivedi and Dwivedi, 2007). The ancient Indians tested for diabetes by observing whether ants were attracted to a person's urine, and called the ailment "sweet urine disease" (Madhumeha). The Korean, Chinese,
and Japanese words for diabetes are based on the same ideographs (腎糖尿) which mean "sugar urine disease". In medieval Persia, Avicenna (980-1037) provided a detailed account on diabetes mellitus in The Canon of Medicine, "describing the abnormal appetite and the collapse of sexual functions and he documented the sweet taste of diabetic urine." Like Aretaeus before him, Avicenna recognized primary and secondary diabetes. He also described diabetic gangrene, and treated diabetes using a mixture of lupine, trigonella (fenugreek), and zedoary seed, which produces a considerable reduction in the excretion of sugar, a treatment which is still prescribed in modern times. Avicenna also "described diabetes insipidus very precisely for the first time", though it was later Johann Peter Frank (1745-1821) who first differentiated between diabetes mellitus and diabetes insipidus (Nabipour, 2003). Although diabetes has been recognized since antiquity, and treatments of various efficacy have been known in various regions since the Middle Ages, and in legend for much longer, pathogenesis of diabetes has only been understood experimentally since about 1900 (Patlak, 2002).

The discovery of a role for the pancreas in diabetes is generally ascribed to Joseph von Mering and Oskar Minkowski, who in 1889 found that dogs whose pancreas was removed developed all the signs and symptoms of diabetes and died shortly afterwards (Von Mehring and Minkowski, 1890). In 1910, Sir Edward Albert Sharpey-Schafer suggested that people with diabetes were deficient in a single chemical that was normally produced by the pancreas—he proposed calling this substance insulin, from the Latin *insula*, meaning island, in reference to the insulin-producing islets of Langerhans in the pancreas (Patlak, 2002). The endocrine role of the pancreas in metabolism, and indeed the
existence of insulin, was not further clarified until 1921, when Sir Frederick Grant Banting and Charles Herbert Best repeated the work of Von Mering and Minkowski, and went further to demonstrate they could reverse induced diabetes in dogs by giving them an extract from the pancreatic islets of Langerhans of healthy dogs (Banting et al., 1922). Banting, Best, and colleagues (especially the chemist Collip) went on to purify the hormone insulin from bovine pancreases at the University of Toronto. This led to the availability of an effective treatment—insulin injections—and the first patient was treated in 1922. For this, Banting and laboratory director MacLeod received the Nobel Prize in Physiology or Medicine in 1923; both shared their Prize money with others in the team who were not recognized, in particular Best and Collip. Banting and Best made the patent available without charge and did not attempt to control commercial production. Insulin production and therapy rapidly spread around the world, largely as a result of this decision. The distinction between what is now known as type 1 diabetes and type 2 diabetes was first clearly made by Sir Harold Percival (Harry) Himsworth (Himsworth, 1936). Other landmark discoveries include (Patlak, 2002):

- Identification of the first of the sulfonylureas in 1942
- Reintroduction of the use of biguanides for Type 2 diabetes in the late 1950s. The initial phenformin was withdrawn worldwide (in the U.S. in 1977) due to its potential for sometimes fatal lactic acidosis and metformin was first marketed in France in 1979, but not until 1994 in the US.
- The determination of the amino acid sequence of insulin (by Sir Frederick Sanger, for which he received a Nobel Prize)
• The radioimmunoassay for insulin, as discovered by Rosalyn Yalow and Solomon Berson (gaining Yalow the 1977 Nobel Prize in Physiology or Medicine) (Yalow and Berson, 1960)
• The three-dimensional structure of insulin (PDB 2INS)
• Dr Gerald Reaven's identification of the constellation of symptoms now called metabolic syndrome in 1988
• Identification of the first thiazolidinedione as an effective insulin sensitizer during the 1990s

Epidemiology

In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double (Wild et al., 2004). Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will likely be found by 2030 (Wild et al., 2004). In 2005 there were about 20.8 million people with diabetes in the United States alone. According to the American Diabetes Association, there are about 6.2 million people undiagnosed and about 41 million people that would be considered prediabetic. The American Diabetes Association point out the 2003 assessment of the National Center for Chronic Disease Prevention and Health Promotion (Centers for Disease Control and Prevention) that 1 in 3 Americans born after 2000 will develop diabetes in their lifetime (American Diabetes Association, 2005). Diabetes mellitus prevalence
increases with age, and the numbers of older persons with diabetes are expected to grow as the elderly population increases in number. The National Health and Nutrition Examination Survey (NHANES III) demonstrated that, in the population over 65 years old, 18% to 20% have diabetes, with 40% having either diabetes or its precursor form of impaired glucose tolerance (Harris et al., 1998).

**Signs, symptoms and diagnosis**

The classical triad of diabetes symptoms is polyuria, polydipsia and polyphagia, which are, respectively, frequent urination, increased thirst and consequent increased fluid intake, and increased appetite. Symptoms may develop quite rapidly (weeks or months) in type 1 diabetes, particularly in children. However, in type 2 diabetes symptoms usually develop much more slowly and may be subtle or completely absent. Type 1 diabetes may also cause a rapid yet significant weight loss and irreducible fatigue. All of these symptoms except weight loss can also manifest in type 2 diabetes in patients whose diabetes is poorly controlled. When the glucose concentration in the blood is raised beyond its renal threshold, reabsorption of glucose in the proximal renal tubule is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Patients (usually with type 1 diabetes) may also initially present with diabetic ketoacidosis (DKA), an extreme state of metabolic dysregulation characterized by the smell of acetone on the patient's breath; a rapid, deep breathing known as Kussmaul breathing; polyuria; nausea; vomiting and abdominal pain; and any of many altered states of consciousness or arousal (such as hostility and mania or,
Diabetic ketoacidosis is a medical emergency and requires immediate hospitalization. A rarer but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration due to loss of body water. Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following: (WHO 1999) fasting plasma glucose level at or above 126 mg/dL (7.0 mmol/l) and/or plasma glucose at or above 200 mg/dL (11.1 mmol/l) two hours after a 75 g oral glucose load as in a glucose tolerance test and/or random plasma glucose at or above 200 mg/dL (11.1 mmol/l).

Prognosis: Patient education, understanding, and participation is vital since the complications of diabetes are far less common and less severe in people who have well-controlled blood sugar levels (Nathan et al., 2005).

Acute complications include Diabetic ketoacidosis (DKA) is an acute and dangerous complication that is always a medical emergency. Low insulin levels cause the liver to turn to fat for fuel (i.e., ketosis); ketone bodies are intermediate substrates in that metabolic sequence. DKA is always a medical emergency and requires medical attention. Ketoacidosis is much more common in type 1 diabetes than type 2; Nonketotic hyperosmolar coma - Hyperosmolar nonketotic state (HNS) is an acute complication sharing many symptoms with DKA, but an entirely different origin and different treatment. A person with very high (usually considered to be above 300 mg/dL (16 mmol/L)) blood glucose levels, water is osmotically drawn out of cells into the blood and the kidneys eventually begin to dump glucose into the urine. This results in loss of water and an
increase in blood osmolarity. If fluid is not replaced (by mouth or intravenously), the osmotic effect of high glucose levels, combined with the loss of water, will eventually lead to dehydration. Electrolyte imbalances are also common and are always dangerous. As with DKA, urgent medical treatment is necessary, commonly beginning with fluid volume replacement. Lethargy may ultimately progress to a coma, though this is more common in type 2 diabetes than type 1; Hypoglycemia or abnormally low blood glucose, is an acute complication of several diabetes treatments. It is rare otherwise, either in diabetic or non-diabetic patients. The patient may become agitated, sweaty, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings akin to dread and immobilized panic. Consciousness can be altered or even lost in extreme cases, leading to coma, seizures, or even brain damage and death. Furthermore, reduced sympatho-adrenal responses can cause hypoglycemia unawareness. The concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes posits that recent incidents of hypoglycemia causes both defective glucose counterregulation and hypoglycemia unawareness. By shifting glycemic thresholds for the sympatho-adrenal (including epinephrine) and the resulting neurogenic responses to lower plasma glucose concentrations, antecedent hypoglycemia leads to a vicious cycle of recurrent hypoglycemia and further impairment of glucose counterregulation.

**Chronic complications**

Vascular disease - Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). The endothelial cells lining the blood vessels take in more glucose than normal, since they don't depend on insulin. They then form more surface
glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under "microvascular disease" (due to damage to small blood vessels) and "macrovascular disease" (due to damage to the arteries). The damage to small blood vessels leads to a microangiopathy, which can cause one or more of the following:

**Diabetic retinopathy**, growth of friable and poor-quality new blood vessels in the retina as well as macular edema (swelling of the macula), which can lead to severe vision loss or blindness.

**Diabetic neuropathy**, abnormal and decreased sensation, usually in a 'glove and stocking' distribution starting with the feet but potentially in other nerves, later often fingers and hands. When combined with damaged blood vessels this can lead to **diabetic foot**.

Diabetic amyotrophy is muscle weakness due to neuropathy.

**Diabetic nephropathy**, damage to the kidney which can lead to chronic renal failure, eventually requiring dialysis. Diabetes mellitus is the most common cause of adult kidney failure worldwide in the developed world.

**Diabetic cardiomyopathy**, damage to the heart, leading to diastolic dysfunction and eventually heart failure.

Macrovascular disease leads to cardiovascular disease, Coronary artery disease, leading to angina or myocardial infarction ("heart attack"), Stroke (mainly the ischemic type), Peripheral vascular disease, which contributes to intermittent claudication (exertion-related leg and foot pain) as well as diabetic foot, Diabetic myonecrosis ('muscle wasting'). Diabetic foot, often due to a combination of sensory neuropathy (numbness or
insensitivity) and vascular damage, increase rates of skin ulcers and infection. Carotid artery stenosis does not occur more often in diabetes, and there appears to be a lower prevalence of abdominal aortic aneurysm. However, diabetes does cause higher morbidity, mortality and operative risks with these conditions (Weiss and Sumpio, 2006). Diabetic encephalopathy is the increased cognitive decline and risk of dementia observed in diabetes. Various mechanisms are proposed, including alterations to the vascular supply of the brain and the interaction of insulin with the brain itself (Gispen and Biessels, 2000).

Classification of Diabetes mellitus

The American Diabetes Association (ADA, 2008) classified diabetes mellitus in a new form as shown in Table 1. The principal two idiopathic forms of diabetes mellitus are known as types 1 and 2. The term "type 1 diabetes" has universally replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes (IDDM). Likewise, the term "type 2 diabetes" has replaced several former terms, including adult-onset diabetes, obesity-related diabetes, and non-insulin-dependent diabetes (NIDDM). Beyond these two types, there is no agreed-upon standard nomenclature. Various sources have defined "type 3 diabetes" as, among others, gestational diabetes, insulin-resistant type 1 diabetes (or "double diabetes"), type 2 diabetes which has progressed to require injected insulin, and latent autoimmune diabetes of adults (LADA or "type 1.5" diabetes) There is also maturity onset diabetes of the young (MODY) which is a group of several single gene (monogenic) disorders with strong family histories that present as type 2 diabetes before 30 years of age.
Table 1: Etiologic classification of diabetes mellitus (ADA, 2008)

| I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency) |
|---------------------------------|---------------------------------|
| A. Immune mediated              | B. Idiopathic                   |

| II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance) |

<table>
<thead>
<tr>
<th>III. Other specific types</th>
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</thead>
<tbody>
<tr>
<td>A. Genetic defects of β-cell function</td>
</tr>
<tr>
<td>1. Chromosome 12, HNF-1α (MODY3)</td>
</tr>
<tr>
<td>2. Chromosome 7, glucokinase (MODY2)</td>
</tr>
<tr>
<td>3. Chromosome 20, HNF-4α (MODY1)</td>
</tr>
<tr>
<td>4. Chromosome 13, insulin promoter factor-1 (IPF-1; MODY4)</td>
</tr>
<tr>
<td>5. Chromosome 17, HNF-1β (MODY5)</td>
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<tr>
<td>6. Chromosome 2, NeuroD1 (MODY6)</td>
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<td>7. Mitochondrial DNA</td>
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<td>8. Others</td>
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<th>B. Genetic defects in insulin action</th>
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</thead>
<tbody>
<tr>
<td>1. Type A insulin resistance</td>
</tr>
<tr>
<td>2. Leprechaunism</td>
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<td>3. Rabson-Mendenhall syndrome</td>
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<td>4. Lipoatrophic diabetes</td>
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<td>5. Others</td>
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<th>C. Diseases of the exocrine pancreas</th>
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<tbody>
<tr>
<td>1. Pancreatitis</td>
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<td>2. Trauma/pancreatectomy</td>
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<tr>
<td>3. Neoplasia</td>
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<tr>
<td>4. Cystic fibrosis</td>
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<tr>
<td>5. Hemochromatosis</td>
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<tr>
<td>6. Fibrocalculous pancreatopathy</td>
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<td>7. Others</td>
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<tr>
<th>D. Endocrinopathies</th>
</tr>
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<tbody>
<tr>
<td>1. Acromegaly</td>
</tr>
<tr>
<td>2. Cushing's syndrome</td>
</tr>
<tr>
<td>3. Glucagonoma</td>
</tr>
<tr>
<td>4. Pheochromocytoma</td>
</tr>
<tr>
<td>5. Hyperthyroidism</td>
</tr>
</tbody>
</table>
6. Somatostatinoma
7. Aldosteronoma
8. Others

E. Drug- or chemical-induced
1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. β-adrenergic agonists
8. Thiazides
9. Dilantin
10. α-interferon
11. Others

F. Infections
1. Congenital rubella
2. Cytomegalovirus
3. Others

G. Uncommon forms of immune-mediated diabetes
1. "Stiff-man" syndrome
2. Anti-insulin receptor antibodies
3. Others

H. Other genetic syndromes sometimes associated with diabetes
1. Down's syndrome
2. Klinefelter's syndrome
3. Turner's syndrome
4. Wolfram's syndrome
5. Friedreich's ataxia
6. Huntington's chorea
7. Laurence-Moon-Biedl syndrome
8. Myotonic dystrophy
9. Porphyria
10. Prader-Willi syndrome
11. Others

IV. Gestational diabetes mellitus (GDM)
3.2 TYPE 1 DIABETES MELLITUS (IMMUNE-MEDIATED DIABETES)

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes, type I diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cells include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD$_{65}$), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective (ADA, 2008).

3.2.1 Pathophysiology of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is an autoimmune disease governed by multiple genetic and environmental risk factors. T1DM is characterized by a permissive immune system that fails to impose tolerance to arrays of self-antigens. Overt diabetes reflects glucose intolerance due to insulin deficiency. It is the end result of prediabetes, with progressive lymphoid infiltration around and then inside pancreatic islets of Langerhans and subsequent destruction of insulin-producing β-cells by autoreactive T lymphocytes. β-cell stress and death in the course of early islet restructuring are thought to provide sensitizing autoantigens, which expand autoreactive T cell pools in pancreatic lymph node (Mathis et al., 2001; Anderson and Bluestone, 2005).
Much of our present understanding about the immunopathology of IDDM results from studies in the non-obese diabetic (NOD) mouse (Delovitch and Singh, 1997). From these studies several mechanisms have been suggested to contribute to β-cell destruction, including delayed type hypersensitivity reactions mediated by CD4+ Th-1 cells reactive with islet antigens (Haskins and McDuffie, 1990), cytotoxic T-cell (CTL)-mediated lysis of islet cells (Wicker et al., 1994), local production of cytokines (TNF-α and IL-1) that directly damage islet cells (K gi et al., 1999), and autoantibodies against islet cells (Tisch et al., 1993, Daniel et al., 1995). The mechanisms contributing to the development of autoimmune reactions against pancreatic β-cells in certain individuals are unclear, but a strong genetic disposition to develop IDDM, including HLA genes is generally accepted (Anjos and Polychronakos, 2004).

Thymic negative selection

The underlying mechanism that initiates disease has been suggested to hinge upon the aberrant selection of autoreactive T lymphocytes that occurs during T cell development (Aoki et al., 2005, Kwon et al., 2005). Immature T cells differentiating in the thymus interact with the major histocompatibility complex (MHC) molecules on thymic epithelium or hematopoietically derived antigen (Ag)-presenting cells (APCs) through their T cell receptors (TCR) (Berg and Kang, 2001). These interactions lead to the ‘positive selection’ of T cells that recognize peptides bound to self-MHC gene products of the thymus. To prevent autoimmune responses, those T cells that possess TCR specific for self-antigens are eliminated in the thymus by ‘negative selection’ (Murphy et al., 1990, Chen, 2004). The
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origins of T1DM are the result of the erroneous survival of autoreactive, diabetogenic T cells that have escaped negative selection.

Table 2: Defects that might hamper positive and negative selection during the induction of central tolerance in NOD mice, BB rats and humans (Rosmalen et al., 2002)

<table>
<thead>
<tr>
<th>Positive selection</th>
<th>Thymic epithelium</th>
<th>Thymic APCs</th>
<th>Thymocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD mice and humans: positive selection bias towards high affinity CD4+ T cells</td>
<td>NOD mice and humans: selection-bias towards high affinity CD4+ T cells</td>
<td>NOD mice and humans: positive selection bias</td>
<td>NOD mice and humans: hyporesponsiveness to TCR stimulation might lead to reduced clonal detection.</td>
</tr>
<tr>
<td>because of poor peptide binding by particular diabetes-associated MHC class II haplotypes</td>
<td>NOD mice: lack of positive selection of regulatory T cells</td>
<td>NOD mice: lack of positive selection of regulatory T cells</td>
<td>NOD mice: Disturbed induction of apoptosis.</td>
</tr>
<tr>
<td>NOD mice: lack of positive selection of regulatory T cells</td>
<td>NOD mice: hyporesponsiveness to TCR stimulation might lead to reduced clonal detection.</td>
<td>BB rats: increased rate of apoptosis.</td>
<td>BB rats: increased rate of apoptosis.</td>
</tr>
<tr>
<td>Negative selection</td>
<td>NOD mice: disturbed reticulum structure with loss of the corticomedullary junction that is essential for negative selection.</td>
<td>NOD mice and humans: antigen-processing defects of peptides for MHC-class I-mediated negative selection of CD8+ T cells.</td>
<td>NOD mice and humans: lack of negative selection of autoreactive CD4+ T cells related to diabetes-associated MHC class II haplotypes</td>
</tr>
<tr>
<td>NOD mice and humans: lack of negative selection of autoreactive CD4+ T cells related to diabetes-associated MHC class II haplotypes</td>
<td>NOD mice and humans: lack of negative selection of autoreactive CD4+ T cells related to diabetes-associated MHC class II haplotypes</td>
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</table>

Peripheral-tolerance induction in diabetes-prone individuals

In addition to the aberrations in central tolerance, several mechanisms of peripheral tolerance also show defects, thus contributing to the loss of self-tolerance observed in diabetes-prone individuals (Table 3). These mechanisms include activation-induced cell death (AICD) and the activation of regulatory T cells.

Shefalee Bhavsar
L. M. College of Pharmacy, Ahmedabad, India
Table 3: Defects that might hamper mechanisms of induction of peripheral tolerance in NOD mice, BB rats and humans (Rosmalen et al., 2002)

<table>
<thead>
<tr>
<th>Activation-Induced cell death (AICD)</th>
<th>Peripheral APCs</th>
<th>T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD mice, BB rats and humans: aberrantly matured APCs with antigen-processing defects result in poor T-cell stimulation, thus hampering AICD.</td>
<td>NOD mice and humans: TCR signalling defects might result in disturbed AICD.</td>
<td></td>
</tr>
<tr>
<td>NOD mice, BB rats and humans: constitutive expression of PGS2 by APCs leads to inhibition of T-cell IL-2-mediated signal transduction.</td>
<td>NOD mice and humans: decreased induction of apoptosis.</td>
<td></td>
</tr>
<tr>
<td>Regulatory T cells</td>
<td>NOD mice, BB rats and humans: aberrant maturation of APCs results in poor stimulation of regulatory T cells.</td>
<td>NOD mice, BB rats and humans: functional deficiency in regulatory NK T cells.</td>
</tr>
<tr>
<td>NOD mice and humans: enhanced levels of IL-12 shift the Th1-Th2 cytokine balance in favour of Th1.</td>
<td>BB rats: lyp mutation results in an absence of regulatory RT+T cells.</td>
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</table>

Activation-induced cell death (AICD)

AICD is achieved upon APC-mediated stimulation of the TCR accompanied by simultaneous ligation of death receptors, such as CD95 and other members of the tumor necrosis factor (TNF) receptor family, expression of which is induced by the previous activation of T cells. Several studies suggest that APCs in diabetes-prone individuals might be unable to fulfill the requirements for optimal T-cell stimulation leading to AICD. Several studies suggest that APCs in diabetes-prone individuals might be unable to fulfill the requirements for optimal T-cell stimulation leading to AICD. An aberrant maturation of Mφs and DCs, resulting putatively in impaired function of APCs, has been observed in NOD mice, BB rats and diabetic humans. BM progenitor cells of granulocytes and monocytes from NOD mice proliferate poorly in response to several myeloid growth factors, including interleukin-3 (IL-3), granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-5, compared
with progenitor cells from normal mice (Feili-Hariri and Morel, 2001). Also, human diabetes patients and high-risk relatives show disturbances in the maturation of APCs, such as impaired yield and function of blood monocyte-derived DCs, and a decreased level of expression of CD80 and CD86 by DCs (Takahashi et al., 1998). Together, these data suggest that the AICD-mediated induction of peripheral tolerance in diabetes-prone individuals is affected at the level of the APC by maturation disturbances, giving rise to PGS2-expressing APCs with Ag-processing defects, resulting in suboptimal stimulation of T cells (Rosmalen et al., 2002).

Regulatory T cells

The development of autoimmune diabetes in humans and animals is associated with deficiencies in certain regulatory T-cell subsets responsible for the maintenance of peripheral tolerance. The T-cell deficiency in BB rats affects particularly the establishment of the regulatory mono-ADP-ribosyltransferase 2 (ART2)/RT6+ T-cell population (Hernandez-Hoyos et al., 1999). Another regulatory T-cell subset, identified as natural killer (NK) T cells, appears to be functionally deficient in NOD mice, BB rats and human type 1 diabetes patients (Wilson et al., 1998; Iwakoshi et al., 1999; Poulton et al., 2001). Thus, certain regulatory T-cell subsets are (functionally) deficient during the development of diabetes. Other regulatory T-cell subsets might not exert their suppressive actions because of insufficient stimulation by APCs, as a consequence of the maturational defects of the latter cells. Indeed, the stimulation of regulatory T cells, as measured in the autologous or syngeneic mixed leukocyte reaction (MLR), is disturbed in both human and animal diabetes. Therefore, an insufficient stimulation of regulatory T cells might be the
consequence of maturational defects in the APC lineages in human and animal diabetes. The function of regulatory T cells in the down-regulation of autoreactive Th1 cells appears to be disturbed during the development of diabetes. Some of the regulatory T-cell subsets, including RT6+ T cells in BB rats and NK T cells in NOD mice, BB rats and humans, are functionally deficient. Other regulatory T-cell subsets are stimulated insufficiently owing to abnormal peripheral APCs expressing diabetes-associated MHC haplotypes (Rosmalen et al., 2002).

Table 4: Characteristics of autoimmune diabetes in NOD mice, BB rats and humans

<table>
<thead>
<tr>
<th></th>
<th>NOD mice</th>
<th>BB rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC association</td>
<td>I-A g required, but not sufficient</td>
<td>RT1a required, but not sufficient</td>
<td>Multiple haplotype are associated</td>
</tr>
<tr>
<td>Incidence</td>
<td>Varying among colonies</td>
<td>Varying among colonies</td>
<td>0.1-0.4%</td>
</tr>
<tr>
<td>Gender distribution</td>
<td>F&gt;M</td>
<td>F=M</td>
<td>F=M</td>
</tr>
<tr>
<td>Age at onset of diabetes</td>
<td>4 months</td>
<td>3 months</td>
<td>Throughout life</td>
</tr>
<tr>
<td>Peri insulitis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Duration of Insulitis</td>
<td>Long</td>
<td>Short</td>
<td>Short to long</td>
</tr>
<tr>
<td>Islet autoantibodies</td>
<td>±</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Ketoacidosis</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leukocyte infiltrates in other tissues</td>
<td>Always</td>
<td>Often</td>
<td>Sometimes</td>
</tr>
</tbody>
</table>

Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. The pathogenesis of autoimmune diabetes is complex, as expected from the >23 genes found associated with the onset of disease (Wicker et al., 2005), both in humans and the non-obese diabetic (NOD) mouse, the most commonly used animal model. However, IDDM is a multi-factorial autoimmune disease for which susceptibility is determined not only by genetic but also by environmental factors as can be seen in monozygotic twins, where the concordance rate for IDDM is only 50% (Barnett et al., 1981).
Role of Cytokines

Cytokines are a category of signaling proteins and glycoproteins that, like hormones and neurotransmitters, are used extensively in cellular communication. While hormones are secreted from specific organs to the blood, and neurotransmitters are related to neural activity, the cytokines are a more diverse class of compounds in terms of origin and purpose. They are produced by a wide variety of hematopoietic and non-hematopoietic cell types and can have autocrine, paracrine and endocrine effects, sometimes strongly dependent on the presence of other chemicals. More than 30 immunologically active cytokines exist and are grouped as interleukins (ILs), interferons (IFNs), tumor necrosis factors (TNFs) and colony stimulating factors (CSFs). Both the production of cytokines by cells and the action of cytokines on cells are complex: a single cell can produce several different cytokines and a given cytokine can act on one or more cell types. Cytokines are characterized by considerable "redundancy", in that many cytokines appear to share similar functions. Generalization of functions is not possible with cytokines. Nonetheless, their actions may be grouped as: Autocrine, if the cytokine acts on the cell that secretes it. Paracrine, if the target is restricted to the immediate vicinity of a cytokine's secretion. Endocrine, if the cytokine diffuses to distant regions of the body (carried by blood or plasma).

Before substantial β-cell destruction occurs in both humans and rodent models of type-1 diabetes a progressive invasion of pancreatic islets by immune cells (insulitis) has been demonstrated. Insulitis is characterized by increased expression of proinflammatory cytokines such as interleukin (IL)-1β and tumour necrosis factor (TNF)-α as well as type I
cytokines i.e. interferon (IFN)-y which are released by infiltrating mononuclear cells like activated macrophages and T-cells (Rabinovitch, 1998). Cytokines like IL-1β, IL-1α and IFN-y are able to mark β-cells from NOD mice for destruction by diabetogenic CD4+ T-cells and remarkably, through a Fas-dependent mechanism (Amrani et al., 2000). It was also shown that cytokine-cultured islets are a more sensitive target for cytotoxic lymphocytes as demonstrated in vitro and in vivo (Kuttler et al., 1994). Moreover, it has been reported that exposure of human and mouse islets to inflammatory cytokines such as IFN-γ, TNF-α and IL-1β can induce moderate levels of ICAM-1 expression in vitro (Vives et al., 1991; Prieto et al., 1992). IL-1β alone caused a great impact on islet cell integrity and function of pancreatic β-cells, the simultaneous action of IL-1β, IFN-γ and TNF-α during an immune attack could be particularly critical for survival of pancreatic β-cells. The marked increase of ICAM-1 on the surface of β-cells could be responsible for an improved binding of effector T-cells to their targets. In addition, IL-1β primes pancreatic β-cells for apoptotic mechanisms mediated by the 'death receptor' Fas. The simultaneous activity of proinflammatory cytokines such as IL-1β and TNF-α as well as type I cytokines like IFN-γ resulted in enhanced Fas expression, which might increase the risk of Fas-mediated β-cell destruction (Wachlin et al., 2003).

Recently, it has been reported that Interferon-α initiates type 1 diabetes in nonobese diabetic mice. There is increased expression of several IFN-α-inducible genes in CD4+ T cells and increased production of IFN-α in the PLNs of 3- to 4-week-old NOD mice. Blockade of IFN-α signalling by an anti-IFNAR1mAb during this period significantly delayed the onset and decreased the incidence of T1DM in NOD mice (Li et al., 2008).
Role of viruses in the pathogenesis of type 1 diabetes mellitus

The first documented association between viruses and type 1 diabetes was the link with mumps virus (Harris, 1898). Before the worldwide use of vaccines against mumps, this virus had been implicated in the pathogenesis of type 1 diabetes. Currently, there are many other viruses associated with type 1 diabetes such as enterovirus, mumps, rubella, measles, chicken pox and rota virus. The only confirmed viral link to human type 1 diabetes is congenital rubella infection. Intrauterine exposure leading to congenital rubella syndrome is associated with diabetes, which may appear many years after the original exposure (Ginsberg-Fellner et al., 1984). Attention has recently been focused upon the role of enteroviruses as a possible agent in the pathogenesis of type 1 diabetes. The coxsackie virus, particularly coxsackie B4, has been implicated as a possible causative agent in type 1 diabetes. Antibodies to these viruses were more prevalent in newly diagnosed type 1 diabetes patients and especially in the 10–18-year-old age group when compared with control subjects (Gamble et al., 1973).

The mechanism of viral-induced diabetes

Many different possible mechanisms may lead to β cell damage by viruses. Viruses may directly infect and destroy β cells of the pancreas or act indirectly by triggering autoimmunity. Viruses such as EMC-D can induce type 1 diabetes by directly infecting and destroying β cells through cytolysis. Although a direct viral infection of the β cells may not be necessary to induce insulitis or diabetes, infection of the surrounding tissue may lead to β cell destruction by the release of immune mediators such as cytokines (Soldevila et al., 1991). In contrast, retroviruses may infect beta cells and induce antigens leading to
specific beta cell autoimmunity. Viruses such as coxsackie virus can infect β cells and induce the expression of IFN-α and subsequently chemokines that will stimulate lymphocytes to home to pancreatic β cells leading to β cell destruction. Viruses are the most potent inducer of IFN-α, other agents such as bacteria, interleukin-2, hypoxia, IFN-γ, polyanions DNA or RNA, and vasoactive intestinal peptide are capable of inducing IFN-α. The viral double-stranded RNA present during infection interacts with Toll-like receptor 3 (TLR-3) activating MyD88 adaptor molecule and the protein kinase activated dsRNA (PKR). Subsequently, other pathways are activated including the NFκB pathway, which leads to apoptosis of the cell demonstrates a schematic diagram outlining the role of virus and IFN-α in the pathogenesis of type 1 diabetes. Apoptosis is a potent inducer of IFN-α expression, but IFN-α itself can induce apoptosis (Casciola-Rosen et al., 1994). Some gene products such as PKR, oligo-adenylate synthase (OAS), and RNAse L appear to contribute to the anti-viral effects after IFN-α activation (Figure 1).
Figure 1: The cellular and molecular pathways of virus and interferon alpha inducing type 1 diabetes. Viral dsRNA activates the toll-like receptor-3 (TLR3) and via various differentiating factors activates the nuclear factor NFκB to induce apoptosis. Viral dsRNA also activates the production of IFN-α in various cells, which is directly cytotoxic to beta cells of the pancreas. IFN-α also induces apoptosis by activating the oligoadenylate synthase (OAS)–RnaseL pathway and the protein kinase R (PKR) pathway. Apoptotic materials induce more IFN-α and activate the immune system.
3.2.2 CURRENT THERAPIES FOR TYPE 1 DIABETES MELLITUS

Type 1 DM is treated with insulin replacement therapy — usually by injection or insulin pump, along with attention to dietary management, typically including carbohydrate tracking, and careful monitoring of blood glucose levels using glucose meters.

Current focus is on cell-based treatments for diabetes because, diabetes is caused by the loss of a single cell type it is amenable to treatment by cell replacement therapy (Jones et al., 2008). Advances in islet transplantation procedures have demonstrated that people with Type 1 diabetes can be cured by human islet transplantation, but the severely limited availability of donor islets has restricted the widespread application of this approach, and driven the search for substitute transplant tissues. Recent experimental studies suggest that three separate sources of tissue show therapeutic potential – xenografts from other species, tissue stem cells and embryonic stem cells (Gaglia et al., 2005; Marzorati et al., 2007). Of these, xenografts are closest to clinical application but there are still major obstacles to be overcome. Insulin-expressing cells have been derived from a number of different stem cell populations but embryonic stem cells offer the major advantage of being able, in principle, to provide the vast numbers of cells required for transplantation therapy (Jones et al., 2008). In a 2007 trial of 15 newly diagnosed patients with type 1 diabetes treated with stem cells raised from their own bone marrow after immune suppression showed that the majority did not require any insulin treatment for prolonged periods of time (Voltarelli et al., 2007).
3.3 TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus (NIDDM) is characterized by peripheral insulin resistance (reduced uptake of glucose from blood into the skeletal muscle - a progressive decline in insulin action), followed by the inability of beta cells to compensate for insulin resistance (pancreatic beta cell dysfunction), and increased endogenous glucose production (liver). Insulin resistance is a characteristic metabolic defect that precedes overt beta cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia. The beta cells normally compensate insulin resistance by secreting more amounts of insulin to maintain the glucose homeostasis. In the course of time, however, this beta cell function gets impaired leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and frank diabetes (Lebovitz and Banerji, 2004). The disease is often associated with obesity and develops when chronic over nutrition conspires with genetic susceptibility to cause impaired insulin signalling, also known as insulin resistance, as well as a relative insulin deficiency of non-autoimmune aetiology. This contrasts with type 1 diabetes, which is caused by the complete absence of insulin secondary to autoimmune destruction of the pancreatic islet β-cells. Insulin normally controls fuel homeostasis through the stimulation of glucose uptake into peripheral tissues and by suppressing the release of stored lipids from adipose tissue. Defective insulin secretion and action therefore leads to multiple metabolic abnormalities in type 2 diabetes, including hyperglycemia due to impaired insulin-stimulated glucose uptake and uncontrolled hepatic glucose production, and dyslipidemia, which includes perturbed homeostasis of
fatty acids, triglycerides and lipoproteins. These chronic increases in circulating glucose and lipid levels can further impair insulin secretion and action and cause other forms of tissue damage by several mechanisms (Muoio and Newgard, 2008).

3.3.1 PATHOPHYSIOLOGY AND MOLECULAR AND METABOLIC MECHANISMS OF INSULIN RESISTANCE

Defects in insulin action result in tissue insulin resistance. Insulin resistance is a metabolic state in which physiologic concentrations of insulin produce less than normal biologic response. A widely held notion is that insulin resistance is a direct consequence of obesity-associated exposure of tissues to elevated dietary nutrients, resulting in the accumulation of toxic metabolic by-products. However, recent works has indicated that other factors may also be important, including inter-organ communication networks that are mediated by peptide hormones and inflammatory molecules (cytokines), and activation of intracellular stress response pathways.

3.3.1.1 ALTERATIONS IN METABOLIC FUNCTION IN LIVER AND MUSCLE

Lipid-derived metabolites begin to accumulate outside of the adipose depots (including skeletal muscle, heart and liver) in response to high-fat diets and the onset of obesity.

Metabolic overload in the liver: A current popular theory of lipid-induced hepatic insulin resistance is that lipid species accumulate as a result of the impairment of fatty acid oxidation, resulting in the redirection of long-chain acyl coAs (LC-coAs) into ER-localized and cytosolic lipid species, such as diacylglycerols (DAGs), ceramides and TGs. This is thought to be regulated mostly by glucose induced increases in the levels of malonyl coA, which serves both as the immediate precursor of de novo lipogenesis and as an important
allosteric inhibitor of carnitine palmitoyltransferase-1 (CPT1), the rate-limiting enzyme for import of LC-coAs into the mitochondria for β-oxidation (McGarry and Banting, 2002). In addition, insulin inhibits the hepatic expression of β-oxidative enzymes by antagonizing the effects of PGC1α (peroxisome proliferator-activated receptor-γ (PPARγ) co-activator-1α) (Li et al., 2007). This role of insulin is maintained even as insulin resistance develops, whereas its role to suppress gluconeogenesis wanes. Together, this sets the stage for development of hepatic steatosis during sustained periods of overfeeding, leading to glucose intolerance. Indeed, infusion of lipids or ingestion of high-fat diets in rodents leads to the accumulation of TGs, LC-coAs, DAGs and ceramides (Griffin et al., 1999; Chavez and Summers, 2003; Chavez et al., 2005). Suppression of mitochondrial glycerol-3-phosphate acyltransferase-1 (GPAT1, the first enzyme in TG synthesis) or acetyl coA carboxylase-2 (ACC2) activity results in increased fatty acid oxidation, lowered DAG levels and reversal of hepatic insulin resistance (Abu8 Elheiga et al., 2003). Pharmacological inhibition of Ser palmitoyltransferase-1 (SPT1) or genetic knockout of dihydroceramide desaturase-1 (Degs1) — both of which are involved in the synthesis of ceramides from the saturated precursor palmitoyl coA — prevented hepatic insulin resistance induced by glucocorticoid administration or infusion of saturated (but not unsaturated) fats in rodents (Holland et al., 2007).

Metabolic overload in muscle: As in liver, intramuscular levels of lipid signalling molecules, such as LC-coAs, DAG and ceramides, positively correlate with TG content and negatively correlate with insulin sensitivity (Shulman, 2000; Holland et al., 2007). However, although a role for these cytosolic lipids in the development of hepatic insulin resistance is well
supported, the link with skeletal muscle is still mainly based on circumstantial evidence. The strongest experimental data come from two recent reports in which tissue levels of ceramide and DAG were altered. Thus, pharmacological or genetic inhibition of ceramide biosynthesis in rodents attenuated muscle insulin resistance caused by infusion of high concentrations of palmitate (Holland et al., 2007). Inhibition of ceramide synthesis did not prevent insulin resistance in response to linoleic acid, suggesting discrete mechanisms for different fatty acids. It is noteworthy that this report highlighted the role of ceramide in liver and suggested that sphingolipid synthesis in other tissues, including muscle, might not be a factor in the early stages of obesity-induced glucose intolerance. Another study showed that transgenic overexpression of diacylglycerol acyltransferase-1 (DGAT1) in skeletal muscle increased TG content and prevented diet-induced insulin resistance, in association with 20–30% reductions in muscle DAG and ceramide levels (Liu et al., 2007). However, muscle β-oxidation was not evaluated, and could have been reduced as a consequence of repartitioning lipids into esterification pathways. Alternatively, recent work has suggested that mitochondrially derived by-products of lipid oxidation (rather than diversion of lipid metabolites into biosynthetic pathways) may have a key role in the development of insulin resistance in skeletal muscle (Muoio and Newgard, 2006). Chronic exposure of muscle to elevated lipids induces an increase rather than a decrease in expression of genes of the fatty acid β-oxidative pathway (Koves et al., 2005; Muoio and Newgard, 2006). In addition, lipid induced upregulation of the enzymatic machinery for β-oxidation in muscle is not coordinated with upregulation of downstream metabolic pathways such as the tricarboxylic acid (TCA) cycle and electron transport chain. This could
lead to incomplete metabolism of fatty acids in the β-oxidation pathway and accumulation of lipid-derived metabolites in the mitochondria (Koves et al., 2005; Muoio and Newgard, 2006). Higher rates of incomplete fat oxidation were observed in isolated mitochondria from insulin-resistant rat skeletal muscles compared with insulin-sensitive muscles, and several long- and medium-chain acylcarnitines accumulated in the muscle of obese rats compared with lean rats (An et al., 2004). These abnormalities were reversed by exercise intervention in mice that were fed on a high-fat diet, in association with increased TCA cycle activity and restoration of insulin sensitivity and glucose tolerance (Koves et al., 2005). Other work has shown that obesity results in impaired switching from fatty acid to carbohydrate substrates during the fasting-to-fed transition and in a coincident reduction in levels of several TCA cycle intermediates (Koves et al., 2008). This phenomenon, known as metabolic inflexibility (Kelley et al., 1999), was apparent at both the whole-body level and in isolated muscle mitochondria. Moreover, knockout mice lacking malonyl CoA decarboxylase (MCD), which promotes CPT1 activity and β-oxidation, had markedly lower acylcarnitine levels in muscle and were protected against diet-induced insulin resistance, despite high levels of LC-CoA (Koves et al., 2008). Transgenic mice with muscle-specific overexpression of PPARα, a nuclear receptor that activates β-oxidative genes, developed both local and systemic glucose intolerance (Koves et al., 2008).

In conclusion, a large body of evidence supports the idea that the impairment of mitochondrial fatty acid oxidation and diversion of lipid species into cytosolic by-products has a major role in the development of hepatic insulin resistance. In contrast, oversupplying lipids to muscle can result both in increased diversion of LC-CoA species into...
cytosolic products such as TG, DAG and ceramide, and in enhanced incomplete fatty acid oxidation owing to transcriptional regulation and increased substrate supply. In the absence of exercise, this increase in fatty acid oxidation is not matched by an increase in TCA cycle activity. As a result, lipid-derived intermediates accumulate in mitochondria, possibly contributing to mitochondrial stress, and ultimately to insulin resistance. Muscle insulin resistance has been linked to a form of mitochondrial dysfunction that is characterized by morphological and metabolic abnormalities, diminished oxidative phosphorylation capacity, reduced activity of the electron transport chain and low expression levels of PGC1α, a master transcriptional regulator of mitochondrial biogenesis (Kelley et al., 1999; Koves et al., 2005). These traits have been observed in association with ageing, inactivity, obesity and type 2 diabetes, and are also evident in young insulin-resistant offspring of parents with diabetes (Muio and Newgard, 2006). Thus, mitochondrial insufficiencies of various origins appear to occur in close association with impaired insulin action.

Metabolic overload in liver and muscle: Effects on Insulin Signalling

The accumulation of lipid-derived metabolites affects the core insulin signalling pathway, members of the protein kinase c (PKC) family are regulated by lipid-derived by-products such as DAG and are therefore implicated. These kinases have been shown to phosphorylate Ser on the insulin receptor and/or its immediate targets, insulin receptor substrate-1 (IRS1) and -2 (IRS2), thereby impairing insulin-receptor-mediated Tyr phosphorylation of IRS1 and, as a consequence, interfering with insulin signaling (Hirosumi et al., 2002; Cohen, 2006; Taniguchi et al., 2006). Moreover, hepatic steatosis induced by
overexpression of GPAT1 in rat liver is associated with increased hepatic DAG levels and activation of PKCe54, whereas Gpot1-knockout mice exhibit decreased PKCe activity in concert with enhanced hepatic insulin sensitivity (Hammond et al., 2005). Furthermore, silencing of hepatic PKCe in liver is sufficient to prevent hepatic insulin resistance caused by short-term high-fat feeding (Samuel et al., 2007). In skeletal muscle, PKCθ has emerged as a candidate mediator of fatty-acid-induced insulin resistance, based on findings that this enzyme is activated by lipid infusion in both humans and rodents, and that PKC θ-knockout mice are protected from insulin resistance that is caused by acute lipid infusion (Kim et al., 2004). However, other reports suggest that PKCθ is required for the maintenance of normal skeletal muscle insulin sensitivity (Serra et al., 2003). Thus, there is more to learn about signalling pathways in lipid-induced muscle insulin resistance, including whether increased fat oxidation in muscle mitochondria leads to oxidative stress and activation of kinases other than PKCs to interfere with insulin signalling.

Finally, an important and recently emerged concept is that excess lipids and other metabolic changes that are associated with overnutrition may trigger stress responses in the ER (Ozcan et al., 2004). In genetic or diet-induced forms of obesity, markers of ER stress are elevated in the liver and adipose tissue. These changes are linked to activation of c-jun n-terminal kinases (JNKs), which phosphorylate IRS1, thereby interfering with insulin action. In fibroblasts from mice that lack inositol-requiring kinase-1 (IRE1), a proximal ER stress sensor, chemical activators of ER stress fail to activate JNK. Overexpression of XBP1 (a downstream transcription factor that modulates the unfolded protein response (UPR) prevents JNK activation in liver cells that are treated with agents
that cause ER stress, and heterozygous deletion of XBP1 increases the susceptibility of mice to diet-induced insulin resistance and diabetes (Ozcan et al., 2004). Moreover, treatment of obese and diabetic mice with 4-phenyl butyric acid and taurine-conjugated ursodeoxycholic acid (which are both small molecule chemical chaperones, and ER-stress relieving agents) resolves hepatic steatosis and restores peripheral insulin sensitivity (Ozcan et al., 2006). Thus, diet-induced insulin resistance appears to be controlled in part by the induction of ER and inflammatory stress responses that connect to the core insulin signalling pathways. Interestingly, several of the enzymes that are involved in lipid esterification pathways are localized to the ER, providing a possible link between hepatic lipid overstorage and the development of ER stress (Muoio and Newgard, 2008).

3.3.1.2 ADIPOCYTE DYSFUNCTION, ADIPOKINES AND INSULIN RESISTANCE

Adipose tissue, when carried around in excessive amounts, predisposes to a large number of diseases, excess adipose tissue predisposes toward the development of insulin resistance. At the interface of energy metabolism and inflammation, adipose tissue also plays a key role in the development of the metabolic syndrome. While storage and release of lipids are major functions of adipocytes, the adipocyte also uses specific lipid molecules for intracellular signaling and uses a host of protein factors to communicate with essentially every organ system in the body. The intensity and complexity of these signals are highly regulated, differ in each fat pad, and are dramatically affected by various disease states. As the master regulator of systemic lipid storage and through secretion of a number of these adipokines, adipose tissue has an influence on many processes, including
energy metabolism, inflammation, and pathophysiological changes such as cancer and infectious disease (Philipp, 2006).

Adipocytes have a regulatory role in the development of insulin resistance because they can produce adipokines (a group of hormones and cytokines) and because their capacity to store excess lipids can become saturated in obesity, resulting in abnormal redistribution of lipids to other organs and tissues. A new appreciation of endocrine functions of adipose tissue began with the discovery that the mutated gene in the ob/ob mouse, which exhibits hyperphagia, hyperlipidemia and insulin resistance, is the cytokine-related molecule leptin (Pelleymounter et al., 1995). The ensuing decade of research has revealed that adipose cells also produce other peptide hormones, including adiponectin (AcrP30), retinol-binding protein-4 (RBP4) and resistin, and proinflammatory cytokines such as interleukin (IL)-6 and tumour necrosis factor-α (TNF-α) (Trujillo and Scherer, 2006). Leptin and adiponectin have been categorized as 'anti-diabetogenic' based on their common capacity to decrease triglyceride (TG) synthesis, stimulate β-oxidation and enhance insulin action in both skeletal muscle and liver. These effects can be explained in part by their common ability to activate 5'-AMP-activated protein kinase (AMPK), an enzyme that responds to a fall in ATP and a rise in AMP levels by activating both glucose and fatty acid oxidation. Interestingly, leptin levels are increased and adiponectin levels are decreased in insulin-resistant obese humans and animals, which suggests that obesity leads to a state of leptin resistance and adiponectin deficiency. The consequences of the absence of fat (lipodystrophy) further underscore the importance of adipose tissue in the normal regulation of insulin action. Animals lacking white adipose tissue have severe hepatic and muscle insulin resistance,
and exhibit large increases in TG stores in both tissues (Reitman and Gavrilova, 2000). Transplantation of normal fat tissue into such mice restores insulin sensitivity (Gavrilova et al., 2000).

The ability of adipose tissue to produce inter-organ regulatory factors is dependent on the metabolic state. Mice with an adipose-specific knockout of the Glut4 glucose transporter have impaired insulin sensitivity in muscle and liver (Abel et al., 2001). The impairment in insulin action is only apparent in tissues in situ and not in excised tissue samples, which implies the participation of a blood borne hormone or metabolite. Furthermore, circulating RBP4 levels are increased in adipose-specific Glut4- knockout mice, and infusion or transgenic expression of RBP4 in normal mice causes insulin resistance (Yang et al., 2005). Interestingly, food deprivation (fasting) also causes a form of insulin resistance and is associated with a decrease in adipose Glut4 expression. This raises the possibility that the original purpose of adipocyte derived insulin-desensitizing molecules, such as RBP4, TNF-α and resistin, may have been to prevent hypoglycemia in the fasted state, which has been subverted to pathophysiology with the advent of overnutrition and sedentary behaviours in modern life (Muoio and Newgard, 2005).

**Leptin**

Leptin levels are increased in insulin-resistant obese humans and animals, which suggests that obesity leads to a state of leptin resistance. Leptin appears to have a crucial role in the 'rescue' of insulin action in diabetes models because leptin infusion ameliorates insulin resistance in lipoatrophic mice (Shimomura et al., 1999), whereas transplantation of fat from leptin-deficient mice into such animals fails to improve insulin sensitivity (Colombo et
Furthermore, leptin administration to humans with severe lipodystrophy partially reverses their insulin resistance and hyperlipidemia (Oral et al., 2002).

**Adiponectin (AcrP30)** Adiponectin is an adipokine that is specifically and abundantly expressed in adipose tissue and directly sensitizes the body to insulin. Hypoadiponectinemia, caused by interactions of genetic factors such as SNPs in the Adiponectin gene and environmental factors causing obesity, appears to play an important causal role in insulin resistance, type 2 diabetes, and the metabolic syndrome, which are linked to obesity. The adiponectin receptors, AdipoR1 and AdipoR2, which mediate the antidiabetic metabolic actions of adiponectin, have been cloned and are downregulated in obesity-linked insulin resistance. Upregulation of adiponectin is a partial cause of the insulin-sensitizing and antidiabetic actions of thiazolidinediones. Therefore, adiponectin and adiponectin receptors represent potential versatile therapeutic targets to combat obesity-linked diseases characterized by insulin resistance. Adiponectin acts as an insulin sensitizer, stimulating fatty acid oxidation in an AMP-activated protein kinase (AMPK) and peroxisome proliferator activated receptor-α (PPAR-α)-dependent manner (Kadowaki et al., 2006).

**Retinol-binding protein-4 (RBP4)** induces insulin resistance through reduced phosphatidylinositol-3-OH kinase (PI(3)K) signalling in muscle and enhanced expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase in the liver through a retinol-dependent mechanism (Yang et al., 2005).

**TNF-α and IL-6** TNFα affects adipocytes profoundly and results in the attenuation of insulin signalling and inhibition of adipogenesis. Thus, TNFα functions to compromise
normal adipocyte functions, including optimal storage of triglycerides. The negative regulation of the nuclear hormone receptor peroxisome proliferator-activated receptor-γ (PPARγ) as a key element in mediating these effects of inflammatory cytokines. Emerging evidence suggests that TNFα can affect PPARγ at multiple levels, including the transcription, translation and turnover of PPARγ mRNA and protein. TNFα treatment of adipocytes activates these transcription factors through stimulation of IKKβ (for NF-κB) and MAP4K4 (for AP1) cascades. TNFα potently decreases expression of numerous adipocyte specific genes and adipogenic transcription factors including C/EBPα and PPARγ (Guilherme et al., 2008).

IL-6 is secreted from adipose tissue during noninflammatory conditions in humans. Omental adipose tissue produces 3-fold more IL-6 than sc adipose tissue. Obesity is expected to result in increased secretion of IL-6, with its detrimental metabolic effects. In contrast, mice lacking the gene encoding IL-6 (IL-6−/−) developed mature-onset obesity and disturbed carbohydrate and lipid metabolism, which were reversible by IL-6 replacement. The anti obesity effect of IL-6 was mainly exerted at the level of the central nervous system, being inactive when administered peripherally (Wallenius et al., 2002). At first glance, this is puzzling. However, IL-6 acts as a terminator as well as a prompter of inflammation. It should again be remembered that cytokines act in cascade, and total depletion of IL-6 might be detrimental because other proinflammatory cytokines (TNF-α) are not adequately down-regulated, as is the case in other knockout models (Wallenius et al., 2002; Guilherme et al., 2008).
Suppressors of cytokine signaling (SOCS) and Insulin resistance Pathways involving the induction of suppression of cytokine signaling (SOCS) proteins (Mooney et al., 2001) and inducible nitric oxide synthase (iNOS) (Perreault and Marette et al., 2001) may be involved in mediating cytokine-induced insulin resistance. Secretion of these proinflammatory proteins, particularly MCP-1 by adipocytes, endothelial cells and monocytes, increases macrophage recruitment and thereby contributes to a feed forward process (Chen et al., 2005).

Adipsin Adipsin is a circulating glycoprotein of the serine protease family, which is predominantly synthesized and secreted by adipose tissue (Cook et al., 1987). This protein, the expression of which shows marked changes in pathophysiological states associated with variations in adipose tissue mass, presents the properties of a putative systemic regulator of energy balance. The adipsin-ASP (acylation stimulating protein) system, therefore, does appear to be involved in the regulation of triglyceride metabolism in adipocytes, the function of this system is to increase the rate of triglyceride synthesis in adipocytes rather than, as was originally suggested, to increase the rate at which lipolysis occurs (Flier et al., 1987). The increase in triglyceride synthesis induced by ASP is achieved by a dual effect. That is, ASP causes translocation of glucose transporters from intracellular vesicles to the cell surface, thereby increasing specific membrane glucose transport (Germinario et al., 1993). By contrast, membrane transport of fatty acids is not directly affected by ASP. However, net fatty acid uptake does increase secondary to stimulation of the enzyme, diacylglycerol acyltransferase, which controls the rate limiting step in the synthesis of a triglyceride molecule (Yasruel et al., 1991). Related to glucose metabolism,
ASP increases glucose transport in adipocytes and myotubes through translocation of Glut1, Glut3 and Glut4 (Germinario et al., 1993; Masiowska et al., 1997; Tao et al., 1997)

3.3.1.3 FATTY ACID BINDING PROTEINS (FABPs)

Fatty-acid trafficking in cells is a complex and dynamic process that affects many aspects of cellular function. Fatty acids function both as an energy source and as signals for metabolic regulation, acting through enzymatic and transcriptional networks to modulate gene expression, growth and survival pathways, and inflammatory and metabolic responses. Intracellular lipid chaperones known as fatty acid-binding proteins (FABPs) are a group of molecules that coordinate lipid responses in cells and are also strongly linked to metabolic and inflammatory pathways. Different members of the FABP family exhibit unique patterns of tissue expression (Table 5) and are expressed most abundantly in tissues involved in active lipid metabolism (Furuhashi and Hotamisligil, 2008).

FABP4 also known as A-FABP Adipocyte FABP, was first detected in mature adipocytes and adipose tissue. This protein has also been termed adipocyte P2 (aP2) because of its high sequence similarity (67%) to peripheral myelin protein 2 (M-FABP/FABP8) (Hunt et al., 1986). Expression of A-FABP (FABP4) is highly regulated during differentiation of adipocytes, and its mRNA is transcriptionally controlled by fatty acids, PPAR-γ agonists and insulin (Haunerland and Spener, 2004; Makowski and Hotamisligil, 2005; Chmurzynska, 2006). A-FABP is the best-characterized isoform among the entire FABP family with the most striking biology. A-FABP-deficient mice exhibited reduced hyperinsulinemia and insulin resistance in the context of both dietary and genetic obesity, but the effect of A-
FABP on insulin sensitivity was not observed in lean mice (Hotamisligil et al., 1996; Uysal et al., 2000).

Table 5: Family of fatty acid-binding proteins (FABPs)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Common name</th>
<th>Alternative names</th>
<th>Expression</th>
<th>Chromosomal location</th>
</tr>
</thead>
<tbody>
<tr>
<td>FABP1</td>
<td>Liver FABP</td>
<td>L-FABP</td>
<td>Liver, intestine, pancreas, kidney, lung, stomach</td>
<td>2p11 6q1 4q32</td>
</tr>
<tr>
<td>FABP2</td>
<td>Intestinal FABP</td>
<td>I-FABP</td>
<td>Intestine, liver</td>
<td>4q28 3q1 2q42</td>
</tr>
<tr>
<td>FABP3</td>
<td>Heart FABP</td>
<td>H-FABP, MDGI</td>
<td>Heart, skeletal muscle, brain, kidney, lung, stomach, testis, aorta, adrenal gland, mammary gland, placenta, ovary, brown adipose tissue</td>
<td>1 p32 4p3 5q36</td>
</tr>
<tr>
<td>FABP4</td>
<td>Adipocyte FABP</td>
<td>A-FABP, aP2</td>
<td>Adipocyte, macrophage, dendritic cell</td>
<td>8q21 3A1 2q23</td>
</tr>
<tr>
<td>FABP5</td>
<td>Epidermal FABP</td>
<td>E-FABP, PA-FABP, mal1</td>
<td>Skin, tongue, adipocyte, macrophage, dendritic cell, mammary gland, brain, intestine, kidney, liver, lung, heart, skeletal muscle, testis, retina, lens, spleen</td>
<td>8q21.13 3A1-3 2</td>
</tr>
<tr>
<td>FABP6</td>
<td>Ileal FABP</td>
<td>II-FABP, I-BABP, gastrointestinal</td>
<td>Ileum, ovary, adrenal gland, stomach</td>
<td>5q33.3-q34 11B1.1 10q21</td>
</tr>
<tr>
<td>FABP7</td>
<td>Brain FABP</td>
<td>B-FABP, MRG</td>
<td>Brain, glia cell, retina, mammary gland</td>
<td>6q22-q23 10B4 20q11</td>
</tr>
<tr>
<td>FABP8</td>
<td>Myelin FABP</td>
<td>M-FABP, PMP2</td>
<td>Peripheral nervous system, Schwann cell</td>
<td>8q21.3-q22.1 3A1 2q23</td>
</tr>
<tr>
<td>FABP9</td>
<td>Testis FABP</td>
<td>T-FABP</td>
<td>Testis, salivary gland, mammary gland</td>
<td>8q21.13 3A2 2q23</td>
</tr>
</tbody>
</table>

aP2, adipocyte P2; I-BABP, ileal bile acid-binding protein; MDGI, mammary derived growth inhibitor; MRG, MDGI-related gene; PA-FABP, psoriasis-associated FABP; PMP2, peripheral myelin protein 2.

Adipocyte/macrophage FABPs, A-FABP and E- FABP act at the interface of metabolic and inflammatory pathways. These FABPs exert a dramatic impact on obesity, insulin resistance, type 2 diabetes, fatty liver disease, atherosclerosis and asthma. The creation of pharmacological agents to modify FABP function may therefore provide tissue-specific or cell-type-specific control of lipid signalling pathways, inflammatory responses and metabolic regulation, thus offering a new class of multi indication therapeutic agents. A-
FABP, offers highly attractive therapeutic opportunities for a broad range of pathologies in metabolic diseases by addressing an evolutionary bottleneck in the metabolic design of humans (Furuhashi and Hotamisligil, 2008).

3.3.1.4 INFLAMMATION & INSULIN RESISTANCE

Metabolic and immune systems are among the most fundamental requirements for survival. Many metabolic and immune response pathways or nutrient- and pathogen-sensing systems have been evolutionarily conserved throughout species. As a result, immune response and metabolic regulation are highly integrated and the proper function of each is dependent on the other. This interface can be viewed as a central homeostatic mechanism, dysfunction of which can lead to a cluster of chronic metabolic disorders, particularly obesity, type 2 diabetes and cardiovascular disease (Hotamisligil, 2006). Obesity, insulin resistance and type 2 diabetes are closely associated with chronic 'inflammation' characterized by abnormal cytokine production, increased acute-phase reactants and other mediators, and activation of a network of inflammatory signalling pathways (Wellen and Hotamisligil, 2005). Indeed, it has been recognized for the past century that high doses of salicylates (aspirin) reverse insulin resistance and diabetes (Kim et al., 2001; Yuan et al., 2001), and salicylates also appear to preserve β-cell function by inhibiting prostaglandin formation (Chen and Robertson, 1979).

The finding that the proinflammatory cytokine TNF-α was able to induce insulin resistance and tumour necrosis factor-α (TNF-α) is overexpressed in the adipose tissue of obese mice provided the first clear link between obesity, diabetes and chronic inflammation (Hotamisligil et al., 1993). This was a revolutionary idea, that a substance produced by fat
— and overproduced by expanded fat — had local and potentially systemic effects on metabolism. The concept of fat as a site for the production of cytokines and other bioactive substances quickly extended beyond TNF-α to include leptin, IL-6, resistin, monocyte chemoattractant protein-1 (MCP-1), PAI-1, angiotensinogen, visfatin, retinol-binding protein-4, serum amyloid A (SAA), and others. Adiponectin is similarly produced by fat, but expression decreases with increased adiposity (Shoelson et al., 2006). While leptin and adiponectin are true adipokines that appear to be produced exclusively by adipocytes, TNF-α, IL-6, MCP-1, visfatin, and PAI-1 are expressed as well at high levels in activated macrophages and/or other cells. TNF-α, IL-6, resistin, and undoubtedly other pro- or antiinflammatory cytokines appear to participate in the induction and maintenance of the subacute inflammatory state associated with obesity. MCP-1 and other chemokines have essential roles in the recruitment of macrophages to adipose tissue. These cytokines and chemokines activate intracellular pathways that promote the development of insulin resistance and T2DM (Shoelson et al., 2006).

The antihyperglycemic effects of salicylates focused attention on IKKβ and NF-κB, while increased adiposity activates both JNK and IKKβ (Hirosumi et al., 2002; Cai et al., 2005). Many of the more typical proinflammatory stimuli simultaneously activate JNK and IKKβ pathways, including cytokines and TLRs (Figure 2). Concordantly, genetic or chemical inhibition of either JNK or IKKβ/NF-κB can improve insulin resistance (Hirosumi et al., 2002; Cai et al., 2005). High-fat diets or obesity result in activation of the transcription factor NF-κB and its targets in the liver, and salicylates suppress NF-κB activity. Overexpression of a constitutively active version of the NF-κB-activating kinase, IκB kinase catalytic subunit-β...
(IKKβ), in the liver of normal rodents results in liver and muscle insulin resistance and diabetes. In addition, both high-fat feeding and IKKβ overexpression increase hepatic production of IL-6, IL-1β and TNF-α, whereas antibody-mediated neutralization of IL-6 in animals fed on a high-fat diet partially restores insulin sensitivity (Cai et al., 2005). Interestingly, deletion of IKKβ in the liver of mice is protective against diet-induced hepatic insulin resistance, although muscle and adipose insulin resistance still develop. However, mice in which IKKβ is selectively knocked out in myeloid cells remain globally insulin sensitive (Arkan et al., 2005). In rodents, macrophage infiltration of adipose tissue is stimulated within 1 week of high-fat feeding and appears to involve increased adipocyte expression of MCP-1, which recruits monocytes to sites of injury and infection (Chen et al., 2005).

The several hypothesized mechanisms that might explain how obesity activates JNK and NF-κB can be separated into receptor and nonreceptor pathways (Figure 2). Proinflammatory cytokines such as TNF-α and IL-1β activate JNK and IKKβ/NF-κB through classical receptor-mediated mechanisms that have been well characterized (Figure 2). JNK and IKKβ/NF-κB are also activated by pattern recognition receptors, defined as surface proteins that recognize foreign substances. These include the TLRs and the receptor for advanced glycation end products (RAGE). Many TLR ligands are microbial products, including LPS and lipopeptides derived from bacteria (Akira et al., 2006). The fact that TLRs recognize microbial lipid conjugates has led to speculation that endogenous lipids or lipid conjugates might also activate 1 or more of the TLRs in obesity, a possibility supported by experiments showing that saturated fatty acids bind and activate TLR4 (Lee et al., 2001).
Figure 2: Potential cellular mechanisms for activating inflammatory signaling in obesity and diabetes mellitus. Obesity and high-fat diet activate IKKβ/NF-κB and JNK pathways in adipocytes, hepatocytes, and associated macrophages. Stimuli that have been shown to activate these pathways during metabolic dysregulation include ligands for TNF-α, IL-1, Toll, or AGE receptors (TNFR, IL-1R, TLR, or RAGE, respectively), intracellular stresses including ROS and ER stress, ceramide, and various PKC isoforms. Obesity-induced IKKβ activation leads to NF-κB translocation and the increased expression of numerous markers and potential mediators of inflammation that can cause insulin resistance. Obesity-induced JNK activation promotes the phosphorylation of IRS-1 at serine sites that negatively regulate normal signaling through the insulin receptor/IRS-1 axis. Examples include serine-302 (pS302) and serine-307 (pS307). By contrast, evidence has not been reported for obesity-induced effects on transcription factors such as AP-1 that are regulated by JNK. IKKβ and/or NF-κB are inhibited or repressed by the actions of salicylates, TZDs, and statins.
Likewise, RAGE binds a variety of ligands, including endogenous advanced glycation end products (AGEs) and a distinct set of microbial products (Ramasamy et al., 2005). AGEs are non-enzymatic adducts formed between glucose and targeted proteins, particularly those with slow rates of turnover. Prolonged hyperglycemia and the accompanying production of excess quantities of AGEs can activate NF-κB.

Inflammation is also closely linked to the pathogenesis of atherosclerosis, suggesting that inflammation might be a common denominator that links obesity to many of its pathological sequelae. Overlapping collections of transcriptionally regulated inflammatory proteins participate in the pathogenesis of these disorders. Signs of inflammation accompany even the earliest accumulation of lipid within the arterial wall, including the upregulation of the cell adhesion molecules P- and E-selectin, ICAM-1, and VCAM-1, which localizes circulating immune cells (Adams and Shaw, 1994; Tedder et al., 1995).

NF-κB regulates many of the proteins that mediate the atherogenic process, in common with the pathogenesis of insulin resistance, suggesting that small increases in obesity-induced inflammation might promote both processes via common mechanisms. This also suggests the corollary that pharmacological decreases in inflammatory activity might coordinately downregulate the production of a number of proteins involved in the pathogenesis of insulin resistance, T2D, and cardiovascular disease (CVD) (Shoelson et al., 2006).
3.3.2 β-CELL FAILURE IN TYPE 2 DIABETES MELLITUS

Although obesity often leads to insulin resistance, only a subset of obese, insulin-resistant individuals progress to type 2 diabetes. In both animal models and humans, the triggering factor is β-cell failure, which involves a decrease in β-cell mass and deterioration of key β-cell functions such as glucose-stimulated insulin secretion (GSIS).

Regulation of insulin secretion in normal islets

In all mammals, including humans, postprandial insulin secretion is regulated in a biphasic manner by nutritional and hormonal signals, but the primary regulator is glucose. Other secretagogues such as free fatty acids, amino acids or the incretin regulator glucagon-like peptide-1 (GLP1) serve as potentiators, requiring a threshold stimulatory level of glucose in the bloodstream before their effects are engaged. Insulin secretion from pancreatic islet β-cells is stimulated by glucose metabolism, which leads to a rise in the ATP: ADP ratio, resulting in closure of ATP-sensitive K⁺ (K_{ATP}) channels, plasma membrane depolarization, activation of voltage-gated Ca²⁺ channels, and Ca²⁺-mediated stimulation of granule exocytosis (Newgard and McGarry, 1995). The K_{ATP} channel-dependent mechanism, also known as the triggering signal, appears to be particularly important for the first, acute phase of insulin release that occurs in the first 10 minutes following glucose stimulation (Henquin et al., 2003). By contrast, in the second and sustained phase of insulin secretion, ATP and Ca²⁺ may have more limited or permissive roles, allowing other glucose-derived second messengers (otherwise known as amplifying signals) to come to the forefront (Henquin et al., 2003; Nenquin et al., 2004). The mitochondrial metabolism of glucose
generates signals other than changes in the ATP: ADP ratio that are important for the control of insulin release.

Figure 3: Signal transduction in β-cells. β-cells have complex mechanisms to enable them to respond to multiple external stimuli (nutrient and non-nutrient) and secrete the appropriate amount of insulin to maintain blood glucose levels. Briefly, glucose is transported into β-cells on the high capacity Glut2 transporter and metabolized within the cell, generating ATP which binds to ATP-sensitive K⁺-channels (K_{ATP}), and leading to closure of the channels. The consequent inhibition of K⁺ efflux leads to β-cell plasma membrane depolarisation and opening of voltage-operated Ca²⁺ channels. Extracellular Ca²⁺ enters β-cells resulting in elevations in intracellular Ca²⁺, activation of Ca²⁺-sensitive downstream signalling pathways and initiation of insulin secretion. Insulin secretion is also stimulated by ligands such as acetylcholine (ACh) and cholecystokinin (CCK) acting at cell surface receptors linked to IP₃ and DAG generation and those such as glucose-dependent insulinotropic peptide (GIP) and glucagon, which elevate intracellular cyclic AMP.
Genetic susceptibility to β-cell failure

β-cell mass is increased in obese non-diabetic humans compared with lean controls, and is decreased in obese patients with impaired fasting glucose or type 2 diabetes (Butler et al., 2003). Similarly, β-cell apoptosis is increased in obese humans with glucose intolerance or diabetes. Genetic background has an important role in determining the susceptibility of β-cells to decompensation and progression to type 2 diabetes. This is demonstrated using rodent models. For example, BTBR/leptin<sup>ob</sup> mice exhibit defective islet proliferation in response to obesity, leading to severe diabetes, whereas C57Bl6/leptin<sup>ob</sup> mice are able to expand β-cell mass and are protected against hyperglycemia (Stoehr et al., 2000). In humans, several forms of maturity onset diabetes of the young (MODY), which are generally considered to be a subclass of type 2 diabetes, are monogenic diseases that involve mutations in important β-cell transcription factors or metabolic regulatory proteins, including hepatocyte nuclear factor-4α (HNF4α; resulting in MODY1), glucokinase (resulting in MODY2), and pancreatic and duodenal homeobox-1 (PDX1; resulting in MODY4) (Winter et al., 1999). These diseases are generally characterized by impaired GSIS and the onset of diabetes at an early age, but represent only 1–2% of the total population of type 2 diabetes cases worldwide. The remaining population with typical or obesity-related type 2 diabetes is thought to comprise a cluster of genetic variations that confer enhanced susceptibility to environmental factors such as overnutrition, obesity and stress. Current evidence suggests that β-cell failure occurs as a combined consequence of metabolic overload, oxidative stress, increased rates of apoptosis, and loss of expression of fundamental components of the insulin granule secretory machinery, but specific
genetic mutations that predispose to these events in patients with non-MODY type 2 diabetes remain to be identified.

**Metabolic overload in β-cells**

Chronic exposure of pancreatic islets to elevated levels of nutrients induces β-cell dysfunction and ultimately triggers β-cell death. Exposure of isolated rodent islets to hyperglycemia for several days raises basal insulin secretion but abrogates insulin secretion in response to stimulatory glucose (Chen et al., 1994; Khaldi et al., 2004). Similarly, exposure of islets to elevated levels of fatty acids does not impair GSIS unless the islets are cultured at or above a threshold concentration of glucose (usually ~8 mM) (Poitout and Robertson, 2002; Prentki et al., 2002). These and other findings have led to the concept of β-cell functional impairment as a consequence of ‘glucolipotoxicity’ rather than as a consequence of exposure to either nutrient alone (Poitout and Robertson, 2002; Prentki et al., 2002). In this model, glucose increases malonyl coA levels, thereby leading to inhibition of CPT1 and fatty acid oxidation, and diversion of lipid metabolites into cytosolic products such as ceramides or esterified lipids, similar to what was described earlier as the commonly held metabolic mechanism of insulin resistance. However, once again, recent studies suggest that the deleterious effects of fatty acids on β-cell function may actually occur as a consequence of increased rather than decreased fatty acid oxidation. Chronic exposure of rodent islets to elevated fatty acids has been reported to decrease PDH activity and glucose oxidation, and it was suggested that this inhibition could prevent the normal glucose-induced increase in ATP:ADP ratio, thereby impairing GSIS (Zhou and Grill, 1994; Zhou and Grill 1995). However, several recent studies have
arrived at different conclusions. Long-term treatment of InS1 cells with oleate caused a modest decrease in glucose oxidation, but glycolytic flux, citrate levels and PDH activity were unchanged (Segall et al., 1999). Furthermore, in the InS1-derived 832/13 cell line and in rat islets, chronic fatty acid treatment did not alter glucose oxidation, but instead induced β-oxidative enzymes and stimulated fatty acid oxidation (Boucher et al., 2004). Pc enzymatic activity has been reported to increase in islets from insulin-resistant, pre-diabetic ZDF rats, leading to the suggestion that this would result in increased pyruvate cycling and increased insulin secretion in these animals (Liu et al., 2002). Because the static measurement of PC activity does not provide information about metabolic flux, 13C NMR was used to measure flux through the relevant metabolic pathways in β-cells that were exposed to elevated fatty acids (Boucher et al., 2004). Chronic exposure of 832/13 cells to fatty acids caused an increase in pyruvate cycling activity at basal glucose levels that paralleled increased insulin release. Thus, rather than suppression of PDH, the emergent mechanism for lipid-induced impairment of GSIS involves activation of PC by its allosteric activator acetyl coA, which rises as a consequence of upregulated fatty acid oxidation in lipid cultured cells (Boucher et al., 2004). Further evidence for the importance of dysregulated pyruvate cycling in mediating lipid induced β-cell dysfunction comes from studies using a membrane-permeant ester of malate, dimethylmalate (DMM). Addition of DMM to β-cell lines and rat islets potentiates GSIS and increases pyruvate cycling activity (Boucher et al., 2004). Inclusion of DMM during insulin secretion assays that were performed on glucose insensitive islets from ZDF rats or lipid-impaired 832/13 cells caused a remarkable improvement in GSIS in both cases. Changes in mitochondrial metabolism...
may synchronize with other effects of chronic lipid exposure in β-cells. Exposure of islets or insulinoma cell lines to elevated fatty acid levels increases uncoupling protein-2 (UCP2) expression, whereas lipid-induced impairment of β-cell function is prevented in islets from UCP2−/− mice (Joseph et al., 2004). Furthermore, UCP2 expression is increased in islets from ob/ob mice, and breeding of UCP2−/− mice with ob/ob mice results in the restoration of first-phase insulin secretion and normalization of blood glucose levels (Zhang et al., 2001). However, the mechanism by which UCP2 might impact β-cell function is not fully resolved. One idea is that UCP2 overexpression causes mitochondrial proton leakage, resulting in impaired ATP production during glucose stimulation (Joseph et al., 2002). It has also been reported that palmitate increases the production of reactive oxygen species (ROS) in normal islets, but not in UCP2−/− islets104. However, another recent study showed no increase in ROS (peroxide) or nitric oxide species (NOS) in rat islets that were chronically exposed to elevated glucose and fatty acid105. Moreover, the addition of antioxidants such as N-acetyl-cysteine (NAC) or pyridoxamine failed to correct lipid-induced impairment of GSIS in these studies, although NAC blocks the toxic effects of chronic exposure of islets to severe hyperglycemia (Robertson and Harmon, 2006).

The role of ER stress pathways in β-cell failure

Mechanisms that underlie the increased rate of β-cell apoptosis and the decline in β-cell mass in type 2 diabetes are incompletely understood. However, several important clues have been revealed from recent work, and the outlines of a possible pathway are emerging. Interestingly, ER stress could have a role. The protein kinase RNA (PKR)-like ER-associated kinase (PERK) is an important regulator of protein translation in mammalian
cells because it phosphorylates and inhibits eukaryotic translation initiation factor-2a (eIF2a). Regulation of PERK-eIF2a is important for linking ER stress to the control of protein translation. Humans and mice that lack PERK have profound β-cell dysfunction and are severely diabetic (Harding et al., 2001), whereas mice with a mutation in the PERK phosphorylation site in eIF2a have fewer β-cells and are diabetic as a result of insulin deficiency (Scheuner et al., 2001). Moreover, feeding of heterozygous Eif2a-mutant mice on a high-fat diet causes distension of the ER lumen in β-cells, a reduction in islet insulin content and diabetes (Scheuner et al., 2005). Although mutations in PERK-eIF2a or other components of the ER stress pathway have not been described in human diabetes, the studies mentioned above suggest a pathway by which chronic exposure of islets to increased nutrient levels could cause the gradual demise of the β-cell. Thus, ingestion of excess calories and increased body weight will require an increase in insulin biosynthesis and secretion in order to maintain fuel homeostasis. As this condition becomes chronic, the biosynthetic demand may eventually overload the protein folding capacity of the ER, leading to activation of the UPR, which in turn leads to activation of PERK and inhibition of protein translation. Continued exposure to overnutrition could then lead to desensitization of the UPR, ultimately uncoupling translational control from protein folding capacity, exactly as occurs in heterozygous Eif2a-mutant mice that are fed on a high-fat diet (Scheuner et al., 2005). The slow, cumulative damage feature of this model is attractive because it could help to explain why humans can remain obese and insulin resistant for long periods of time before β-cell decompensation finally causes the transition to full-blown diabetes.
Role of amyloid fibrils in β-cell failure

Finally, the deposition of toxic amyloid fibrils may be a further mechanism that links overnutrition and hyperstimulation of the islet β-cell to eventual β-cell decompensation and failure. Sections of islets taken from humans with type 2 diabetes contain amyloid fibril deposits, which are now known to be comprised of islet amyloid polypeptide (IAPP), also known as amylin (Cooper et al., 1987; Westermark et al., 1987). Amylin is synthesized and secreted from islet β- and d-cells; in humans, non-human primates and cats, it has a propensity to form amyloid fibrils owing, in large part, to the hydrophobicity of amino acids 20–29 in the protein. By contrast, rodents have three Pro substitutions in this region of amylin and, therefore, rodent amylin does not form amyloid fibrils. Thus, early studies with transgenic mice overexpressing rodent amylin were not revealing, but more recent studies in rodent models of human amylin overexpression demonstrate the development of an islet pathology that is similar to that of human diabetes. In one recent example, human amylin overexpression led to increased rates of β-cell apoptosis, diminished first-phase insulin secretion and decreased β-cell mass, ultimately resulting in the onset of glucose intolerance and then diabetes (Matveyenko and Butler, 2006). Again, the gradual accumulation of amyloid deposits would be consistent with the observation of prolonged β-cell compensation in many obese and insulin-resistant individuals before the transition to diabetes as the β-cell fails. This mechanism could work in concert with metabolically induced impairment of glucose sensing and cumulative ER stress to create a ‘perfect storm’ that causes β-cell decompensation (Muoio and Newgard, 2008).
3.4 INSULIN SIGNALLING PATHWAYS

Binding of insulin to the insulin receptor (IR) elicits autophosphorylation of the IR, leading to the binding of various scaffold proteins, including the insulin receptor substrate (IRS) proteins, but also Src-homology-2-containing protein (SHC), and the c-Cbl (Cbl) proto-oncogene, among others (Cohen, 2006; Taniguchi et al., 2006). Phosphorylation of these scaffold proteins by the IR, in turn, engages various signalling pathways. IRS family members seem to have particularly important roles in the control of metabolic fuel homeostasis. Thus, IRS1 is the key mediator of insulin-stimulated glucose uptake and activation of anabolic pathways in muscle and adipose tissue, whereas the anabolic effects of insulin in the liver are mainly directed by IRS2. Phosphorylation of IRS1 and IRS2 leads to their association with the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K). This interaction recruits the p110 catalytic subunit of PI3K to the plasma membrane, resulting in conversion of phosphatidylinositol-4, 5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3). PtdIns(3,4,5)P3 facilitates additional signaling events by binding to phosphoinositide-dependent protein kinase-1 (PDK1), PDK2 and Akt (also known as protein kinase B (PKB)). Colocalization of PDKs and Akt facilitates activation of Akt by phosphorylation at Thr308 (PDK1) and Ser473 (PDK2), leading to phosphorylation of downstream targets such as glycogen synthase kinase-3 (GSK3) and the AS160 Rab GTPase-activating protein, which in turn interacts with the small GTPase RAB10 to facilitate translocation of glucose transporter 4 (Glut4)-containing vesicles to the cell surface (Sano et al., 2007). These actions of insulin promote glucose uptake and storage under anabolic conditions. This insulin-triggered signalling cascade can
be attenuated or 'tuned down' by various regulators. Thus, the initiating event in the cascade, autophosphorylation of IR, is reversed by the protein Tyr phosphatase-1B (PTB1B, also known as PTPN1), and Tyr kinase activity of the IR is inhibited by suppressor of cytokine signalling-1 (SOCS1) and SOCS2. Tyr phosphorylation and activation of IRS proteins is opposed by Ser phosphorylation of these proteins in response to overnutrition and activation of stress pathways. Accumulation of PtdIns(3,4,5)P3 and signalling through PDK1–PDK2–Akt can also be downregulated by the activity of the lipid phosphatase PTEN (phosphatase and tensin homologue on chromosome-10), which converts PtdIns(3,4,5)P3 to PtdIns(4,5)P2 (Lazar and Saltiel, 2006). The growing understanding of the core elements of the insulin signalling pathway and its modulators provides opportunities for therapy. For example, knockout of Ptpn1 in mice reduces insulin resistance and improves glucose homeostasis (Delibegovic et al., 2007; Xue et al., 2007) and pharmaceutical companies are currently searching for selective inhibitors of this enzyme.

Figure 4: Insulin signaling in cells.
3.5 PI3K/AKT PATHWAY IN DIABETES MELLITUS

The phosphatidylinositol 3-kinase (PI3K) pathway and antagonistic signaling elements such as the phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10), are crucial elements of the signaling cascade triggered by insulin and growth factors. On the one hand, the PI3K pathway serves to protect cells against apoptosis. This effect may be important for the survival of tumour cells. As a matter of fact, the PI3K pathway is constitutively activated in many tumour cells and thus presumably participates in the generation of tumours. On the other hand, the pathway plays a pivotal role for the effects of insulin, and impaired signaling through PI3K may predispose to the development of diabetes. In the past few years some mechanisms mediating the activation of PI3K and its effectors kinases PDK (phosphatidylinositol-dependent kinase), protein kinase B (PKB/Akt), atypical protein kinases C (aPKC) and SGK (serum and glucocorticoid inducible kinase) have been disclosed. Moreover, a variety of downstream targets of PI3K have been identified, including transport molecules and transcription factors. However, the complexity of PI3K-dependent cellular signaling precluded an integrated view of this important pathway (Cohen, 2006).

Downstream signalling of PI3K

Insulin and growth factors exert many of their cellular effects through activation of phosphoinositide 3-kinase (PI3K), which phosphorylates phosphatidylinositol 4,5 bisphosphate (PtdIns(4,5)P2) to generate the second messenger PtdIns(3,4,5)P3. PtdIns(3,4,5)P3 triggers the activation of downstream signaling pathways by recruiting signaling proteins containing pleckstrin homology (PH) domains to the plasma membrane.
PtdIns(3,4,5)P3 is metabolised in two ways. It can either be dephosphorylated at the 3 position by the PTEN tumour suppressor phosphatase to generate PtdIns(4,5)P2 or at the 5 position by the SHIP1 and SHIP2 phosphatases to generate PtdIns(3,4)P2. Several PH domain-containing proteins such as PKB, PDK1 and DAPP1, bind PtdIns(3,4,5)P3 and PtdIns(3,4)P2 with similar affinity (Cohen, 2006). To date only the Tandem PH domain-containing Protein-1 (TAPP1), TAPP2 and the PX domain of the phagocytic p47 (phox) cytosolic subunit required for activation of NADPH oxidase, possess the properties of binding PtdIns(3,4)P2 specifically. However, TAPP1 binds PtdIns(3,4)P2 with over 1000-fold higher affinity than does the PX domain of p47(phox) (Busjahn et al., 2002). Stimulation of cells with insulin and growth factors frequently results in a transient production of PtdIns(3,4,5)P3, whose levels peak after a few minutes and decline thereafter (Alessi, 2001).

Regulation of the PI3K dependent serine kinases aPKC and PKB

The serine kinases of the PKB family and the atypical PKC (aPKC) play crucial roles in overlapping or identical signaling cascades. The PKB family consists of three members while there are two atypical PKCs. Within both families the members show strong homology to each other and have a similar structural organization. A common feature of these kinases is their activation by the immediate upstream kinase PDK-1. While insulin dependent activation of PKBs is generally accepted, activation of aPKC is less clear (Alessi, 2001). In contrast to classical or novel PKC activated by binding of diacylglycerol and/or calcium, respectively, aPKC do not possess either of these binding domains. Their activation therefore occurs after phosphorylation by PDK-1 at the membrane which is
insulin dependent. Consequently, several studies find an increased kinase activity for aPKC in tissues or cell lines after insulin stimulation (Anderson et al., 1998). In addition to phosphorylation of numerous substrates involved in different signaling cascades, several regulatory proteins binding directly to the kinases have been described. The proteins CTMP and Trb2/3 bind to the carboxyl-terminus or in the centre of PKB, respectively, and both are having a negative effect on the kinase activity. While in vivo data on CTMP have not been published, Trb3 seems to be especially important in the liver where its expression level is upregulated 10-20 fold in fasted animals. Similarly, the PRK-2 protein is cleaved during apoptosis and the carboxyl-terminal fragment binds to PKB, thereby inhibiting its phosphorylation and activation (Alessi, 2001). By contrast, Tcl1, APPL and Ft-1 have been described as proteins that enhance the kinase activity. For aPKC, also several binding proteins are known (Busjahn et al., 2002). They include the p62 protein (ZIP) that connects aPKC to TNF-R signaling. Well known in developmental biology are interacting proteins identified in C. elegans, Par4 and -6. Whereas Par4 inhibits the kinase activity of aPKCs, Par6 regulates the localization of the kinase and increases its activity. The increased kinase activity leads to enhanced phosphorylation of IRS-1 which downregulate the PI3K cascade. In addition, aPKC directly can phosphorylate PKB and thereby inhibit its kinase (Alessi, 2001).

AKT (Protein Kinase B)

The serine/threonine kinase Akt, also known as protein kinase B (PKB), is a central node in cell signaling downstream of growth factors, cytokines, and other cellular stimuli and is one of the most important and versatile protein kinases at the core of human physiology.
and disease. Aberrant loss or gain of Akt activation underlies the pathophysiological properties of a variety of complex diseases, including type-2 diabetes and cancer (Manning and Cantley, 2007).

Substrate Specificity

The three Akt isoforms (Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ) have extensive homology to protein kinases A, G, and C within their kinase domains and are, therefore, members of the AGC kinase family. Following the identification of a residue on the kinase GSK3 as the first direct target of Akt in cells (Cross et al., 1995), experiments with peptides containing variants of this sequence defined the minimal recognition motif of Akt as R-X-R-X-X-S/T-B (Alessi et al., 1996b), where X represents any amino acid and B represents bulky hydrophobic residues. The critical requirement for R residues at both the −5 and −3 positions (that is, 5 and 3 residues, respectively, N-terminal to the phosphoacceptor site) on peptides efficiently phosphorylated by Akt distinguishes the substrate specificity of Akt from that of two other mitogen-stimulated AGC kinases, RSK (MAPKAP-K1) and S6K1 (p70S6K), which can better tolerate K at these positions.

In these “peptide-bashing” experiments, more subtle Akt preferences were also uncovered for other residues surrounding the phosphorylation site (such as a preference for T at −2). Structural insights into the molecular interactions dictating the substrate selectivity of Akt have been provided by a high-resolution crystal structure of Akt bound to this GSK3 peptide substrate (Yang et al., 2002).
Akt Substrates and Functions

A careful search of the literature finds over 100 reported nonredundant Akt substrates, of which approximately 25% do not contain the minimal requirements for an Akt site (e.g., R-X-R-X-X-S/T). Furthermore, many of those that do contain this motif have not been characterized further than in vitro kinase assays. In the literature there are 18 substrates for which there have been multiple independent published reports.

Cellular Metabolism

In response to growth factors, Akt signaling regulates nutrient uptake and metabolism in a cell-intrinsic and cell-type-specific manner through a variety of downstream targets. One of the most important physiological functions of Akt is to acutely stimulate glucose uptake in response to insulin. Akt2, the primary isoform in insulin-responsive tissues, has been found to associate with glucose transporter 4 (Glut4)-containing vesicles upon insulin stimulation of adipocytes (Calera et al., 1998), and Akt activation leads to Glut4 translocation to the plasma membrane (Kohn et al., 1996). Although the precise molecular mechanisms by which Akt stimulates Glut4 translocation are still being intensely studied, the Rab-GAP AS160 (also known as TBC1 domain family member 4; TBC1D4) has emerged as an important direct target of Akt involved in this process (Eguez et al., 2005; Sano et al., 2007). Five putative Akt sites are phosphorylated on AS160 in response to insulin, of which S588 and T642 score the highest using the Scansite program. Importantly, mutation of these two sites to alanine significantly blocks insulin-stimulated Glut4 translocation (Sano et al., 2003). The other candidate Akt substrates involved in various steps of Glut4 translocation have been identified, including PIKfyve (Berwick et al., 2004). Glut1 is the
main glucose transporter in most cell types, and unlike Glut4, it appears to be regulated primarily through alterations in expression levels. Activation of mTORC1, through Akt-mediated phosphorylation of TSC2 and PRAS40, can contribute to both HIFα-dependent transcription of the Glut1 gene and cap dependent translation of Glut1 mRNA (Zelzer et al., 1998; Taha et al., 1999).

Akt activation can also alter glucose and lipid metabolism within cells. Upon entry into the cell, glucose is converted to its active form glucose 6-phosphate through the action of hexokinases. Akt has been demonstrated to stimulate the association of hexokinase isoforms with the mitochondria, where they more readily phosphorylate glucose, but the direct target of Akt responsible is currently unknown (Robey and Hay, 2006). Glucose 6-phosphate can be stored by conversion to glycogen or catabolized to produce cellular energy through glycolysis, and Akt signaling can regulate both of these processes. Particularly important in muscle and liver, Akt-mediated phosphorylation and inhibition of GSK3 prevents GSK3 from phosphorylating and inhibiting its namesake substrate glycogen synthase, thereby stimulating glycogen synthesis. Akt activation also increases the rate of glycolysis (Elstrom et al., 2004), and this is probably a major factor contributing to the highly glycolytic nature of tumor cells. Akt’s ability to enhance the rate of glycolysis is due, at least in part, to its ability to promote the expression of glycolytic enzymes through HIFα (Semenza et al., 1994; Majumder et al., 2004; Lum et al., 2007). In a cell-context-dependent manner, Akt-mediated phosphorylation and inhibition of FOXO1 also contribute to glucose homeostasis, as FOXO1 promotes hepatic glucose production and regulates the differentiation of cells involved in metabolic control (Accili and Arden, 2004).
In hepatocytes, Akt can also inhibit gluconeogenesis and fatty acid oxidation through direct phosphorylation of S570 on PGC-1α (Li et al., 2007), which is a coactivator that can coregulate genes with FOXO1 and other transcription factors. Akt signaling regulates lipid metabolism through phosphorylation and inhibition of GSK3. As described above, phosphorylation of substrates by GSK3 often targets them for proteasomal degradation, and GSK3 has been shown to promote degradation of the sterol regulatory element-binding proteins (SREBPs), which are transcription factors that turn on the expression of genes involved in cholesterol and fatty acid biosynthesis (Sundqvist et al., 2005). Therefore, Akt-mediated inhibition of GSK3 promotes SREBP stability and enhances lipid production. As discussed above, Akt has also been proposed to directly activate ACL (Berwick et al., 2002), with the caveat that the relevant phosphorylation site on this enzyme does not meet the minimal consensus for an Akt site. As intense interest in the role of the PI3K-Akt pathway in metabolic diseases and tumor metabolism continues, it is likely that Akt signaling will be found to impinge on many other areas of central metabolism.

**Glucose Transporter4 (Glut 4)**

The rate-limiting step in the uptake and metabolism of glucose by insulin target cells is glucose transport, which is mediated by specific glucose transporters of the plasma membrane. In normal muscle cells and adipocytes, the glucose transporter isoform is Glut4, a 12-transmembrane domain protein that facilitates transport of glucose in the direction of glucose gradient (Douen et al., 1990; Barret et al., 1999). Insulin promotes Glut4 incorporation into plasma membrane, and this translocation from intracellular
compartments appears to fail in the insulin resistance present in some form of diabetes (Klip et al., 1990; King et al., 1992; Zierath et al., 1996; Garvey et al., 1998).

Figure 5: Signaling Pathways Leading to Glut4 Translocation. Insulin signaling through the PI 3-kinase pathway and muscle contraction through both elevated AMP/ATP ratios and intracellular [Ca^{2+}] leads to activation of downstream protein kinases (Akt, aPKCζ, AMPK, CaMKII cPKC) that phosphorylate putative effectors that modulate steps in the Glut4 trafficking pathways. AS160 is one such substrate that appears to negatively regulate an early step in Glut4 exocytosis. Negative regulation of these pathways by fatty acids, cytokines, and endoplasmic reticulum stress responses are observed in obesity and diabetes, contributing to insulin resistance.

Other glucose transporter isoforms have been identified among which Glut1 is the main glucose transporter in most cell types, and unlike Glut4, it appears to be regulated primarily through alterations in expression levels; while Glut2 is present mainly in liver and kidney and Glut3 in neurons and cardiac tissues, Glut5 is a fructose transporter present in small intestine and sperm (Huang and Czech, 2007).
Protein tyrosine phosphatases (PTP)

The mechanism by which PtdIns(3,4,5)P3 declines after prolonged insulin and growth factor stimulation is poorly defined. PtdIns(3,4)P2 which is generated after PtdIns(3,4,5)P3 production, could serve as a specific negative feedback signal, recruiting to the plasma membrane phosphatases that could dephosphorylate receptors and adaptor subunits leading to inactivation of PI3K and thus to a reduction in the level of PtdIns(3,4,5)P3. In support of this model, recent studies have indicated that the TAPP1 and TAPP2 adaptor proteins interact with the Protein Tyrosine Phosphatase-L1 (PTPL1 (PTPL1, also known as FAP-1, PTP1E, PTP-BAS and PTPN13)). TAPP1 and TAPP2 possess a PDZ binding motif at their C-terminus that interacts directly with a PDZ domain on PTPL1 (Datta et al., 2000). Localisation studies have revealed that PTPL1 bound to TAPP1 is present in the cytosol, and following stimulation of cells with agonists, which elevates the level of PtdIns(3,4)P2, the PTPL1 –TAPP1 complex is recruited to the plasma membrane (Datta et al., 2000).

Overexpression of PTPL1 in a breast cancer cell line was also recently induced the dephosphorylation of the insulin-receptor-substrate protein-1 (IRS1), resulting in inhibition of PI3K regulated cell growth and survival responses (Debonneville et al., 2001). Taken together, these results provide evidence that the PI3K pathway may be a physiological target for the PTPL1 phosphatase. There is significant evidence that the PTP1B tyrosine phosphatase participates in the dephosphorylation of the insulin receptor and therefore negatively regulates the insulin-signaling pathway. This is also confirmed by the finding that mice lacking PTP1B are significantly sensitised to insulin (Dijkers et al., 2000). These discoveries have stimulated significant interest in developing inhibitors of
PTP1B for the treatment of type 2 diabetes. As in tissues of mice lacking PTP1B, the insulin-signaling pathway is still switched off following removal of insulin, other tyrosine phosphatases are also likely to be involved in dephosphorylating the insulin receptor. The crystal structure of the catalytic domain of PTPL1 has recently been solved by our laboratory (Embark et al., 2004), and interestingly revealed a second positively charged phosphotyrosine binding pocket located near the PTPL1 catalytic site. PTP1B is the only other tyrosine phosphatase that has thus far to be shown to possess a second phosphotyrosine binding pocket, and is required for PTP1B to dephosphorylate the activated insulin receptor that contains two adjacent phosphotyrosine residues. Kinetic and mutagenesis analysis indicates that PTPL1 also efficiently dephosphorylates the insulin receptor with similar catalytic efficiency as PTP1B and that the second phosphotyrosine pocket is required for optimal catalytic efficiency (Embark et al., 2004).

The current hypothesis is that a pool of endogenous PTPL1 is maintained in the cytoplasm of unstimulated cells through its interaction with TAPP1. Following prolonged activation of PI3K, significant levels of PtdIns(3,4)P2 accumulate at the membrane leading to the recruitment of the PTPL1-TAPP1 complex to this location. PTPL1 then dephosphorylates the insulin receptor and/or adaptor protein(s), thereby inducing the inactivation of PI3K. Thus, PtdIns(3,4)P2 could trigger the activation of a negative feedback loop to switch off PI3K through its recruitment of PtdIns(3,4)P2 to the plasma membrane (Cohen, 2006).
The role of PI3K pathway in the regulation of beta cell ion channels and insulin release

Ion channels play a pivotal role in the regulation of insulin secretion. $K_{\text{ATP}}$ channels are responsible for the resting membrane potential in unstimulated cells (Anderson et al., 1998; Alessi, 2001). Glucose leads to closure of $K_{\text{ATP}}$ channels with subsequent cell depolarization, opening of voltage dependent $\text{Ca}^{2+}$ channels, $\text{Ca}^{2+}$ entry and insulin release. Elevated cytosolic $\text{Ca}^{2+}$ is the main although not the sole stimulus for the exocytotic release of insulin. In concert with $K_{\text{ATP}}$ channels, the activity and regulation of $\text{Ca}^{2+}$ channels, $K^+$ channels others than $K_{\text{ATP}}$ channels and of $\text{Cl}^-$ channels modulate secretion (Anderson et al., 1998). Multiple receptor-activated pathways may be involved in the regulation of these ion channels. Elevated cytosolic $\text{Ca}^{2+}$ activates $K^+$ channels which limits $\text{Ca}^{2+}$ influx. PKC and phospholipids modulate $K^+$ channel activity. Adenyl cyclase activation by GLP1, glucagon or GIP increases the opening time of Ca2+ channels and antagonizes Kv channel opening. The signaling pathways regulating insulin release and peripheral insulin action include PI3K. Early experiments using low concentrations of wortmannin, a potent PI3K inhibitor, suggest a negative feedback regulation of insulin release by PI3K (Cohen, 2006). In pancreatic beta cells, PI3 kinase stimulates pathways involved in cell proliferation and apoptosis rather than secretion. These pathways are especially activated under pathophysiological conditions, i.e. diabetes mellitus when the rate of cell death is increased and proliferation changed. However, cross talks between signaling pathways regulating secretion with that regulating cell proliferation have been described. Most evident is the effect of GLP-1, a well known potentiator of insulin secretion, which activates adenylyl cyclase (Bortul et al., 2003). GLP-1 reduces the
apoptotic rate and augments cell proliferation of insulin secreting cells. GLP-1 may interfere with PI3K pathways via cAMP mediated effects on CREB (Debonneville et al., 2001). Little is known about the role of PI3K dependent signaling pathways in regulation of ion channels relevant for insulin secretion. Considering the role of PI3K pathway in the regulation of ion channels, the effects of PI3K could at least partially be mediated by altered channel function. The diabetogenic glucocorticoids upregulates both, Kv1.5 channels and SGK1 (serum and glucocorticoid inducible kinase 1) expression in beta cells (Embark et al., 2004). SGK1 decreases channel recycling from the plasma membrane and thus increases K⁺ channel activity at the plasma membrane. Glucocorticoids also decrease mRNA for GLP-1 receptors in endocrine tissue (Embark et al., 2004).

**PI3K-dependent modulation of insulin signal transduction by serine phosphorylation of IRS-1**

Several pathophysiological stimuli have been identified which impair the insulin signal transduction. These include elevated glucose concentrations, elevated free fatty acids, TNF-α, IL-6, angiotensin II and prolonged hyperinsulinemia (Alessi, 2001; Cohen, 2006). Recent studies show that these stimuli induce serine phosphorylation at specific sites at the IRS-1 molecules leading to impaired insulin signal transduction. Various serine kinases have been shown to be activated by the stimuli causing impaired insulin signaling and leading to increased serine phosphorylation of IRS-1; these include JNK and PKC. The phosphorylation of Ser-318 is dependent on PI3K since it is inhibited by specific PI3K inhibitors. On the other hand, this serine phosphorylation regulates insulin signal transduction via the PI3K pathway. Together, the data demonstrate that activation of PI3K
is necessary for serine phosphorylation of IRS-1 and that PI3K activity itself is modulated by this serine phosphorylation. However, the detailed mechanism and regulation of this crosstalk and its functional significance is unknown (Alessi, 2001; Cohen, 2006).

3.6 CURRENT THERAPIES FOR TYPE 2 DIABETES MELLITUS

A universal antidote does not exist for all of the metabolic abnormalities that are embodied in type 2 diabetes. Several drugs (Sulfonylureas and Meglitinides) that target the $K_{atp}$ channel complex to stimulate insulin secretion are available for the treatment of $\beta$-cell dysfunction, but these compounds tend to be transiently efficacious and are associated with hypoglycemia. Therapies that are based on glucagon-like peptide-1 (GLP1) — including more efficacious or long-acting analogues of the native peptide or inhibitors of the GLP1-degrading enzyme DPP-IV — are attractive because of the low risk of hypoglycemia, given that GLP1 is entirely glucose-dependent as an insulin secretagogue. Recent studies comparing islets from type 2 diabetic individuals and normal controls have revealed drastically decreased expression of multiple proteins of the insulin exocytosis pathway, and strongly impaired glucose-stimulated insulin secretion (GSIS). Remarkably, GLP1 can almost completely restore GSIS in the diabetic islets (Ostenson et al., 2006). GLP1 has also been ascribed an antiapoptotic and $\beta$-cell-regenerative function (Baggio and Drucker, 2007). The extent to which GLP1 administration to humans engages these regenerative effects remains to be established. In the realm of insulin resistance, the biguanide metformin and the thiazolidinediones are the most commonly used medications. Metformin activates 5'-AMP-activated protein kinase (AMPK) to stimulate glycolysis and fatty acid oxidation. Thiazolidinediones are PPAR$\gamma$ ligands and, in addition to
activating AMPK, stimulate adipogenesis and the redistribution of lipids from liver and muscle into adipose tissue. However, weight gain and fluid retention are common side-effects; hepatotoxicity (Kohroser et al., 2000) and an increased risk of heart disease (Nissen and Wolski, 2007) have also been associated with members of this class of compound. Because overnutrition is a central driver of the disease, one potential strategy is to reduce food intake. It has been suggested that antagonists of the cannabinoid receptor-1, which regulates central appetite control in response to endogenous ligands, may fulfill this purpose (Bray and Ryan, 2007). However, these antagonist drugs have incompletely characterized peripheral effects, and have been associated with increased nausea, dizziness and depressed mood. Nevertheless, strategies that restore energy balance, or that combat fundamental pathogenic mechanisms such as ER stress (Ozcan et al., 2006), may ultimately prove to be globally efficacious and deserve further investigation.
3.7 PHARMACOGNOSY, PHYTOCHEMISTRY AND PHARMACOLOGICAL STUDIES OF

**HELICTERES ISORA** Linn. (Kirtikar and Basu, 1981; Satyavati et al., 1987)

**Scientific Name:** Helicteres isora Linn.

**Family:** Sterculiaceae

**Scientific Classification:** Kingdom- Plantae, Division- Magnoliophyta, Order- Malvales, Family- Sterculiaceae, Subfamily- Helicteroideae, Genus- Helicteres.

**Parts used:** Roots, Fruits and Bark

**Description:** Helicteres isora is a large shrub or small tree up to 5 meters in height with grey bark and young shoots clothed with stellate hairs. Roots are cylindrical to somewhat tortuous in shape, 4mm to 2cm wide with very few lateral roots being very thin and branched. Outer surface is warty, shows lenticels, longitudinal ridges, exfoliated at places and earthy brown in colour. Inner surface is creamish brown in colour. Fracture is hard, odor is characteristic and taste is bitter.

![Picture 1: Pictures of Helicteres isora Linn. (A) Plant (B) Flowering twig and (C) Roots](image)

**Common Names:** Ail, Atmura, Bid, Daruphal, Dhanmad, East Indian Arrow Tree, Kayyuna, Kiules, Marorphali, Marrorphali, Mriga-Shringa, Argoti, Popid, Valambiri, and Valampiri.

Shefalee Bhavsar

L. M. College of Pharmacy, Ahmedabad, India
Distribution: Widely distributed from southern China to India (common in central and western India), Burma, Malaya, South East Asia, Australia, West Indies, Central Peninsula.

Vernacular names:

<table>
<thead>
<tr>
<th>Languages</th>
<th>Vernacular Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabic</td>
<td>altwaallatu, maror phali.</td>
</tr>
<tr>
<td>English</td>
<td>Kaivum fibre.</td>
</tr>
<tr>
<td>Hindi</td>
<td>bhendu, jonkaphal, kapasi, maraphali, marodphali, maraphali, marori, marorphali, marosi.</td>
</tr>
<tr>
<td>Kannada</td>
<td>bhootakaru, bhutakaru, edamuri, kadukalnaru, kalyuri, kavargi, kempukaveri, murudi, narukolu, thunshulla, yadamuri, bhoota karulu, chuchuja, kaadukalnaru, kaiyyi uri, kavargi, kempu kavari, kowri, maradaddi shingi, nakheeja, yedamuri, karurimara, yedamuri.</td>
</tr>
<tr>
<td>Malayalam</td>
<td>ishvaramuri, isora-murri, itampirivalampiri, kaivalanara, kaiyuna, valampiri, valumbari, valampiri, kayyuna.</td>
</tr>
<tr>
<td>Marathi</td>
<td>maedasingi, kevani, muradasinge, kewan, muradsing.</td>
</tr>
<tr>
<td>Persian</td>
<td>kisht-bur-kisht, kishtburkisht, pechaka.</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>avaratani, avartani, avartaphala, avartiphala, avarttani, avarttani, avatarini, avurtunnie, awartaki, awartaki, awartani, mrgasrngi, mrigashinga, mrigashinga, mrigashringa, murva, murva.</td>
</tr>
<tr>
<td>Tamil</td>
<td>vadampiri, valamburi, valampiri, valampuri, valampuri, kay, tirukupalai, valampurikkay, valambiri, valumbirikai, valampurikai, valumbirikai, valamburi, vadampiri, kaiva, valambiri, edampuri, karuval, kasavu, vattachi, valampuri, cattikatci, iracarcapalam, lampam, panippalli, paniyam, pirapicam, pirimurrukku, pirivuppapi, tirukupalai, valampurikkay.</td>
</tr>
<tr>
<td>Telugu</td>
<td>adasamanti, adasyamali, adavi-camanti, gubadarra, guvalada, kavanchi, nulitada, nuliti, peddasamanti, sadala, valumbari, gubathada, peddasamala, adavichamanti.</td>
</tr>
<tr>
<td>Urdu</td>
<td>marorphali, marodphalli, maror phali.</td>
</tr>
</tbody>
</table>
Uses in traditional medicine

In India, dried bark and leaf of *H. isora* mixed with decoction of Gymnema sylvestre leaves are used in diabetes (Khan and Singh, 1996), while hot water extract of bark and seed or whole plant is also used for diabetes (Jain and Sharma, 1967). *H. isora* is mentioned as antigalactogogue, expectorant, antidiarrhoeal, antidysenteric and carminative (Kapoor and Kapoor, 1980; Mudgal and Pal, 1980; Singh et al., 1984; Nagaraju and Rao, 1990). Fruits of *H. isora* are soaked in boiling mustard oil, cooled and rubbed on body of an infant with rickets (Girach et al., 1994). In India, fruits are also used to treat infantile diarrhea and earache (Jain, 1989). Ramchandran and Nair (1981) reported the use of leaves of *H. isora* by natives of Cannanore district, Kerala for skin ailments including eczema. In Rayalseema area of Andhra Pradesh, root powder is mixed with turmeric powder and applied externally for cuts and wounds (Nagaraju and Rao, 1990). In Karnataka, decoction of stem bark made with pepper is used for cough and throat infections (Bhandary et al., 1995). Fruits and roots are also used in polio and cholera by the tribal of Khunti area of Bihar (Topho and Ghosh, 2003). In Saudi Arabia, *H. isora* is also used as traditional medicine. Hot water extract of *H. isora* is used as antispasmodic, nerve tonic, antipyretic, antidysenteric, antiparalytic, antidiarrhoal (Al-Yahya, 1986). In Thailand, fresh root is crushed and used as a poultice in inflammation (Panthong et al., 1986). In Malaysia, dried fruit is eaten as an aphrodisiac in male while hot water extract of dried fruit is given to female after childbirth (Burkill, 1966). The roots and bark have been used as expectorant, demulcent, astringent, antigalactagogue, a cure for scabies and to lessen griping. The roots and the bark are expectorant, demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhoea.
and dysentery (Prajapathi et al., 2003). Juice of the root is used in emphysema, stomach
afflictions and diabetes. Fruits are demulcent, mildly astringent and useful in griping and
flatulence (Kirtikar and Basu, 1981). *H. isora* is used in emphysema, snakebite and
diabetes (Singh et al., 1984).

Previously reported studies

Phytochemical studies of *H. isora*

Singh et al. (1984) isolated tetratriacontanol, 3β-sitosterol and tetratriacontanoic acid from
the leaves of *H. isora*. Satake et al. (1999) reported the presence of 4'-0-β-D-
glucopyranosyl rosarinic acid, 2R-O-(4'-0-β-D-glucopyanosyl caffeoyl)-3-(4-
hydroxyphenyl) lactic acid (4'-0-β-D-glucopyranosylisorinic acid), and rosaminic acid.
Various triterpenes like α-amyrin, β-amyrin, bauerenol acetate, betulic acid, taraxerone,
friedelin, friedelinol, isorin, lupeol, oleanolic acid are also reported to be present in *H.
isora* (Dan and Dan, 1988; Qu et al., 1991). Dan and dan (1988) estimated diosgenin
(0.33%) in Free State as well as glycosidic form, and found not to be admixed with other
steroidal sapogenins like other sources of diosgenin. From the roots, Cucurbitacin B and
isocucurbitacin B were isolated and reported to possess cytotoxic activity (Bean et al.,
1985). From the fruits, neolignans, helisterculins A and B and helisorin were isolated,
showing weak inhibitory activity against reverse transcriptase from avian myeloblastosis
virus (Yasuhiro et al., 1999). Tezuka et al. (2000) isolated six neolignans, the helicterins and
elucidated their structures by spectral analysis. Helicterins are dimeric (7.5', 8.2')-
neolignans with a bicycle (2.2.2) octane C-framework. Kamiya et al. (2001) reported the
presence of five flavonoid glucurononides from fruits of *H. isora* mainly isoscutellarein 4'-
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methyl ether 8-O-β-D glucuronide, isoscutellarein 4'-methyl ether 8-O-β-D glucuronide
6"-n-butyl ester, isoscutellarein 4'-methyl ether 8-O-β-D glucuronide2" sulfate,
isoscutellarein 4'-methyl ether 8-O-β-D glucuronide2", 4"-disulfate, isoscutellarein 8-O-β-
D glucuronide2", 4"-disulfate.

Pharmacological activities of H. isora

Dhawan and Saxena (1985) reported the uterine stimulant effect of H. isora in rat. Pohocha and Grampurohit (2001) reported the antispasmodic activity of H. isora in vitro on guinea pig ileum against acetylcholine, histamine and BaCl₂. The antispasmodic activity was also studied in vivo by observing the gastrointestinal motility in mice.

Chakrabarti et al. (2002) reported that ethanol extract of the roots of H. isora produced significant hypoglycemic effect in C57BL/KsJ-db/db mice, reduced the lipid levels in high fat diet fed hamster and lowered triglyceride levels in hypertriglyceridemic swiss albino mice.

Venkatesh et al. (2003; 2004) reported that H. isora improved glucose tolerance in glucose-induced hyperglycemic rats, produced antidiabetic effect in alloxan induced type 1 diabetes mellitus; the extracts showed a significant anti-diabetic activity comparable with that of glibenclamide and H. isora do not have antihyperglycemic activity in normal rats.

Kumar et al. (2006a, 2006b) reported that H. isora aqueous bark extract produced significant hypoglycemic effect in streptozotocin induced type 1 diabetes mellitus in rats.