International Textbook of Diabetes Mellitus
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As the epidemic of diabetes continues to expand in parallel with the rapid spread of obesity, healthcare providers strive to find interventions to reduce the morbidity, mortality, and rising costs associated with this devastating disease, which ravages both the micro- and the macrovasculature. Although the increase in incidence of type 2 diabetes may be attributed to the expanding girth of the population coupled with a lack of physical activity, the marked increase in the incidence of type 1 diabetes remains unexplained. Our knowledge of the cellular, biochemical, and molecular etiology of impaired insulin action and beta-cell failure has expanded enormously, but the genetic basis of both type 1 and type 2 diabetes and their associated complications is still by and large undefined. Despite the introduction of multiple new classes of antidiabetic agents for the treatment of type 2 diabetes, and newer insulin preparations, insulin delivery systems, and glucose-sensing devices for the management of type 1 diabetes, glycemic control is suboptimal in approximately half of all diabetic patients and the excess risk for macrovascular complications is largely unexplained. In many parts of the world, these treatment advances are not available and instituting behavioral modification programs at the societal and individual level is proving to be inadequate in curbing the growing epidemic of obesity. Whether the introduction of novel weight loss medications will stem the tide of obesity remains to be determined.

The fourth edition of the *International Textbook of Diabetes Mellitus* will continue to be the most widely referenced textbook of diabetes worldwide and draws upon the expertise of leading basic scientists, clinicians, educators, and healthcare professionals globally to provide the most updated information on advances in diabetes research and clinical care. This information will be an invaluable resource and provide the practicing physician, as well as the basic scientist and clinical investigator, with the requisite resources to advance them to the frontiers of biomedical research in the fields of diabetes, metabolism, and obesity and to provide them with state-of-the-art knowledge to optimize clinical care for their diabetic patients.

Ralph A. DeFronzo, MD
SECTION I

Epidemiology
CHAPTER 1
Classification of diabetes mellitus and other categories of glucose intolerance

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Key points
• The classification and diagnosis of diabetes is based on etiology and not on pharmacologic treatment.
• Diagnoses of diabetes are made using fasting plasma glucose, 2-hour postchallenge of glucose or HbA1c.
• Differentiation between type 1 and type 2 diabetes is usually straightforward but can be difficult among obese children and adults.
• Precise diagnoses of certain monogenic diabetes using genetic testing can be useful as the outcomes can influence treatment decisions.
• A range of commonly used drugs such as statins and glucocorticoid steroids can lead to the development of diabetes.

Introduction

A critical requirement for orderly epidemiologic, genetic and clinical research, and indeed for the management of diabetes mellitus and other forms of glucose intolerance is an appropriate classification system. Furthermore, a hallmark in the process of understanding the etiology of a disease and studying its natural history is the ability to identify and differentiate its various forms and place them into a rational etiopathologic framework. While there have been a number of sets of nomenclature and diagnostic criteria proposed for diabetes, no systematic categorization existed until the mid 1960s [1]. Now diabetes mellitus is recognized as being a syndrome, a collection of disorders that have hyperglycemia and glucose intolerance as their hallmark, due either to insulin deficiency or to impaired effectiveness of insulin’s action, or to a combination of these.

Historical perspective and current classifications

Previous classifications

In 1965, an Expert Committee on Diabetes Mellitus published the first World Health Organization (WHO) report on diabetes classification [1]. The report includes one of the first attempts at international consensus on a classification. They decided to classify diabetes: “… based on the age of recognized onset, which seemed to be the only reliable means of classification for universal use.”

The report also recognized certain specific types of diabetes including brittle, insulin-resistant, gestational, pancreatic, endocrine, and iatrogenic diabetes. Since then, several pathogenic mechanisms have been described and long-term studies have shown different courses and outcomes of different types of diabetes.

A revised classification of glucose intolerance, was formulated by the National Diabetes Data Group (NDDG) [2]. This was amended and adopted in the second report of the WHO Expert Committee in 1980 [3] and in a modified form in 1985. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them insulin-dependent diabetes mellitus (IDDM) or type 1, and non-insulin-dependent diabetes mellitus (NIDDM) or type 2 [3]. In the 1985 Study Group Report, the terms type 1 and type 2 were omitted, but the classes IDDM and NIDDM were retained and a new class of malnutrition-related diabetes mellitus (MRDM) was introduced [4]. The 1985 WHO classification was essentially based on clinical descriptions, with a specific focus on the pharmacologic management of patients (i.e., insulin-dependent, non-insulin-dependent, gestational). The question as to whether certain clinical forms
of diabetes (such as the so-called “tropical diabetes”) had been
given adequate priority to correct hierarchic order that was
raised many years before probably led to the introduction of
MRDM, although more precise epidemiologic data and a better
assessment were needed, and called for.

Both the 1980 and 1985 reports included other types of
diabetes and impaired glucose tolerance (IGT) as well as
gestational diabetes mellitus (GDM). The 1985 classification
was widely accepted and used internationally, and represented
a compromise between clinical and etiological classifications.
Furthermore, it permitted classification of individual patients
in a clinically useful manner even when the specific etiology
was unknown. The 2011 American Diabetes Association
(ADA) [5] classifications or staging of diabetes still include
clinical descriptive criteria but a complementary classification
according to etiology is recommended by both organizations.

In 1999, the WHO incorporated an approach developed by
Kuzuya and Matsuda [6], which clearly separated the criteria
related to etiology from those related to the degree of deficiency
of insulin or insulin action, and defined each patient on the basis
of these two sets of criteria (Figure 1.1). It is now well established
that diabetes may progress through several clinical stages during
its natural history, quite independent of its etiology. The clinical
staging reflects this and, indeed, individuals may move from
one stage to another stage in both directions (Figure 1.1). Even
if there is no information concerning the underlying etiology,

<table>
<thead>
<tr>
<th>Types</th>
<th>Normoglycemia Normal glucose tolerance</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Autoimmune</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Idiopathic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Predom. insulin resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Predom. insulin secretory defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other specific types</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Genetic defects of β-cell function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Genetic defects of insulin action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Diseases of exocrine pancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Endocrinopathies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Drug or chemical induced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational hyperglycemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGT and/or IFG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not insulin requiring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin: for control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin: for survival</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Current classification**

The current classification allows for various degrees of hyper-
glycemia in individuals irrespective of the disease process. These
are glycemic stages ranging from normoglycemia (normal glu-
cose tolerance) to hyperglycemia where insulin is required for
survival. All individuals with the disease can be categorized
according to clinical stage [7]. The stage of glycemia may
change over time depending on the extent of the underlying
disease processes. As shown in Figure 1.1, the disease process
may be present but may not have progressed far enough to
cause hyperglycemia. The etiological classification is possible
as the defect or process which may lead to diabetes may be
identified at any stage in the development of diabetes, even at
the stage of normoglycemia. As an example, the presence of
islet cell antibodies (ICA) and/or antibodies to glutamic acid
decarboxylase (anti-GAD) [8] in a normoglycemic individual
indicates the autoimmune process, which underlies type 1
diabetes, is present, although the individual may or may not
ultimately develop diabetes [7,9]. For type 2 diabetes, there are
few useful highly specific indicators, though the presence of risk
factors such as obesity indicates the likelihood of developing
type 2 diabetes. Hopefully, future research will reveal some
specific markers of the type 2 diabetes disease process.

![Figure 1.1 Disorders of glycemia: etiologic types and clinical stages. Source: World Health Organization 1999 [7]. Reproduced with permission of the WHO.](image-url)
The same disease process can cause various degrees of impaired glucose metabolism such as impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT) without fulfilling the criteria for the diagnosis of diabetes [7]. Weight reduction, exercise and/or oral hypoglycemic therapy can achieve satisfactory glycemic control in some persons with type 2 diabetes. These persons, therefore, do not require insulin initially but may do so much later in their course as β-cell function deteriorates. Some persons require insulin for adequate glycemic control at an earlier stage in type 2 diabetes but could survive without it. By definition these persons have some residual insulin secretion. Patients with extensive β-cell destruction (minimal residual insulin secretion) do require insulin for survival and this is the hallmark of type 1 diabetes [7,9].

The classification by etiological type (Table 1.1) results from improved understanding of the causes of diabetes, although this is still far from complete, particularly for type 1 diabetes.

The terms “insulin-dependent diabetes mellitus,” “non-insulin-dependent diabetes mellitus” and their acronyms “IDDM” and “NIDDM” have been removed from classifications. These terms were very confusing and frequently resulted in misclassification, as patients were classified on the basis of their treatment, and indeed their age, rather than on pathogenesis. In the current classification, the terms “type 1” and “type 2” are retained (using Arabic rather than Roman numerals) [7].

Type 1 includes those cases attributable to an autoimmune process (although the basic precipitating cause of this process is still unknown), as well as those with β-cell destruction for which neither an etiology nor a pathogenesis is known (idiopathic). Those forms of β-cell destruction or failure to which specific causes can be assigned (e.g. cystic fibrosis, mitochondrial defects) are not included in this type of diabetes. These issues are discussed in greater detail later.

Type 2 includes the common major form of diabetes which results from defect(s) in insulin secretion and/or from insulin resistance, and often a combination of both. Malnutrition-related diabetes (MRDM) is no longer part of the WHO classification [7]. Of its two subtypes, protein-deficient pancreatic diabetes (PDPD or PDDM) needs more studies for a better definition. The other former subtype of MRDM, fibrocalculous pancreatic diabetes (FCPD), is now classified as a disease of the exocrine pancreas labeled “fibrocalculous pancreatopathy”, which may lead to diabetes.

Impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG) are classified as stages of impaired glucose regulation, since they can be observed in any hyperglycemic disorder.

Gestational diabetes is a state of glucose intolerance first recognized during pregnancy which usually resolves after delivery but is associated with later increased long-term risk of type 2 diabetes. It encompasses the groups formerly classified as gestational impaired glucose tolerance (GIGT) and gestational diabetes mellitus (GDM) [7].

### TABLE 1.1 Etiologic classification of disorders of glycemiaa [7]

<table>
<thead>
<tr>
<th>Type 1</th>
<th>β-cell destruction, usually leading to absolute insulin deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autoimmune</td>
</tr>
<tr>
<td></td>
<td>Idiopathic</td>
</tr>
<tr>
<td>Type 2</td>
<td>(may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance)</td>
</tr>
<tr>
<td>Other specific types</td>
<td>see Table 1.3</td>
</tr>
<tr>
<td>Genetic defects of β-cell function</td>
<td></td>
</tr>
<tr>
<td>Genetic defects in insulin action</td>
<td></td>
</tr>
<tr>
<td>Diseases of the exocrine pancreas</td>
<td></td>
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<tr>
<td>Endocrinopathies</td>
<td></td>
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<tr>
<td>Drug- or chemical-induced</td>
<td></td>
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<tr>
<td>Infections</td>
<td></td>
</tr>
<tr>
<td>Uncommon forms of immune-mediated diabetes</td>
<td></td>
</tr>
<tr>
<td>Other genetic syndromes sometimes associated with diabetes</td>
<td></td>
</tr>
<tr>
<td>Gestational diabetes**</td>
<td></td>
</tr>
</tbody>
</table>

*As additional subtypes are discovered it is anticipated that they will be reclassified within their own specific category.
**Includes the former categories of gestational impaired glucose tolerance and gestational diabetes.

Source: World Health Organization 1999 [7]. Reproduced with permission of the WHO.

### DIABETES TYPES

**Type 1 process**

Type 1 indicates the processes of β-cell destruction that may ultimately lead to diabetes in which insulin is required for survival in order to prevent the development of ketoacidosis, coma, and death. This category comprises:

- **Immune-mediated diabetes mellitus**: This is the classical form of type 1 diabetes, which can occur at any age, and results from a cell-mediated autoimmune destruction of the pancreatic β cells. The type 1 process is characterized by the presence of ICA, anti-GAD, islet antigen 2 (IA2) or insulin autoantibodies which identify the autoimmune process associated with β-cell destruction [9]. Other autoimmune disorders such as Grave’s disease, Hashimoto’s thyroiditis and Addison’s disease may be associated with type 1 diabetes mellitus [9].

The rate of β-cell destruction is quite variable, typically being rapid in children and slower in adults. Typically, type 1 diabetes requires insulin therapy from the time of presentation in both adults and children, but a slowly progressive form, latent autoimmune diabetes in adults (LADA), is well described [8]. Blood glucose in LADA can initially be controlled by lifestyle change and oral hypoglycemic agents, and may therefore masquerade as type 2 diabetes. However, in comparison to the typical patient with type 2 diabetes, LADA patients are leaner and
progress much more rapidly to requiring insulin. Importantly, markers of autoimmunity (most commonly anti-GAD antibodies) are present, and therefore LADA falls within type 1 autoimmune diabetes.

- Idiopathic: There are some forms of type 1 diabetes which have no known etiology, and no evidence of autoimmunity. Some of these patients have permanent insulinopenia and are prone to ketoacidosis [10]. This form is more common among individuals of African and Asian origin [11].

### Type 2 process

Type 2 diabetes is the commonest form of diabetes and is characterized by disorders of insulin resistance and insulin secretion, either of which may be the predominant feature. Both are usually present at the time diabetes is clinically manifest. Insulin levels may be normal or even elevated at the time when diabetes is diagnosed. However, in the setting of insulin resistance, these levels are inadequate to maintain normoglycemia. This relative insulin deficiency is what differentiates diabetic insulin-resistant individuals from normoglycemic insulin-resistant individuals. Indeed, it is noteworthy that, to date, the majority of the genes that have been associated with type 2 diabetes are related to insulin secretion, and not to insulin resistance [12].

At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive [13]. Type 2 diabetes is frequently asymptomatic and undiagnosed for many years because the hyperglycemia is often not severe enough to provoke noticeable symptoms [14]. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. Type 2 diabetes is a very heterogeneous disorder and there are certainly many different causes of this form of diabetes. However, it is likely that the number of patients placed in this category will decrease in the future as identification of specific pathogenic processes and genetic defects permit better differentiation and a more definitive classification. Although the specific etiologies of type 2 diabetes are not known, autoimmune destruction of the pancreas does not occur and patients do not have any of the other specific causes of diabetes listed in Table 1.2.

Most patients with the type 2 process of diabetes are overweight or obese, and obesity itself causes insulin resistance. Many of those not obese by traditional criteria, for example body mass index, may have an increased percentage of body fat distributed predominantly in the abdominal region [13]. Ketoacidosis seldom occurs in type 2 diabetes and when seen, it usually arises in association with the stress of another illness such as infection. Ketosis-prone atypical diabetes, also referred to as ketosis-prone type 2 diabetes is characterized by presentation with severe hyperglycemia and ketoacidosis requiring immediate insulin therapy [15]. More than 50% of these individuals will revert to an insulin-free near-normoglycemia.

### Table 1.2 Other specific types of diabetes [7]

<table>
<thead>
<tr>
<th>Genetic defects of β-cell function</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNF1A MODY</td>
</tr>
<tr>
<td>HNF4A MODY</td>
</tr>
<tr>
<td>HNF1B MODY</td>
</tr>
<tr>
<td>GCK MODY</td>
</tr>
<tr>
<td>MDA 3243 MIDD</td>
</tr>
<tr>
<td>KCNJ11 PNDM</td>
</tr>
<tr>
<td>KCNJ11 DEND</td>
</tr>
<tr>
<td>6q24 TNDM</td>
</tr>
<tr>
<td>ABC28 TNDM</td>
</tr>
<tr>
<td>INS PNDM</td>
</tr>
<tr>
<td>WFS1 Wolfram syndrome</td>
</tr>
<tr>
<td>FOXF3 IPEX syndrome</td>
</tr>
<tr>
<td>Elf2AK3 Wolcott–Rallison syndrome</td>
</tr>
</tbody>
</table>

### Genetic defects in insulin action

| INS Type A insulin resistance   |
| INSR Leprechaunism              |
| INSR Rabson–Mendenhall syndrome |
| LMNA FPLD                      |
| PPPR A2 FPLD                   |
| AGPAT2 CGL                     |
| BSCL CGL                       |
| Lipoatrophic diabetes          |

### Diseases of the exocrine pancreas

| Fibrocalculous pancreatopathy   |
| Trauma / pancreatectomy         |
| Neoplasia                       |
| Cystic fibrosis                 |
| Hemochromatosis                 |
| Others                          |

### Endocrinopathies

| Cushing syndrome                |
| Aldosteronoma                   |
| Acromegaly                      |
| Pheochromocytoma                |
| Glucagonoma                     |
| Hyperthyroidism                 |
| Somatostatinoma                 |
| Others                          |

### Drug- or chemical-induced (see Table 1.3)

### Infections

| Congenital rubella             |
| Cytomegalovirus                |
| Others                         |

### Uncommon forms of immune-mediated diabetes

| Insulin autoimmune syndrome (antibodies to insulin) |
| Anti–insulin receptor antibodies                       |
| “Stiff man” syndrome                                    |
| Others                                                  |

### Other genetic syndromes (see Table 1.4)

Notes: Nomenclature: the gene name is followed by the clinical syndrome with the gene number designated using the HUGO convention. MODY maturity onset diabetes of the young MIDD maternally inherited diabetes and deafness PNDM permanent neonatal diabetes mellitus DEND development delay epilepsy TNDM transient neonatal diabetes mellitus

Source: World Health Organization 1999 [7]. Reproduced with permission of the WHO.
within weeks or months with multiorgan insulin resistance not dissimilar to type 2 diabetes [16]. This condition is commonly found in sub-Saharan Africa and African migrants and is referred to as “Flatbush diabetes”[17].

The risk of developing type 2 diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM, in those with hypertension or dyslipidemia, and its frequency varies between different ethnic subgroups [7]. Type 2 diabetes is often associated with strong familial, likely genetic, predisposition but the genetics of type 2 diabetes are quite complex and not clearly defined [18]. Some patients who present a clinical picture consistent with type 2 diabetes have been shown to have antibodies similar to those found in type 1 diabetes.

Although diagnosis in most patients with type 2 diabetes is made in adult years, the disease is now increasingly seen in adolescents and even children, especially in a background of high obesity prevalence. At presentation, ketosis or even ketoacidosis, may occur in this younger age group and insulin is often required in the initial management. However, once the acute metabolic disturbance is rectified, insulin can often be withdrawn, and glycemic control achieved with lifestyle measures and oral pharmacotherapy.

**Other specific types**

The other specific types of diabetes are less common and can be broadly classed as genetic, exocrine pancreatic, endocrine, and drug-induced causes [7]. A more comprehensive breakdown is provided in Table 1.2 and the more common types are discussed briefly later.

**Classification of genetic disorders**

With ongoing advances in the study of molecular genetics, there has been considerable progress in the identification of specific subtypes of diabetes of genetic origin. Through this work, it has been shown that the clinical subgroups are heterogeneous and there has been recognition of several novel, genetic-based syndromes associated with diabetes. The progress in our ability to examine genes to arrive at a diabetes diagnosis has improved treatment for these patients [19] and thus genetic diagnosis has become a key part of clinical management in many countries.

**Genetic defects of β-cell function**

The diabetic state may be associated with monogenic defects in β-cell function. These forms are characterized by onset of mild hyperglycemia during childhood or early adulthood, and include maturity-onset diabetes of the young (MODY), permanent neonatal diabetes (PNDM), transient neonatal diabetes (TNDM), and many other insulin-deficient syndromes with a myriad of other clinical features [7]. The most well characterized of these is MODY. MODY is inherited in an autosomal dominant pattern and typically presents before the age of 25 years. While the condition results from β-cell dysfunction, it is not always insulin dependent. Molecular genetic testing can define a diagnosis in 1–2% of all diabetic patients with monogenic diabetes. Advances in this field have led to the identification of the genes associated with many clinically identified subgroups of diabetes and explained clinical heterogeneity in conditions defined by age of diagnosis, for example neonatal diabetes and MODY. Molecular genetic tests are now available to help define the diagnosis, and importantly alter prognosis and optimize treatment of children, young adults and their families with diabetes.

Several mutations associated with MODY have been identified to date, of which the most common genetic subtypes are: GCK MODY, HNF1A MODY, HNF4A MODY, and IPF1 MODY [19]. These are listed in Table 1.2. Among these subtypes, the HNF1A MODY subtype is the most common and results in a progressive and marked hyperglycemia with a high risk of microvascular and macrovascular complications [20], but these patients respond well to sulfonylureas [21]. Subtype HNF4A is similar to HNF1A but patients have marked macrosomia and transient neonatal hypoglycemia [22]. The other subtype, GCK MODY, is a milder form of diabetes, characterized by a mild fasting hyperglycemia that is generally lifelong with little deterioration with age and does not requirement treatment [23,24].

In children less than 6 months of age, diabetes is more likely to be monogenic than autoimmune type 1 diabetes [25]. However, in approximately 50% of these infants, the diabetes is transient (TNDM) [24]. Further to the specific genetic types mentioned here, there are also many subtypes of neonatal diabetes which present as a result of multisystem clinical syndromes [26]. For example, Wolfram syndrome, also referred to as DIDMOAD, is inherited by autosomal recessive trait, is a monogenic multisystem syndrome, and is characterized by marked β-cell dysfunction [27].

Point mutations in mitochondrial DNA have been found to be associated with diabetes and sensori-neural deafness [28] and lead to a condition known as maternally inherited diabetes and deafness (MIDD). Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families. Usually such traits are inherited in an autosomal dominant pattern [29] and the resultant carbohydrate intolerance is mild.

**Genetic defects in insulin action**

Genetic defects in insulin action are rare, and the associated metabolic abnormalities may range from hyperinsulinemia and modest hyperglycemia to severe symptomatic diabetes resulting in death [30]. Acanthosis nigricans may be present in some of these individuals. This syndrome was termed type A insulin resistance in the past. In such patients, diabetes only occurs when there is no β-cell response to the insulin resistance.
Two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance are called leprechaunism and the Rabson–Mendenhall syndrome [31]. A heterogeneous group of disorders of lipid storage characterized by lipodystrophy, in which insulin resistance is a common feature, has also been described [32].

**Diseases of the exocrine pancreas**

Pancreatitis, trauma, infection, pancreatic carcinoma, and pancreatectomy are some of the acquired processes of the pancreas that can cause diabetes. Any process that diffusely injures the pancreas may cause diabetes [33]. With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than a simple reduction in β-cell mass [34]. Hemochromatosis will also damage β cells and impair insulin secretion [35]. Fibrocalculus pancreaticopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcification on X-ray and ductal dilatation. Pancreatic fibrosis and calcified stones in the exocrine ducts are found at autopsy [36].

**Endocrinopathies**

Insulin action can be antagonized by several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine). Diseases associated with excess secretion of these hormones can cause diabetes (e.g., acromegaly, Cushing syndrome, glucagonoma and pheochromocytoma) [7]. These forms of hyperglycemia resolve when the hormone excess is removed. Somatostatinoma and aldosteronoma-induced hypokalemia, can cause diabetes at least in part by inhibiting insulin secretion [37]. Hyperglycemia generally resolves following successful removal of the tumor.

**Drug-or chemical-induced diabetes**

Insulin secretion may be impaired by many drugs. They may not, by themselves, cause diabetes but may precipitate diabetes in persons with insulin resistance [38]. Pancreatic β-cell destruction may occur with the use of certain toxins such as Vacor (a rat poison) [39], pentamidine [40], and some immunosuppressive drugs. Among these β-cell toxic agents, the most commonly used are the immunosuppressive agents of which the calcineurin inhibitors (e.g., tacrolimus and cyclosporin) are the main culprits. While the main action of calcineurin inhibitors in inducing diabetes is by reducing insulin secretion by pancreatic β cells, these drugs may also increase insulin resistance [41]. There is good evidence to suggest that there is greater potential of tacrolimus to induce diabetes compared with cyclosporine [42]. Diabetes induced by these drugs may be permanent due to β-cell destruction, or may only occur while the drug is being taken, with recovery between treatment cycles [42].

Studies involving other immunosuppressive agents such as mycophenolate mofetil and sirolimus are few and results are inconsistent. Clinical studies have shown that daclizumab seems to have a neutral effect [43]. Patients receiving interferon alpha have been reported to develop diabetes associated with islet cell autoantibodies and, in certain instances, severe insulin deficiency [44].

There are also many drugs and hormones that can impair insulin action. The list shown in Table 1.3 is not all-inclusive, but reflects the more commonly recognized drug-, hormone-, or toxin-induced forms of diabetes and hyperglycemia. Among these, there are several commonly used diabetes-inducing drugs that deserve special mention. These include the HMG CoA reductase agents (statins), glucocorticoid steroids, anti-HIV agents and antipsychotic drugs.

**HMG CoA reductase agents**

HMG CoA reductase agents (statins) are commonly used drugs which have been purported to cause diabetes. Sattar et al. [45] reported that statin use compared to placebo increased risk of diabetes in a meta-analysis of 13 placebo-controlled trials. Another meta-analysis comparing intensive dose statin use with moderate statin therapy in five trials showed that the risk of developing diabetes was greater at higher statin doses [46]. The mechanism as to how statins cause diabetes is not known, but it has been suggested that these drugs may affect muscle and liver insulin sensitivity resulting in an increased diabetes risk [46]. It has also been suggested that the observed relationship between statins and diabetes is due to confounding as there is a tendency of individuals who take statins to have a high inherent risk of diabetes. Despite the increased risk of diabetes associated with statin use, a risk–benefit analysis has shown the beneficial nature of statins for cardiovascular disease (CVD), which outweighs the risk of diabetes associated with statin use [47].

**Antipsychotic agents**

There is accumulating evidence supporting an association of certain psychiatric conditions with type 2 diabetes which can be attributed to side-effects of treatment and a high baseline risk of diabetes in this patient group [48].

### Table 1.3 Drug or chemical-induced diabetes

<table>
<thead>
<tr>
<th>Drug or chemical-induced diabetes</th>
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<tbody>
<tr>
<td>Nicotinic acid</td>
</tr>
<tr>
<td>Glucocorticoids</td>
</tr>
<tr>
<td>Thyroid hormone</td>
</tr>
<tr>
<td>Alpha-adrenergic agonists</td>
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<tr>
<td>Beta-adrenergic agonists</td>
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<tr>
<td>Thiazides</td>
</tr>
<tr>
<td>Dilantin</td>
</tr>
<tr>
<td>Pentamidine</td>
</tr>
<tr>
<td>Vacor</td>
</tr>
<tr>
<td>Interferon-alpha therapy</td>
</tr>
<tr>
<td>Statins</td>
</tr>
<tr>
<td>L-asparagine</td>
</tr>
<tr>
<td>Antipsychotic drugs, e.g. clozapine,</td>
</tr>
<tr>
<td>Highly active antiviral therapy, e.g. protease inhibitors</td>
</tr>
<tr>
<td>Others</td>
</tr>
</tbody>
</table>

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**Table 1.3 Drug or chemical-induced diabetes**

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</tr>
<tr>
<td>Others</td>
</tr>
</tbody>
</table>
can be induced by the use of atypical antipsychotics including clozapine, olanzapine, risperidone, quetiapine, ziprasidone, and aripiprazole. These drugs have a direct effect of raising blood glucose and also lead to weight gain, [48] which subsequently may increase blood glucose levels.

Clozapine and olanzapine have been associated with a higher risk of diabetes than other antipsychotic agents in several studies [48]. These drugs have been associated with new-onset diabetes, exacerbation of pre-existing diabetes, and presentations with complications such as ketoacidosis. The data on risperidone and quetiapine in the studies mentioned earlier show inconsistent findings [48].

Atypical antipsychotics may have an independent effect on insulin sensitivity. Studies comparing insulin sensitivity in patients taking clozapine, olanzapine, or risperidone showed that those in clozapine and olanzapine groups had significantly decreased insulin sensitivity compared to risperidone groups. While there is generally less long-term data on aripiprazole and ziprasidone, a comparison of olanzapine and aripiprazole use in schizophrenic patients showed an increase in glucose in the olanzapine group [48].

**Anti-HIV agents**

Diabetes is fourfold more common in HIV-infected men exposed to highly active antiretroviral therapy (HAART) than HIV-negative men. Although most of the diabetes observed in this group is type 2 there has been a recent report of autoimmune diabetes and the development of anti-GAD antibodies after immune system recovery post HAART therapy [49], which suggests that type 1 diabetes can also arise in this group from treatment.

HAART is based on the use of a class of drugs known as protease inhibitors (PIs) and include atazanavir, darunavir, saquinavir, and ritonavir. PIs have been shown to increase insulin resistance and reduce insulin secretion, by interfering with GLUT-4 mediated glucose transport. PIs interfere with cellular retinoic acid-binding protein type 1 which interacts with peroxisomal proliferator-activated gamma (PPARγ) receptor. Inhibition of PPARγ promotes adipocyte inflammation, release of free fatty acids and insulin resistance [49]. Hyperglycemia resolves in almost all patients when PIs are discontinued [49] and all PIs do not have the same metabolic effects, with some drugs having a worse adverse effect than others.

Apart from HAART, another class of anti-HIV drugs associated with diabetes are the nucleoside analogs (reverse transcriptase inhibitors) (NRTIs) [50] especially when used for long periods of time [51]. The risk of diabetes is highest with stavudine, but the risk is also significant with zidovudine and didanosine. Proposed mechanisms include insulin resistance, lipodystrophy, and mitochondrial dysfunction [51]. It is postulated that PIs confer acute metabolic risks, while NRTIs confer cumulative risks of diabetes in predisposed, exposed persons. The use of both classes of drugs may be additive for diabetes risk [51].

**Glucocorticoids**

Glucocorticoids are the most common cause of drug-induced diabetes. They are used in the treatment of many medical conditions but are mostly prescribed for their anti-inflammatory effects [52]. They act through multiple pathways at the cellular and molecular levels, suppressing the cascades that would otherwise result in inflammation and promoting pathways that produce anti-inflammatory protein [53]. The mechanism by which glucocorticoids cause diabetes is thought to be mainly via insulin resistance, but there is also some evidence of effects on insulin secretion [54].

The effect of glucocorticoids is mainly on nonfasting glucose rather than fasting glucose levels [52], but there is uncertainty as to whether this reflects a relationship with clock time (perhaps linked to dosing times), or to a predominant effect on postprandial blood glucose levels.

**Infections**

Certain viruses have been associated with β-cell destruction. Diabetes occurs in some patients with congenital rubella [55]. Coxsackie B, cytomegalovirus, and other viruses (e.g. adenovirus and mumps) have been implicated in inducing diabetes [56–58].

**Uncommon but specific forms of immune-mediated diabetes mellitus**

Diabetes may be associated with several immunologic diseases with a pathogenesis or etiology different from that which leads to the type 1 diabetes process. Postprandial hyperglycemia of a severity sufficient to fulfill the criteria for diabetes has been reported in rare individuals who spontaneously develop insulin autoantibodies. However, these individuals generally present with symptoms of hypoglycemia rather than hyperglycemia [59]. The “stiff man syndrome” is an autoimmune disorder of the central nervous system, characterized by stiffness of the axial muscles with painful spasms. Affected people usually have high titers of anti-GAD and approximately one third to one half will develop type 1 diabetes [60].

Anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor thereby reducing the binding of insulin to target tissues [61]. However, these antibodies can also act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia [62]. Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases [63].

**Other genetic syndromes associated with diabetes**

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down syndrome, Klinefelter syndrome, and Turner syndrome. These and other similar disorders are listed in Table 1.4.
Diabetes is commonly observed in cystic fibrosis patients. While it shares features of type 1 and type 2 diabetes, cystic fibrosis-related diabetes (CFRD) is a distinct clinical entity. It is primarily caused by insulin insufficiency, although fluctuating levels of insulin resistance related to acute and chronic illness and medications such as bronchodilators and glucocorticoids also play a role [64]. Since blood glucose levels within the IGT range appear to have an adverse effect on lung function, it has been suggested that diagnostic criteria for CFRD should be lower than that for other forms of diabetes, but data are currently inadequate to make this change [64]. CFRD is not associated with atherosclerotic vascular disease, despite the fact that individuals with cystic fibrosis nowadays can have a lifespan well into the 50s and 60s.

There are several distinct clinically defined subgroups of diabetes where an etiology has not yet been defined. In recognition of this, during the most recent WHO consultation, it was recommended that a category of “unclassified” or “nonclassical phenotype” be available.

**Diabetes in children and youth**

Type 1 diabetes in children and youth is typically characterized by weight loss, polyuria, polydipsia, blurring of vision, very high plasma glucose concentrations, and ketonuria. The diagnosis is usually very clear with high random glucose values, and there is rarely a need to investigate with an oral glucose tolerance test (OGTT). Type 2 diabetes in children is associated with milder symptoms and is often associated with obesity. In these cases, diagnosis is made using any one of OGTT, fasting plasma glucose, or HbA1c, with preference for HbA1c as there is no requirement to fast. However, there is still debate as to the use of the latter in children [65].

Classification of diabetes in youth poses special problems. Although type 1 diabetes remains the most common form of diabetes in youth of European background, type 2 diabetes is increasingly common, especially among adults at particularly high risk of type 2 diabetes. With the increase in obesity over the last 20 years, there has been an increase in type 2 diabetes in children especially among ethnicities at high risk as well as an increase in the number of children with type 1 who are overweight. Type 2 diabetes may also be present in youth with ketosis or ketoacidosis, which serves only to compound the problem further. While a practical delineation between these may be the use of insulin, it can no longer be assumed that those on insulin are type 1. Other investigations which could provide insight include measurement of C-peptide, characteristic type 1 antibodies, for example anti-GAD antibodies, and the monitoring of endogenous insulin secretion over time [17].

There has also been an increase in the number of children and adolescents with a mixture of the two types of diabetes, that is, subjects who are obese and/or with signs of insulin resistance as well as being positive for markers of autoimmunity to β cells. These cases present a problem under the current classification as they present with an overlapping phenotype of both type 1 and type 2 diabetes and have been referred to as hybrid diabetes, double diabetes, or latent autoimmune diabetes in youth (LADY) [66]. In such children, presentation of double diabetes is similar to LADA in adults. However, unlike LADA, little is known about the prevalence of double diabetes or the prevalence and significance of autoimmune markers in children. In addition, whether autoimmune-positive youth with double diabetes progress more rapidly to insulin dependence than those with type 2 diabetes without is not known. This is particularly important as these children/youth could be at risk for complications associated with β-cell dysfunction, as well as macro- and microvascular complications of type 2 diabetes. It has been suggested that the current classification of diabetes should be revised to include this new phenotype [66].

Another challenge among young people is the possibility of misdiagnosis of monogenic diabetes as type 1 and type 2. As noted previously, monogenic diabetes results from the inheritance of mutation(s) in a single gene that regulates β-cell function or less commonly in genes related to insulin resistance.

The clinical characteristics of a child with monogenic diabetes compared to children and youth with type 1 and type 2 are shown in Table 1.5. Monogenic diabetes should be considered in a child initially diagnosed as type 1 who has been diagnosed at less than 6 months of age, has a family history of diabetes with a parent affected, evidence of endogenous insulin production outside the “honeymoon” phase of diabetes with detectable C-peptide, and the absence of pancreatic islet autoantibodies (measured at diagnosis) [67].

In children with an initial diagnoses of type 2, a diagnosis of monogenic diabetes should be considered in the following circumstances: when the child is not obese or other diabetic family members have weight in the normal range, and the child does not have acanthosis nigricans; when the child is from an ethnic group with a low prevalence of type 2 diabetes and when there is no evidence of insulin resistance with normal fasting C-peptide levels [24,68].

In scenarios when monogenic diabetes is misdiagnosed as type 1 or 2, the afore-mentioned criteria should be considered as a

---

**Table 1.4** Other genetic syndromes sometimes associated with diabetes

<table>
<thead>
<tr>
<th>Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Down syndrome</td>
</tr>
<tr>
<td>Friedreich’s ataxia</td>
</tr>
<tr>
<td>Huntington’s chorea</td>
</tr>
<tr>
<td>Klinefelter syndrome</td>
</tr>
<tr>
<td>Lawrence–Moon–Biedl syndrome</td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
</tr>
<tr>
<td>Porphyria</td>
</tr>
<tr>
<td>Prader–Willi syndrome</td>
</tr>
<tr>
<td>Turner syndrome</td>
</tr>
<tr>
<td>Wolfram syndrome</td>
</tr>
<tr>
<td>Others</td>
</tr>
</tbody>
</table>
Table 1.5  Clinical characteristics of type 1 diabetes, type 2 diabetes, and monogenic diabetes in children and adolescents [67]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Type 1 Polygenic</th>
<th>Type 2 Polygenic</th>
<th>Monogenic Monogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age on onset</td>
<td>6 months and older</td>
<td>Usually pubertal (or later)</td>
<td>Often postpubertal except glucokinase and neonatal diabetes</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>Most often acute, rapid</td>
<td>Variable; from slow, mild (often insidious) to severe</td>
<td>Variable (may be incidental in glucokinase)</td>
</tr>
<tr>
<td>Autoimmunity</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ketosis</td>
<td>Common</td>
<td>Uncommon</td>
<td>Common in neonatal diabetes, rare in other forms</td>
</tr>
<tr>
<td>Obesity</td>
<td>Population frequency</td>
<td>Increased frequency</td>
<td>Population frequency</td>
</tr>
<tr>
<td>Acanthosis nigricans</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Frequency (% of all diabetes in young people)</td>
<td>Usually &gt;90%</td>
<td>Most countries &lt;10%, Japan 60–80%</td>
<td>71–3%</td>
</tr>
<tr>
<td>Parents with diabetes</td>
<td>2–4%</td>
<td>80%</td>
<td>90%</td>
</tr>
</tbody>
</table>

whole rather than individually and are not absolute. DNA testing is now also available for diagnosis of monogenic diabetes.

**DIAGNOSTIC CRITERIA**

Diabetes is characterized by hyperglycemia, and thus diagnostic tests focus on establishing elevated blood glucose levels [69]. A casual blood glucose, fasting glucose or an OGTT of 75 grams may be performed. For children, the oral glucose load is proportional to body weight at 1.75 g per kg body weight. Recently, HbA1c has been added as an acceptable and reliable means of diagnosing diabetes (discussed later). The cutpoints for the diagnosis of diabetes are listed in Table 1.6.

In the absence of symptoms clearly attributable to diabetes, a diagnosis should not be based on a single measurement, but requires results within the diabetes range on two separate days.

The most notable change in diagnostic criteria in recent years is the recommendation by the ADA and WHO to use HbA1c for diagnosis of diabetes. A summary of the evolution of this decision is described in the following section.

**Diagnosis of diabetes using HbA1c**

HbA1c is a hemoglobin variant primarily composed of glycohemoglobin, which is formed by the nonenzymatic attachment of glucose to hemoglobin [70]. It was first identified in 1968 by Rabar, who noted it was associated with diabetes. By 1980, its clinical utility as a marker of glycemic control had been recognized. By the 1990s, supported by strong evidence from two studies, the Diabetes Control and Complications Trial [71] and the United Kingdom Prospective Diabetes Study [72], and the development of new high throughput methods and improved coefficients of variation (CV), HbA1c had become the cornerstone marker in the monitoring of diabetes. In more recent years, a US national glycohemoglobin standardization program has been established and the International Federation of Clinical Chemistry (IFCC) has taken the lead to ensure that HbA1c assays are standardized. In 2011, the units of reporting were also changed from percentage points to IFCC mmol/mol. After a period of dual reporting, HbA1c will be reported in mmol/mol in many countries.

With some improvement in the assay and standardization of HbA1c, together with evidence from key trials demonstrating the importance of intensive glycemic control (as reflected by HbA1c levels) in reducing the risk of microvascular complications of diabetes, the move from using glucose for diagnosis to HbA1c had begun to gather support. However, concerns over standardization of the HbA1c assays and over other factors that may affect HbA1c continued to dampen the enthusiasm for use of HbA1c for diagnosis. In the last 10 years, however, several developments have resulted in the incorporation of HbA1c into the diagnostic armamentarium. There has been significant improvement in the assays of HbA1c [73], analysis from eight different studies showed that HbA1c is as strongly related to the presence of diabetic retinopathy as are blood glucose levels [74], and HbA1c is strongly predictive of macrovascular outcomes and mortality [75,76].

The advantages of using HbA1c for diagnosis are clear. Firstly, HbA1c has far less day-to-day biological variation than fasting or 2-hour glucose [77]. Secondly, HbA1c is stable for one week at room temperature after collection while glucose is susceptible to glycolysis despite the use of fluoride oxalate to preserve the sample. Thirdly, unlike glucose measurement, there is no requirement for the patient to fast. Finally, glucose
levels are also susceptible to modification by short-term lifestyle intervention while HbA1c reflects glycemia over a period of 3 to 4 months.

The major disadvantage of HbA1c is that there are a number of nonglycemic conditions that interfere with the assay. In particular, alterations of red blood cell turnover (e.g., kidney failure, hematocrit deficiencies, hemolysis, acute blood loss, pregnancy, and erythropoietin therapy) may affect the relationship between HbA1c and recent glycemia. The other important disadvantage is the need for a laboratory to use an IFCC aligned assay and be part of a standardization program, which may not be possible in developing countries.

Cutpoints of HbA1c have been set using similar methods to those adopted for the setting of blood glucose criteria. Cross-sectional data from 47,364 individuals from 12 countries reported that the threshold for diabetes-specific retinopathy was 6.3% (45 mmol mol⁻¹), with an optimal decision limit of 6.5% [74]. This latter cutpoint has been adopted by the ADA and WHO as an appropriate cutpoint for diabetes.

Although support for the use of HbA1c for diagnosis of diabetes has increased over the years, several questions about its suitability remain. For example, what should be the appropriate HbA1c ranges for pre-diabetes or intermediate glycemia? The ADA suggested that 5.7–6.5% (39–48 mmol mol⁻¹) should be used [5] to indicate intermediate glycemia while the WHO [78] suggested that levels of HbA1c below 6.5% may indicate intermediate glycemia but were reluctant to indicate a precise lower cutpoint. An international expert committee suggested that those with HbA1c between 6.0–6.5% (42–48 mmol mol⁻¹) could be considered at high risk, and should be targeted for diabetes prevention activities [79].

A further concern about moving from glucose to HbA1c to diagnose diabetes is that we will observe a change in prevalence of diabetes, as an elevated HbA1c does not identify exactly the same individuals as does an elevated blood glucose. It should, however, be noted that a similar discrepancy in individuals identified also applies to diagnosis by fasting glucose compared to diagnosis by 2-hour plasma glucose in the OGTT.

In general, the use of HbA1c for diagnosis of diabetes results in a lower prevalence of diabetes with the magnitude of the difference between blood glucose-based prevalence and HbA1c-based prevalence varying widely between populations [80].

Diagnosis of diabetes using HbA1c is now recommended by both the ADA and WHO as detailed in Table 1.6. As discussed earlier, it is important to ensure that the HbA1c assay used meets stringent quality assurance test and is aligned with the IFCC standardization program. It is also important to ensure that there are no clinical conditions that preclude its accurate measurement.
Table 1.7 Diagnostic criteria for gestational diabetes mellitus [82]

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting venous PG</td>
<td>≥5.1 mmol L⁻¹</td>
</tr>
<tr>
<td>1-h venous PG</td>
<td>≥10.0 mmol L⁻¹</td>
</tr>
<tr>
<td>2-h venous PG</td>
<td>≥8.5 mmol L⁻¹</td>
</tr>
</tbody>
</table>

One or more of these values must be abnormal for the diagnosis of GDM.

**Diagnosis of gestational diabetes mellitus**

The diagnosis of gestational diabetes mellitus has been traditionally based on glucose tolerance levels measured between 24 and 28 weeks of gestation [7] using an OGGT. These guidelines have been modified due to the evidence from the Hyperglycemia and Adverse Pregnancy Outcome Study (HAPO) [81]. HAPO was a large, prospective, blinded, multinational study showing a strong and continuous relationship of maternal glycemia at 24–28 weeks with neonatal outcomes of increased birth weight and increased cord-blood serum C-peptide levels, and to increased cesarean section delivery rates in the mothers. Based on these data, new GDM guidelines [82] were proposed which have since been adopted internationally. Diagnostic criteria for gestational diabetes are shown in Table 1.7.

**Other glucose tolerance categories**

**Impaired glucose regulation (impaired glucose tolerance and impaired fasting glycemia)**

Impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG) are categorized as stages in the natural history of disordered carbohydrate metabolism. They occur in all individuals as they progress from normal to diabetes, but since the transition through these states is rapid in type 1 diabetes, they are rarely identified in such individuals. Therefore, nearly all of the literature dealing with IGT and IFG is concerned with issues relating to type 2 diabetes, such as risk of developing type 2 diabetes and CVD.

IFG and IGT represent a metabolic state intermediate between normal glucose homeostasis and diabetes. The pathophysiologic aspects of hyperglycemia of each category are somewhat different. IGT is associated with muscle and liver insulin resistance and thus IGT is often associated with the metabolic or insulin resistance syndrome [7], while IFG is usually related to insulin secretory deficits.

A meta-analysis suggested that there is a positive relationship between IFG/IGT and diabetes which varies across ethnicity and age [83]. This study showed that individuals with combined IGT and IFG had the highest risk of future diabetes. In terms of sensitivity and specificity for the subsequent development of diabetes, the sensitivity of IFG as originally defined at 6.1 mmol L⁻¹ (110 mg dL⁻¹) is less than that of IGT in most populations [84], but the specificity of IFG is greater [85]. IGT is more common than IFG using 6.1 mmol L⁻¹ (110 mg dL⁻¹) in most populations but it should be noted that the sensitivity and specificity of both IGT and IFG is entirely dependent on the cutpoints selected, and not on any inherent differences between FPG and 2hPG [86]. If IGT and IFG are defined such that they have similar prevalence to each other, they then have the same predictive values for subsequent diabetes [86]. Further, with the ADA cutpoint of IFG (5.6 mmol L⁻¹ or 100 mg dL⁻¹ — see later), the sensitivity of IFG is similar to IGT, but the specificity falls.

Thus, neither the risk of developing diabetes nor the sensitivity and specificity for future diabetes seem to differ enough between IGT and IFG to suggest one category is more useful than the other. In reality, in most populations IGT is more prevalent than IFG (if IFG is defined as FPG of 6.1–6.9 mmol L⁻¹ (110–125 mg dL⁻¹)), and thus it identifies a greater proportion of those who will develop diabetes. Furthermore, although at the lower cutpoint of IFG of FPG 5.6–6.9 mmol L⁻¹ (100–125 mg dL⁻¹), the prevalence of IFG approaches that of IGT, the two groups remain limited in their overlap. Relying on only a FPG will not identify the same proportion of individuals at risk compared to undertaking an OGTT.

The relationship between IGT and IFG and CVD is well studied in meta-analyses. In a review of the evidence from 27 studies [87], IFG (at both cutpoints) and IGT were both associated with a significantly increased risk of approximately 20% of CVD.

Diagnosis of IGT and IFG categories has been traditionally made by measuring blood glucose levels, either in the fasting state (for IFG) or during an OGGT (for IGT) (see Table 1.6 for cutpoints). Since individuals with IFG may have diabetes, it is recommended that those who are found to have IFG should have an OGTT to exclude diabetes [7].

Whilst IGT has been part of the classification of glucose intolerance for many years, IFG was only added in 1997, with a lower cutpoint of 6.1 mmol L⁻¹ (110 mg dL⁻¹). However, in 2002, the ADA proposed a new cutpoint of IFG of 5.6 mmol L⁻¹ (100 mg dL⁻¹), as this maximized the sensitivity and specificity for predicting future diabetes [88]. On review of the same evidence, the WHO decided not to adopt this new cutpoint, as it significantly increased the number of people being labeled as abnormal, but without evidence that so doing would improve outcomes [69].

The purpose of defining other categories of glucose intolerance or prediabetes is to identify a group of the population at increased risk for the development of both diabetes and CVD, so that interventions (lifestyle and pharmacologic) can be applied to reduce these risks. IGT and IFG are considered risk factors for diabetes and CVD.

In summary, longitudinal data show that IFG and IGT are rather similar to each other in their ability to predict future diabetes and CVD. However, since the populations of
IFG and IGT have limited overlap with each other, undertaking the OGTT to identify those with IGT provides the opportunity to identify a greater proportion of the at-risk population.

**Normoglycemia**

The notion underpinning setting a normal category of glucose is that people with values below the upper limit of normal are at no or only “normal” risk of developing diabetes or its micro- and macrovascular complications [5,7,84,89]. Since the risks of future development of diabetes and CVD are related to blood glucose across most of its spectrum, and well into any normal ranges that have been set, such notions of “normal” blood glucose should be interpreted very cautiously. The actual setting of the cutpoint indicating normoglycemia over the years has undergone considerable changes. The early classification in 1985 by US NDDG that was adopted by WHO had set diabetes at a fasting glucose of 7.8 mmol L⁻¹ (140 mg dL⁻¹) and those under this threshold were labeled “normoglycemia”. In 1997, upon the availability of new data, the ADA, with support from WHO reset the cutpoint of a “normal” fasting plasma glucose from 7.8 mmol L⁻¹ (140 mg dL⁻¹) to 6.0 mmol L⁻¹ (110 mg dL⁻¹) [5,7,89]. In 2002–2003, the ADA recommended that the cutpoint be 5.5 mmol L⁻¹ (100 mg dL⁻¹) [88].

**Summary**

The classification of diabetes is an evolving process. As the research into diabetes is a continuing and dynamic process and epidemiologic and clinical studies are in progress, there may well be revision and refinement of the classification system. This is especially important given the recent recommendation to use HbA1c to diagnose diabetes and the caveats to its use. As more knowledge emerges about the etiology of cases currently positioned in the type 2 process category, modification and refinement may be necessary.

**References**


CHAPTER 2

Epidemiology and risk factors for type 1 diabetes mellitus

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Key points

- The incidence of type 1 diabetes has been increasing progressively over the last half century by 3–5% per year.
- Type 1 diabetes results from a chronic autoimmune destruction of the pancreatic β cells with a preclinical period marked by the presence of autoantibodies to pancreatic β-cell antigens.
- Though genetic markers can identify varying risk, it is only once autoimmunity has begun that a high positive predictive value is achieved.
- Multiple islet autoantibodies are present in the great majority of prediabetics.
- Studies indicate that genetic factors alone cannot explain the etiology of type 1 diabetes.
- Seasonality, increasing incidence and epidemics of type 1 diabetes suggest a critical role of environmental factors, such as infections with certain viruses and effects of early childhood diet.
- In the absence of renal disease, the long-term mortality risk in type 1 diabetes is not increased compared with the general population.

Introduction

Type 1 diabetes (T1DM) is one of the most prevalent severe chronic diseases of childhood, affecting more than 170,000 children in the United States, an increase of 23% since 2001 [1]. In the US, more than 25,000 children are diagnosed annually with 1:200 children and 1:100 adults diagnosed with T1DM during the lifespan [2]. T1DM is the leading cause of end-stage renal disease, blindness, and amputation, and a major cause of cardiovascular disease and premature death in the general population. Annually, in the US, an estimated 70–200 children die at the onset of diabetes [3] with 30% of children who develop diabetes presenting with ketoacidosis. For unknown reasons, the incidence of T1DM has been increasing progressively over the last half century by 3 – 5% per year [4]. In addition the percentage of children expressing the highest risk HLA genotype (DR3/4-DQ2/8) has dramatically decreased over the past 50 years [5], apparently reflecting increased disease penetrance of lower risk haplotypes. The high incidence, associated severe morbidity, mortality, and associated healthcare expenditures make T1DM a prime target for prevention. Population-based epidemiological studies as well as family studies and clinical trials have provided new insights into the pathogenesis and natural history of T1DM. Such studies are essential for appropriate diagnosis and for evidence-based programs of prevention and treatment.

Prevalence and incidence

The prevalence of T1DM, that is the proportion of people in the population who have the disease at a given point in time, is determined not only by disease incidence but also by case survival, which may vary markedly in populations. The prevalence of β-cell autoimmunity appears to be roughly proportional to the incidence of T1DM in different populations. In contrast, the prevalence of β-cell autoimmunity in first-degree relatives of T1DM persons does not differ a lot between high and low risk countries for T1DM. The prevalence of T1DM in children aged less than 15 years ranges from 0.05 to 0.3% in most European and North American populations [6].

Incidence is the rate at which new cases of disease appear in the population and is usually expressed as the annual number of new cases per 100,000 persons. Incidence of T1DM varies by geographic location, ethnicity, age, gender, and time. The incidence of T1DM is increasing worldwide both in low and high incidence populations (Figure 2.1) [7]. The average annual incidence is the
highest in Finland where it has increased to 64 per 100,000 per year in 2005 [8] while the current mean incidence is 18/100,000 person-years in Australia [9].

**Geographic location**

One of the most striking characteristics of T1DM is the large geographic variability in the incidence [10–13]. The incidence rates of T1DM vary from 0.73/100,000 per year in China [14] to 60/100,000 per year in Finland. A child in Finland is 100 times more likely to develop diabetes than a child in China. A clear difference appears between the Northern and Southern Hemisphere with countries below the equator having a lower incidence; in contrast above the equator the disease is common. The largest intracontinental variation in incidence appears to be in Europe. There are also noticeable within-country variations in incidence rates, which can only be partially explained by racial composition of the population.

The geographic and ethnic variation in T1DM risk may reflect either different pools of susceptibility genes or different prevalence of causative environmental factors or a combination of both.

**Race and ethnicity**

There are also striking racial differences in T1DM risk within the same population, although they are not as important as the geographic differences. In the United States, non-Hispanic whites are about one and a half times as likely to develop T1DM as African Americans or Hispanics [15,16]. There are similar differences reported in Europe, where France has lower rates than do Britain and Scandinavia.

Interestingly, there is also evidence that migrants from a country with low incidence of T1DM soon have the higher rate incidence of the country where they live; for example South Asian children living in the UK have similar overall rates of T1DM compared to indigenous white children [17]. Genetic factors are unlikely to explain such a rapid change, implying an influence of environmental factors in disease etiology.

**Age and gender**

T1DM incidence peaks at the ages of 2, 4–6 and 10–14 years, perhaps due to alterations in the pattern of infections or increases in insulin resistance. Few cases of T1DM develop in the first year of life. The main incidence peak occurs at puberty, with females having a pubertal peak about 1 year earlier. There is a decrease in incidence after puberty for both sexes [18]. In Sweden, the age-specific incidence rates vary from 5–20/100,000 person-years in adults aged 15–35 years and decline with age. Several studies indicate a stable or decreasing trend of T1DM among young adults [18], while, in contrast, a study from Italy (lower-risk population) found that the incidence from 1984 to 1996 increased not only in children but also in young adults [19].

In general, males and females have similar risk of T1DM [20]. In lower-risk populations, such as Japan or African Americans, there is a female preponderance, while in high-risk groups, there is a slight male excess [18]. Interestingly, even within Europe, populations with an incidence higher than 20/100,000 (Sardinia, UK, Italy, Finland, Norway) had male excess, whereas those with a lower rate (the Baltic countries, Macedonia, Yugoslavia, Romania) had female excess [21].

**Increasing incidence and seasonality**

There is evidence for marked variations in the incidence of T1DM over time, both seasonally and annually. In the Northern Hemisphere, the incidence declines during the warm summer months; similarly in the Southern Hemisphere, the seasonal pattern exhibits a decline during the warm months of December and January, implicating a climatic factor. This seasonal pattern appears to occur predominantly in older children [16], suggesting that factors triggering diabetes may be related to school attendance. Most population-based registries have shown increasing T1DM incidence over time [22,23]. Several studies have observed periodic outbreaks superimposed on a steady secular increase in incidence.

While the increase in T1DM incidence has affected all age groups, several studies have reported a particular increase among the youngest children [24]. In the youngest age group (0–4 years) an increase of 11% and 24% per year was reported in, respectively, the UK and Switzerland [25]. On the contrary, some studies show the highest increase in incidence in children 10–14 years old [13].

In the EURODIAB ACE Study Group, representing most European countries and Israel, the rates of increase were 6.3% (4.1–8.5%) for children aged 0–4 years, 3.1% (1.5–4.8%) for 5–9 years, and 2.4% (1.0–3.8%) for 10–14 years [23].

**β-Cell autoimmunity and risk factors**

Type 1A diabetes results from a chronic autoimmune destruction of the pancreatic β cells, probably initiated by exposure of a genetically susceptible individual to some environmental
agent(s). This preclinical period is marked by the presence of autoantibodies to pancreatic β-cell antigens such as insulin, GAD65 (Glutamic Acid Decarboxylase), ICA512 (called also IA-2) or ZnT8 (Zinc Transporter 8), and precedes the onset of hyperglycemia by a few years. Several prospective studies have reported that these autoantibodies can appear early in childhood and the presence of two or more of these antibodies is highly predictive for the development of diabetes [26]. However, the etiology of the autoimmune process and β-cell destruction is not known. The islet cell antibody (ICA) assay, using immunofluorescence and pancreatic tissue has been notoriously difficult to standardize and has been replaced by a combination of radioassays for autoantibodies to insulin [27], GAD and IA-2. These tests have been shown to be quite sensitive and predictive in relatives of T1DM patients [26] and in the general population. Several prospective cohort studies investigate early genetic and environmental factors contributing to risk of β-cell autoimmunity and progression to diabetes in relatives of patients with T1DM and in a general newborn population [28,29].

Prediction of T1DM during the preclinical period offers opportunities for prevention of T1DM. Prediction at present is based upon genetic, immunologic, and metabolic information (Figure 2.2). Though genetic markers can identify varying risk, it is only once autoimmunity has begun (marked by the presence of multiple autoantibodies to pancreatic β-cell antigens such as insulin, GAD65, IA-2 or ZnT8) that a high positive predictive value (>90%) can be achieved, and multiple autoantibodies are present in the great majority of prediabetics [30,31].

**Family history of T1DM**

Concordance rates for T1DM in monozygous twins (MZ) with long-term follow-up is greater than 50% [32], compared to 6–10% in dizygous (DZ) twins, which is similar to what is found in non-twin siblings. With long-term (>30 years) follow-up, at least 2/3 of initially discordant MZ twins develop persistent β-cell autoantibodies and/or diabetes.

Among first-degree relatives, siblings are at a higher risk (5–10% risk by age 20) than offspring; offspring of diabetic fathers are at a higher risk (~12%) than offspring of diabetic mothers (~6%) [33]. It is not clear why offspring of diabetic mothers are at lower risk compared to offspring of fathers, with one study reporting that presence of transplacental islet autoantibodies may decrease risk [34]. It is very likely that both environmental factors and genetic susceptibility are essential for development of T1DM, but the environmental factors may be ubiquitous and thus not be a major determinant of which individual develops diabetes given genetic susceptibility.

**HLA class II susceptibility genotypes**

The estimated risk of developing T1DM for general population children is 1/300 while the risk for children who have the HLA-DR3/4,DQB1*0201,DQB1*0302 genotype is approximately 1:15–1:25 (Table 2.1) [35]. Only 2.4% of the general population carries this genotype compared to 25–40% of T1DM patients. Deschamps and coworkers examined the predictive value of HLA typing in a study of 536 siblings of diabetic probands in France [36]. The risk of T1DM after 8 years, estimated by life table analysis, was 10% for siblings who were HLA identical with the probands, 3–4% for siblings with either DR3 or DR4, and 16% for those with HLA-DR3/DR4 genotype. Recently, there has been evidence for additional susceptibility loci within or linked to the MHC independent of HLA-DR/DQ, such as HLA class I alleles [37,38]. In the DAISY (Diabetes Autoimmunity Study in the Young) cohort, siblings of children with T1DM who have HLA-DR3/DR4-DQB1*0302 and are identical by descent for both HLA haplotypes with their diabetic proband sibling had a 65% risk for developing islet autoantibodies by age 7 years and a 50% risk of developing diabetes by age 10 years [39]. This strongly suggests that additional (non-DR,DQ) MHC-linked genes determine T1DM risk. On the other hand, DAISY general population children with DR3/DR4-DQB1*0302 who lack protective alleles DPB1*0402 and DRB1*0403 have a risk of 20% of activating islet autoimmunity [40]. Only 25% of autoantibody positive new onset children presenting to the Barbara Davis Center for Childhood Diabetes in Denver during this past decade have the HLA-DR3/4 genotype [41].

![Figure 2.2](image_url) **Figure 2.2** Risk factors and prediction tools for type 1 diabetes.

| Table 2.1 Risk, by the age of 20 years, of type 1 diabetes and β-cell autoimmunity in the general population and family members of T1DM patients |
|-----------------|-----------------|-----------------|
| **Risk Group** | **Type 1 Diabetes** | **Pre-Diabetic Autoimmunity** |
| **General population** | All HLA genotypes 1:300 | 1:30–1:100 |
| | HLA-DR3/4,DQB1*0302 1:15 | 1:10 (7) |
| **Family members** | Maternal offspring 1:50 | 1:15 |
| | Paternal offspring 1:15 | 1:5 |
| | Siblings (all) 1:12–1:35 | 1:5 |
| | Monozygotic twins 1:3 | 1:1 (7) |
| | HLA-identical siblings 1:4 | 1:2 (7) |

Source: Rewers M, Norris J. Chapter 9 - Epidemiology of Type I Diabetes (Revised 11/3/2010). In: Eisenbarth GS: *Type 1 Diabetes: Cellular, Molecular and Clinical Immunology* [Online book]. Available at http://www.barbaradaviscenter.org
Several HLA haplotypes dominantly protect, including HLA-DQA1*0102, DQB1*0602, DRB1*1401, DQA1*0101, DQB1*0503 and DRB1*0701, DQA1*0201, DQB1*0303 [42]. Too few HLA-DQB1*0602 individuals expressing multiple islet autoantibodies have been studied to know the exact magnitude of protection once autoantibodies are present, but 1% of new-onset patients expressing islet autoantibodies have DQB1*0602 versus 20% of the general US population.

Other genetic factors associated with type 1 diabetes

More than 40 non-HLA susceptibility gene markers have been confirmed [43]. At present, polymorphisms of the insulin gene and PTPN22 gene contribute most to diabetes risk after HLA alleles [44]. Adding high-risk alleles of these genetic markers to HLA Class II genotyping can increase risk, but even for these loci with odds ratios between 1.7 and 2.0, the effect is small.

Recent studies indicate that specific single nucleotide polymorphisms (e.g. PTPN22) [45] of non-MHC genes are associated with dramatic differences in T-cell signaling. For instance, the PTPN22 allele associated with diabetes risk is associated with decreased T-cell receptor signaling (gain of function). It is known that a subset of individuals without the specific polymorphism have similar phenotypes, and it is possible that, as diabetes-associated lymphocyte phenotypes are defined, they will contribute to disease prediction [46].

Islet autoantibodies

The first large-scale studies of the prediction of T1DM relied upon the detection of cytoplasmic islet cell autoantibodies (ICA) assays based on indirect immunofluorescence. High titer cytoplasmic ICA is most often associated with the presence of multiple islet autoantibodies and therefore a high risk of progression to diabetes [47]. Screening for risk of T1DM now utilizes “biochemical” autoantibody assays for specific islet autoantigens. These include autoantibodies to insulin (IAA), glutamic acid decarboxylase (GAD), IA-2 (ICA512) and most recently ZnT8 [48]. Individuals having a single positive autoantibody (insulin, GAD, IA-2, or ZnT8 autoantibodies) are at low risk for progression to T1DM. Single positive autoantibodies can be a nonreproducible false positive result (e.g. switched sample), transient, or represent the presence of an autoantibody reacting with the specific autoantigen that does not confer increased risk of diabetes (e.g. low affinity insulin autoantibodies) [49]. Individuals expressing two or more positive autoantibodies, especially on multiple tests over time, are at very high risk of progressing to diabetes. This high risk may result from autoimmunity spreading to other autoantigens whose targeting increases destruction or from the high statistical specificity of expression of multiple autoantibodies. The Diabetes Autoantibody Standardization Program (DASP) workshop aims to improve and standardize measurement of autoantibodies associated with T1DM across laboratories [50].

Different islet autoantibodies have been associated with different risks of progression, with IA-2 autoantibodies most often associated with expression of other biochemical autoantibodies and high risk [51]. Of note, insulin autoantibodies are extremely high at the onset of diabetes in young children while usually negative in individuals first presenting with diabetes after age 12. There is a log-linear inverse relationship between the levels of insulin autoantibodies and the age of onset of diabetes [52]. In DAISY, 89% of children who progressed to diabetes expressed ≥2 autoantibodies with cumulative incidence of 74% by age 10 for those expressing three autoantibodies (Figure 2.3). In children expressing ≥2 autoantibodies, there is no significant difference in progression to diabetes between relatives and general population subjects.

Autoantibody screening among relatives

The initial large screening studies found approximately 3% of first-degree relatives to be positive for ICA. With analysis of biochemical autoantibodies, it has become evident that the presence of ICA in the absence of GAD65 or ICA512 autoantibodies is associated with a low risk of progression to diabetes [26,53]. The cumulative risk of developing diabetes within 15 years is only 2.8% for individuals with ICA but without GAD or ICA512 (IA-2) autoantibodies versus 66% for those with ICA and either or both GAD or ICA512 autoantibodies.

As in many studies, in the DAISY study, the incidence of islet autoantibodies is much higher in first-degree relatives, compared to the general population. The risk by age 10 is particularly high in the HLA-DR3/4,DQB1*0302 positive siblings (43%) and offspring (34%) and moderate-risk siblings (19%) [54]. In the DAISY children without a first-degree relative with T1DM, the incidence of persistent islet autoantibodies by the age of 9 years is 10.6%, 5.5% and 3.4% in, respectively, high-, moderate- and average-HLA risk groups. The HLA genotype and having a diabetic relative, but not gender or ethnicity, predict development of islet autoantibodies.
Islet autoantibodies appear early in life. The BABYDIAB, DIPP, and DAISY studies have demonstrated that a significant proportion of first-degree relatives progressing to T1DM before age 15 develop islet autoantibodies before their 2nd birthday [31,55,56]. Although IAA usually appear first, a significant percentage of children followed from birth initially express GAD65 autoantibodies, while IA-2 and ZnT8 autoantibodies usually develop later.

Autoantibody screening in the general population
Islet autoantibody screening studies have included mainly first-degree relatives; however, 90% of T1DM cases occur in individuals with no family history of T1DM. Several ongoing studies have followed children from birth for the development of islet autoantibodies. The two studies with the longest follow-up are the DAISY study from Denver Colorado and the DIPP study in Finland [57]. More recently, the multicenter international TEDDY study (The Environmental Determinants of Diabetes in the Young) [58] has enrolled over 8600 high-risk general population children identified by newborn screening into a prospective follow-up for islet autoantibody measurements from birth.

While the risk of developing islet autoantibodies is 3–4 times higher in relatives than in the general population with the same HLA-DR/DQ genotypes, the persistent presence of islet autoantibodies portends similar high risk of progression to diabetes for both relatives and the general population [52,59]. Higher titer and higher affinity of the autoantibodies as well as presence of autoantibodies to multiple autoantigens predict higher risk of diabetes [60].

Measurement of autoantibodies in adult-onset diabetes
After the age of about 15 years, measuring the incidence of T1DM is complicated by misclassification of some patients as type 2 diabetics. It has been estimated that at least 37% of T1DM is diagnosed after the age of 19 years and 15% after the age of 30 years [61]. New cases of T1DM presenting in adult life tend to have a longer duration of symptoms before diagnosis and higher C-peptide levels remaining at diagnosis compared with those presenting in childhood, suggesting a slower rate of β-cell destruction. However, C-peptide levels in islet autoantibody positive adult diabetic patients are still significantly lower than in those with type 2 diabetes. Assays for GAD65 autoantibodies have been the most useful in identifying latent autoimmune diabetes in adults (LADA).

Between 5 and 10% of patients with a diagnosis of gestational diabetes have positive islet autoantibodies and the great majority progress to T1DM [62]. Among adults diagnosed with type 2 diabetes and participating in the UKPDS, the proportion of patients with ICA and GAD65 decreased with increasing age at diagnosis: from 21% in patients aged 25–34 to 4% in those aged 55–65 for ICA and from 34% to 7% for GAD65 [63]. Most (94%) of patients with ICA and 84% of those with GAD65 required insulin therapy by 6 years, compared with 14% of those without the antibodies.

Measurement of β-cell function
Direct measurement of the functional mass of islet cells is currently not possible. The first-phase insulin response (FPIR) to intravenous glucose can help predict progression to diabetes among individuals with positive islet autoantibodies [64,65]. The FPIR is usually calculated as the sum of the 1- and 3-min insulin levels measured after glucose is administered intravenously at 0.5 g per kg body weight. Low FPIR has been defined as below the 1st, 5th or 10th percentile of the distribution in nonobese healthy subjects. Subjects from the Joslin family study with FPIR below the first percentile on the first test had a 3-year diabetes-free survival rate of 13% compared with 78% for the group with higher FPIR [66]. The FPIR is severely depressed at the time of detection of islet autoantibodies in many young children. In the DPT-1 study, loss of the FPIR was strongly associated with diabetes [64]; the average time from the fall of FPIR below the 1st percentile to the onset of diabetes was 1.8 years. A recent study from the Belgian registry reported similar results relative to first-phase insulin secretion with hyperglycemic clamps amongst individuals with IA-2 autoantibodies [67].

The oral glucose tolerance test (OGTT), performed in clinical trials of T1DM prevention largely to formalize the diagnosis of clinical diabetes, has long been known to have some value in predicting progression to T1DM among subjects with islet autoantibodies [68]. DPT-1 reported that a risk score based on age, BMI, and OGTT indexes (glucose and C-peptide values), without use of IVGTTs or additional autoantibodies, accurately predicted T1DM in ICA-positive relatives [69]. The likelihood of progression to diabetes increased with mild fasting or post oral glucose-load dysglycemia.

In 1993, Leech and coworkers reported that HbA1c measured by high-performance liquid chromatography may be slightly but significantly higher in nondiabetic ICA-positive teenagers compared to ICA negative controls [70]. More recently, the DAISY study has demonstrated that HbA1c steadily increases within the normal range over a few years preceding diabetes onset and may therefore be useful in early detection of T1DM [71]. Among children who have persistent islet autoimmunity, increase in HbA1c predicted increased risk of progression to T1DM, with a hazard ratio of 4.8 for each 0.4% (SD) increase in HbA1c, independent of random glucose and number of autoantibodies.

Recently, increased HbA1c level has been added by the joint American Diabetes Association (ADA), International Diabetes Federation, and European Association for the Study of Diabetes International Expert Committee (IEC) as a diagnostic tool for diabetes with a recommended threshold of 6.5% for diagnosis of diabetes on two tests [72]. This threshold was established based on research conducted in adults with type 2 diabetes. However, studies in adolescents at high risk for T1DM suggest that HbA1c > 6.5% is a specific but not sensitive early indicator for T1DM [73].
Through the Diabetes Complications and Control Trial (DCCT), it has been shown that patients who have sustained production of C-peptide have lower rates of severe hypoglycemia, microalbuminuria, and retinopathy [74,75]. In the Diabetes Prevention Trial-Type 1 (DPT-1), post challenge C-peptide levels begin to decrease appreciably in the 6 months before diagnosis and continue to decrease within 3 months after diagnosis [76]. Recent data from the TrialNet study show a biphasic decline in C-peptide during the first 2 years post diagnosis, with a higher rate of fall during the first year [77].

Environmental factors
Twin [78] and family studies indicate that genetic factors alone cannot explain the etiology of T1DM. Seasonality, increasing incidence and epidemics of T1DM as well as numerous ecological, cross-sectional and retrospective studies suggest a critical role of environmental factors, such as infections with certain viruses (especially enteric infections in early life) and effects of early childhood diet. Natural history studies that follow children at increased risk of T1DM provide the best opportunity to study environmental triggers.

Viruses: Herpes viruses, mumps, rubella, and retroviruses [79] have been implicated. Viral infections appear to initiate autoimmunity and perhaps also precipitate diabetes in subjects with autoimmunity. ICA or IAA have been detected after mumps, rubella, measles, chickenpox, Coxsackie, ECHO-4, and rotavirus [80] infections.

While several viruses have been linked to T1DM, studies have provided the strongest overall evidence for enteroviruses, although results have been somewhat conflicting [81]. In one longitudinal birth cohort study (DAISY), progression from islet autoimmunity to T1DM seemed to increase after an enterovirus infection [82]. In the Finnish type 1 Diabetes Prediction and Prevention (DIPP) study, enteroviral infections are seen more frequently in prediabetic children and prior to the onset of islet autoimmunity, implying a temporal relationship between enterovirus infections and the induction of β-cell autoimmunity [83]. Although several studies report that enteroviruses may play a role in the pathogenesis of T1DM, the evidence that enterovirus infections are associated with initiation or progression of islet autoimmunity is still inconsistent [84].

Effect of childhood infections and daycare exposure: Early infectious exposure may play a role in the development of immunoregulatory mechanisms that protect against diabetes. Social mixing through attendance at daycare in early infancy appears to confer protection against the development of childhood diabetes [85]. Although several other well-designed case-control studies show a statistically significant protective effect of day care exposure on T1DM [86], meta-analysis reveals too much heterogeneity to accept overall synthesis [87].

Improvement in hygiene: Genetic models are unable to explain the apparent temporal changes in the incidence of T1DM [88]. Alternative explanations look at environmental factors and some invoke the congenital rubella model. Briefly, increased hygiene in the Western world has led to a decline in immunity to common infections among women in child-bearing age. These women are more likely to develop viremia during pregnancy resulting in congenital persistent infection of β cells and early onset T1DM in the offspring. This model could explain both the increasing incidence of diabetes and the decreasing age of disease onset.

Routine childhood immunization: Neither type nor quantity or timing of vaccinations, including BCG vaccine, HIB vaccine, diphtheria, tetanus and pertussis vaccine, measles, mumps and rubella vaccine, hepatitis B vaccine, varicella vaccine, or tick-born encephalitis vaccine, have been associated with the development of islet antibodies and diabetes [86,89,90]. At least two studies even showed a possible protective effect of the measles-mumps-rubella vaccine [91].

Perinatal factors: Environmental risk factors may play a role early in life, possibly in utero. Several studies have investigated perinatal determinants for developing T1DM. Offsprings of a T1DM parent have an increased risk of developing diabetes, the risk being higher if the father has diabetes. Although the relation of maternal age and birth order to risk of T1DM is complex, several studies found that maternal age over 35–40 years [92] and/or increasing birth order [93] were associated with an increase in T1DM. There also seems to be a relatively weak but significant association between increasing birth weight and increasing risk of T1DM [94,95], although several other case-control studies have not found any association [96].

Interestingly, several studies suggest that early weight gain and/or rapid linear growth are risk factors for development of not only type 2 but also T1DM in children [97].

Dietary factors: Cow’s milk or wheat introduced at weaning trigger insulitis and diabetes in animal models perhaps through a molecular mimicry [98]. Breast-feeding may be viewed as a surrogate for the delay in the introduction of diabetogenic substances present in formula or early childhood diet. Several human studies suggested an association between short duration of breast-feeding and increase in T1DM [99,100], while cohort studies failed to find an association between cow’s milk and β-cell autoimmunity [90,101] (Figure 2.4). Interestingly, a study from Finland suggested that current cow’s milk consumption was more closely linked to prediabetic autoimmunity and diabetes than infant exposure [102]. To resolve this controversy, a dietary intervention trial to prevent T1DM by a short-term elimination of cow’s milk from infant diet (TRIGR) is underway [103].

Concerning the introduction of cereals, the DAISY [104] and the German BabyDiab [105] studies found that early introduction of gluten before the age of 3 months increases the risk of development of β-cell autoimmunity (Figure 2.4). However, delaying gluten exposure until the age of 12 months in the BabyDiet cohort did not substantially reduce the risk for islet autoimmunity in genetically at-risk children [106].
Vitamins and dietary supplements: Studies in vitro have shown that vitamin D3 is immunosuppressive or immunomodulating and studies in experimental models of autoimmunity have shown vitamin D to be protective \[107\]. Results from clinical studies have been somewhat conflicting. While a European study reported a protective effect of vitamin D supplementation in infancy against T1DM \[108, 109\], the DAISY cohort study did not show any association between vitamin D intake or 25(OH)D levels throughout childhood with the risk of islet autoimmunity or progression to T1DM \[110\] (Figure 2.4). On the other hand, maternal intake of vitamin D through food during pregnancy was associated with a protective effect on the appearance of islet autoimmunity in DAISY offspring \[111\]; however, these findings were not confirmed in a more recent Finnish study (DIPP) \[112\]. A study in Norway \[109\] found that cod liver oil taken during pregnancy was associated with reduced risk of T1DM, suggesting that vitamin D and/or the omega-3 fatty acids in the cod liver oil have a protective effect. In the prospective DAISY study, dietary intake of omega-3 fatty acids and the omega-3 fatty acid content of erythrocyte membranes were associated with reduced risk of islet autoimmunity \[113\] (Figure 2.4). However, neither intake nor membrane levels of omega-3 or omega-6 fatty acids were associated with risk of developing T1DM in those children with islet autoimmunity \[114\]. A Nutritional Intervention to Prevent T1D (NIP) is currently underway to examine whether nutritional supplements with docosahexaenoic acid (DHA), given during the last trimester of pregnancy and the first few years of life, can prevent development of islet autoantibodies \[115\].

Gene–environment interaction in clinical type 1 diabetes
T1DM is likely caused by an interactive effect of genetic and environmental factors within a limited age-window. While both the susceptibility genes and the candidate environmental exposures appear to be quite common, the likelihood of these causal components meeting within the susceptibility age-window is low. To investigate the environmental causes of T1DM, the study subjects have to be screened for known susceptibility gene markers so that gene–environment interactions can be accounted for.

Interaction between HLA Class II alleles and viral infection:
Susceptibility to diabetogenic enteroviruses in humans appears to be genetically restricted by HLA-DR and DQ alleles \[116\]. However, the allelic specificity is controversial and may depend on the viral type and epidemicity. In general, the HLA-DR3 allele, present in most patients with T1DM, is associated with viral persistence.

Interaction between HLA Class II alleles and infant diet:
Few studies to date have examined a possibility of an interaction between the HLA genes and dietary exposures \[117\]. The epidemiologic data are limited, but suggest that an early exposure to cow’s milk in relatives with HLA-DR3/4,DQB1*0302, DR3/3 or DRx/4,DQB1*0302 is not associated with development of β-cell autoantibodies \[101\].
Established type 1 diabetes

Clinical onset
In industrialized countries, 20–40% of T1DM patients younger than 20 years present with diabetic ketoacidosis [118]. After adjusting for age, gender, ethnicity, diabetes type, and family history of diabetes, diabetic ketoacidosis at diagnosis was associated with lower family income, less desirable health insurance coverage, and lower parental education [3]. Younger children present with more severe symptoms at diagnosis, because children younger than 7 years old have lost on average 80% of the islets, compared to 60% in those 7–14 years old and 40% in those older than 14 years [119]. Case fatality in industrialized countries ranges between 0.4–0.9% [120]. Both diabetic ketoacidosis and onset death are largely preventable, because most of the patients have typical symptoms of polyuria, polydipsia, and weight loss 2–4 weeks prior to diagnosis. The diagnosis is straightforward in almost all cases, based on the symptoms, random blood glucose over 200 mg/dL−1 and/or HbA1c >7%.

Traditionally, nearly all children with newly diagnosed T1DM were hospitalized. More recently, an increasing proportion of new-onset children have been managed on an outpatient basis, especially in urban centers with specialized diabetes education and treatment facilities. Hospitalization at onset does not improve short-term outcomes such as re-admission for diabetic ketoacidosis or severe hypoglycemia [118], if adequate family education and follow-up is available on outpatient basis.

Remission (honeymoon period)
Shortly after clinical onset, most T1DM patients experience a transient fall in insulin requirement due to improved β-cell function. Total and partial remissions have been reported in, respectively, 2–12% and 18–62% of young T1DM patients [118]. Older age and less severe initial presentation of T1DM and low or absent ICA or IA-2 [121] have been consistently associated with deeper and longer remission. Evidence relating GAD autoantibodies [121,122], non-Caucasian origin, HLA-DR3 allele, female gender, and family history of T1DM to a less severe presentation, greater frequency of remission, and slower deterioration of insulin secretion is inconclusive. Most studies agree that preserved β-cell function is associated with better glycemic control (lower HbA1c) and preserved α-cell glucagon response to hypoglycemia.

The natural remission is always temporary, ending with a gradual or abrupt increase in exogenous insulin requirements. Destruction of β-cells is complete within 3 years of diagnosis in most young children, especially those with the HLA-DR3/4 genotype. It is much slower and often only partial in older patients [123], 15% of whom have still some β-cell function preserved 10 years after diagnosis.

Acute complications
Acute complications of T1DM (diabetic ketoacidosis, hypoglycemia, and infections) are described in detail in other chapters. The risk of hospital admission for acute complication is 30/100 patient-years in the first year of the disease and 20/100 patient-years in the subsequent 3 years [118]. An estimated 26% of the patients have at least one episode of severe hypoglycemia within the initial 4 years of diagnosis. The incidence of severe hypoglycemic episodes varies between 6 and 20 per 100 person-years, and increases with younger age, longer duration of diabetes, intensity of insulin treatment, lower levels of HbA1c, and in older children with presence of underinsurance and psychiatric disorders [118,124]. The incidence of ketoacidosis is about 8 per 100 person-years and increases with age in girls; the risk of ketoacidosis also increases with higher HbA1c, higher reported insulin dose, and in older children with limited access to care due to underinsurance and presence of psychiatric disorders [124]. Interestingly, most of ketoacidosis and/or hypoglycemic episodes occur among 20% of children who have recurrent events.

Morbidity and mortality
Insulin treatment dramatically prolongs survival but it does not cure diabetes. Excess mortality seems to be lowest in Scandinavia, intermediate in the US, and highest in countries where T1DM is rare, for example, Japan, probably due to a combination of the quality of care and access. On the other hand, 40% of the patients survive over 40 years and a half of these have no major complications. Several studies have shown that survival in T1DM has improved over time [125]. The Pittsburgh Epidemiology of Diabetes Complications (EDC) study cohort has recently shown that the life expectancy for those diagnosed 1965–1980 was 15 years greater than participants diagnosed 1950–1964, a difference that persisted regardless of sex or pubertal status at diagnosis [126]. Both the Finnish Diabetic Nephropathy (FinnDiane) study and the Pittsburgh EDC study report that in the absence of renal disease and microalbuminuria, the long-term mortality risk in T1DM is not increased compared with the general population [127].

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Age- and sex-specific prevalence of type 2 diabetes in different ethnic groups

Type 2 diabetes mellitus (T2DM) is now taking its place as one of the main threats to human health in the twenty-first century [1]. In 1921, Dr Elliot Joslin was already concerned that according to his count there had been a doubling of diabetes in three decades [2]. The impact of T2DM is increasingly felt around the world, with its prevalence rising dramatically over recent decades. The World Health Organization (WHO) estimated that there were 150 million people aged 20 years and older living with diabetes in 2000, and by 2025, this will have risen to 300 million (Figure 3.1). There will be a 42% increase, from 51 to 72 million, in developed countries and a 170% increase, from 84 to 228 million, in developing countries [3]. The top 10 countries with the highest estimated number of people with diabetes in 2025 are listed in Table 3.1.

Diagnostic criteria for diabetes

In the past two decades there have been several important developments, which have had significant impact on the definition of diabetes and thereby on the assessment of its magnitude. In 1979, the 2-h 75-g oral glucose tolerance test (OGTT) was proposed as a standard test for diagnosis of diabetes by the National Diabetes Data Group (NDDG) [4], and endorsed by WHO in 1980 [5]. In 1985, WHO made a minor modification to the diagnostic criteria [6]. This has created order out of the confusion in the diagnostic criteria for diabetes. Before that enormous variations existed in diagnostic cutoff values for fasting as well as after glucose loading. The glucose load varied between 50 and 100 g or was body weight related. The differences in glucose assay methods, glucose load, and the time after loading made the comparison between different studies difficult. Near-universal adoption of the WHO criteria has had a significant influence on epidemiologic studies of diabetes.

In 1997, a revision of the diagnostic criteria was approved by the American Diabetes Association (ADA) [7] and adopted by WHO Consultation in 1999 [8]. The major changes were lowering the positive cutoff value of fasting venous plasma glucose from 7.8 mmol L$^{-1}$ (140 mg dL$^{-1}$) to 7.0 mmol L$^{-1}$ (126 mg dL$^{-1}$). The positive cutoff value for 2-h plasma glucose remained unchanged, that is, 11.1 mmol L$^{-1}$ (200 mg dL$^{-1}$) and over. For epidemiologic studies and for routine clinical practice, the ADA did not recommend the primary use of OGTT, but WHO Consultation still retained the OGTT as the standard test procedure. The prevalence data assembled in this chapter are estimated mainly according to the WHO 1999 criteria [8], except for those noted otherwise.

Conversion factors for glucose concentrations

Glucose concentration can be measured using different blood specimens such as venous plasma glucose, capillary whole blood glucose, and venous whole blood glucose. The cutoff points for the classification of stages of glucose abnormalities from different specimens are different. Currently, there are no internationally accepted conversion factors for glucose concentrations in the literature. Recently, conversion factors for changing glucose concentrations between different blood samples were developed on the basis of data from a Finnish study and applied them in the DECODE study (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) [9–11]. The equations were derived based on 294 matched samples of whole blood (capillary and serum) glucose and plasma glucose concentrations drawn from a standard 75-g OGTT in 74 individuals at 0, 30, 60, and 120 min at the
Chapter 3

Figure 3.1 Number of people with diabetes mellitus, 1995–2025. Source: Adapted from King et al. [3].

Table 3.1 Top 10 countries for estimated number of adults with diabetes, 1995 and 2025

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>1995 (millions)</th>
<th>Country</th>
<th>2025 (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>19.4</td>
<td>India</td>
<td>57.2</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>16.0</td>
<td>China</td>
<td>37.6</td>
</tr>
<tr>
<td>3</td>
<td>United States</td>
<td>13.9</td>
<td>U.S.</td>
<td>21.9</td>
</tr>
<tr>
<td>4</td>
<td>Russian Federation</td>
<td>8.9</td>
<td>Pakistan</td>
<td>14.5</td>
</tr>
<tr>
<td>5</td>
<td>Japan</td>
<td>6.3</td>
<td>Indonesia</td>
<td>12.4</td>
</tr>
<tr>
<td>6</td>
<td>Brazil</td>
<td>4.9</td>
<td>Russian Federation</td>
<td>12.2</td>
</tr>
<tr>
<td>7</td>
<td>Indonesia</td>
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<td>Mexico</td>
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</tr>
<tr>
<td>8</td>
<td>Pakistan</td>
<td>4.3</td>
<td>Brazil</td>
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<tr>
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<td>Egypt</td>
<td>8.8</td>
</tr>
<tr>
<td>10</td>
<td>Ukraine</td>
<td>3.6</td>
<td>Japan</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>All other countries</td>
<td>49.7</td>
<td></td>
<td>103.6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>135.3</td>
<td></td>
<td>300.0</td>
</tr>
</tbody>
</table>

Source: Adapted from King et al. [3].

Diabetes and Genetic Epidemiology Unit, National Public Health Institute in Finland. The relationships between glucose concentrations as measured by the different methods used were estimated. The formulas derived are as follows:

Venous plasma glucose (mmol L⁻¹)

= 0.558 + 1.119 × whole blood glucose (mmol L⁻¹)

Venous plasma glucose (mmol L⁻¹)

= 0.102 + 1.066 × capillary blood glucose (mmol L⁻¹)

Venous plasma glucose (mmol L⁻¹)

= −0.137 + 1.047 × serum glucose (mmol L⁻¹)

Age- and sex-specific plasma glucose concentration

The age- and sex-specific mean fasting and 2-h plasma glucose after 75-g glucose load were estimated in general Caucasian populations in Europe, and in Chinese, Japanese, and Indians in Asia, who did not have prior history of diabetes. The participants are included in the DECODE and the DECODA (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Asia) studies, the two largest epidemiologic studies for diabetes in Europe and Asia, with a total of 15,606 subjects from Europe [10] and 19,845 subjects from Asia [12]. The data presented here are based on the pooled data from 13 DECODE and the 11 DECODA participating cohorts. A standard OGTT was carried out in all populations, and subjects with prior history of diabetes were not included in the analysis. The plasma glucose concentrations rose with age and reached a peak at 60–69 years of age and then started to decline in Indians but continued to increase after 70 years of age in Europeans (Figure 3.2). In each age group, the mean 2-h plasma glucose was significantly higher for Indians than for Chinese and Japanese, and the same was also true for fasting plasma glucose in most of the age groups (Figure 3.2). The mean fasting and 2-h glucose concentrations did not differ between Chinese and Japanese except at 40–49 years of age where the glucose values were higher in the Japanese. The mean glucose levels were lower in Europeans than in Asians younger than 70 years, whereas they were higher in Europeans than in Asians 70 years or older. The mean glucose levels were similar in Asian men and women. In Europe, the mean fasting glucose concentration was higher in men than in women at 30–69 years of age but after 70 years of age, it was higher in women. The 2-h glucose was higher in women than in men throughout the age range. Two-hour glucose increased more with age than did fasting glucose.

Age- and sex-specific prevalence of diabetes

Europe

The prevalence of diabetes has been estimated by applying the WHO 1999 criteria [8] for 13 European and 11 Asian cohorts participating in the DECODE and the DECODA studies. In Europe, the age-specific prevalence of diabetes rose with age up to 70s and 80s in both men and women [10] (Figure 3.3). In most of the studies, the prevalence was less than 10% in subjects younger than 60 years and between 10 and 20% at 60–79 years of age. They were higher in Malta than in other populations. The prevalence of isolated postload hyperglycemia (2-h glucose ≥11.1 mmol L⁻¹ and fasting glucose <7.0 mmol L⁻¹) increased more with age than did isolated fasting hyperglycemia (fasting glucose ≥7.0 mmol L⁻¹ and 2-h glucose <11.1 mmol L⁻¹), particularly in women.

A recent German study using WHO 1999 criteria [8] showed that the prevalence of diabetes in Germany in 2000 was 16.7% in men and 8.6% in women at 55–59 years of age and 23.1% in men and 17.0% in women at 70–74 years of age [13]; that is, rates are within the variation reported for the DECODE study: In the recent Turkish Diabetes Epidemiology Study (TURDEP-II) undertaken in 2010, the prevalence of diabetes was 16.5% and increased with age, reaching a peak of 37.7% in urban men at 70–74 years and 43.6% in urban women.
at 75–79 years of age [14]. These rates are higher than those reported by an earlier Turkish study in 1997–1998 [15].

Compared with most of the other racial and ethnic groups worldwide, where age- and sex-specific prevalence of diabetes has been reported, Europeans have a moderate to low prevalence of diabetes [10].

**United States**

In the United States, the prevalence of diabetes varies considerably among different ethnic groups. The prevalence was 1.9 times greater in Latino Americans and 1.6 times in African Americans than in Whites of the same age in the Third National Health and Nutrition Examination Survey (NHANES III) [16]. In NHANES III, where a single fasting plasma glucose \( \geq 7.0 \text{ mmol L}^{-1} \) was applied for the diagnosis of diabetes, the prevalence of diabetes in US Whites in 1988–1994 was 5.9% in men and 4.8% in women at 40–49 years of age and reached a peak of 19.2% in men and 16.6% in women at 75 years or older [16]. The prevalence of undiagnosed diabetes according to the same fasting glucose criteria at a comparable age range of 40–59 years was higher in US Whites than in most of the female and in half of the male European populations participating in the DECODE study [10]. The rates were higher in Mexican Americans than in US Whites, and were higher than seen in any of the populations included in the DECODE study.

The Pima Indians have the highest prevalence of diabetes in the world, being 50% at 30–64 years of age, estimated using 2-h postload glucose test alone [17]. The prevalence of diabetes in Native Hawaiians (Polynesian population of Hawaii) in two rural communities was estimated using WHO 1985 criteria [18]. At 30–39 years of age, the prevalence was 6.5% in women and 10.7% in men, reaching a peak of 34.6% in women and 40.0% in men at 70 years or older. Other Native American tribes also have a higher prevalence of diabetes than Caucasoids do.

**Central and South America**

The age-standardized prevalence of diabetes using 2-h glucose criteria alone was investigated in a Brazilian population in Sao Paulo in 1987 and in a Colombian population in Bogota in 1988–1989 [17]. The prevalences in both populations were similar, around 7% for men and 9% for women. The age-specific prevalence of diabetes in a Mexican population in Mexico City
was 4.2% in men and 3.2% in women at 35–39 years of age and reached 23.1% in men and 41.7% in women at 60–64 years of age [17].

**Australia**
The Australia Diabetes, Obesity and Lifestyle Study (AusDiab) took place in 1999–2000 applying WHO 1999 criteria mainly in Caucasoids. The prevalence of diabetes increased with age, from 2.7% in men and 2.2% in women at 35–44 years of age to 23.5% and 22.7% at 75 years or older [19]. These rates were higher than those in most of the DECODE populations [10].

**Asia and Pacific Islands**

**Asia** The prevalence of diabetes varies markedly among Asian populations. In those participating in the DECODA study, it rises with age up to 70s and 80s in Chinese and Japanese, and in Indian men and women with age up to 60s and then declines [12] (Figure 3.4a,b). The age- and sex-specific prevalence and the peak prevalence of diabetes were higher in cohorts from India and Singapore than in most of the Chinese and Japanese cohorts. In Chinese and Japanese, the prevalence was less than 10% at 30–49 years of age; the peak prevalence was less than 20% in most of the cohorts and none exceeded 30%. In contrast, in India and Singapore the prevalence was over 10% among people aged 40–49 years, and over 30% among those aged 50–69 years for most of the cohorts. The urban Chinese and Japanese had significantly higher prevalences of diabetes than their rural counterparts at 40–69 years of age in men and at 50–79 years of age in women (Figure 3.4). The prevalence of diabetes in Korea was within the range observed in China and Japan [20].

Type 2 diabetes was found at a relatively younger age in Pakistan and the prevalence reached the peak in the age group 55–64 years [21–23]; the prevalence pattern was similar to that in India. In a rural community the prevalence was over 13% at 35–44 years of age, with the highest prevalence of 30% in men at 65–74 years of age [22], indicating diabetes has already become a major health threat in Pakistan as in India.

In the late 1990s, King et al. carried out a series of surveys on diabetes in Uzbekistan and Mongolia using WHO 1985 criteria [6]. In Uzbekistan, the prevalence of T2DM was relatively low in people younger than 45 years, around 1%, and 10–15% at 65 years or older [24]. It was relatively rare in people younger than 45 years, around 1%, and 10–15% at 65 years or older. The prevalence in Mongolia [25] was relatively low with a peak of less than 5% at the age of 65 years or older, comparable to the prevalence reported from China in 1994 [26]. Taking into account the high positive cutoff value of 7.8 mmol L\(^{-1}\) for fasting glucose for diabetes in the WHO 1985 criteria, the prevalence reported in these studies would be somewhat higher if the current cutoff value of 7.0 mmol L\(^{-1}\) were used. Because different diagnostic criteria have been applied, the results from some of these studies cannot be compared directly with those from the DECODA study [12].

Compared with the European populations included in the DECODE study [10], the age-specific prevalence of diabetes in urban Chinese and Japanese was slightly higher than that in Europeans at 30–69 years of age, but was lower than that in Indians. In the elderly population, however, the peak prevalence was higher in a few European populations than in Indians, such as in Maltese, in Finnish women in Oulu, and in women living in the Canary Islands, Spain. The age at which the peak prevalence
Figure 3.4 Age- and sex-specific prevalence of diabetes in Asia men (a) and women (b) in the DECODA study. DMF, diabetes determined by FPG $\geq 7.0\text{ mmol L}^{-1}$ and 2hPG $< 11.1\text{ mmol L}^{-1}$; DMP, diabetes determined by 2hPG $\geq 11.1\text{ mmol L}^{-1}$ and FPG $< 7.0\text{ mmol L}^{-1}$; DMF&DMP, diabetes determined by FPG $\geq 7.0\text{ mmol L}^{-1}$ and 2hPG $\geq 11.1\text{ mmol L}^{-1}$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, for the difference between urban and rural Chinese and Japanese; +$p < 0.05$, +++$p < 0.001$, for the difference between Chinese and Japanese combined and Indians. Source: Adapted from the DECODA Study Group [12].
of diabetes was reached was similar for Europeans, Chinese, and Japanese (over 70 years), while Indians had their highest prevalence at the age up to 60s and then started to decline. These differences in prevalence in the elderly (women, in particular) are probably due to selective mortality associated with diabetes. The recent survey in China showed a marked increase in the prevalence of T2DM; it was 9.7%, representing an estimated 92.4 million adults in China with diabetes in 2007 [27].

In all Asian populations included in the DECODA study, the prevalence of isolated fasting hyperglycemia (fasting plasma glucose ≥ 7.0 mmol L⁻¹ and 2-h plasma glucose < 11.1 mmol L⁻¹) did not increase with age (Figure 3.4). The prevalence of isolated 2-h hyperglycemia (2-h plasma glucose ≥ 11.1 mmol L⁻¹ and fasting plasma glucose < 7.0 mmol L⁻¹) tended to increase with age in Chinese and Japanese but not in Asian Indians [12].

Pacific Islands There are remarkable differences in the prevalence of diabetes among the Melanesia, Micronesian, and Polynesian populations of the Pacific Islands. According to the 2-h postload glucose criteria alone [6], an age-standardized diabetes prevalence of more than 40% was revealed in the Micronesia population of Nauru in the 1980s [28,29]. The prevalence of diabetes in the Melanesia population of Papua New Guinea had been reported close to 0% in highland populations [30], whereas in the urbanized Koki people the age-standardized rate exceeds 40%, approaching that of Nauru [29], exhibiting an extreme urban–rural gradient. Intermediate rates are seen in other Pacific Island populations. In the Polynesia population of the Western Samoa, the crude prevalence rates were 3.4 and 8.7% in rural and urban populations, respectively. By 1991, these rates had risen to 6.5 and 9.0% in two rural communities and to 16% in the urban settings of Apia [31]. A recent study in the Polynesia population of Tonga in 1998–2000 using 2-h OGTT showed that the peak prevalence of diabetes reached 20% in men and 40% in women aged 60 years or older [32].

Middle East The prevalences of diabetes in Arabian countries, calculated according to the WHO 1985 criteria [6], have been reported to be high [33–35]. It is relatively low before the age of 30 years and starts to increase during the 40s, with the highest in the oldest age group (Figure 3.5). In a rural Palestinian village, in 1996 the prevalence of diabetes was less than 4.0% in people younger than 40 years but increased to 11.0% at 40–49 years of age, with a peak of 21.7% in men and 31.6% in women at 60–65 years of age [35]. The prevalence in Palestine was similar to that in rural Saudi Arabia, but both were lower than that in urban Saudi Arabia [33]. The prevalence of T2DM in 1995–1996 in Kuwait was recalculated using ADA 1997 criteria [7]. It was lower than 3% at age 20–29 years, around 9% at age 30–39 years and higher than 15% at age 40–49 [36]. Diabetes is prevalent in all Arab countries despite the differences in economic status among these countries, indicating that genetic susceptibility and cultural factors may play an important role in the development of the disease.

The age-specific rate ranged from 8% at 40–44 years of age to 25% at 60–64 years of age in Israeli Jews [17].

Africa In subjects aged 30–64 years, the age-standardized prevalence of diabetes using 2-h glucose alone has been reported to be higher in Hindu and Muslim Indians living in Mauritius [37] and Tanzania [38], around 10% in Tanzania and 13–18% in Mauritius. The age-standardized rate was very low in Bantu in Tanzania, less than 1% in women and 0.9–3.3% in men [39]. The prevalence was 8% in Tunisia [17]. It is interesting to note that the age-standardized prevalence was much higher in

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**Figure 3.5** Prevalence of diabetes in men and women in three Arabian countries. (For a color version of this figure, please see the color plate section.)
Chinese living in Mauritius than that in Da Qing in China in the mid 1980s [17,37], indicating the importance of impact of the environmental factors.

**In Native People: Mapuche and Aymara in Chile and in Siberia in Russian Federation**

The native people who still practice their traditional lifestyle and undertake considerable physical activity have extremely low prevalences of diabetes despite their high prevalence of obesity [40,41]. Among Aymara the prevalence of diabetes in 1997 was almost undetectable despite the fact that 13% of the men and 24% of the women had a body mass index (BMI) higher than 30 kg m\(^{-2}\) [40]. Similar findings have been reported among native Mapuche [41,42]. The indigenous groups in northern Siberia also showed a very low prevalence, being less than 1% in 1994 [43]. This suggests that a healthy lifestyle with much physical activity provides protection from the development of diabetes. A recent report from Mapuche [41] showed that the prevalence in 1998 was higher than that reported 15 years ago, as was the prevalence of obesity [42], suggesting a possible impact of lifestyle changes on the trends in prevalence of diabetes.

**Previously undiagnosed diabetes**

The proportion with previously undiagnosed diabetes varies with age. It seems to be highest at 30–39 years of age (70–80%), and lowest in the elderly (around 40%) [10,12]. The only exception was seen in European women where the proportion of undiagnosed diabetic cases was around 40–45% in all age groups. The proportion of undiagnosed diabetes was higher in European men than in European women. In Asians, it was slightly higher in women than in men in the youngest age groups.

**Prevalence of impaired glucose tolerance and impaired fasting glycaemia in different ethnic groups**

A category of nondiabetic fasting hyperglycaemia was defined only recently, by the ADA in 1997 [7] and adopted also by WHO in 1999 [8], and named impaired fasting glycaemia (IFG). It was introduced by consensus to define impaired glucose homeostasis intermediate between diabetes and normal glucose homeostasis and to be analogous to impaired glucose tolerance (IGT), but without any epidemiologic evidence of possible risks associated with it. Since the introduction of the category of IFG, prospective studies have examined the relationship between IFG and future morbidity and mortality with a comparison to IGT, and shown that the risk of cardiovascular disease (CVD) morbidity and mortality is higher for IGT than for IFG [44–47]. Thus far the data are scarce on the risk of progression to diabetes in subjects with IFG as compared with those with normal fasting glucose or those with IGT. A few studies, which have examined the issue, agree that the risk of developing diabetes is high in subjects with either IFG or IGT and highest in those with both IFG and IGT, as compared with subjects with normal fasting and normal 2-h glucose [48–52]. At present neither IFG nor IGT is considered a clinical entity, but as a risk category for the future development of diabetes [53]. Each represents a metabolic state intermediate between normal glucose homeostasis and diabetic hyperglycemia, and they were combined and defined formally as impaired glucose regulation (IGR) by WHO Consultation in 1999 [8]. All studies agree that only IGT but not IFG is a risk factor for CVD.

The prevalences of IGT and IFG in Europe and Asia were reported recently by the DECODE and the DECODA Study Groups [8,12].

**Europe**

The prevalence of IGR rose with age in each study [10] (Figure 3.6). In most of the study populations, the prevalence of IGR was less than 15% at 30–59 years of age and between 15 and 30% after 60 years of age. The prevalence of IGT increased linearly with age, but the prevalence of IFG did not (Figure 3.6). The increase in the prevalence of undiagnosed diabetes and IGR in the elderly population resulted mainly from the proportionately larger increase in postload hyperglycemia than in fasting hyperglycemia.

**Asia**

The prevalence of IGR rose with age up to the 70s and 80s in most of the study cohorts [12] (Figure 3.7a,b). The increase was graded with aging in Chinese, Japanese, and Singaporean populations, as observed in Europeans, but not in Indians where the prevalence of IGR started to increase by the age of 30–39 years and did not change much with increasing age. The peak prevalences of IGR were not different among different populations, but the age-specific prevalence of IGR was higher in Indians than in Chinese and Japanese at 30–49 years of age for both men and women. In the urban populations the prevalence was higher than in the rural populations aged 40–69 years in men and 50–59 years in women in the Chinese and Japanese populations (Figure 3.7). The difference in the prevalence pattern in different ethnic groups may not be completely explained by living environments and geographic locations, suggesting that genetic differences also play a role.

IGT was more prevalent than IFG in almost all age groups in Asian subjects (Figure 3.7). The prevalence of IGT increases with age whereas IFG does not. This pattern is consistent with that among the European populations [10]. The concordance for IFG and IGT was very poor in all populations, particularly in Asians [10,12,54–56]. The finding that postload hyperglycemia was more prevalent in the elderly in Europe and Asia is consistent with the report from NHANES III [57]. Thus, the prevalence of undiagnosed diabetes and IGR would be underestimated to a large extent, especially in female and elderly populations, if only fasting glucose determination were used. The primary purpose of population-based testing for blood glucose is to detect previously undiagnosed diabetes and IGR in order to apply early intervention to reduce the serious
diabetic complications and to prevent progression from IGT to diabetes as demonstrated by the recent diabetes prevention trials [58–62].

**Sex differences in prevalence of diabetes, IGT, and IFG**

The ratio of prevalences of glucose abnormality between men and women has been estimated in many studies, but so far there has been no consistent trend [16,17]. In the DECODE study, we found there is a clear pattern in the prevalence of postload hyperglycemia and the prevalence of fasting hyperglycemia by sex [10]. Undiagnosed diabetes and IFG defined by isolated fasting hyperglycemia was more common in men than in women at 30–69 years of age, whereas the prevalence of isolated postload hyperglycemia was higher in women than in men and was particularly high in the elderly population [10]. In the DECODA study, sex difference was not as clear as in Europe. The prevalence of IFG also seems higher in Chinese and Japanese men than in women, whereas it was higher in Indian women than in men. IGT was more prevalent in Chinese and Japanese women than in men, but such a difference was not observed in Indians [12]. Sex differences in the prevalence of diabetes and IGR depend on how the prevalence was estimated, by fasting or by postload hyperglycemia, on the age distributions, and on the ethnic groups. Asian Indians, who have a very high risk of diabetes, show abnormalities in fasting glucose values at an earlier age than other populations.

**The ratio of IGT to diabetes**

The ratio of IGT to diabetes has been reported to decrease as prevalence of diabetes rises [17] and may have some predictive value in determining the stage of a glucose intolerance epidemic within a population [63]. When the ratio is high but the prevalence of diabetes is low, the early stage of a diabetes epidemic may be occurring [17]. The age- and sex-specific ratios of IGR to diabetes according to the newly revised diagnostic criteria for diabetes [8] are shown in Figure 3.8a for Asian and Figure 3.8b for European populations [10,12]. The ratio of IGR to diabetes declined when the prevalence of diabetes increased in both Asian and European populations.

**Secular trends in prevalence of type 2 diabetes**

Accumulating evidence shows that the prevalence of diabetes is increasing with time over recent decades. This upward trend has been seen primarily in developing countries [3,64] (Figure 3.1). A series of studies in the southern Indian city of Chennai showed a steady increase in the prevalence of diabetes in the
Figure 3.7 Age- and sex-specific prevalence of impaired glucose regulation in the DECODA study populations in men (a) and women (b). Isolated-IFG, FPG 6.1–6.9 mmol L\(^{-1}\) and 2hPG <7.8 mmol L\(^{-1}\); Isolated-IGT, 2hPG 7.8–11.0 mmol L\(^{-1}\) and FPG <6.1 mmol L\(^{-1}\); IFG&IGT, FPG 6.1–6.9 mmol L\(^{-1}\) and 2hPG 7.8–11.0 mmol L\(^{-1}\). * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\), for the difference between urban and rural Chinese and Japanese; + \(p < 0.05\), +++ \(p < 0.001\), for the difference between Chinese and Japanese combined and Asian Indian populations combined. Source: Adapted from the DECODA Study Group [12].
Indian population. The age-standardized prevalence increased from 8.2% in 1988–1989 [65] to 11.6% in 1994–1995 [66] and reached 13.5% in 2000 [67], a 65% increase within the two decades. During the last two decades a considerable amount of information has been obtained from China. Studies conducted in China between 1980 and 1990 consistently show low diabetes prevalence rates of approximately 1.5% or less, even in urban populations such as in Shanghai in 1980 [68–71]. The prevalence of diabetes in Shanghai in 1980 was close to 1%. In rural Guangdong province it was 0.33% [69]. Studies undertaken in the late 1990s, however, indicate sharply rising prevalence rates in China [72–74] and the rates estimated at the beginning of the current century show that diabetes in an urban Chinese population in mainland China [75] is already as prevalent as in Hong Kong and Taiwan in the mid 1990s (Figure 3.9) [76–78]. In 2007, the prevalence of T2DM in China was almost 10% indicating a three-fold increase in three decades [27]. It Turkey, the prevalence of T2DM doubled during a 12-year interval from 1998 to 2010 [24]. The prevalence of both diabetes and its microvascular complications in a Pacific Island population (20 years or older) of Western Samoa was examined in 1978 and 1991 [31]. In 1978, the crude prevalence rates were 3.4 and

Figure 3.8 Age- and sex-specific scatter plot of ratios of IGR to diabetes against the prevalence of diabetes (diagnosed and undiagnosed) separately for 11 Asian (a) and 13 European (b) studies. Source: Adapted from the DECODE [10] and the DECODA [12] Study Groups.
8.7% in rural and urban populations, respectively. By 1991, these rates had risen to 6.5 and 9.0% in two rural communities and to 16.0% in the urban setting of Apia.

Recent studies indicate that diabetes prevalence continues to increase even in developed countries. According to the data from the US NHANES II and NHANES III surveys, using ADA criteria, the prevalence of T2DM in the US adult population aged 40–74 years of age increased from 8.9% in the period 1976–1980 to 12.3% by 1988–1994. A similar increase was found when WHO criteria were applied (11.4 and 14.3%) [16]. In Australia, the total prevalence of diabetes had increased from 3.4 to 7.2% from 1981 to 1999–2000 and the difference persisted after adjustment for BMI [19] (Figure 3.10). In an adult Norwegian population [79], the crude prevalence of known diabetes in men increased from 2.6% in 1984–1986 to 3.3% in 1995–1997, an increase of 24%, but the increase was not found in women. Over the same time period, an increase of 86% in the prevalence of obesity defined by BMI ≥30 kg m\(^{-2}\) was observed in men, which was much higher than the increase of 38% in women. In a Danish study of a 60-year-old cohort over a 22-year period an increase of 58% in men and 21% in women in the prevalence of diabetes was observed, which was fully explained by a concurrent increase in BMI [80]. Rising trends in the prevalence of diabetes and obesity have also been reported in other European countries [81,82]. In addition to the increase in obesity, reduced physical activity resulting from changes in work-related activity and sedentary lifestyle has contributed to the rising trend in T2DM.

Prevalence, which reflects the accumulation of the patients at any given time, can be influenced by many factors such as an increase in the number of new cases and a reduction in the mortality attributed to the disease. There is evidence that mortality in diabetes has declined in men in the United States [83]. Thus, an increase in prevalence could be a result of an improved survival of diabetic subjects. However, studies also show an increasing trend in diabetes incidence due to the increase in obesity and decrease in exercise. Knowler et al. [84] compared the incidence rates over two 10-year time periods, 1965–1975 and 1975–1985, in Arizona Pima Indians, and found that over the 10-year period the incidence rates increased by 50% in most age and sex groups. The San Antonio Heart Study revealed an increasing secular trend in the 7- to 8-year incidence of T2DM occurring from 1987 to 1996 in Mexican American and non-Hispanic Whites [85]. Therefore, both increased incidence and decreased mortality among diabetic subjects have contributed to the increased trend in the prevalence of diabetes.

**Type 2 diabetes in children**

**Prevalence and incidence**

Type 2 diabetes was historically a rare occurrence in children but recent studies have reported marked increases in the prevalence of T2DM in children. Type 2 diabetes was first reported in a population-based study in 1979 of American Indians children in Arizona [86]. This American Indian community has one of the highest rates of T2DM in adults and obesity in both adults and children [87]. After 30 years of follow-up in this population, the youngest age of onset of T2DM was 4 years and the prevalence of T2DM in 15–19-year-old children increased from 2.4 to 3.8% in boys and from 2.7 to 5.3% in girls [88]. Data from the Indian Health Service (IHS) in the United States confirmed an increase in the prevalence of diabetes in American Indian populations in the United States, with a 68% increase in the prevalence of diagnosed diabetes in American Indian and Alaska Natives adolescents between the ages of 15 and 19 years between 1990 and 1998. Although these IHS estimates were for all diabetes and not only T2DM, T1DM is very rare in some of these American Indian populations [89,90].

The increase in T2DM is not limited to the American Indians. Very few population-based studies have been conducted in other racial and ethnic groups but results from diabetes
Table 3.2  Selected studies of type 2 diabetes in children and adolescents

<table>
<thead>
<tr>
<th>Study and reference</th>
<th>Years</th>
<th>Race/ethnicity</th>
<th>Age (years)</th>
<th>Sample size</th>
<th>No. of cases</th>
<th>Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population-based studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Mexico, USA [136]</td>
<td>1991–1992</td>
<td>Navajo Indians</td>
<td>12–19</td>
<td>142</td>
<td>2</td>
<td>14.1 [0–33.5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15–19</td>
<td>530</td>
<td>27</td>
<td>50.9 [32.2–69.6]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10–19</td>
<td>U</td>
<td>7</td>
<td>0 for boys and 36.0 for girls</td>
</tr>
<tr>
<td>Taiwan [102]</td>
<td>1993–1999</td>
<td>Thai</td>
<td>School age children</td>
<td>2,932,855</td>
<td>2066</td>
<td>0.08 in boys and 0.12 in girls</td>
</tr>
<tr>
<td>Clinic-based studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indian Health Services, USA [89]</td>
<td>1996</td>
<td>American Indians, all Americans</td>
<td>0–14</td>
<td>402,580</td>
<td>518</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15–19</td>
<td>111,239</td>
<td>498</td>
<td>4.5</td>
</tr>
<tr>
<td>United Kingdom [100]</td>
<td>1993–2000</td>
<td>British</td>
<td>0–18</td>
<td>261,811</td>
<td>21</td>
<td>0.038</td>
</tr>
<tr>
<td>Clinic-based study [96]</td>
<td>1981–1990</td>
<td>Libyan</td>
<td>0–14</td>
<td>11,846</td>
<td>12</td>
<td>0.5 (0.26–0.87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15–34</td>
<td>75,836</td>
<td>1027</td>
<td>70.9 (66.6–75.5)</td>
</tr>
<tr>
<td>Cincinnati, OH, USA [92]</td>
<td>1994</td>
<td>Whites and African Americans</td>
<td>0–19</td>
<td>U</td>
<td>U</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10–19</td>
<td>U</td>
<td>U</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10–19</td>
<td>U</td>
<td>U</td>
<td>33 (in 994)</td>
</tr>
<tr>
<td>San Diego, CA, USA [105]</td>
<td>1993–1994</td>
<td>Whites, Hispanics, and African and Asian Americans</td>
<td>0–16</td>
<td>160</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>San Antonio, TX, USA [106]</td>
<td>1990–1997</td>
<td>Whites, Hispanics</td>
<td>U</td>
<td>560</td>
<td>101</td>
<td>18</td>
</tr>
<tr>
<td>Ventura, CA, USA [103]</td>
<td>1990–1994</td>
<td>Hispanics</td>
<td>0–17</td>
<td>31</td>
<td>14</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>1996–1999</td>
<td>Thai</td>
<td>0–14</td>
<td>39</td>
<td>7</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Note: Numbers in italics are estimates. NHANES III = National Health and Nutrition Examination Survey III; U = unknown data.

<table>
<thead>
<tr>
<th>Study and reference</th>
<th>Years</th>
<th>Race/ethnicity</th>
<th>Age (years)</th>
<th>Sample size</th>
<th>No. of cases</th>
<th>Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>registries, case reports, and cross-sectional studies from Canada [91], Cincinnati [92], Japan [93–95], Libya [96], Thailand [97], United Kingdom [98–100], India [101], Taiwan [102] and numerous case reports from the United States [103–107] have all indicated a significant increase in the prevalence of T2DM in children, although all these studies did not use a standard method to differentiate between type 1 and type 2 diabetes (see Table 3.2). The incidence of T2DM in African American adolescents in Cincinnati increased 10-fold from 0.7 to 7.2 per 100,000 over a 12-year period [92]. The proportion of T2DM in children diagnosed with diabetes has increased from 2–4% in 1992 to 8–45% in the last decade [108]. The proportion of type 1 to type 2 diabetic children is highly variable according to their different age and racial and ethnic groups (see Table 3.2).</td>
<td></td>
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</tr>
</tbody>
</table>
**Characteristics and diagnosis**

The majority of children with T2DM are non-Caucasian and in one review about 94% of cases were from minority groups [109]. The cause of this increased risk in non-Caucasian groups is unclear but will involve genetic predisposition and cultural and environmental risk factors. African American [110,111] and Hispanic [112] children, for example, have been shown to have higher insulin resistance than do non-Hispanic White children.

The majority of T2DM cases occur in overweight and obese children and they may have clinical signs of insulin resistance, such as acanthosis nigricans. The prevalence of T2DM in these children is higher in girls who may be two to six times more likely to have T2DM. The mean age of onset is around puberty in most populations.

Cases may present with classic signs and symptoms of diabetes but the disease may have an insidious onset and may only be detected by opportunistic screening. In a series from a referral center in Cincinnati, 32% of children with T2DM were discovered by opportunistic screening [92]. Therefore, underreporting of cases of T2DM in children may be as common as in adults.

The differentiation of T2DM from T1DM may be difficult in some cases (see Table 3.3). Currently, the mainstay of differentiating diabetes in children is inadequate and includes the use of clinical characteristics such as obesity, severity of onset, use of insulin, age of onset, diabetic ketoacidosis, and family history of diabetes. With the rising prevalence of obesity in children, more T1DM cases are presenting with obesity [113]. In addition, some cases of T2DM may present with diabetic ketoacidosis [114,115]. Diabetes-related autoantibodies that include the glutamic acid decarboxylase (GAD), tyrosine phosphatase-like molecule (IA2), islet cell antibody (ICA), and C-peptide (which is co-secreted with insulin) concentrations are currently being investigated as a means of improving the differentiation of diabetes type in children. The use of diabetes autoantibodies is limited by problems with assay methodology and the transient nature of some of these autoantibodies in cases of T1DM [116,117]. The use of C-peptide to differentiate the type of diabetes in children is made especially difficult by varying levels of residual β-cell function in type 1 diabetic cases. Current efforts are underway in a CDC (Centers for Disease Control) and NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) sponsored study; the SEARCH for Diabetes in Youth Study, to develop cutoff points and algorithms that include diabetes autoantibodies and C-peptide concentration which may be used to diagnose and differentiate diabetes in children.

**Risk factors**

Very few studies have examined the risk factors for T2DM in children. Some of the established risk factors for adult T2DM, discussed in other sections of this chapter, may play a role in the development of T2DM in children. Overweight and obesity are some of the strongest risk factors for insulin resistance and T2DM in adults. The rising prevalence of overweight in children may thus play a role in the risk of T2DM in children. In the United States, the prevalence of overweight in children has increased sharply over the last three decades to 15% in a 1999–2000 survey [118]. Over 20% of Hispanic and African American children are overweight compared to 12% in non-Hispanic White children [119]. This difference in prevalence of obesity may in part explain the higher prevalence of T2DM in ethnic minority children such as American Indians, African Americans, and Hispanics in the United States [88,92,103] and Asian Indians in the United Kingdom [100].

Most cases of T2DM occur around puberty. The reason for this increased risk around puberty is unclear but may be related to the physiologic 30% increase in insulin resistance observed in children as they go through Tanner stages II to IV [120–124]. This physiologic increase in insulin resistance is thought to be related to increased growth hormone production during puberty [121,122] but not related to the increase of sex hormones [121]. In the presence of increased insulin resistance produced by over-weight and physical inactivity the reduction in insulin resistance around puberty may precipitate T2DM in these children especially if they have inadequate compensatory insulin secretion (see Figure 3.11).

Prenatal and early childhood events may also increase the risk of developing T2DM in children. Among siblings from the same nuclear family, the child of a mother who had diabetes during pregnancy has a threefold greater risk of developing early onset diabetes than their sibling who was born before the mother became diabetic [125]. This increased risk is mainly due to exposure to diabetes in utero since these siblings share the same environment and a similar probability of inheriting the same genetic composition.

Low birth weight and disproportionate growth in utero have been shown to be associated with both T2DM and insulin resistance [126,127]. Breast-feeding is also protective against obesity and T2DM [128–130] especially when sustained for a long durations.

---

**Table 3.3 Challenges in the classification of diabetes in children**

<table>
<thead>
<tr>
<th>Type 1 diabetes</th>
<th>Type 2 diabetes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not overweight</td>
<td>Overweight or obese</td>
<td>Rising obesity means &gt;30% of type 1 are obese</td>
</tr>
<tr>
<td>Severe onset</td>
<td>Insidious</td>
<td>Presentations may vary</td>
</tr>
<tr>
<td>Weight loss</td>
<td>Some</td>
<td>Presentation may vary</td>
</tr>
<tr>
<td>Polyuria</td>
<td>Polyuria</td>
<td>Common to both</td>
</tr>
<tr>
<td>Polydipsia</td>
<td>Polydipsia</td>
<td>Common to both</td>
</tr>
<tr>
<td>Insulinopenia</td>
<td>No insulinopenia</td>
<td>Not present in all type 1 diabetes in honeymoon phase</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td>No Autoantibodies</td>
<td>Usually occur in type 1, absence does not exclude type 1</td>
</tr>
<tr>
<td>No insulin resistance</td>
<td>Insulin resistance</td>
<td>Usually occur in type 2</td>
</tr>
</tbody>
</table>
Complications and comorbid conditions
The development of T2DM in children is alarming. In adults, T2DM is associated with the development of complications such as retinopathy, renal disease, and CVD. Recent reports of T2DM in children suggest that diabetes carries a similar risk for the development of complications in early adulthood. In a study on the development of complications in Pima Indian children with T2DM, the rate of development of nephropathy was as severe in children as in adults over 30 years of follow-up [131]. The rate of development of retinopathy was, however, lower in cases that developed diabetes in childhood. A small series of young adults from the First Nation in Canada who had been diagnosed with T2DM before their 17th birthday were traced and followed up for the prevalence of diabetic complications and adverse outcomes by their 36th birthday. In this small series 9% had died, and 6.3% were already on dialysis and their blood glucose control was poor [132].

At diagnosis, a number of children have comorbid conditions such as dyslipidemia and hypertension both of which are risk factors for CVD [133]. Cardiovascular risk is strongly related to duration of diabetes [134] and T2DM in children will increase their risk to develop CVD later on in life.

The apparent rise in the prevalence of T2DM in childhood is presenting new challenges in the management and classification of diabetes in children. Adolescents are more likely to be noncompliant and may find it more difficult to follow the lifestyle modifications and treatment regimen that would be necessary to control blood glucose [108,135]. Furthermore, very few medications have been approved for use in treating type 2 diabetic children. Insulin and metformin are the only medications currently licensed for treatment of T2DM in children in the United States.

Modifiable risk factors for type 2 diabetes

Obesity
Obesity and weight gain have consistently been shown to be the one of the strongest modifiable risk factors for diabetes [140–142]. The ratio of a person’s weight in kilograms divided by the square of their height in meters called the body mass index (BMI) has been used in numerous studies as a surrogate for obesity. In a representative sample of the US population, each unit increase in BMI was associated with a 12% increased risk of T2DM [143]. Compared to people with BMI <22 kg/m² those with BMI of 25–27 kg/m² had 2.75 times the risk of diabetes, and each kilogram increase in body weight over 10 years was associated with a 4.5% increase in diabetes risk. Numerous studies have found similar results in different populations [144,145], but the magnitude of risk associated with a given BMI may differ across populations. The distribution of weight and weight gain are also important risk factor for diabetes. Central obesity, that is, deposition of fat in the trunk and abdominal areas, has been shown to be a strong risk factors for diabetes. The surrogate measures of central obesity include circumference of waist, waist-to-hip ratio, and waist-to-thigh ratio. More recent technological advances like dual-energy X-ray absorptiometry (DEXA) and magnetic resonance imaging (MRI), and computer tomography have made it possible to measure subcutaneous and intra-abdominal fat. Intra-abdominal fat has been shown to increase the risk of insulin resistance and diabetes in a number of studies in different populations [145–148] and this effect may be independent of the effects of total body obesity [149,150]. Obesity always occurs when energy intake exceeds energy expenditure.

Physical activity
Physical activity has been consistently reported to be inversely related to future risk of diabetes in most populations. Higher levels of physical activity are associated with a lower risk of diabetes in observational studies [151]. Increased physical activity reduces the risk of obesity but its effect on diabetes risk has been shown to be independent of its effect on body weight. Numerous studies have shown that exercise is related to acute and long-term improvements in insulin sensitivity and reduction in insulin concentrations [152,153]. Several epidemiologic studies in diverse populations [154–161] confirm a dose–response relationship between levels of physical activity and diabetes incidence. One study among Finnish men [160] found a strong gradient of diabetes risk associated with intensity of physical activity regardless of duration, but most evidence indicates that the total amount (i.e., number of days or minutes) of physical activity per week is a more important determinant.

Sedentary lifestyle
Sedentary lifestyle has also been shown to be a risk factor for both diabetes and obesity. Prolonged television (TV) watching as a surrogate of a sedentary lifestyle has also been reported to be a risk factor for diabetes [162,163]. Compared to men spending 0–1 h per week watching TV, those spending 2–10 h had 66% higher risk of diabetes, and the risk increased progressively to 187% higher incidence among those spending >40 h per week [162]. In women, every 2 h per day increment in TV watching was associated with a 23% increase in risk of
obesity and 14% increase in risk of diabetes [163]. Furthermore, each 2-h increment in sitting at work was associated with a 5% increase in diabetes risk. It is estimated that a relatively active lifestyle (<10 h per week of TV watching and ≥30 min per day of brisk walking) can prevent 43% of new cases of diabetes [163].

Evidence from intervention studies
Results from the first diabetes prevention trials have clarified the role of physical activity as a risk factor for diabetes. Results from the Malmö trial in Sweden [164], Da Qing trial in China [58], Diabetes Prevention Study (DPS) in Finland [59], and the Diabetes Prevention Program in the United States [60] have all shown that moderate physical activity reduces the progression of IGT to diabetes by 30–58%. In these studies moderate physical activity was included in a lifestyle intervention arm of the trials with dietary changes that include reduction in caloric intake and in the percentage of dietary fat and increase in fiber intake (Table 3.4). In most of these studies it is difficult to discern the individual effect of the dietary intervention as it was combined with modifications in physical activity.

Dietary factors
Diet is a phenomenon that is made up of complex interactions between numerous foods and nutrients that are highly correlated and are determined by personal preference, cultural heritage, and socioeconomic factors. Diet is traditionally measured using questionnaires and food diaries. A number of studies have examined the relationship between diabetes and different aspects of diet including absolute intake of nutrients, nutrient intake as a percentage of total energy, dietary patterns, and bioavailability characteristics of foods like glycemic index.

Nutrients and diabetes risk
Dietary fat Animal studies and clinical human studies suggest several plausible mechanisms relating diet to the etiology of T2DM. High-fat diets have been associated with obesity [165], increased body fat for a given weight [166], and altered fat distribution [166]. Furthermore, alterations in cell membrane composition induced by the composition of dietary fat could alter membrane fluidity and/or insulin-mediated signal transduction as well as subsequent insulin action [167].

The results from epidemiologic studies on the relationship of total fat and T2DM have been controversial. In general, most of the migrant [168,169] and retrospective [170,171] studies show a positive relationship between high-fat, low-carbohydrate diets and T2DM, whereas results from prospective studies from different populations have been mixed [172–174] and not as consistent.

The effect of different subtypes of fat such as saturated, polyunsaturated, monounsaturated fat [175,176] and ω-3 fatty acids [177–179] on diabetes risk has also been investigated. An extensive review [180] concluded that while neither total fat nor total carbohydrate as proportions of total energy play a major part in the development of T2DM, specific types of fat and carbohydrate are important. For example, they concluded that (1) higher intake of polyunsaturated fat and long-chain n-3 fatty acids (fish oil) may be beneficial, and (2) higher intake of saturated fat and trans-fatty acids may be deleterious.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Age (years)</th>
<th>Interventions</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malmö [164]</td>
<td>181 Swedish men with IGT</td>
<td>47–49</td>
<td>Diet, physical activity, and training</td>
<td>50% with IGT reverted to normal; blood sugars normalized in 50% with diabetes</td>
<td>6-year follow-up; participants were not randomized</td>
</tr>
<tr>
<td>Da Qing [58]</td>
<td>577 Chinese men and women with IGT</td>
<td>&gt;25 (mean age 44.9)</td>
<td>Diet only, exercise only, and diet + exercise</td>
<td>Diet only: 31% ↓ in diabetes risk; exercise only: 46% ↓; diet + exercise 42% ↓</td>
<td>6-year follow-up; randomization by clinic</td>
</tr>
<tr>
<td>Diabetes Prevention Study (DPS) [59]</td>
<td>522 overweight Finnish men and women with IGT</td>
<td>Middle age (mean age 55)</td>
<td>Increased physical activity, increased fiber intake, and reduced fat intake</td>
<td>58% decrease in diabetes risk</td>
<td>3.2-year follow-up</td>
</tr>
<tr>
<td>DPP [60]</td>
<td>3324 overweight US men and women with IGT</td>
<td>Mean age 50.6</td>
<td>Lifestyle (increased physical activity, weight loss, reduced fat intake), and drug (metformin)</td>
<td>Lifestyle 58% ↓ in diabetes risk; metformin 31% ↓</td>
<td>2.8-year follow-up; subjects from several different race/ethnic groups. Lifestyle interventions were effective in all race/ethnic and age groups</td>
</tr>
</tbody>
</table>

Note: IGT = impaired glucose tolerance.
The glycemic index of food is a measure of the postprandial excursion of glucose as a result of the ingestion of a fixed amount of food. Foods with high glycemic index cause greater excursions of glucose. The postprandial excursions are dependent on the rate of absorption of glucose, which in turn is dependent on a number of factors including the type of carbohydrate (simple or complex) and the amount of fiber. Food with lower glycemic index has been associated with a lower risk of developing diabetes in some studies [182,184], but others have failed to show any association between the glycemic index of foods and the risk of developing diabetes [185,186].

Fiber intake
High-fiber intakes have been related to a reduction in the risk of diabetes in some populations [182,184,187–190]. The mechanism by which high fiber reduces the risk of diabetes is unknown. High-fiber diets usually have a lower glycemic index but some studies that have shown a relationship between fiber intake and diabetes have failed to demonstrate a beneficial effect of the glycemic index of the diet [185]. Thiamine and vegetable protein are found in high concentrations in foods that are rich in fiber and have also been shown to reduce 2-h glucose concentration in women and may explain some of the association between fiber intake and diabetes [191,192].

Vegetable and fruit intake
Higher intakes of vegetables have been shown to be associated with a reduction in the risk of diabetes [172,173,193]. The component of vegetables that confers lower risk has not been clearly identified but may include antioxidants such as carotenoids and tocopherols [194], higher fiber intake, and vitamins [172,195,196]. The effect of fruit consumption on diabetes risk has been inconclusive [173,193].

Dietary patterns and scores
Studies have examined the effect of the whole composition and quality of diet on diabetes risk using various scores and dietary patterns identified by principal component and factor analyses [197,198] on diabetes risk. The results from these studies have shown that a diet that has relatively higher intakes of vegetables and fruits and lower intakes of fat-rich foods has been associated with a reduction in diabetes risk [197,198].

Self-rated dietary patterns can be used to discriminate differences in the nutrient composition in diet. Pima Indians who self-reported a diet more similar to their traditional Pima diet had a lower risk of developing diabetes over a 12-year period [199] when compared to a more Westernized dietary preference. The traditional Pima diet had a higher fiber and lower fat intake when compared to the more Westernized diet. The traditional diets of most indigenous populations have a higher intake of fiber and lower glycemic index [200,201] than the more recent Westernized diets that most of these populations have adopted.

Nontraditional modifiable risk factors for type 2 diabetes

Inflammation
Although insulin resistance and relative insulin deficiency represent the main characteristics of T2DM, the underlying mechanism responsible for these abnormalities remains largely unknown. On the basis of hypothesis that both T2DM and atherosclerosis may generate from a “common soil” [202,203], more recently a growing body of evidence points out that inflammation may constitute the common factor that leads to the development of both these diseases. One of the most used markers of subclinical inflammation is the C-reactive protein (CRP). A number of cross-sectional studies have shown that increased concentrations of CRP are associated with abnormalities characterizing the metabolic syndrome, including obesity, insulin resistance, low HDL cholesterol, and hypertriglyceridemia [204–206]. In addition, prospective studies have demonstrated that high levels of CRP increase the risk of developing T2DM [207–212]. These findings have opened new avenues for understanding the pathogenesis of T2DM and, eventually, for preventing the disease. Indeed, if, as these data have indicated, subclinical inflammation is an important determinant of T2DM, then the use of anti-inflammatory drugs may prevent diabetes. In this regard, it is noteworthy that in patients with T2DM high-dose aspirin reduces insulin resistance and improves glucose tolerance [213].

Smoking
Numerous prospective studies have indicated that smoking is associated with the development of diabetes. In the US Nurses’ Health Study, women who smoked at least 25 cigarettes per day compared to those who never smoked had a relative risk of developing diabetes of 1.42, even after controlling for known risk factors [214]. Similar results were shown in men [215]. These findings were confirmed by Will et al. in a prospective study involving over 275,000 men and 434,000 women from the US Cancer Prevention Study [216]. Among those who smoked at least two packs per day at baseline, men had a 45% higher diabetes rate than men who had never smoked; in women the comparable increase was 74%. More important, quitting smoking reduced the rate of diabetes to that of nonsmokers after 5 years in women and after 10 years in men. In support of these findings are cross-sectional data on the association between cigarette smoking and hemoglobin A1C (HbA1C)
from the East Anglian component of the European Prospective Investigation into Cancer (EPIC-Norfolk) [196]. In this study, current smokers had highest mean HbA1C concentrations, lowest levels were observed in never smokers, and intermediate in former smokers. HbA1C levels also correlated in a dose–response manner both with the number of cigarettes smoked per day and with total amount of smoking as measured by pack-years. This association persisted even after adjusting for potential confounders including BMI, waist-to-hip ratio, physical activity, and dietary variables. However, to better assess the role of smoking as a determinant of T2DM we need cohort studies to further confirm that this association exists in different populations.

There is evidence that smoking is associated with insulin resistance [217,218]. In a study by Facchini et al. [217] chronic smokers compared to nonsmokers had significantly higher plasma triglycerides and lower HDL-cholesterol levels and higher insulin concentrations after a 75-g oral glucose challenge. Well-designed clinical studies of the effects of acute and chronic smoking on insulin resistance are needed to elucidate the mechanism by which smoking induces insulin resistance. It is plausible that oxidative stress caused by smoking may induce endothelial dysfunction, resulting in insulin resistance into muscle and liver.

The possibility that smoking may play a causal role in the development of T2DM has important implications for prevention. Both diabetes and smoking are conditions common enough that even a small increase in the risk of diabetes associated with smoking may have an important public health impact.

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SECTION II

Physiology of pancreatic function
CHAPTER 4
Development and maintenance of the islet β cell

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Key points
• The islets of Langerhans are composed of five hormone-secreting cell types: α cells produce glucagon, β cells produce insulin, δ cells produce somatostatin, PP cells produce pancreatic polypeptide, and ε cells produce ghrelin.
• The pancreas is derived from the merger of distinct buds that are induced dorsally and ventrally from the foregut endoderm by the adjacent mesoderm.
• The extensively branched structure of the mature pancreas is attained by continued growth and remodeling of the primitive tubular epithelium in both dorsal and ventral buds.
• The branching epithelium becomes organized into “tip” and “trunk” domains, within which progenitor cells reside.
• The “secondary transition” is the phase during mid-pancreas development that is marked by the differentiation of exocrine cells and the major wave of islet cells.
• A number of transcription factors expressed broadly in early pancreas development later become restricted to specific endocrine cell types and subsequently acquire additional functions in these cell types.
• The state of β-cell mass and function is a large determinant of glucose and lipid homeostasis.
• β cells exhibit progressively slower rates of turnover as a mammal ages.

Introduction
The discovery of insulin by Banting and Best in the early 1920s marked the beginning of a new era of diabetes research that focused on the study of insulin and the biology of the hormone-producing cells of the pancreas. In the ensuing decades, although much was learned regarding the synthesis, structure, and action of insulin, the developmental origins of insulin-producing β cells remained ambiguous. In the 1970s, the long-held belief that β cells might arise from the neural crest was refuted by elegant interspecies cell transplantation studies, which demonstrated that radiolabeled quail neural crest cells do not populate pancreatic endocrine tissues in host chick embryos [1]. This seminal finding, and the advent of techniques to manipulate the mouse genome, has resulted in a greater understanding of how the pancreas develops and matures. The lessons from developmental biology have also elicited speculation that maintenance of endocrine cell mass in the adult pancreas follows a similar paradigm, whereby a pool of proliferative progenitor cells differentiates as needed to repopulate the endocrine compartment. It is becoming increasingly recognized that processes that maintain or increase β-cell mass rely on signals distinct from those in the developing pancreas, and therefore the mechanisms of cell specification and maintenance differ depending upon the age of the organism. This chapter reviews the fundamentals of pancreas organogenesis, proceeds with an overview of cytodifferentiation with an emphasis on the β cell, and concludes with a review of β-cell growth and maintenance in the adult pancreas.

Pancreas development
The pancreas is a compound digestive gland, comprised of both exocrine and endocrine components that secrete digestive enzymes and hormones, respectively. The exocrine component makes up approximately 95% of pancreas mass, and consists of acinar cells and duct cells. Acinar cells produce and secrete proteases, lipases, amylases, and nucleases that are necessary for the digestion of nutrients. Duct cells form a tubular network throughout the pancreas, and also secrete mucins and fluids that flush the acinar secretions to the intestine. The mature endocrine component of the pancreas, organized into structures known as the islets of Langerhans, comprises ~2%
Figure 4.1 Differentiation of pancreatic β cells in vivo. Schematic representation of the five subdivisions of pancreatic development and some of the major secreted signaling factors acting during the transition between each stage. Important tissue markers are listed above each schematic drawing. (1) specification of definitive endoderm, (2) patterning of primitive foregut tube, (3) induction of dorsal and ventral pancreatic buds, (4) growth and branching of pancreatic endoderm, (5) cytodifferentiation and islet morphogenesis. Source: Adapted from Mayhew CN, Wells JM. Current Opinion in Organ Transplantation 2010;15:54–60. Reproduced with permission of LWW.

of the total organ mass. In the adult, islets of Langerhans are composed primarily of five discrete hormone secreting cell types: α cells produce glucagon, β cells produce insulin and islet amyloid polypeptide, δ cells produce somatostatin, PP cells produce pancreatic polypeptide, and ε cells produce ghrelin. β Cells comprise the majority (60–80%) of the cells that make up the islet in vertebrate animals. The progression of pancreas development in vertebrates can be segmented into five major events (summarized in Figure 4.1): (1) induction of definitive endoderm, (2) formation of the primitive gut tube and patterning of endoderm into organ-specific progenitor zones, (3) induction of dorsal and ventral pancreatic buds, (4) outgrowth, branching, and fusion of the pancreatic buds, and (5) cytodifferentiation.

**Induction of the definitive endoderm**

During early development, pluripotent stem cells in the gastrula stage embryo evolve into the multipotent progenitor cells of the three primary germ layers known as ectoderm, mesoderm, and definitive endoderm. Broadly speaking, mesodermal derivatives provide support and movement, whereas ectodermal derivatives provide for sensation of, and protection from, the environment. The definitive endoderm is the innermost germ layer of metazoan embryos, and its derivatives mediate exchanges with the environment, including nutrient absorption and gas exchange. Definitive endoderm gives rise to the pancreas, liver, intestine and other digestive organs, as well as the thyroid and parathyroid glands and the respiratory tract.

Model systems must be employed to study the mechanisms of development. Considering its evolutionary proximity to humans and the wealth of sophisticated tools for genetic manipulation, the mouse (Mus musculus) is the most pervasively utilized model organism. However, much of what is known about endoderm induction and morphogenesis has been learned through the study of lower vertebrates. Model systems such as the zebrafish (Danio rerio), the African clawed frog (Xenopus laevis), and the chicken (Gallus gallus) each exhibit species-specific experimental advantages (see Table 4.1).

During gastrulation, cells delaminate from the epiblast cell layer, and ingress through a transitory structure called the primitive streak in amniotes (e.g. mammals and birds) or the marginal zone in fish and frog, and emerge as endoderm cells. Although the morphogenetic events of gastrulation vary widely in model organisms, these events are regulated by a conserved set of regulatory molecules. The most fundamental endoderm induction signal is Nodal, a secreted TGFβ-like molecule that is highly conserved in amniotes, amphibians, and fish. Nodal signaling is necessary and sufficient in all vertebrates to trigger a genetic cascade leading to endoderm formation [2]. Nodal is expressed at the site of gastrulation, where it initially generates a transitory mesendodermal precursor cell population. This population is subsequently subdivided into mesoderm and endoderm by the morphogenetic properties of Nodal, with the highest levels of Nodal signaling producing endoderm. Expression of the gene encoding Nodal is initiated in divergent ways depending upon the species. In mice, the gene is induced by WNT signaling at the interface of embryonic and extra-embryonic tissues [3], whereas in zebrafish and frogs it is induced by maternally provided factors that are localized to the site of Nodal induction [4,5]. However, in all vertebrates studied,
### Table 4.1 Characteristics of vertebrate model systems

<table>
<thead>
<tr>
<th></th>
<th>mouse (Mus musculus)</th>
<th>chicken (Gallus gallus)</th>
<th>frog (Xenopus laevis)</th>
<th>zebrafish (Danio rerio)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genome size</strong></td>
<td>3.3 × 10^9 bp</td>
<td>1.1 × 10^9 bp</td>
<td>3.1 × 10^9 bp</td>
<td>1.5 × 10^9 bp</td>
</tr>
<tr>
<td><strong>Gestation time</strong></td>
<td>19 days</td>
<td>21 days</td>
<td>~5 days</td>
<td>~5 days</td>
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<tr>
<td><strong>Generation time</strong></td>
<td>9–11 weeks</td>
<td>18–25 weeks</td>
<td>1–2 years</td>
<td>10–12 weeks</td>
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<td>(egg to egg interval)</td>
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<td><strong>Lifespan</strong></td>
<td>2–3 years</td>
<td>1–8 years</td>
<td>5–25 years</td>
<td>2–4 years</td>
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<td><strong>Imaging and embryological manipulations</strong></td>
<td>Difficult</td>
<td>Simple</td>
<td>Simple</td>
<td>Intermediate difficulty</td>
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<td></td>
<td>Fragile embryo</td>
<td>Robust embryo</td>
<td>Robust embryo</td>
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<td>Excellent for</td>
<td>Excellent for</td>
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<td>micromanipulation</td>
<td>micromanipulation</td>
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<tr>
<td><strong>Blastula diameter</strong></td>
<td>60–80 μm (small)</td>
<td>1–2 mm (large)</td>
<td>1–1.3 mm (large)</td>
<td>~800 μm (intermediate)</td>
</tr>
<tr>
<td><strong>Transgenesis</strong></td>
<td>Simple transgenesis</td>
<td>Transgenesis is possible</td>
<td>Transgenesis is possible</td>
<td>Simple transgenesis with common approaches</td>
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<td>with common approaches</td>
<td>but inefficient</td>
<td>but inefficient</td>
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<td>Not widely practiced</td>
<td>Not widely practiced</td>
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<td><strong>Imaging</strong></td>
<td>Internal development</td>
<td>External development</td>
<td>External development</td>
<td>External development</td>
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<td>(in utero)</td>
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<td>in vitro culture of</td>
<td>Windowing of eggs</td>
<td>Opacity of embryos</td>
<td>Transparent embryos and</td>
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<td>embryos and explants</td>
<td>allows limited live</td>
<td>limits deep imaging</td>
<td>fluorescent transgenes</td>
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<td></td>
<td>feasible over short</td>
<td>imaging</td>
<td></td>
<td>facilitate live/time</td>
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<td></td>
<td>time periods.</td>
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<td>lapse imaging</td>
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<tr>
<td><strong>Forward genetics</strong></td>
<td>Mutable with common</td>
<td>Not genetically tractable</td>
<td>Not genetically tractable</td>
<td>Mutable by common methods</td>
</tr>
<tr>
<td>(find phenotype--&gt;map genotype)</td>
<td>methods (i.e., ENU, virus)</td>
<td></td>
<td>Long egg-to-egg period makes screening time consuming</td>
<td>Strong history of genetic screens.</td>
</tr>
<tr>
<td></td>
<td>Growing collection of</td>
<td></td>
<td></td>
<td>Intermediate difficulty in mapping mutations</td>
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<td></td>
<td>ENU-induced alleles</td>
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<td></td>
<td>Easy mapping of ENU mutations</td>
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<td></td>
<td>Relatively time</td>
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<td></td>
<td>consuming, inefficient, and costly</td>
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<td><strong>Reverse genetics</strong></td>
<td>Facile homologous</td>
<td>Antisense morpholino</td>
<td>Antisense morpholino</td>
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<td>(alter genotype--&gt;study phenotype)</td>
<td>recombination in ES cells</td>
<td>oligos</td>
<td>feasible but</td>
<td>Gene misexpression via DNA or</td>
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<td></td>
<td>Many targeted alleles</td>
<td>Viral misexpression of</td>
<td>complicated, due to</td>
<td>RNA injection</td>
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<td>and Cre stains</td>
<td>genes</td>
<td>allotetraploid genome</td>
<td>Evolving tools for targeted</td>
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<td>Antisense morpholinos</td>
<td></td>
<td>Produce 100s of</td>
<td>mutagenesis and homologous</td>
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<td>used rarely</td>
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<td>synchronous embryos</td>
<td>recombination</td>
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<td>Produce 100s of synchronous</td>
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<td>embryos</td>
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</table>

Nodal expression is enhanced by high levels of WNT signaling and maintained by a positive paracrine feedback loop [6]. The targets of Nodal include the Mix-like (Mixl) family of homeobox genes, the Foxa family of forkhead genes, and the high mobility group box gene Sox17. Together, these genes comprise an essential transcription factor network that stabilizes the endodermal fate while simultaneously segregating it from mesoderm [7,8].

**Patterning of the gut tube into organ domains**

All endoderm generated during gastrulation is not equivalent: cells acquire positional identity upon specification, which is dependent upon the time at which the cells are specified. The foregut endoderm is specified first, followed by midgut, then hindgut. As gastrulation concludes, the foregut expresses unique spatial markers, including Hhex, which is directly induced by Nodal, as well as Sox2 and Foxa2. These three transcription factors are essential for the development of foregut organs [2,9,10]. Moreover, the antero-posterior (A-P) patterning of the endoderm is dictated by the release of several signaling molecules from the mesoderm. In addition to Nodal, which induces anterior endoderm at the highest levels of expression and posterior fates at progressively lower levels, a core set of secreted signaling molecules are encountered by migrating cells. Fibroblast growth factors (FGFs), Wingless/Int (WNTs), bone
morphogenetic proteins (BMPs), and retinoic acid (RA) are secreted from a posterior location, serve to suppress anterior fates, and to partition further the gut tube into regions that express genes encoding the crucial transcription factors Pdx1 (intermediate gut region) and Cdx1/2/4 (hindgut region) [3,8]. Moreover, as discussed later, this set of signaling molecules acts iteratively within the pancreatic endoderm throughout development to generate the mature pancreas.

Immediately after formation, the germ layers are arranged as three flat, stacked sheets of cells. The endoderm rapidly transitions to a tubular morphology and becomes enveloped by mesoderm. In amniotes, the morphological transformation begins with the folding together of the lateral edges of the anterior and posterior ends of the sheet to form foregut and hindgut pockets. The lateral edges of the endoderm sheet are then fused together by zipper-like morphogenetic behavior at the ventral midline that progressively seals from the anterior and posterior ends; this process ultimately generates a hollow gut tube. In zebrafish and frogs, the endodermal sheet first forms a solid rod through mass endodermal cell migration toward the midline. This rod later hollows by the mechanism of cavitation, which may involve lumen-directed fluid transport.

The foregut endoderm gives rise to multiple organs, including pancreas, liver, thyroid, and lung. The gut tube becomes subdivided into organ forming regions through reciprocal interactions between the endodermal cells and the adjacent mesenchyme, a process requiring FGF, BMP and RA signaling pathways. Explant studies using uncommitted mouse foregut originally revealed the role of FGF emanating from the septum transversum mesenchyme. Low levels of FGF led to expression of Pdx1 in ventral pancreatic progenitors, intermediate levels led to hepatic fates, whereas the highest levels specified lung and thyroid. Mouse genetic experiments later confirmed this model in vivo, and the requirement for FGF is conserved in both zebrafish and frogs [4,5,11]. BMP signals, which also originate from the septum transversum mesenchyme in mammals and from the lateral plate mesoderm in fish, regulate pancreatic specification [6,12]. BMP signals repress pancreatic development from a common pool of progenitors [7,8,13]. RA signaling biases endoderm to more posterior fates, and may have a role in partitioning the expression domains of the genes encoding Pdx1 (Pancreatic transcription factor 1a), two transcription factors essential for proper development of all pancreatic cell types [16]. The ventral bud is specified by low-level FGF signaling from the mesenchyme in the absence of BMP signaling, as described earlier. Dorsal bud fate is specified when FGF2 and Activin signals secreted from the notochord mitigate Sonic Hedgehog (Shh) expression in the dorsal midline of the gut tube [17]. In contrast to the suppressive role of Shh in early pancreatic bud induction, Shh later plays a positive role in the expansion of the pancreatic epithelium and in β-cell function [18]. Additionally, dorsal bud induction requires RA [19] as well as yet unidentified factors secreted from the dorsal aorta. In contrast to amniotes and frogs, dorsal fate specification likely occurs by a divergent mechanism in zebrafish, as Shh has a positive regulatory role in dorsal bud induction [20]. Furthermore, signaling from the dorsal aorta does not appear to have a fundamental role in zebrafish, as cloche mutants of zebrafish that lack all vasculature still demonstrate normal bud induction [21].

**Induction of the dorsal and ventral pancreatic buds**
The pancreas is derived from the merger of distinct buds that are induced dorsally and ventrally from the foregut endoderm by the adjacent mesoderm. In all vertebrates, both dorsal and ventral buds are marked by expression of Pdx1 and Ptft1a (Pancreatic transcription factor 1a), two transcription factors essential for proper development of all pancreatic cell types [16]. The ventral bud is specified by low-level FGF signaling from the mesenchyme in the absence of BMP signaling, as described earlier. Dorsal bud fate is specified when FGF2 and Activin signals secreted from the notochord mitigate Sonic Hedgehog (Shh) expression in the dorsal midline of the gut tube [17]. In contrast to the suppressive role of Shh in early pancreatic bud induction, Shh later plays a positive role in the expansion of the pancreatic epithelium and in β-cell function [18]. Additionally, dorsal bud induction requires RA [19] as well as yet unidentified factors secreted from the dorsal aorta. In contrast to amniotes and frogs, dorsal fate specification likely occurs by a divergent mechanism in zebrafish, as Shh has a positive regulatory role in dorsal bud induction [20]. Furthermore, signaling from the dorsal aorta does not appear to have a fundamental role in zebrafish, as cloche mutants of zebrafish that lack all vasculature still demonstrate normal bud induction [21].
pancreas divisum, the genetic pathways underlying this process are not well understood [29].

**Cytodifferentiation in the developing pancreas**

As stated earlier, the early evaginating pancreatic buds are made up of progenitor cells that express Pdx1 and Ptf1a. These early pancreatic progenitor cells will induce the specification of multipotent progenitor cells (MPCs), which direct derivative cell populations toward a particular fate. The early developing pancreatic buds are marked by the appearance of cells with low-level digestive enzyme production and an initial wave of glucagon- and insulin-expressing cell types, a period referred to as the “primary transition” of pancreas formation. The term “secondary transition” is applied to the phase during mid-pancreas development that is marked by the differentiation of exocrine cells and the major wave of islet cell formation [30]. The secondary transition is characterized by a dramatic increase in cells expressing acinar digestive enzymes, as well as a large increase in cells producing endocrine hormones including insulin, glucagon, ghrelin, somatostatin, or pancreatic polypeptide. Preceding this major wave of differentiation, the secondary transition also encompasses the emergence of the MPCs and the establishment of the pre-acinar and bipotent duct/endocrine cell populations from which the differentiated exocrine, and endocrine or duct cells derive, respectively (Figure 4.2).

In recent years, a network of genes has been identified whose products specify the development of the different cell types (see Table 4.2). The importance of these genes has largely been identified by lineage tracing studies and targeted mutations in mouse models, and has led to two important concepts in pancreatic cytodifferentiation. First, the products of many of these genes function in a “cell-autonomous” manner, meaning that their expression level in a given cell type alters the fate and function of that cell. Second, misexpression of specific genes in magnitude or in a spatial (cell- or domain-specific) or temporal (time of development-specific) manner can redirect developing progenitor cells to cell fates they would otherwise not have adopted. With respect to the latter concept, the study of cell-autonomous factors has the potential to identify means through which other cell types might be converted to β cells for the treatment of different forms of diabetes.

**Tip versus trunk domains**

The stratification of the pancreatic epithelium and the resulting formation of microlumens is an essential morphological change that occurs during the primary transition. The subsequent remodeling of the ductal plexus and branching of the epithelium continues throughout embryonic pancreas development [26]. Epithelial branching leads to the morphogenesis of different domains, which become most apparent at the beginning of the secondary transition. There is mounting evidence that MPCs exist within these emerging epithelial domains. In particular, Melton and colleagues proposed the identity of the MPCs as those cells expressing the factors Ptf1a, Pdx1, c-Myc, and Cpa1 [31]. This concept stemmed from a genome-wide transcription factor analysis in mouse pancreas tissue at embryonic day (E) 14.5, whereby gene expression patterns were identified to segregate into particular domains of the developing pancreas. Specifically, patterns emerged

![Figure 4.2 Cytodifferentiation in the developing pancreas. The tip domain houses MPCs, which give rise to pre-acinar cells, and bipotent duct/endocrine cells (gray) that subsequently reside in the trunk of the branching epithelium and give rise to duct cells (white) or hormone-producing endocrine progenitor cells (colored cells). In the mouse, endocrine progenitor cells express Neurog3 and were determined to be unipotent, such that each Neurog3+ endocrine progenitor is destined to become a particular hormone-expressing endocrine cell type, including insulin-expressing β cells, glucagon-expressing α cells, somatostatin-expressing δ cells, ghrelin-expressing ε cells, and pancreatic polypeptide-expressing PP cells. Some of the transcription factors responsible for the development of each specific islet cell type are outlined.](image-url)
that could be grouped into five domains: pan-epithelium, tip, trunk, mesenchyme, and vasculature. Genes discovered to be expressed in the tip domain later segregated into differentiated acinar cells, whereas genes expressed in the trunk domain were identified in the ducts or differentiated endocrine cells. Taken together, data from multiple studies suggest that MPCs residing in the tip domain will give rise to pre-acinar cells, destined to become exocrine tissue, and bipotent duct/endocrine cells that reside in the trunk of the branching epithelium (Figure 4.2).

**Endocrine versus exocrine cell fate decision**

It is in the early developing pancreatic domain, when progenitor cells are multipotent, that the endocrine versus exocrine decision is made. In the mouse, Ptf1a is located in the early pancreatic progenitor cells and over time becomes restricted to expression in the branching tips and then differentiated acinar cells [32].

At the beginning of pancreas development the transcription factors Nkx6.1 and Nkx6.2 are co-expressed in the MPCs before becoming restricted and separated in their expression pattern. The Nkx6 factors and Ptf1a have been noted to function antagonistically in the decision between endocrine and exocrine cell fates, such that Nkx6 factors promote the endocrine decision whereas Ptf1a promotes the exocrine decision [33].

The endocrine versus exocrine cell fate decision is also influenced by the level of expression of the transcription factor Neurog3 (Neurog3) in the progenitor cells. Specifically, a high level of Neurog3 is required for commitment to the endocrine fate [34]. Moreover, Notch signaling is used in the trunk domain to subdivide this compartment between endocrine and ductal cells via a lateral inhibition mechanism. Neurog3 upregulates expression of the Notch ligand Delta-like 1 (Dll1) in endocrine progenitors, which activates the Notch pathway in neighboring cells thereby repressing their differentiation into endocrine cells.

**The endocrine progenitor cell**

The culmination of many studies has confirmed that in the developing mouse pancreas, the transcription factor that defines the endocrine progenitors is Neurog3. Neurog3-null mice exhibit absence of endocrine cells in the pancreas, and such mice develop neonatal diabetes and die shortly after birth [35]. During mouse pancreas development a subset of hormone-expressing cells is observed as early as E9.5, whereas the major wave of endocrine differentiation occurs during the secondary transition. Lineage tracing experiments using genetically-engineered mouse reporter lines identified that, regardless of when the endocrine cell differentiates, all hormone-expressing cells are derived from cells that previously expressed Neurog3 [36,37].

The process of endocrine differentiation has also been linked to the morphological process of delamination of the progenitor cells from the pancreatic epithelium. Interestingly, the delamination of progenitor cells is initiated in the cells that express
Neurog3 [38]. Moreover, the subsequent differentiation into different endocrine cells types is influenced by the timing of Neurog3 expression. Specifically, altering the temporal expression of the gene encoding Neurog3 in the mouse influences the competence of progenitor cells to differentiate into the specific endocrine cell types, such that earlier expression produces almost exclusively α cells, whereas later expression produces varied ratios of all hormone-expressing cell types [39].

Previous models of pancreas development suggested that each Neurog3-expressing cell could give rise to any subsequent differentiated endocrine cell type. However, this perspective has been challenged by lineage tracing experiments using genetically-altered mice, which demonstrated that each Neurog3-expressing endocrine progenitor cell is in fact unipotent, and therefore destined to become a particular single-hormone expressing endocrine cell type [40] (Figure 4.2). The implication of this discovery is that the transcription factor “code” responsible for the differentiation of each hormone-expressing cell type may be delineated before endocrine progenitors are specified.

Clearly, the expression of Neurog3 is of great significance to the development and differentiation of endocrine cells in the mouse. However, the effect of loss of this transcription factor in other species is not identical to the mouse. For example, in zebrafish Neurog3 is not observed in the pancreas [41]. Homozygous mutations in Neurog3 have been identified in humans, resulting in congenital malabsorptive diarrhea and childhood-onset diabetes [42,43], but without congenital loss of pancreatic endocrine cells (as seen in the mouse). Nevertheless, the absence of enteroendocrine cells was noted in these individuals.

Other transcription factors are also expressed in the early endocrine cell population, and genetic deletion studies identified these factors to be crucial to endocrine cell differentiation. In particular, the transcription factor Isl1 (Isl1) is expressed in all mature, non-replicating islet cell types. Interestingly, Isl1 expression is also observed in the mesenchyme that surrounds the early dorsal pancreatic bud. The dorsal pancreatic mesenchyme does not form in Isl1-deficient mouse embryos, leading to a loss of exocrine differentiation in the dorsal pancreas; the pancreas is also devoid of all islets in these mice. Loss of the transcription factor Pax6 in the mouse leads to death shortly after birth. The pancreas of Pax6-deficient animals is devoid of α cells and has marked reductions in β, δ, and PP cells [44]. Whereas a human mutation in the ISL1 gene has been identified in a patient with type 2 diabetes [45], no link to diabetes has been observed in humans with mutations in the PAX6 gene. Therefore similar to Neurog3, the functional importance between lower organisms and humans may not be completely conserved for genes involved in endocrine differentiation.

**What makes a β cell?**

The insulin-producing β cell is perhaps the most intensely studied endocrine cell type, largely because of the implications for understanding the pathogenesis and treatment of diabetes. Many mouse models have clarified the factors necessary for β-cell differentiation, development, and maturation (see Table 4.2). One such factor is the basic helix-loop-helix transcription factor Neurod1. Interestingly, Neurod1 is expressed in all endocrine cell types except the somatostatin-producing δ cell, and targeted disruption of the Neurod1 gene in mice results in severe reduction in α and β cells, and in neonatal diabetes owing to β-cell apoptosis [46]. The Maf family of transcription factors is also involved in the pathway of α- and β-cell differentiation. Both MafA- and MafB-deficient mouse models have pancreatic phenotypes. Loss of MafB leads to perinatal lethality and, although the total endocrine cell mass is unaffected, the pancreas shows reduced numbers of α and β cells [47]. By contrast, mice with a targeted deletion of gene encoding MafA are born viable and with normal islet cell numbers, but demonstrate β-cell dysfunction with advancing age, leading to glucose intolerance and diabetes [48]. In these mice, β-cell genes, including those encoding insulin, Neurod1, and Glut2, are significantly reduced. Owing to its importance in β-cell function, MafA is considered as a marker for mature, functional β cells.

Interestingly, a number of factors expressed broadly in early pancreas development become restricted to specific endocrine cell types during the secondary transition and acquire additional function in the differentiation or maturation of these cell types. One particular example is Pdx1. Whereas mice with a homozygous deletion of the gene encoding Pdx1 are born without a pancreas, haploinsufficiency of Pdx1 results in glucose intolerance [49,50]. Virtually identical phenotypes are observed in humans with respective homozygous and heterozygous mutations in Pdx1 [44]. After the pancreas is fully developed, Pdx1 is expressed primarily in β cells and is necessary for β-cell function, including transcriptional activation of several β-cell genes [51]. Nkx2.2 is another transcription factor that shows expression in the early pancreatic progenitors but becomes restricted to specific endocrine cell populations later in development [52]. Loss of Nkx2.2 in the mouse results in the complete absence of differentiated insulin-producing β cells, a significant decrease in α and PP cells, and a concomitant increase in ghrelin-expressing c cells [53].

Mouse models have also identified specific factors necessary for the differentiation of the glucagon-expressing α cell. In particular, deletion of the transcription factor Arx results in hypoglycemia and neonatal lethality. Given that Arx is expressed in all endocrine cells except the β cell, the pancreas of the Arx-null mouse has altered endocrine cell ratios: a complete absence of α cells and an increase of β and δ cells. Conversely, the mis-expression of the Arx gene in either Pdx1- or Pax6-expressing cells results in a loss of β and δ cells and an increase in α and PP cells [48]. Moreover, compound mutants have demonstrated the complexity of transcription factor interactions and the importance of Arx function to endocrine cell development. Specifically, deletion of the Arx and Pax4 genes result in the loss of α and β cells but an increase in δ cells [48], and the Arx/Nkx2.2
compounds and their effects on the pancreas.

The evidence for the relevance of transcription factors to human pancreas development and disease is highlighted by the discovery that mutations in a number of transcription factors identified to be important in pancreas development in the mouse also display pancreas-related phenotypes in the human. Mutations or deletions of many of these factors have been established as the cause of monogenic forms of diabetes known as maturity-onset diabetes of the young (MODY) [54], or as the cause of human syndromes that include diabetes [42,43,55–58] (Table 4.2).

**Translating pancreas development into cell-based therapies for diabetes**

The knowledge gained from decades of pancreas development research has stimulated the translational pursuit of engineering insulin-producing \( \beta \) cells *in vitro* for therapeutic purposes. Specific extrinsic factors, including GGF, RA, and inhibitors of BMP or Shh signaling, have been applied to mouse and human embryonic stem cells in culture to successfully drive these malleable cells toward a pancreas fate [54] (see Figure 4.3). More recently, success in the creation of pluripotent, embryonic-like stem cells from somatic cells has opened the possibility of generating patient-specific \( \beta \) cells as cell replacement therapies for diabetes [55]. To date, however, such techniques have resulted in compound or mixed populations of hormone-producing cells, which have little or no capacity for glucose-stimulated insulin secretion when generated wholly *in vitro*, suggesting that many intrinsic and/or extrinsic factors involved in the cytodifferentiation of pancreatic progenitors remain to be identified.

In the mouse, the genetic manipulation of certain intrinsic factors, that is, Pdx1, Neurog3, MafA [59], or the induction of differentiation of pancreatic progenitors remain to be identified. In the mouse, the genetic manipulation of certain intrinsic factors, that is, Pdx1, Neurog3, MafA [59], or the induction of severe pancreatic injury [60], has also identified the capacity of differentiated cells in the pancreas to be “reprogrammed” to \( \beta \) cells. Therefore the continued merging of developmental biology research with *in vitro* differentiation technology may produce the long awaited therapeutic cure for diabetes.

**Postnatal \( \beta \)-cell growth and maintenance**

Following the secondary transition, the total mass of the pancreas increases substantially, but \( \beta \) cells comprise only about 1–2% of this cellular mass in the mature adult pancreas. Despite this relatively small percentage, the states of \( \beta \)-cell mass and function represent perhaps the greatest determinants in overall glucose and lipid homeostasis in virtually all types of metabolic disorders [61]. Two fundamental concepts regarding postnatal \( \beta \) cells have emerged over the past two decades: (1) although \( \beta \) cells were considered to be a postmitotic cell type in an adult mammal, it is now understood that they indeed exhibit a slow rate of turnover that decreases with age [62], and (2) the mass and function of \( \beta \) cells (and therefore the balance between new cell formation and death) can be dynamically altered to an extent to compensate for the physiologic or pathologic state of the organism [60]. A major focus and area of controversy has been the mechanisms that underlie postnatal \( \beta \)-cell growth and maintenance. From the discussion in the preceding sections on prenatal development, it is evident that formation of most \( \beta \) cells during embryogenesis occurs through a process known as neogenesis, in which new cells arise from the differentiation of stem or progenitor cells. Although some studies suggest the existence of multipotent stem cells within the postnatal rodent pancreatic epithelium, such cells normally do not give rise to significant numbers of new \( \beta \) cells in adult animals.

**Neonatal \( \beta \)-cell turnover**

The neonatal period between birth and weaning in rodents is characterized by a high rate of \( \beta \)-cell turnover and net increase in \( \beta \)-cell mass. Turnover is defined as the dynamic formation and loss of cellular mass [62]. Because there is no

![Figure 4.3](image_url) *in vitro* differentiation of pancreatic \( \beta \) cells. A schematic representation of a five-step embryonic stem (ES) cell differentiation protocol that mimics *in vivo* events of pancreatic organogenesis and differentiation through addition of secreted signaling factors. Important tissue markers that are shared with development of embryonic tissues are listed below each stage. (1) ES are guided through the mesendoderm (ME) stage to definitive endoderm (DE), then (2) to primitive foregut tube (GT), (3) posterior foregut (PF), and (4) pancreatic endoderm (PE). (5) The factors regulating the final stage of differentiation into endocrine cells (EN), cannot be performed effectively *in vitro*, and cells are transplanted into a murine host to complete cytodifferentiation by receiving physiologic cues. Additional abbreviations: FBS, fetal bovine serum; Cyc, cycloamine (SHH antagonist); Nog, Noggin (BMP antagonist); B27, defined tissue culture supplement. Source: Kroon E, et al. *Nature Biotechnology* 2008;26:443–452. Reproduced with permission of Nature Publishing Group.
longitudinal, noninvasive way to measure β-cell turnover in a given animal, the techniques that estimate β-cell turnover are based on cross-sectional studies from cohorts of animals that analyze steady-state β-cell mass, new β-cell formation (primarily by replication), and β-cell death. Nonetheless, studies using thymidine analog (BrdU) incorporation estimate that in the neonatal rat the rate of β-cell replication is as high as ~20% new cells per day at 2 days of age, and falling to about ~10% new cells per day by the time of weaning [63]. By contrast, replication rates in adult rats and mice are much lower, in the range of 0–2% new cells per day [63,64]. Although replication is thought to be the primary source of new β-cell formation during this period, studies of thymidine analog incorporation cannot detect specific contributions from neogenesis, which is thought to play a role during the neonatal period [65]. Balancing this rate of replication is, in part, the rate of apoptosis that appears to be elevated during the neonatal period, with the frequency of apoptotic cells rising as high as ~4% (compared to less than 0.4% in adult rats) [63]. However, it should be noted that the true rate of β-cell death is very difficult to measure because dead/dying cells may be cleared more rapidly than can be measured by tissue morphometry, and other forms of death (necrosis) are not typically measured.

The foregoing studies in rodents appear to also reflect the dynamics of β-cell turnover in humans. Based on autopsy studies, the replication rate of β cells appears highest in children, especially infants (coincident with increases in β-cell mass during early life), then declines in adulthood [66]. Taken together, these studies suggest a dynamic remodeling of β cells and their mass in the neonatal/early postnatal period, and mechanisms underlying the increase in β-cell replication rate have been the focus of intense investigation [65]. The growth factors insulin and insulin-like growth factors (IGFs) are obvious candidates, given the autonomous production of both by β cells in the early postnatal period. Elimination of IRS-2 (a key protein in growth factor signaling) results in the failure to maintain β-cell mass in the face of increasing insulin resistance as mice age, suggesting a potential β-cell growth-promoting effect of this signaling cascade [67]. However, elimination of either of the two genes encoding mouse insulin, the insulin receptor in β cells, or the IGF-1 receptor in β cells in mice does not affect neonatal replication or accrual of β-cell mass [68]. Curiously, key cell cycle activators (Cyclins D1 and D2 and Cdk4) also appear dispensable for neonatal β-cell replication, but not for maintenance of β-cell mass in adulthood [69]. These results collectively suggest that the early signals driving replication of β cells during embryogenesis differ from signals that drive accrual of β-cell mass in early life (see later). The physiologic significance of the high neonatal β-cell turnover is a matter largely of speculation. Considering that neonatal islets show diminished or absent responsiveness to glucose-stimulated insulin secretion suggests that the neonatal turnover may be important for the eventual refinement and maturation of β cells [62]. Intriguingly, it has been suggested that this early turnover of β cells may result in exposure of β-cell autoantigens and trigger the pathogenesis of type 1 diabetes in susceptible individuals [70], although more recently this hypothesis has been challenged in mouse models [71].

β-Cell growth with aging

For many years it was speculated that β cells, much like neurons, were postmitotic and that their turnover in the mammal was minimal or zero. Over the last two decades, studies in mice have suggested a more dynamic picture, wherein β-cell mass can change in response to physiologic states such as growth, pregnancy, and obesity [72]. Following weaning in rodents, there is considerable increase in β-cell mass that reflects the increase in body mass and an adaptation to the needs for increased insulin release. β-Cell mass is reflective of the changes in β-cell formation, individual β-cell size, and β-cell death. Whereas replication can be fairly reliably estimated using thymidine analog incorporation strategies, the rate of death is much more difficult to measure, primarily because dying β cells are cleared from the islet rapidly and therefore are difficult to detect. Thus, in studies of mature rodents, the turnover of β cells is estimated in large part from rates of replication. Studies in rats have shown that β-cell mass increases in a near-linear fashion with body weight [63,73]. The replication rate of β cells declines with age (to ≤2% per day in 20 month-old animals), but does not approach zero, and β-cell volume increases with age. These results suggest a low, but clearly measurable, turnover of β cells in adult rats and a β-cell lifespan in the order of 1–3 months. Using a continuous BrdU labeling strategy in mice, a much lower rate of replication has been estimated in adult mice, leading to the conclusion that β-cell turnover is near zero [72]. Human β-cell mass accrual and replication rates are significantly more difficult to estimate, largely because cross-sectional data from a genetically diverse population must be extrapolated using static markers of replication (e.g. Ki67 or BrdU). Nevertheless, data from human autopsy samples suggest that there is accrual of β-cell mass with increasing body mass in children, with progressively decreasing potential for replication with age [66,74].

Compensatory β-cell growth: adaptation to demand

The concurrent growth of β-cell mass with growth of the organism is but one example of the capacity of β cells to adapt to the increasing metabolic demands of peripheral tissue. Physiologic states of tissue resistance to insulin action, such as obesity and pregnancy, pose similar challenges to the β cell. It was recognized as early as the 1930s from autopsy studies that the average size of the islets of Langerhans increases as humans become overweight [75]. Similar findings are seen in a variety of mouse, rat, pig, and other animal models of obesity, where both β-cell size and number are reportedly increased. The increase in β-cell mass in response to obesity reflects an adaptation to the increased insulin demands imposed by the resistance to insulin action in liver, muscle, and fat. Pregnancy
imposes a challenge on the β cell similar to obesity, as pregnancy causes a state of tissue resistance to insulin. In either obesity or pregnancy, inherent defects that prevent increases in β-cell mass and insulin release may be the underlying causes for the development of diabetes. For example, in mouse models haploinsufficient for the gene encoding Pdx1, there is impaired compensation for insulin resistance in terms of both β-cell mass and function, with ensuing glucose intolerance and diabetes [76]. Similarly, humans with heterozygous mutations of Pdx1 (a disorder known as maturity-onset diabetes of the young 4, or MODY4) develop diabetes with age, typically in adolescence or early adulthood [54]. In these individuals, it is thought that β-cell compensation for linear growth and/or age-related insulin resistance is impaired. Pdx1 is crucial not only in the regulation of genes encoding β-cell proteins that are important in insulin secretion, such as the glucose transporter Glut2, glucokinase, and insulin, but also in the regulation of genes that are downstream of the growth-promoting insulin receptor/insulin-like growth factor 1 (IGF-1) receptor signaling cascade [51].

Origins of new β cells in the adult: neogenesis, transdifferentiation, and replication

Considering the relatively small mass of β cells with respect to overall body mass, there has been a vigorous attempt over the last decade to define better the potential sources of new β cells in the growing mammal and to harness such sources for the creation of new β cells for those who are deficient. As discussed in the foregoing section, new β-cell formation was largely estimated by rates of β-cell replication, but excluded potential contribution from neogenesis. Therefore, if neogenesis were a major contributor to new β-cell formation, then rates of β-cell turnover were substantially underestimated. Speculation that a precursor β cell, or a true MPC, exists in the pancreas arose from early observations in rat models that new insulin-positive cells emanated from cells within proliferating [77]. The question of the origin of new β cells in models of pancreas regeneration has been addressed using lineage tracing analysis in mice to show that β cells arise almost exclusively by replication of preexisting insulin-positive cells rather than via neogenesis [78], a finding confirmed in subsequent studies in mice [79]. However, these findings do not exclude the possibility that a rare, insulin-positive cell type with high proliferative capacity (i.e., a cell type that would not be defined as a mature β cell) has the ability to serve as a MPC, or that under certain conditions other cell types within the pancreas (i.e., facultative stem cells) have the capacity to differentiate to β cells. Thus, investigators continue to posit the existence of these alternative cell types in the pancreas whose differentiation into mature β cells may recapitulate a pathway of transcription factor expression similar to that seen in development [80].

Because all pancreatic epithelial cell types arise from a common Pdx1-positive precursor, it has been proposed that mature pancreatic cells of either exocrine or endocrine origin may have the capacity to directly differentiate into β cells without the need for de-differentiation into a precursor form (a process known as “transdifferentiation”). In this respect, although lineage tracing analyses have all but ruled out the possibility that mature acinar cells transdifferentiate under normal conditions to β cells in mice [81], the ectopic expression of the key developmental transcription factors Pdx1, Neurog3, and MafA in acinar cells enables a program that allows their conversion to insulin-expressing cells [59]. Similarly, under specific experimental conditions in mice, mature α cells have the capacity to transdifferentiate into β cells [48]. Taken together, these studies reinforce the theme that different mature cell types of the pancreas that arise from a common origin have the capacity to exhibit phenotypic characteristics of one another, and leave open the possibility that under specific conditions such cell types may transdifferentiate to offset loss of β-cell mass.

Whether any of the mechanisms discussed earlier—neogenesis or transdifferentiation—play a role in human β-cell replenishment remains uncertain. Interestingly, studies in vitro suggest the potential existence of precursor cell types in the human pancreas [80], but it is unknown whether and to what extent such cells give rise to β cells normally in humans. To date, the best available data indicate that replication of preexisting β cells is the likely mechanism for accrual of β-cell mass during human growth [66,74,82].

Regulators of β-cell growth: growth factors and cell cycle regulators

A host of circulating factors appears crucial in the stimulation of early postnatal β-cell growth. As the growth of β cells closely parallels the growth of the organism during this early phase, it is relevant to note that nutrients, particularly glucose, remain among the most important factors contributing to β-cell replication during this period. Thus, intravenous glucose infusions for even short time periods (96 hours), which only mildly increase serum glucose concentrations, result in fivefold increases in β-cell replication in young mice [83]. Although an effect of glucose to directly stimulate β-cell replication has been proposed, it is possible that its effect may be caused by its stimulation of insulin release from β cells, such that insulin in an autocrine manner serves as the mitogen. Insulin and insulin-like growth factor 1 are classic growth factors that signal through related transmembrane receptors with associated receptor tyrosine kinases. Mice lacking the insulin receptor in β cells display impaired insulin release associated with reduced β-cell mass, whereas mice lacking the IGF-1 receptor in β cells display impaired insulin release without associated loss of β-cell mass. Interestingly, loss of both the insulin receptor and IGF-1 receptor in β cells results in severe reductions in β-cell mass and frank diabetes [68]. These data suggest that the insulin and IGF-1 signaling pathways function in distinct, but complementary ways, notwithstanding that both receptors share similar downstream signaling molecules (insulin receptor substrate proteins, phosphatidyl inositol-3 kinase, and protein
kinase B). In recent years, a host of other growth factors has also been shown to positively influence β-cell replication and/or function (see Table 4.3), and include factors released not only from the islet, but also from a variety of organs, such as bone (osteocalcin), the anterior pituitary (growth hormone, prolactin), gut (glucagon-like peptide 1), fat (leptin, adiponectin), and brain (serotonin). Whereas these metabolites and growth factors can directly or indirectly impact β-cell replication, it should be noted that their effects are much greater in younger mice and humans and much less so as aging occurs.

Although the effects of the aforementioned growth factors result in enhanced β-cell replication and insulin release, the pathways leading to activation of cellular replication machinery differ depending upon the factor [68]. Nevertheless, all factors ultimately impinge upon the components of the cell cycle. Transit through the cell cycle requires the β cell to exit the resting state (G0) and traverse G1, S, G2, and M states [82]. For the most part, replication of β cells is largely driven by factors that control the G1/S transition of the cell cycle. Genetic manipulation studies in mice have emphasized the importance of not only activators of the G1/S transition, but also inhibitors, such that the balance between the two appears to regulate the overall drive for β-cell replication. Cyclins and cyclin-dependent kinases (Cdks proteins) are major activators of β-cell replication. Cyclins and Cdks negatively regulate the major pocket protein known as pRb, which functions as a “molecular brake” on the G1/S transition. Cyclins and Cdks appear crucial in the accrual of early postnatal β-cell mass, but interestingly not in the generation of β-cell mass in the embryo. Mice homozygous null for the gene encoding CyclinD2 or Cdk4 exhibit no alterations in β-cell mass at birth, but show loss of mass accrual with age [84,85]. Similarly, loss of the gene encoding CyclinD1 gene does not affect embryogenesis, but heterozygosity of the CyclinD1 gene in combination with homozygous loss of CyclinD2 results in even further loss of β-cell mass with age and severe, life-threatening diabetes [84]. The cyclin-dependent kinase inhibitors (CKIs) — including p15Ink4b, p16Ink4a, p18Ink4c, p19Ink4d, p21Cip, p27Kip1, and p57Kip2 — are major negative regulators of β-cell proliferation, and their actions appear to predominate in later life, where these factors may be responsible for inhibition of β-cell proliferation in aging mammals [82].

Recent studies have clarified the human islet G1/S cell cycle protein expression pattern [86]. Whereas murine and human islets differ in their expression of the G1/S cell cycle activator Cdk4 (humans express Cdk6), they have virtually all G1/S CKIs in common. This latter observation may be crucial in the understanding of why β-cell replication is so dramatically reduced in aging humans. A particularly intriguing target in this respect is p16Ink4a, whose expression in β cells is up-regulated as mice age, and may serve as a target to prevent the age-induced limitations in β-cell mass [87]. The potential for β cells to undergo uncontrolled replication as a result of deregulation of G1/S cell cycle proteins is dramatically emphasized by mutations in the gene encoding Menin in both mice and humans. Menin is a tumor suppressor transcription factor that negatively regulates the expression of p18Ink4c and p27Kip1, and its absence or mutation results in the tumorigenic transformation of a variety of endocrine tissues (including β cells) in a syndrome known as multiple endocrine neoplasia 1 (MEN1) [88].

**Conclusions and areas of future study**

In the early twentieth century, the discovery of insulin dramatically transformed the treatment of diabetes mellitus. Indeed, it was thought that the administration of insulin might reduce stress and allow for the time necessary to regrow new β cells, a consequence that was never observed. In the ensuing decades, the incidence of type 2 diabetes rose to dramatic proportions, and as a result the quest for β-cell-based therapies for diabetes has seen broader appeal. As discussed, more recent research has led to dramatic insights into pancreas and β-cell development, and into the postnatal life cycle of the β cell. Although most of these insights derive from studies in lower animal species, their applicability to the treatment of human diabetes mellitus has risen to the forefront of discussion in recent years. Importantly, we know now that although β cells have the capacity to expand in the postnatal period, in humans the window for such expansion may be limited to the first 2–3 decades of life, and thereafter the ability to compensate for...

**Table 4.3** List of major factors that promote growth and function of β cells

<table>
<thead>
<tr>
<th>Category</th>
<th>Factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolites</td>
<td>Glucose</td>
<td>[89]</td>
</tr>
<tr>
<td></td>
<td>Amino acids</td>
<td>[90]</td>
</tr>
<tr>
<td>Pituitary/Neuropeptides</td>
<td>Growth hormone</td>
<td>[91]</td>
</tr>
<tr>
<td></td>
<td>Prolactin/placental lactogen</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>Melanin concentrating hormone</td>
<td>[93]</td>
</tr>
<tr>
<td>Neurotransmitters</td>
<td>Serotonin</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>GABA</td>
<td>[95]</td>
</tr>
<tr>
<td>Adipokines</td>
<td>Leptin</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>Adiponectin</td>
<td>[97]</td>
</tr>
<tr>
<td>Gut-related factors</td>
<td>GLP-1</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>GIP</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>Gastrin</td>
<td>[99]</td>
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<tr>
<td></td>
<td>CCK</td>
<td>[100]</td>
</tr>
<tr>
<td>Growth factors</td>
<td>Insulin</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>IGF-1</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>EGF/Betacellulin</td>
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<tr>
<td></td>
<td>FGF</td>
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<tr>
<td></td>
<td>VEGF</td>
<td>[102]</td>
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<tr>
<td></td>
<td>HGF</td>
<td>[92]</td>
</tr>
<tr>
<td>Bone-related factors</td>
<td>PTHrP</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>Osteocalcin</td>
<td>[103]</td>
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</tbody>
</table>
physiologic stressors (such as obesity) diminishes with age. As such, strategies for therapies for diabetes in the future may well focus on ways to enhance β-cell replication or to engineer new β cells. With respect to the latter, studies of embryonic development have enabled important strides in generating β-like cells from primitive biologic precursors (e.g., human embryonic stem and induced pluripotent cells). Yet, these engineered cells do not exhibit the full phenotypic spectrum of true β cells, such as the ability to release insulin in response to a physiologic glucose challenge. The knowledge that all cells of the pancreas arise from a common progenitor has raised awareness that plasticity of fully differentiated pancreatic cell types may be much greater than originally thought. In this respect, studies of transdifferentiation of other abundant pancreatic cell types (such as α cells or acinar cells) to β cells in vivo may hold promise for the treatment of diabetes, but to date no clear examples of human cell transdifferentiation have emerged. As the burden of diabetes increases, the need to translate research from lower animals to humans increases, and in the coming years it is likely that the generation of better model systems that mimic the human condition will become a greater priority.

References


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CHAPTER 5
Pancreatic morphology in normal and diabetic states

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Key points
• The pancreas comprises exocrine and endocrine compartments, which produce and release digestive enzymes and glucoregulatory hormones, respectively.
• The pancreatic islet is predominantly composed of endocrine (β, α, δ, F, and ε) cells, but is also supplied by an extensive capillary network and receives both parasympathetic and sympathetic innervation.
• The morphologic arrangement of these numerous cell types within the islet, and intercellular communication between them appears to be critical for normal endocrine function.
• The islet extracellular matrix is an emerging site of regulation of β-cell function and survival, and is disrupted or degraded in diabetes.
• In type 1 diabetes, an almost complete obliteration of β cells occurs, predominantly via T-cell-mediated autoimmune destruction.
• In type 2 diabetes, pancreas weight is relatively normal, but loss of β cells also occurs, albeit to a lesser extent than in type 1 diabetes.
• β-Cell loss also occurs in cystic fibrosis-related diabetes, suggesting that exocrine pancreatic abnormalities can have profound effects on the islet.
• In type 2 diabetes, the etiology of β-cell demise is complex. Nutrient excess, amyloid deposition and inflammation have all been proposed as underlying mechanisms of β-cell loss.

Pancreatic anatomy and morphology

The pancreas is located in the upper abdominal cavity, in close proximity to both the duodenum and spleen (Figure 5.1(a)). In humans, pancreatic weight ranges from 40 – 150 g [1,2]. Embryonically, the pancreas develops from two separate buds of the primitive foregut, yielding duodenal (ventral) and splenic (dorsal) lobes [3]. Once developed, these constitute the head and body/tail, respectively. These regions of the pancreas are similar, aside from some differences in the distribution and composition of pancreatic islets (discussed later).

Functionally, the pancreas comprises two independent compartments, the exocrine and endocrine pancreas, which derive from common endodermal precursor cells during development [3]. The exocrine pancreas accounts for the vast majority of pancreatic mass and consists of lobules, each comprising acini that connect into a network of ducts (Figure 5.1(b)). Acinar cells demonstrate a characteristic morphology at the electron microscopy level including electron-dense zymogen granules and an extensive endoplasmic reticulum (ER) network. This ultrastructural organization is consistent with the chief function of the exocrine pancreas, namely to secrete digestive enzymes including amylase and trypsin via the pancreatic ductal system into the gut. The endocrine pancreas comprises islets of Langerhans, roughly spherical structures that contain hormone-producing cells. Islets constitute only a small minority of pancreatic mass but are critically important for metabolism throughout the body, and especially for maintenance of glucose homeostasis. This was first shown in 1890 by studies in which pancreatectomy in dogs resulted in diabetes [4]. In the human pancreas, it has been estimated that there are one to two million islets scattered throughout the exocrine pancreas, which together comprise approximately 2% of pancreatic mass [5,6]. While the function of these two pancreatic compartments differ significantly, they exist in close proximity to one another, and evidence exists for interactions between them. Specifically, islets are distributed throughout the exocrine pancreas. Islet hormone-rich blood perfuses the exocrine pancreas [7], and the islet hormones, insulin and pancreatic polypeptide, have been shown to stimulate amylase secretion from the exocrine pancreas, while glucagon inhibits amylase secretion [8–10]. This suggests that the functional status and viability of the islet can influence function/viability of the exocrine pancreas and potentially vice versa.
Chapter 5

Islet composition and morphology

Despite the relatively small size of pancreatic islets, each comprising approximately 2000–4000 cells [11], every one is a complex mini-organ, containing numerous cell types (Figure 5.1(c)). Endocrine cells are, not surprisingly, the most abundant islet cell type and are characterized at the ultrastructural level by the presence of numerous secretory granules that contain endocrine hormones packaged and stored ready for immediate release in response to the appropriate stimulus. Additionally, endocrine cells contain a high density of mitochondria and abundant ER (although not so dense as the surrounding acinar cells). These endocrine cells are subcategorized based on their predominant hormone constituent, as follows.

Insulin-producing β cells are the predominant islet cell type. They were first identified in 1907 by silver staining [12] and were the second islet endocrine cell type to be described. β Cells comprise 50–80% of islet volume, depending on the species in question; rodent islets typically have a higher proportion of β cells (around 60–80%) [13,14], while nonhuman primate and human islets have a lower relative proportion (around 50–60%) [13–19] (Figure 5.2). The distribution of β cells within islets also differs among species. In rodents β cells are predominantly located in the core of the islet [13,14,19], while in nonhuman primate and human islets they are more evenly distributed throughout the islet (Figure 5.1(c)) [13,14,18]. Regardless of their location within the islet, for all species β-cell secretory granules have a characteristic appearance at the electron microscope level, with an electron-dense core that arises due to the formation of insulin hexamers which are cross-linked with zinc [11]. This granule core is surrounded by an electron-lucent “halo” [11]. Release of insulin from β cells is critically important in regulating blood glucose levels, predominantly acting to suppress hepatic glucose production and to enhance glucose uptake in insulin-sensitive tissues such as skeletal muscle and adipose tissue. Mechanisms of insulin release are discussed in detail in Chapters 7, 8, and 9. In addition to insulin, β cells also produce islet amyloid polypeptide (IAPP), which is co-localized with insulin in secretory granules and is therefore co-secreted with insulin [20].

Glucagon-producing α cells were actually the first islet endocrine cell type to be described and are so called due to the alcohol fixation (“A”-cells) that was used to identify them [12].
These form the second most abundant endocrine cell type in the islet, but are far less abundant than \( \beta \) cells. In rodent islets, they account for around 15% of islet area [19], whereas in humans they are more abundant (around 35%; [13]) (Figure 5.2). Also, like \( \beta \) cells, \( \alpha \) cells differ in their distribution within islets across species. In rodents, they are located almost exclusively in the mantle of the islet [13,14,19], while in human and nonhuman primate islets they are more evenly distributed throughout the islet (Figure 5.1(c)) [13,14,18]. Unlike \( \beta \) cells, whose abundance in islets is relatively constant throughout the pancreas, islets in the head of the pancreas are richer in \( \alpha \) cells than those in the tail [19]. Glucagon-containing secretory granules differ in their appearance compared to insulin-containing granules, in that they lack an electron lucent halo. Thus, the entire secretory granule in the \( \alpha \) cell is electron dense [21]. The hormonal effects of glucagon act to oppose those of insulin, stimulating hepatic glucose production, and being a critical mediator of the restoration of normal glucose levels following hypoglycemia. Glucagon secretion and its effects are described in Chapter 10.

**Somatostatin-producing \( \delta \) cells** comprise the third islet endocrine cell type, being lower in abundance (<10% of islet cells) than either \( \beta \) or \( \alpha \) cells (Figure 5.2). Their distribution within islets is similar to that of \( \alpha \) cells, and in rodent islets \( \delta \) cells occur predominantly in the mantle of the islet, while in primates and humans they are more evenly distributed throughout [13,14,18]. While few studies have systematically assessed \( \delta \)-cell distribution in islets throughout the pancreas, \( \delta \)-cell number does not appear to be markedly altered in islets from different regions of the pancreas [19]. Ultrastructurally, somatostatin-containing granules are homogeneous in appearance, similar to \( \alpha \)-cell granules, but slightly less dense. The effects of islet-derived somatostatin are thought to be largely paracrine in nature due to its short half-life [22]. Somatostatin acts on receptors present on \( \beta \) and \( \alpha \) cells, inhibiting both insulin and glucagon release.

**Pancreatic polypeptide-producing \( F \) cells** are less abundant still than \( \delta \) cells, contributing on average less than 5% of islet cells. Again like \( \alpha \) cells, their distribution differs throughout the pancreas, with islets in the head of the pancreas containing most of the pancreatic polypeptide-producing \( F \) cells [19,23]. Thus, islets in this region of the pancreas can contain up to 20% pancreatic polypeptide-containing cells, at the expense of \( \alpha \) cells, which are decreased in this region. Conversely, in the rest of the pancreas, \( F \) cells only account for a 1–3% of islet cells [19,23] (Figure 5.2). \( F \)-cell granules are small, but similar in appearance to \( \beta \)-cell granules, containing an electron-dense core and electron-lucent halo. The release of pancreatic polypeptide from islets serves as an indicator of vagal outflow [24]. However, the function of pancreatic polypeptide, once released, is unclear. Some studies have suggested a role in inhibition of pancreatic enzyme and bile secretion, while others have suggested a role in modulation of food intake and energy expenditure [9,25].

**Ghrelin-producing \( \varepsilon \) cells** constitute the final known population of islet endocrine cells. These were first described during embryonic development [26], and were originally thought to reflect a population of multihormone positive cells. However, they have been shown to be present in adult islets, and are now considered a *bone fide* islet cell type [26]. Several studies have shown that ghrelin can suppress insulin release in humans and rodents [27,28]. However, whether this occurs due to a paracrine effect of islet-derived ghrelin or due to circulating ghrelin, which is largely produced in the stomach, is unclear at this time.

**Islet vasculature.** The islet is richly vascularized (Figure 5.3); while islets comprise only ~2% of pancreas volume, they receive approximately 15% of the blood flow [29]. Arterioles enter the islet and branch into tortuous capillaries, which have been suggested to contact almost every endocrine cell in the islet. These then converge on collecting venules outside the islet [30]. Blood flow through the islet is thought to occur in two distinct patterns: first, arterioles penetrate the islet core and blood flow then emanates from the center of the islet outward. Second, blood flow can also proceed from one side of the islet to the other. These two patterns of blood flow appear to occur in islets from the same pancreas, with the former pattern being more frequent [31,32]. The concept that islet blood flows predominantly from the center to the mantle of the islet suggests that \( \beta \) cells are perfused

![Figure 5.3](image.png) Scanning electron micrograph of a corrosion cast of a rat islet showing its extensive capillary network. Source: Adapted from Bonner-Weir 1982 [30]. Reproduced with permission of American Diabetes Association.
first [33]. Thus, α cells are exposed to high concentrations of insulin as they lie downstream of β cells in the islet vasculature, which tonically inhibit glucagon secretion. δ Cells are thought to be downstream of both β and α cells, consistent with the notion that somatostatin’s effects to inhibit insulin and glucagon secretion occur in a paracrine, rather than endocrine manner. While the distribution of endocrine cells within nonhuman primate or human islets differs from that of rodents, it is likely that the same directional vascular supply exists [34,35].

Interestingly, there are differences in islet vascular density among species. Rodent islets contain a dense network of small capillaries [30,31], while human islets appear to contain fewer, larger capillaries (Hull, Brissova, Powers, unpublished observation) [36]. However, the functional consequence of this difference in capillary density is unknown. Islet capillaries are lined by a highly fenestrated endothelium, with islet endothelial cells containing around 10 times more fenestrae than capillaries in the neighboring exocrine pancreas [37]. This fenestration allows rapid exchange of nutrients and oxygen between blood and islet cells. However, in contrast to the liver which contains open fenestrae in its endothelium, islet endothelial fenestrae are gated; that is, covered by a glycocalyx, a semipermeable layer composed predominantly of the polysaccharide heparan sulfate [38]. This suggests some selectivity exists with respect to the molecules that can readily pass in and out of the islet capillary, although this is poorly understood. While the islet vasculature is critically important for providing adequate blood flow, supplying nutrients to islet cells and facilitating delivery of islet hormones to peripheral tissues, islet capillaries also provide important signals for normal islet endocrine growth and survival [39–41]. Finally, a vascular basement membrane, a specialized form of extracellular matrix, exists between islet capillaries and endocrine cells [36,37,40,42–44]. While this extracellular matrix comprises predominantly collagen IV and laminins, it also contains a complex array of other proteins and proteoglycans including heparan sulfate proteoglycans, nidogens and hyaluronan which provide both structural support along with critical signals to both islet endothelial cells and endocrine cells, and participates in maintenance of normal function and proliferation of islet cells [40,45,46].

Islet innervation. The islet receives extensive autonomic input, via both sympathetic and parasympathetic branches of the autonomic nervous system [47]. These nerves do not form classical synapses with islet endocrine (or other) cells, but form terminals that release neurotransmitters in close proximity to islet cells which in turn act as important regulators of islet endocrine hormone release [47]. Islet innervation and its functional consequences are reviewed in detail in Chapter 9. Morphologically, islet innervation mirrors that of vascularization, with nerve fibers running parallel to islet capillaries [48]. Accordingly, rodent islets containing numerous, fine nerve fibers [48], while in contrast, human islets contain fewer, larger nerve fibers [49] (Figure 5.4).

Interactions among islet cell types. This highly ordered distribution of islet cell types has functional consequences. Islet cell types interact with one another by a number of different mechanisms including direct cell–cell contact, release of paracrine signals or via the extracellular matrix. For example, signaling via gap junctions is important for coordinating insulin release [50], while autocrine and paracrine signals such as GABA or somatostatin can enhance or suppress islet hormone release from neighboring endocrine cells [22,51,52]. Exposure of islets to neurotransmitters (reviewed in Chapter 9) or endothelial-derived factors such as hepatocyte growth factor, laminin or thrombospondin-1 can stimulate β-cell secretory

![Figure 5.4 Innervation of a mouse (a) and human (b) islet, visualized using the axonal marker synapsin I/II. Source: Adapted from Rodriguez-Diaz 2011 [49]. Reproduced with permission of Elsevier.](image-url)
function and/or replication [41,45,53]. Conversely, β cells produce factors such as vascular endothelial growth factor, which are essential for islet endothelial cell viability and function [40,41]. Culture of β cells on extracellular matrix has profound effects to enhance islet cell proliferation, survival and function, suggesting another mechanism by which endocrine cells can be influenced by the islet vasculature [40,54]. Thus, changes in the abundance or organization of any one of the multiple islet cell types, or in exocrine pancreatic viability and function, likely has significant consequences for islet health and function and ultimately for glucose homeostasis.

**Islet β cell regenerative potential**

As discussed in Chapter 4, development and organization of islet endocrine cells occurs during embryogenesis and the early post natal period [3]. In rodents, continued β-cell expansion can occur into adulthood [55]. However, the ability of rodent β cells to replicate is severely limited with increasing age [56]. In humans, there is some plasticity in islet volume, particularly in very young individuals [57], but there is very limited regenerative potential of β cells in adults [57,58]. Physiologic stimuli such as insulin resistance, obesity, and pregnancy have been reported to result in increased β-cell mass in humans and rodents [59–62]; however, pancreatectomy, which is known to stimulate β-cell regeneration in young rodents [56,63], does not result in increased β-cell replication in older rodents [56] or adult humans [64]. Altering islet volume appears to be an exquisitely regulated process; in line with the requirement for maintenance of the organization of islet cell types, β-cell replication in rodents during pregnancy is preceded by islet angiogenesis (expansion of islet capillaries), suggesting that expansion of the islet vascular supply is required to allow expansion of the endocrine cell population [41]. Maintenance of the appropriate proportions and organization of islet endocrine cell populations in the face of islet expansion also occurs in response to high fat feeding in mice [65]. Islet innervation is also increased under conditions of high fat feeding and insulin resistance [66,67]. However, despite the ability of the islet cell population to expand in response to some physiologic stimuli, the limited capacity for β-cell expansion, especially with age, becomes a major problem in disease states where β cells are targeted for destruction.

**Disturbances in pancreas/islet morphology in diabetes**

**Type 1 diabetes**

Type 1 diabetes (T1DM) is classically associated with autoimmune destruction of β cells [68] (Figure 5.2). However, the pancreas is more broadly affected, with overall pancreas size being decreased in individuals with this form of diabetes [68,69], and loss of exocrine tissue occurring close to areas of immune infiltration [70]. β-Cell destruction is largely a T-cell-mediated process, involving mainly CD8+ cells, but also including CD4+ cells and other immune cells such as macrophages and B cells [71,72]. Lymphocytic infiltration of islets is well documented in animal models [73,74]. However, the degree of infiltration can vary widely even among islets from the same animal in various stages of diabetes development [73,74]. Further, the extent of leukocyte infiltration in humans appears to be less than that seen in animal models, while the variability in affected islets is similar [70,72,75]. In human T1DM, insulitis is primarily reported in individuals with recent onset disease [70,76], although it has been detected in patients 8 years following diagnosis [72,76]. That insulitis occurs predominantly around the time of disease onset is consistent with the clinical observation that the largest decline in C-peptide responses occurs between 6 months prior to and 12 months following disease diagnosis [77,78]. Despite the variability in detectable insulitis, autoimmune destruction appears to result in eventual elimination of the majority of β cells [70,79]. However, β cells can persist for many years into the course of the disease [79,80] and low levels of β-cell replication have been documented in some [81], but not all studies [82]. Further, there is evidence for residual insulin release many years after the development of hyperglycemia [83]. This raises the possibility that β-cell destruction may not be complete and that regeneration may be possible. A recent study documenting the efficacy of stem cell therapy in rapidly reversing T1DM, in many cases up to 36 months of follow-up, provides support for this concept [84].

While β-cell destruction is widespread in T1DM, non-β-cell islet populations, particularly α cells, appear to be spared the autoimmune destruction [70]. However, despite the persistence of α cells in T1DM, their function is undoubtedly dysregulated. Specifically, meal-stimulated glucagon responses are exaggerated [85], while glucagon release in response to hypoglycemia is markedly impaired [86]. These abnormalities may be due to the lack of oscillating insulin levels, which would normally act to regulate glucagon release [87]. However, the lack of glucagon response to hypoglycemia is likely also impacted by the early loss of sympathetic nerve terminals in islets, which has been demonstrated in rodent models of T1DM [88,89] and in human T1DM [90]. This islet neuropathy is selective, with islet parasympathetic innervation appearing to be normal, at least in rodent models of T1DM [90].

Whether islet capillary density is altered in T1DM is currently unknown. Recently however, significant alterations in the extracellular matrix closely apposed to the islet vasculature have been described in human T1DM and animal models thereof [36,42]. Degradation of peri-islet extracellular matrix has been shown to correlate with leukocyte infiltration and β-cell loss in human T1DM and NOD diabetic mice [36,42]. Interestingly, however, once insulitis is resolved, peri-islet extracellular matrix is regenerated, even in the absence of insulin-positive cells, providing further support for a role of leukocytic infiltration in
the degradation of this extracellular matrix. Altered localization of the extracellular matrix component hyaluronan [91,92] and increased production of extracellular matrix degrading enzyme heparanase [93] have also been described in association with lymphocytic infiltration of islets in NOD diabetic mice, in common with other autoimmune diseases [94]. The contribution of these changes in extracellular matrix to diabetes onset and progression are not fully understood at present, but they may be important in allowing leukocytes to gain access to the islet, and are an active area of investigation.

**Type 2 diabetes**

Macroscopically, the pancreas appears largely unchanged in T2DM. Fibrosis in the exocrine pancreas has been described [95], suggesting some abnormality in the exocrine pancreas, but this has not been widely studied. In contrast, the presence of morphologic abnormalities in islets from subjects with T2DM has long been established. More than a century ago, Opie described decreased cell number and accumulation of what was later identified as islet amyloid [96]. Subsequently, it was confirmed that islet β-cell volume is decreased in T2DM [59,97], an observation has been reproduced in numerous studies, across several ethnic groups [15–17] (Figure 5.2). Butler et al. additionally showed that β-cell volume is also decreased in subjects with impaired fasting glucose, with the extent of reduction being intermediate between that of subjects with T2DM and nondiabetic controls [15]. Overall, the extent of β-cell loss reported varies widely among studies (0–63% reduction), most likely due to the variability of β-cell volume among subjects [16,17] and also to the site of sampling [16]. Similar to the situation in T1DM, islet α-cell mass has been shown to be maintained in T2DM, resulting in a relative increase in the α:β cell ratio [2,95,98]. In animal models, islet glucagon and pancreatic polypeptide immunoreactivity have been reported to be similar or increased relative to nondiabetic animals [99,100], while somatostatin immunoreactivity is more variable, being reportedly increased, similar or decreased in comparison to nondiabetic animals [99–101].

Alterations in density and/or morphology of islet capillaries have been described in a variety of rodent models of diabetes. Early in the course of hyperglycemia, distorted islet capillary morphology is present and with more advanced diabetes, loss of capillary density occurs and is frequently associated with islet fibrosis [102–108]. No published studies have been performed on human pancreas specimens, but our unpublished data suggest that while islet capillary morphology is distorted, islet capillary density is not decreased in T2DM relative to nondiabetic controls (Brissova, Powers, Hull, unpublished observation). Decreased islet innervation has also been reported in animal models of T2DM [109], but has not been determined in humans with the disease. Abnormalities in islet extracellular matrix have also been documented in human T2DM and animal models thereof. These include accumulation of islet amyloid, which comprises the aggregated form of the β-cell peptide IAPP [17,95,110], and islet fibrosis occurring due to fibrillar collagen deposition [111,112].

**Influence of exocrine pancreas abnormalities on islet morphology and function**

Diseases affecting the exocrine pancreas are associated with diabetes. Acute pancreatitis has been associated with glucose intolerance and impaired insulin release, but this disturbance seems to be temporary [113], suggesting that exocrine pancreas abnormalities can impair islet function. In cases of chronic pancreatitis whose primary disease etiology is exocrine in nature, diabetes is present in the majority of cases [114]. However, pancreatitis is also more common in individuals with T2DM [95,115], making the link between exocrine disease and the subsequent onset of diabetes less clear.

Cystic fibrosis is an autosomal recessive disorder, arising due to one of several mutations in the cystic fibrosis transmembrane receptor (CFTR), a chloride channel, with disease onset usually occurring in childhood [116]. Lung disease is the primary manifestation of CFTR mutation. However, with improved treatment including lung transplantation, survival has significantly improved in recent years; as a result, other complications of cystic fibrosis are now more common. Pancreatic involvement, namely significant exocrine pancreas fibrosis is the second most common feature of cystic fibrosis, after lung pathology. Accordingly, cystic fibrosis-related diabetes complicates a large proportion of cystic fibrosis cases [117]. This form of diabetes does not seem to include underlying autoimmunity, suggesting its etiology differs from that of T1DM [118].

Unlike T2DM, insulin resistance does not appear to be a major underlying cause [119,120]. However, defective insulin release has been clearly demonstrated [119,120]. This is accompanied by decreased islet β-cell volume, which has been documented in several studies [121,122]. The mechanisms of β-cell loss in cystic fibrosis-related diabetes remain unclear, although islet amyloid deposition is also present in this population [123], suggesting that at least certain aspects of islet pathology share features with T2DM. Thus, some mechanisms that may explain β-cell loss in T2DM, and which are discussed later, are likely also pertinent to cystic fibrosis-related diabetes.

**Mechanisms of β-cell loss in type 2 diabetes**

While alterations in several islet cell types have been reported in T2DM or animal models thereof, only β cells have reproducibly been shown to be reduced [15–17,59,96,97]. Decreased β-cell volume in T2DM is associated with an increase in β-cell apoptosis [15,17], which occurs without a compensatory increase in β-cell replication due at least in part to the limited regenerative capacity of adult human islets [57,58]. Thus, mechanisms that result in β-cell apoptosis or other forms of β-cell death appear
to be critical for loss of β cells in T2DM. This process has been widely studied, and numerous mechanisms have been implicated.

**Chronically elevated glucose and/or free fatty acids**

Type 2 diabetes is characterized by increased circulating nutrients including glucose and free fatty acids (FFA). The literature clearly shows that chronic exposure of β cells to elevated glucose results in impaired β-cell function [124–126], but the data regarding cellular toxicity in response to this nutrient are more mixed. Exposure of cultured β-cell lines or islets to high glucose can, in some cases, result in increased β-cell death [127–132]. This may result from oxidative stress, activation of Fas receptor-mediated or mitochondrial apoptosis and may involve thioredoxin interacting protein (TXNIP) [127–129]. However, this effect of glucose to induce apoptosis is not a universal finding; several studies have demonstrated that the “toxic” effects of chronic hyperglycemia to impair β-cell function are reversible even after several weeks in culture [133,134]. Further in vitro and in vivo studies exposing β cell to elevated glucose have shown beneficial effects with glucose-promoting survival signals, suppressing apoptosis [135] or resulting in increased β-cell replication [136–138].

Exposure of islets/β cells to increased FFA levels alone, or in the presence of hyperglycemia results in impaired insulin release [139–141]. Culture of β cells in the presence of increased FFA, particularly palmitate, can also result in β-cell apoptosis [132,142–144]. This has been shown to occur via some of the same mechanisms as glucose-induced apoptosis, namely oxidative stress, ER stress and activation of the mitochondrial apoptosis pathway, and additionally may require increased ceramide or nitric oxide levels. However, similar to the observations with elevated glucose, high fat feeding or lipid infusions in vivo result in increased β-cell mass, as a result of increased β-cell replication [62,138].

Thus, taken together, the effects of nutrient excess appear to be more detrimental to β-cell secretory function rather than clearly inducing β-cell death, and some of these effects may be reversible.

**Islet amyloid**

As mentioned, amyloid deposition occurs in islets in the majority of subjects with T2DM, as well as in subjects with cystic fibrosis-related diabetes [17,95,110,123]. Accumulation of islet amyloid occurs due to the aggregation of the normally soluble β-cell peptide IAPP, which is then deposited in the islet extracellular matrix, between islet capillaries and β cells. This aggregation only appears to occur under conditions of diabetes or islet dysfunction, with islet amyloid being relatively rare in individuals without diabetes even in individuals with extremely high circulating levels of IAPP [110,145,146]. The underlying cause of this IAPP aggregation is unclear, but may involve impaired processing of IAPP from its precursor proIAPP [147–149] and/or interaction between IAPP and extracellular matrix components, principally heparan sulfate proteoglycans [150–153]. Human autopsy studies have yielded somewhat conflicting results, but the literature clearly demonstrates that the extent of islet amyloid deposition is associated with decreased β-cell volume [17,154] and increased β-cell apoptosis [17] (Figure 5.5). Studies using cultured human islets and transgenic animals expressing human IAPP (mouse and rat IAPP are not amyloidogenic) have further elucidated the mechanism(s) by which IAPP aggregation may elicit β-cell toxicity. Culture of human or transgenic mouse islets under conditions that favor amyloid formation, for example high glucose, result in amyloid-induced oxidative stress and increased β-cell apoptosis, thereby leading to a reduction in β-cell area [155–160]. This β-cell loss can occur via activation of the cell surface death receptor Fas [161], or cJun N-terminal kinase (JNK) and downstream activation of apoptosis [162]. Additionally, when human IAPP aggregation and thereby amyloid formation is

![Figure 5.5 Relationship between islet amyloid deposition and decreased β-cell area (a) (r = -0.76, p < 0.001), and increased β-cell apoptosis (b) (r = 0.56 and p < 0.01) in human autopsy pancreas specimens from subjects with type 2 diabetes (circles) and nondiabetic controls (triangles). Source: Adapted from Jurgens 2011 [17]. Reproduced with permission of Elsevier.](image-url)
inhibited by Congo red [157] or overexpression of the enzyme neprilysin [163] β-cell apoptosis is reduced, suggesting IAPP aggregation is an important mediator of β-cell toxicity. Some, but not all, studies have demonstrated that expression of human IAPP results in an ER stress response [164–166]. However, this appears to be related to the magnitude of IAPP overexpression, and does not occur at physiologic levels of human IAPP, nor does it differ between human islets from individuals with T2DM who do or do not have amyloid deposits [166]. Finally, recent data have shown that human IAPP in its aggregated form is proinflammatory, eliciting cytokine and chemokine production from macrophages/dendritic cells [167,168]. Further, islet IL-1β expression may be increased in conditions of amyloid deposition [112,161,167], suggesting a novel mechanism by which islet amyloid may result in β-cell death.

**Islet inflammation**

As discussed earlier, inflammation in the islet has long been established as a hallmark of T1DM. Islet infiltration and release of molecules such as proinflammatory cytokines have clearly been implicated in β-cell death in this form of diabetes [169]. In T2DM, the concept that low-grade, chronic inflammation exists, most likely associated with insulin resistance, is a relatively new idea. As this field of research has emerged, so too has the hypothesis that inflammation in the islet may play a role in β-cell death in T2DM [112]. However, this remains a controversial area. Evidence in favor of a role for islet inflammation includes reports of increased islet production of interleukin 1β following chronic high glucose culture of human islets [127]. Islet interleukin 1β production has also been suggested in models of islet amyloid formation [112,161,167]. Activation of signaling pathways associated with the innate immune response (namely toll-like receptors) has been shown to occur in β cells in response to agents such as FFA or lipopolysaccharide [170,171]. This activation can lead to β-cell toxicity and death, suggesting that inflammation may play a role in the demise of the β cell in T2DM.

**Summary and future directions**

The morphology of the pancreas and pancreatic islet is complex, and disturbances in pancreas and islet volume/arrangement that occur in diabetes are multifactorial. Loss of β cells is a common feature of type 1-, type 2-, and cystic fibrosis-related diabetes. However, the mechanisms that underlie this pathology differ significantly among the various types of diabetes. Our understanding of how β-cell destruction occurs in type 1 and type 2 diabetes has been improved by a large number of studies, but we still have much to learn about how this occurs. Emerging areas of interest include understanding how changes in islet vasculature, innervation, and extracellular matrix contribute to derangements in islet morphology, which may in turn shed new light on the causes of β-cell loss in diabetes.

**Acknowledgments**

This work was supported by the Department of Veterans Affairs (Seattle Division VA Puget Sound Health Care System, Seattle, WA, USA) and National Institutes of Health grants DK088082 (RLH), DK017047 and DK075998 (SEK).

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Pancreatic morphology in normal and diabetic states

86 Pancreatic morphology in normal and diabetic states.


CHAPTER 6

Insulin gene expression and biosynthesis

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Key points
• Glucose stimulates insulin gene transcription and insulin mRNA stability.
• Glucose regulates the binding of key transcription factors to the insulin gene, notably Pdx-1, MafA, and NeuroD1/Beta2.
• Chronic hyperglycemia and dyslipidemia impair insulin gene expression via Pdx-1 and MafA.
• Glucose stimulates translation of the mRNA into proinsulin and processing of proinsulin into mature insulin.
• Proinsulin is processed in the immature granule compartment by successive cleavage at two dibasic sites by the prohormone convertases PC2 and PC1/3.
• Chronic nutrient excess and hyperinsulinemia in type 2 diabetes impose a high secretory demand to the β cell which is not adequately matched by an increase in biosynthetic capacity.

Introduction

The unique property of the pancreatic β cell is its ability to secrete insulin to enable circulating glucose levels to be maintained within a narrow physiologic range, despite wide fluctuations in energy intake and expenditure. It is able to sense the glucose concentration in the extracellular milieu, and adapt its insulin secretion rate via a complex interplay between nutrients, hormones, and neuronal signals. Whereas the minute-to-minute regulation of insulin secretion occurs at the level of exocytosis of pre-formed insulin, adaptation to long-term changes in the environment also involves regulated changes in the transcription rate of the insulin gene, translation of the mRNA, and processing of the proinsulin molecule into fully mature insulin. These processes are coordinately regulated by glucose (Figure 6.1) under normal circumstances, and their perturbation leads to β-cell dysfunction and type 2 diabetes.

The first section of this chapter focuses on the structure of the insulin gene, normal regulation of its transcription, and dysregulation under pathologic circumstances. In the second section, the successive steps leading from translation of the insulin mRNA molecule to the storage of mature insulin into readily releasable secretory granules are presented, as well as the metabolic regulation of these processes. Space limitations prevent us from exhaustively citing the work of all investigators who contributed to this field. The reader is encouraged to refer to the cited review articles for complete reference lists.

Insulin gene expression

Structure of the insulin gene

The insulin gene is specifically expressed in pancreatic β cells, although low levels of expression have been detected in the brain [1], the thymus [2], and in the yolk sac during fetal development [3]. The gene’s sequence is highly conserved throughout evolution, and is present as a single copy in most species, including humans, where it is located on chromosome 11 between the genes for tyrosine hydroxylase and insulin-like growth factor 2. In rodents, there are two nonallelic insulin genes (I and II), resulting from duplication of the original insulin II gene [4]. The human insulin gene contains three exons and two introns. The first intron is within the 5’-untranslated region, whereas the second intron interrupts the C-peptide coding sequence. The mature preproinsulin mRNA is 446 base pairs (bp) long.

Pancreatic β-cell-enriched gene transcription is controlled by a number of proteins, including both positive-acting islet-enriched transcription factors including Pax6, Pancreatic and duodenal homeobox-1 (Pdx-1), Neurogenic differentiation 1 (NeuroD1/Beta2), and v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) [5,6], and ubiquitously...
Insulin gene expression and biosynthesis

Figure 6.1 Various levels of glucose regulation of insulin gene expression. Glucose stimulates nuclear translocation of Pdx-1; promotes Pdx-1 and MafA phosphorylation and binding to the insulin promoter; and stimulates transcription of the insulin gene, pre-mRNA splicing, translation, and mRNA stability.

distributed transcriptional activators responsive to specific signaling pathways (e.g., activating transcription factor-2 responsive to Ca\(^{2+}\) signaling [7]; nuclear factor of activated T cells (NFAT) responsive to Ca\(^{2+}\) [8]; kinases such as extracellular-regulated kinases (ERK)1/2 [9]; the transcriptional repressors CCAAT/enhancer-binding protein beta (C/EBP\(\beta\)) [10] and c-Jun [11]; phosphatases (e.g., calcineurin [12]); and coactivators such as p300 [13]. Insulin gene expression is principally controlled by a highly conserved region lying approximately 340 bp upstream of the transcription initiation start, termed the enhancer/promoter control region [14,15]. Considerable progress has been made in defining the many different cis- and trans-acting factors that ensure precise transcriptional regulation, with the focus here on describing the \(\beta\)-cell-enriched transcription factors most pertinent to metabolically regulated expression, specifically Pdx-1, NeuroD1/Beta2, and MafA.

Pdx-1 is a homeodomain protein that plays a major role in pancreatic \(\beta\)-cell development and function [16,17]. It primarily binds as a monomer to the conserved AT-rich A3 box (-201/-196 bp) and activates insulin transcription, although this protein also appears to act as a repressor in other gene contexts [18]. Pdx-1 is produced early in rodent pancreatic progenitors, and is essential to acinar, ductal, and islet endocrine cell formation [5]. Both homozygous and heterozygous mutations in the PDX-1 gene have been identified in humans, which lead, respectively, to complete agenesis of the pancreas and a form of maturity-onset diabetes in the young known as MODY 4 [5].

NeuroD1/Beta2 is a basic helix-loop-helix (bHLH) transcription factor, which binds at the conserved insulin E1 (-100/-91 bp) site in a complex with ubiquitously expressed E-box proteins. NeuroD1-null mice die of severe diabetes shortly after birth due to its crucial role in \(\beta\)-cell formation [19]. Moreover, deletion of NeuroD1 specifically in adult \(\beta\) cells in vivo causes glucose intolerance and loss of expression of many genes associated with cell maturation and function [20]. Mutations in human NEUROD1 predisposes one to another form of maturity-onset diabetes in the young, MODY 6 [21], presumably because of its importance in the production and maintenance of fully functional glucose-responsive \(\beta\) cells.

The MafA activator is a basic leucine zipper protein, which binds as a dimer to the conserved insulin C1/RIPE3b1 (-118/-107 bp) element. MafA, and the only additional islet-synthesized large Maf family member, MafB, are expressed unusually late in pancreatic cell development in relation to other islet-enriched transcription factors. In rodents, MafB is principally present in developing \(\alpha\) cells and \(\beta\) cells, and then becomes restricted to \(\alpha\) cells soon after birth [22,23]. In contrast, MafA is only found in \(\beta\) cells, with expression first detected during the principal wave of insulin-positive cell production at embryonic day 13.5 in mice [24]. In human islets, MafB is not
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mice, although gene transcription [42]. Thus, the global β gene transcription, with phosphorylation (i.e., insulin cells but also co-produced with MafA in cells, and becomes in vivo β embryos was reduced insulin and glucagon hormone and gene transcription, which relies on the cooperative α gene transcription [29]. In addition, SUMOylation of gene transcription factors [30]. Finally, Pdx-1 contains at least two sites of stability and is correlated with an increase in insulin protein, increases its nuclear localization as well as its protein acetyl-transferase p300, hyperacetylation of histone H4, and glucose levels, which promotes association with the histone deacetylases HDAC-1 and HDAC-2 to downregulate insulin gene expression under low, non-insulin stimulating glucose concentrations [28]. However, the HDAC-1/2 interaction is prevented at elevated glucose concentrations stimulating insulin secretion. Many different signaling pathways have been shown to regulate the nucleo-cytoplasmic shuttling and transactivation potential of Pdx-1 under these conditions, including glycogen synthase kinase 3 (GSK3), p38/stress-activated protein kinase, phosphatidylinositol 3-kinase (PI3K), atypical protein kinase C isoforms, mitogen-activated protein kinase (MAPK), and Per-Amt-Sim (PAS) kinase [6]. Glucose also appears to regulate the interaction of Pdx-1 with various transcriptional coregulators. Thus, Pdx-1 is associated with the histone deacetylases HDAC-1 and HDAC-2 to downregulate insulin gene expression under low, non-insulin stimulating glucose concentrations [28]. However, the HDAC-1/2 interaction is prevented at elevated glucose levels, which promotes association with the histone acetyl-transferase p300, hyperacetylation of histone H4, and insulin gene transcription [29]. In addition, SUMOylation of Pdx-1 increases its nuclear localization as well as its protein stability and is correlated with an increase in insulin promoter activity [30]. Finally, Pdx-1 contains at least two sites of O-GlcNAcylation that increase its DNA binding activity [31,32]. In addition, glucose-induced phosphorylation of NeuroD1 regulates its nuclear localization and transactivation. However, precisely how the regulation is imposed is unknown. Treatment of pancreatic β cells with the MAPK/ERK kinase inhibitor PD98059 blocks nuclear NeuroD1 translocation, conditions that also impact Pdx-1 phosphorylation and insulin gene transcription [33]. In addition, high glucose levels induce O-GlcNAcylation of NeuroD1 [34], which appears to be important for its translocation into the nucleus. Exactly how these distinct modifications control NeuroD1 activity in islet β cells in vivo still needs to be explored.

Increased MafA protein and DNA activity regulates glucose-dependent insulin gene transcription, with phosphorylation modulating insulin enhancer binding [35]. In addition, the transactivation potential of MafA is potentiated by GSK3-mediated phosphorylation within the N-terminal region which allows recruitment of the P/CAF coactivator [36]. However, precisely how phosphorylation regulates these properties of MafA remains to be determined.

Glucose regulation of insulin mRNA stability
In addition to its major effects on transcription of the insulin promoter, glucose markedly stabilizes preproinsulin mRNA. Indeed, the half-life of the message has been estimated to increase from 29 hours to 77 hours when switched from low to high glucose. Two elements located in the 5'-untranslated region of the mRNA molecule have been proposed as mediators of the glucose-stabilizing effect, the conserved UUGAA sequence and the pyrimidine-rich sequence (insPRS) [37]. Stabilization appears to involve binding of a polypyrimidine tract-binding (PTB) protein to the insPRS, as binding was induced by glucose and prevented upon mutating the core PTB binding site.

Glucose regulation of the insulin gene in vivo
Variations in the amount of insulin mRNA at any given time represent the net effect of metabolic, hormonal, and neuronal stimuli on insulin gene transcription and mRNA stability. From the in vitro effects described earlier, it is predicted that increases in blood glucose should rapidly elevate preproinsulin mRNA levels in the endocrine pancreas (due to rapid stimulation of transcription), whereas a decrease in blood glucose would be followed by a slow decline in preproinsulin mRNA levels (due to reduced transcription and the long half-life of the message). Indeed, early studies in rats showed that 4 days of starvation are necessary to detect significant decreases in preproinsulin mRNA, which returned to basal values only 6 hours after re-feeding [38] or 12 hours after glucose injection [39]. The delay in the disappearance of the message upon fasting appears to be directly due to the stability of the mRNA, as insulin-induced hypoglycemia is followed by a rapid (2-hour) decrease in the level of precursors for insulin mRNA [40].

An additional level of complexity in the regulation of insulin gene expression in vivo is the potential interaction between β cells and non-β (i.e., α, δ, and PP) cells within the intact islet. Indeed, it is known that individual β cells behave differently from one another in terms of insulin secretion than in the context of the islet [41], a phenomenon which has also been demonstrated at the level of insulin gene transcription [42]. Thus, the global response measured in an entire islet represents the integration of

Physiologic regulation of insulin gene expression
Glucose regulation of insulin gene transcription factors
Pdx-1 is mainly localized at the nucleus periphery at low glucose concentrations (1–2 mM) in β cells, and becomes phosphorylated and shuttles into the nucleus in response to concentrations stimulating insulin secretion. Many different signaling pathways have been shown to regulate the glucose-regulated insulin secretion was compromised in adults. Overall, a highly sophisticated network of transcription factors provides the infrastructure for precisely regulating insulin gene transcription, which relies on the cooperative and synergistic interactions between transcription factors and recruited coactivators. Significantly, these factors are also crucial in regulating many other β-cell genes, resulting in a variety of distinct developmental and adult phenotypes in studies of total and conditional transcription factor knockout mice. In the following section, the effect of increasing glucose levels, the most physiologically impactful mediator of β-cell function on Pdx-1, NeuroD1, and MafA will be presented (Figure 6.1).
the individual responses. This fact should be kept in mind when interpreting experiments performed in clonal cell lines, which lack the level of regulation provided by islet architecture and the neighboring non-β cells.

**Regulation of insulin gene expression by glucagon-like peptide-1 (GLP-1)**

GLP-1 is an incretin hormone which strongly potentiates glucose-induced insulin secretion and is the target of a number of type 2 diabetes drugs [43]. Most of the biologic effects of GLP-1 are mediated by its G protein-coupled receptor expressed on the β-cell surface [44]. GLP-1 stimulates insulin gene expression by various mechanisms. First, it directly activates the cyclic-AMP (cAMP) response element (CRE) within the 5’-proximal control sequences of the insulin gene, by a mechanism which seems at least partly independent from protein kinase A activation [45]. Second, it can augment the glucose-stimulated binding activity of Pdx-1 [46]. Third, it can stimulate transcription of the PDX-1 gene (the promoter of which also contains a CRE) [47]. Fourth, GLP-1 potentiates glucose-induced insulin gene transcription by activating NFAT (of which there are three binding sites on the rat insulin I promoter) via calcium/calmodulin-dependent protein phosphatase 2B (calcineurin) activation in response to a rise in intracellular calcium [48].

**Physiologic inhibitors of insulin gene expression**

Several physiologic inhibitors of insulin secretion also impair expression. Epinephrine and somatostatin, two hormones acting through G protein-coupled receptors and known inhibitors of insulin release [49], also inhibit the rate of insulin gene transcription [50]. In addition, somatostatin decreases the stability of insulin mRNA [51]. Glucagon, a key hormone in the counterregulatory response to hypoglycemia in vivo, stimulates expression of the inducible cAMP early repressor, which inhibits insulin gene transcription [52]. Finally, the adipocyte-secreted hormone leptin decreases insulin gene expression [53]. Thus, physiologic modulators of insulin secretion coordinately inhibit insulin gene expression, thereby ensuring that the long-term biosynthetic rate of insulin matches the secretory demand.

**Short-term regulation of insulin gene expression by insulin**

Whether insulin has a functionally relevant role in the regulation of insulin transcription and biosynthesis remains a debated issue [54,55]. Based on the observation that a rapid (within minutes) transcriptional response to glucose in insulin-secreting cells was mimicked by depolarizing agents and exogenous insulin and was suppressed by inhibiting insulin release or the PI3 kinase pathway, it was proposed that insulin acts in an autocrine manner to stimulate insulin gene transcription by binding to the insulin receptor [54]. However, subsequent studies have failed to detect significant changes in preproinsulin mRNA levels upon short-term exposure to glucose and the physiologic relevance of an autocrine positive feedback on the β cell has been questioned [55].

**Dysregulation of the insulin gene**

There is convincing evidence that abnormalities in insulin gene sequence or function play a role in pancreatic β-cell dysfunction in type 2 diabetes. Abnormalities in the insulin gene structure consist of rare control region mutations, while insulin gene expression appears to be reduced by metabolic conditions associated with the diabetic state.

**Polymorphisms of the insulin gene**

The diabetes susceptibility gene IDDM2 has been mapped to the insulin variable number of tandem repeats (VNTR), a highly polymorphic region located 360 bp upstream of the transcription initiation site in the human insulin gene [56]. VNTRs are classified as class I, II, or III, depending on the number of tandem repeats. Whereas the short class I VNTR gene predisposes to type 1 diabetes, the long class III allele is protective. Precisely how VNTR polymorphisms confer susceptibility to or protection from diabetes remains uncertain, although recent evidence clearly suggest that the VNTR determines expression levels of insulin in the thymus and, in turn, the numbers of insulin-specific autoreactive T cells [56].

**Glucotoxicity and the insulin gene**

The glucotoxicity hypothesis proposes that chronic hyperglycemia is deleterious to β-cell function by contributing to the deterioration of insulin secretion [57]. These adverse effects of chronically elevated glucose levels include, but are not limited to, impairment of insulin gene expression in insulin-secreting cells, isolated rat and human islets, and animal models of diabetes [58]. The molecular mechanisms underlying glucotoxicity at the insulin gene involve decreased expression of Pdx-1 and MafA (Figure 6.2), as well as increased expression of C/EBPβ which directly binds the NeuroD1/Beta2, thereby preventing formation of the NeuroD1/Beta2:E47 activator complex required for insulin E1 stimulation [59]. In addition, binding of a C/EBPβ-NFAT complex at the A2C1 element of the insulin promoter under glucotoxicity prevents the formation of the MafA-NFAT complex at that site required for normal glucose stimulation of insulin transcription [60].

The biochemical mechanisms whereby chronically elevated glucose impairs insulin gene expression have received considerable attention in the past few years. The prevailing hypothesis is that high glucose induces the excessive production of reactive oxygen species (ROS) and the formation of advanced glycation end-products (AGE) [61,62]. This hypothesis is supported by in vivo observations. For example, treatment of Zucker diabetic fatty (ZDF) rats with the antioxidant N-acetylcysteine normalizes plasma glucose levels and restores insulin secretion, insulin content, and preproinsulin mRNA levels [63]. Similarly, overexpression of glutathione peroxidase-1 in db/db mice reversed hyperglycemia and restored MafA nuclear localization [64].
Oxidative stress-mediated impairment in Pdx-1 binding activity is prevented by overexpression of a dominant-negative c-jun N-terminal kinase (JNK), and is mimicked by overexpression of wild-type JNK [65]. In addition, chronic exposure to elevated glucose levels may lead to dedifferentiation with loss of genes associated with β-cell function and overexpression of genes normally repressed in differentiated β cells [66,67]. For instance, the c-myc transcription factor is upregulated in diabetic islets [68] and is induced by high glucose in normal islets [69]. In turn, c-myc can inhibit insulin gene transcription [70] by competing for NeuroD1/Beta2 binding at the E-box [71].

Endoplasmic reticulum (ER) stress has also been proposed to contribute to the mechanisms of glucotoxicity independently from oxidative stress [72]. However, alleviation of ER stress by chemical chaperones in glucose-cultured islets improves insulin secretion but not intracellular insulin content, suggesting that ER stress may be involved in defective insulin secretion but not impaired insulin biosynthesis under glucotoxic conditions [73].

**Glucolipotoxicity and the insulin gene**

Like chronic hyperglycemia, hyperlipidemia has been proposed to contribute to β-cell dysfunction in type 2 diabetes [74]. Most of the deleterious effects of chronically elevated lipid levels on the β cell require the concomitant presence of hyperglycemia, a phenomenon referred to as glucolipotoxicity [75]. Amongst its many functional consequences, glucolipotoxicity impairs insulin gene expression via a transcriptional mechanism that involves de novo synthesis of ceramide and defective function of Pdx-1 and MafA [76,77]. Importantly, defective Pdx-1 function and insulin gene expression are also observed in an in vivo model of glucolipotoxicity in rats following a 72-h infusion of glucose and Intralipid, a lipid emulsion which raises circulating...
fatty acid levels when co-infused with heparin [78,79]. It is interesting that glucotoxicity and glucolipotoxicity both affect Pdx-1 and MafA function, albeit by different mechanisms: glucotoxicity alters Pdx-1 mRNA expression [80] and MafA nuclear localization [64], while in glucolipotoxicity Pdx-1 is retained in the cytosolic compartment while MafA mRNA expression is reduced [77] (Figure 6.2).

How de novo ceramide synthesis from palmitate, in the presence of elevated glucose, alters the function of Pdx-1 and MafA and leads to defective insulin gene expression remains unknown. One possible candidate is the serine/threonine kinase PAS kinase, which regulates glucose-induced insulin gene transcription [81]. In insulin-secreting cells and isolated islets, we observed that overexpression of PAS kinase protects from the negative effects of palmitate on the insulin gene [82]. Recent data suggest that this could be mediated by PAS kinase inactivation of GSK3β (via phosphorylation at Ser9) and alleviation of GSK3β-mediated serine phosphorylation of Pdx-1 and proteasomal degradation ([83] and M. Semache, G. Fontés, S. Fogarty, C. Kikani, M. B. Chawki, J. Rutter and V. Poitout, unpublished data). A second candidate mediator of ceramide inhibition of the insulin gene is c-jun N-terminal kinase (JNK). In support of this possibility, palmitate was shown to activate JNK in β cells which results in a decrease in insulin gene transcription [84].

Relevance to human type 2 diabetes
Recent studies in human islets support the notion that defective insulin gene expression may play a role in human type 2 diabetes. First, in islets isolated from pancreata of 13 type 2 diabetic cadaveric organ donors high levels of oxidative stress markers as well as low levels of glucose-induced insulin secretion, reduced insulin mRNA, but increased levels of Pdx-1 and FOXO1 mRNAs have been observed [85]. Second, nuclear expression of MafA is decreased in human diabetic islets [86]. Third, DNA methylation of the insulin promoter is increased in type 2 diabetic patients and correlates negatively with insulin gene expression and positively with hemoglobin A1c levels [87].

Insulin biosynthesis
Introduction
The previous section outlines that insulin gene transcription is a highly controlled process. The product of this process, pre-proinsulin mRNA, is unusually stable in pancreatic β cells and it is further stabilized as glucose concentrations increase [37]. As such, there is normally an abundant source of preproinsulin mRNA in the β-cell cytosol available for translation. Actually, it is the specific regulation of preproinsulin mRNA translation that is the predominant control mechanism for insulin production in the β cell under normal circumstances. This enables the β cell to rapidly replenish insulin stores back to optimal levels, after they have been depleted by stimulated insulin secretion, and is more economic energy-wise to the β cell, since translational control of insulin production bypasses the need for insulin gene transcription and preproinsulin mRNA maturation.

Structure of the insulin molecule
The primary structure A- and B-chain of insulin itself has been known for close to 50 years [88]. However, it was not until at least 10 years after this discovery that it was realized insulin is actually synthesized as a single polypeptide chain precursor molecule, preproinsulin (Figure 6.3) [88]. The N-terminal signal peptide (24 amino acids) is cleaved cotranslationally to yield proinsulin. The proinsulin molecule is a 12-kDa single chain polypeptide that encompasses the B-chain (30 amino acids) and the A-chain (21 amino acids) of insulin joined by the connecting peptide, C-peptide (Figure 6.3). Proinsulin to insulin conversion occurs by cleavage at two dibasic amino acids sequences by the B-chain/C-peptide and C-peptide/A-chain junctions to release the C-peptide moiety yielding the insulin molecule with the two independent disulphide-linked A- and B-chains correctly aligned [88].

Proinsulin biosynthesis: translation and translocation
Essentially, translation of preproinsulin mRNA to preproinsulin protein occurs in a fashion typical of most eukaryotic mRNAs destined to enter the cell’s secretory pathway [88–90]. During the translation process, the emerging signal sequence of preproinsulin binds the signal recognition particle (SRP) that then docks to the SRP-receptor, which is an integral ER membrane protein. This locates the preproinsulin mRNA/ribosomal translational complex to the ER, which is the major site of proinsulin biosynthesis in the β cell. As SRP binds to the SRP receptor, the nascent signal peptide of the newly forming preproinsulin dissociates and is transferred to another ER integral membrane protein, the signal sequence receptor (SSR). SSR is part of a “translocation pore” that facilitates transport of the newly forming preproinsulin polypeptide across the ER membrane into the ER lumen, marking the entrance of the newly synthesized preproinsulin into the β-cell’s secretory pathway. The signal peptide is cleaved by another RER protein, the signal peptidase, resulting in the nascent proinsulin molecule located to the ER lumen. There, the proinsulin molecule undergoes appropriate folding, assisted by the molecular chaperons and formation of disulfide bonds catalyzed by ER disulfide isomerase activity [88].

Proinsulin biosynthesis: effectors and stimulus-response coupling mechanisms
Proinsulin biosynthesis is translationally controlled by certain nutrients, neurotransmitters, and hormones, but glucose is the most physiologically relevant [91]. This translational control response to glucose is rapid. Significant glucose-induced proinsulin biosynthesis can be observed after a 20–30 min lag period that reaches a maximum rate (~20–30-fold increase above basal) by 60 min [91]. Of the peptide hormones that stimulate
proinsulin biosynthesis, perhaps the incretins, GLP-1 and GIP, are the most physiologically relevant. GLP-1/GIP do not increase proinsulin biosynthesis translation in their own right, but potentiate glucose-induced proinsulin biosynthesis as they do glucose-induced insulin secretion [92]. Epinepherine is also worthy of mention for specifically inhibiting glucose-induced proinsulin biosynthesis as it does glucose-induced insulin secretion [91].

Unlike that for glucose-induced insulin secretion, the secondary signals that lead to an increase in proinsulin biosynthesis are less well defined. Glucose metabolism is required for glucose-induction of both proinsulin biosynthesis and insulin secretion, but several lines of evidence indicate that the stimulus-response coupling mechanism for these β-cell functions are quite distinct [91,92]. For example, sulfonylureas stimulate and diazoxides inhibit glucose-induced proinsulin biosynthesis as it does glucose-induced insulin secretion [91].

Proinsulin biosynthesis: translational control mechanism

Glucose modestly increases general protein synthesis in the β cell ~1.5–2-fold. However, the effect of glucose on proinsulin synthesis translation is much greater, and can reach ≥10-fold stimulation above basal [91,92]. This indicates a specific effect of glucose on translational control of proinsulin biosynthesis. Such specific control of glucose-induced proinsulin biosynthesis in the β cell resides in cis-elements in the 5' and 3'-untranslated regions (UTRs) of preproinsulin mRNA itself [92]. In the 3'-UTR of preproinsulin mRNA, just downstream of the polyadenylation signal, there is a highly conserved primary sequence containing a UUGAA cis-element core, that has been reported to be involved in glucose-regulated preproinsulin mRNA stability in addition to the pyrimidine-rich sequence (insPRS) [37,92] (see earlier). There is some degree of cooperativity between the preproinsulin mRNA 5'- and 3'-UTRs for the specific glucose-induced translational control of proinsulin biosynthesis, but it appears that the 5'-UTR preproinsulin mRNA has the major influence [92]. There is also a conserved cis-element that is required for glucose-induced translational control of proinsulin biosynthesis, named ppIGE (for preproinsulin glucose element) [92]. The ppIGE has a highly conserved ppIGE palindromic core of GUCx_CUG or GUUx_UUG (where n ≤ 4 bases). A cytosolic protein trans-acting factor...
(ppIE-BP) binds to this translational control ppIGE cis-element of preproinsulin mRNA in a glucose-dependent manner, but the identity of the ppIGE-BP has yet to be revealed [92]. It should be noted that proinsulin is only one of a small subset of β-cell proteins (~50 in all) [92] whose biosynthesis is regulated by glucose at the translational level. These are mostly β-granule proteins, including the proinsulin processing endopeptidases, PC2 and PC1/3 [92]. Indeed, the ppIGE is also conserved in the 5′-UTR of the majority insulin secretory granule proteins’ mRNAs, and thus, the glucose-induced specific translational control of proinsulin biosynthesis and that for insulin secretory granules can be coordinated and is the principal control mechanism for insulin secretory biogenesis in β cells [92]. The glucose-induced translational control of the proinsulin processing endopeptidases, proPC2 and proPC3, provides a means whereby proinsulin conversion is not compromised upon increased proinsulin biosynthesis [92].

**Transport of proinsulin from the ER to Golgi apparatus**

After proinsulin is translocated into the lumen of the ER, it is then delivered in transport “COP-coated vesicles” to the cis-Golgi apparatus [93] (Figure 6.4). Up until relatively recently, it was thought that newly synthesized proinsulin was passed from the cis-Golgi network “stack” via the medial- to the trans-stack of the Golgi apparatus stacks in “COP”-coated vesicles, but a landmark study conducted in β cells using electron microscope tomography showed that the Golgi apparatus is actually one continuous organelar compartment, and not a series of stacks [94]. As such, newly synthesized proinsulin traverses through the lumen of the β cell’s Golgi apparatus to the trans-Golgi network (TGN) [94], where it accumulates in clathrin-coated regions [93]. This is the site of secretory granule biogenesis (Figure 6.4). The means by which newly synthesized proinsulin (and other select proteins destined to the β granule), is specifically targeted to sites of β-granule biogenesis in the TGN remains a matter of debate [95]. However, it is known to be a highly efficient process, with >99% efficient of newly synthesized proinsulin sorted to the β granule and regulated secretory pathway under normal conditions [91,95].

Generally, analogous to other neuroendocrine cells, the process of β-granule biogenesis should also require other factors including intraluminal acidic pH 6.5, Ca⁡²⁺, ATP, GTP-hydrolysis cytosolic proteins and perhaps protein tyrosine phosphorylation [88,91,93,95]. Although β-granule biogenesis occurs in limited clathrin-coated regions of the TGN [93], the role that clathrin itself plays is unclear although is likely involved in the process of a newly formed immature β granule “budding off” the TGN. An immature β granule then undergoes a maturation process [93,95]. Maturation of β granules involves proinsulin conversion, progressive intragranular acidification, loss of the clathrin-coated regions, and formation of hexameric insulin crystals [91,93]. Acidification provides the correct intragranular pH (pH 5.0–5.5) for proinsulin processing to proceed [91], and optimal insulin crystal formation around insulin’s isoelectric point (pKi 5.3) [88]. Delivery of newly synthesized proinsulin to an immature β granule occurs around 30–40 min posttranslation where proinsulin processing begins and is >90% completed ~3 h later [91,93] (Figure 6.4).

**Proteolytic enzymes of proinsulin conversion**

The major site for processing of proinsulin to biologically active insulin is the immature secretory granule compartment of the β cell [91,93] (Figure 6.4). Production of insulin (and C-peptide) occurs via limited proteolysis of the proinsulin precursor molecule, which is catalyzed by two Ca⁡²⁺-dependent endopeptidases, PC2 and PC1/3 and a Ni²⁺-dependent exopeptidase, CP-H [88,91]. There are two dibasic sites on the human proinsulin molecule: Arg⁶¹, Arg⁶² and Lys⁶⁴, Arg⁶⁵, that signal limited endoproteolytic cleavage of proinsulin to excise the C-peptide moiety and to generate insulin with its disulphide-linked A- and B-chains correctly aligned (Figure 6.3). Endoproteolytic peptide bond cleavage of proinsulin occurs on the carboxylic side of the Arg⁶¹, Arg⁶² or Lys⁶⁴, Arg⁶⁵, followed by rapid and specific exopeptidic removal of the newly exposed basic amino acids by CP-H [88,91]. The two distinct β-granule proinsulin-processing endopeptidase activities were originally discovered as Ca⁡²⁺-dependent with an acidic pH optimum and were later identified as the PC1/3 and PC2 endopeptidase genes [88,91].

A scheme of proinsulin conversion is illustrated in Figure 6.5. Proinsulin conversion could occur by two possible routes. Either PC2 first cleaves on the carboxylic side of Lys⁶⁴-Arg⁶⁵ to yield a split 65,66 proinsulin intermediate, followed by CP-H trimming of the newly exposed lysine and arginine residues to yield des 64,65 proinsulin. Then PC1/3 can then cleave des 64,65 proinsulin at Arg⁶¹, Arg⁶², which together with CP-H trimming of the exposed arginine residues, yields insulin and C-peptide (Figure 6.5). Alternatively, PC1/3 first could cleave at the carboxylic side of Arg⁶¹, Arg⁶² to yield a split 32,33 proinsulin intermediate, followed by CP-H trimming of the revealed arginine residues to yield des 31,32 proinsulin. PC2 can then cleave des 32,33 proinsulin at Lys⁶⁴-Arg⁶⁵, which together with CP-H trimming of the lysine and arginine residues, yields insulin and C-peptide (Figure 6.5). However, PC2 has a much stronger preference for the des 31,32 proinsulin substrate than proinsulin, whereas PC1/3 has an equivalent preference for proinsulin or des 64,65 proinsulin substrates [88,91]. As such, in humans, the sequential processing of proinsulin via des 31,32 proinsulin is the predominant route, where PC1/3 cleaves intact proinsulin first, followed by PC2 cleavage of des 31,32 proinsulin (Figure 6.5). This is consistent with the presence of the des 31,32 proinsulin conversion intermediates in the human circulation but negligible levels of des 64,65 proinsulin [91].

PC2, PC1/3 and CP-H are expressed in most neuroendocrine cells where they are involved in posttranslational processing of other prohormone precursors [88]. The role of these proteolytic enzymes in proinsulin conversion has been substantiated in
Figure 6.4 Cell biology of proinsulin trafficking and processing. The site of preproinsulin biosynthesis is on the ribosomes of the ER. The signal peptide is cleaved co-translationally enabling proinsulin translocation into the ER lumen. The newly synthesized proinsulin is then transported to the cis-Golgi and transported through the stacks of the medial and trans-Golgi to clathrin-coated regions of the trans-Golgi network (TGN). Immature β granules bud off the TGN which is the major site of proinsulin conversion (~30 min posttranslationally). Proinsulin conversion proceeds as a β granule matures. Mature granules form the intracellular insulin storage compartment of the β cells and do not undergo Ca\(^{2+}\)-dependent exocytosis unless triggered by an appropriate stimulus.

The left panel indicates the intracellular compartments in which proinsulin is sequentially transported through, and the right panel indicates the kinetics of the preproinsulin biosynthetic/processing/secretory process in these compartments.

various gene-deletion studies. PC2, PC1/3 or CP-H deficiencies render multiple endocrine deficiencies. PC2 knockout mice have defective proinsulin processing with increased levels of the split proinsulin conversion intermediate des 31,32 proinsulin, consistent with PC2 preferentially cleaving at the Lys\(^{64}\), Arg\(^{65}\) site on proinsulin, and the preferred sequential proinsulin processing route [88] (Figure 6.3). Indeed, PC2 null mice have a polyendocrine phenotype the most obvious being fasting hypoglycemia and glucose intolerance due to a deficiency of circulating glucagon levels rather than increased insulin levels [88]. A human mutation of both PC1/3 alleles exists, which results in negligible PC1/3 activity [88]. This generates a complicated phenotype of multiple endocrine disorders due to general abnormal prohormone processing, one of which is very low insulin levels and high proinsulin levels, together with abnormal glucose homeostasis, consistent with defective proinsulin processing [88]. A very similar phenotype is found in the PC1/3 null transgenic mouse model [88]. Finally, the obese Fat/Fat mice have been found to have a mutation in the CP-H gene resulting in negligible CP-H activity [91]. These CP-H null animals are hyperproinsulinemic, suggesting that CP-H trimming off of basic amino acids after PC2 and
**Figure 6.5** Enzymatic proteolytic conversion of proinsulin. There are two potential pathways of proteolytic conversion of proinsulin to insulin. Either PC2 first cleaves proinsulin at Lys⁶⁴-Arg⁶⁵ to yield split 65,66 proinsulin, followed by CP-H trimming of the newly exposed lysine and arginine residues to yield des 64,65 proinsulin. PC3 can then cleave des 64,65 proinsulin at Arg⁳¹, Arg⁳², which together with CP-H trimming of the exposed arginine residues, yields insulin and C-peptide. Alternatively, PC3 cleaves at Arg⁳¹, Arg⁳² to yield a split 32,33 proinsulin, followed by CP-H trimming of the revealed arginine residues yields des 31,32 proinsulin. PC2 can then cleave des 32,33 proinsulin at Lys⁶⁴-Arg⁶⁵, which together with CP-H trimming of the lysine and arginine residues, yields insulin and C-peptide. In human β cells, the route via des 31,32 proinsulin predominates as illustrated by the larger size of this pathway.

PC1/3 endopeptidic cleavage accelerates proinsulin proteolytic maturation through to insulin [91]. Moreover di-arginyl insulin (that has >50% reduced biological activity) rather than insulin is produced indicating the role that CP-H plays in trimming basic amino acids during the proinsulin conversion process [91].

**Regulation of proinsulin conversion**

PC2 and PC1/3 are Ca²⁺-dependent enzyme activities with an acidic pH 5–5.5 optimum [91]. Fortunately, the β granule contains an intraorganellar environment of 1–10 mM free Ca²⁺ and acidic pH 5.5, which ideally suits the requirements for optimal PC2, PC1/3 and CP-H activities within this organelle. This also ensures that insulin is produced mainly in the intracellular β-granule compartment in which it is stored [91]. To render PC2 and PC1/3 fully active for proinsulin processing in a newly formed β granule, it follows that activation of the proton-pumping ATPase and Ca²⁺-translocation proteins [91] are key regulatory events to control proinsulin conversion.

Both PC2 and PC1/3 are initially synthesized as preproprotein precursor molecules themselves, with the “pre” signal peptide region enabling translocation into the RER lumen during translation as with the signal peptide region of proinsulin (see earlier). ProPC2 and proPC1/3 are transported to β granules along with their proinsulin substrate in the β cell’s secretory pathway, and undergo maturation beginning in the TGN [91]. However, unlike proinsulin, proPC2 and proPC1/3 are thought to be accompanied by individual chaperon molecules, 7B2 and proSAAS, respectively, that specifically inhibit these endopeptidases’ activity [91]. Proteolytic cleavage of 7B2 by PC2 in the TGN/immature β-granule compartment alleviates the inhibition on proPC2 promoting its maturation and activation to mature PC2. Indeed, 7B2 has an important role in controlling PC2 activity in vivo. The 7B2 knockout mouse has multiple neuroendocrine disorders, similar yet more severe than the PC2 null mouse [91]. In contrast, the role of proSAAS in regulating proinsulin processing is doubtful, since proSAAS null mice have normal insulin production and proSAAS is not highly expressed in β cells [96,97].

As previously indicated, the biosynthesis of proPC2 and proPC1/3 is stimulated predominately at a translational level coordinately with that of proinsulin [88,91,92]. In the long term (>12 h), glucose also regulates PC2 and PC1/3 gene transcription in parallel with the preproinsulin gene [91]. Thus, it seems that proinsulin conversion is adaptable to changes in glucose by coordinate regulation of the endopeptidases that catalyze processing [91,92].
The mature β-granule storage pool
A mature β granule is retained from anywhere between a few hours to several days, awaiting transport to the β cell’s plasma membrane and exocytosis under stimulatory conditions, characteristic of a regulated secretory pathway [88,92] (Figure 6.4). It should be noted that under normal conditions, the storage compartment of insulin in mature β granules far exceeds the compartment undergoing transport/exocytosis, so that during a 1-h stimulation by glucose only ~1–2% of the insulin content of a primary islet β cell is secreted [92]. The insulin content of a β cell is kept at a relatively constant level under normal physiologic conditions where secreted insulin is rapidly replaced at the biosynthetic level. However, in the long term there is also an additional regulatory component that maintains insulin stores at optimal levels, via insulin degradation [92]. The half-life of a β granule is several days, but if it is not used for exocytosis it is eventually degraded by fusion with lysosomal compartments by autophagy (also known previously as crinophagy) [92].

Dysfunctional proinsulin processing in diabetes
In type 2 diabetes where there is hyperinsulinemia to compensate for peripheral insulin resistance, an increased proportion of the secreted insulin is actually proinsulin or split proinsulin conversion intermediates (mostly des 31,32 proinsulin) so that it is also a hyperproinsulinemic state [91]. It is possible that genetic defects in the proinsulin conversion enzyme genes or the insulin gene itself hamper proinsulin conversion, resulting in an increased proportion of proinsulin secreted. However, such genetic mutations are very rare, yet hyperproinsulinemia is a common trait of type 2 diabetes [91]. As such, an increased proportion of secreted proinsulin likely occurs as a consequence of β-cell secretory dysfunction in type 2 diabetes [91].

In common obesity-linked type 2 diabetes there is chronic hyperglycemia and dyslipidemia [88,91,92]. As a consequence, the β cell is working very hard, with both proinsulin synthesis and insulin secretion are upregulated in an attempt to compensate for peripheral insulin resistance. Normally in β cells there is preferential exocytosis of newly formed β granules, but under such chronic stimulation from hyperglycemia/hyperlipidemia newly synthesized proinsulin is not retained long enough to be fully converted to insulin and C-peptide, and as a consequence a greater proportion of proinsulin (as well as des 31,32 proinsulin) is secreted [88,91]. It should also be noted that chronic dyslipidemia adversely affects secretory capacity of β cells. Elevated fatty acid levels increase the amount of insulin secreted from the β cell, but in contrast, fatty acids modestly inhibit glucose-induced proinsulin biosynthesis, which in turn markedly decreases insulin content of islet β cells in vivo [92]. A similar situation might also be envisaged with the prolonged use of sulfonylureas, which though potent inducers of insulin secretion, do not stimulate proinsulin synthesis and decrease insulin content [92], thus also reducing the insulin secretory capacity of the β cell. In general, the chronic hyperglycemia and dyslipidemia in obesity-linked type 2 diabetes are constantly making the β cell work harder to produce sufficient insulin to compensate for increased metabolic load and peripheral insulin resistance [91,92]. But in the long run this eventually leads to β-cell dysfunction of which the hyperproinsulinemia is symptomatic. Interestingly, if the β cell in type 2 diabetes patients is allowed to rest, the β-cell secretory dysfunction in vivo is reduced. This emphasizes the importance of protecting β-cell mass and function in the treatment of obesity-linked type 2 diabetes [88,91,92].

Acknowledgments

Work from our laboratories cited in this chapter was supported by the following grants: R01 DK-050610, R01 DK-055267, and the JDRF and Brehm Coalition (C.J. Rhodes); R01 DK-058096 and the Canada Research Chair in Diabetes and Pancreatic Beta-cell Function (V. Poitout), R01 DK 50203, R01 DK-055091 and R01 DK-042502 (R. Stein).

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