Insulin and Glucagon

Secondary article

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Insulin and glucagon are reciprocal hormones which regulate plasma glucose concentrations. Together, these hormones maintain blood glucose in a narrow range, despite wide variances in glucose production and utilization by the body.

Article Contents

Physiological Importance of Glucose Homeostasis

Glucose is the primary external source of energy used by the cells of the body. A continuous supply of glucose must be provided by the bloodstream to the various organs to ensure their proper function and survival. In the resting state, the body requires 12 g glucose per hour, with the brain utilizing 50% of the total. Under a variety of physiological conditions, such as exercise, glucose usage can rise dramatically. If plasma glucose levels fall too low (hypoglycaemia), cellular death can occur. However, a chronic increase in blood glucose levels (hyperglycaemia) also can result in organ damage. Therefore, plasma glucose levels must be maintained in a narrow range of around $5\,\text{mmol}\,\text{L}^{-1}$, which is considered the physiological 'set point'. Liver and skeletal muscle are the two most important tissues involved in maintaining glucose homeostasis. Following a meal 80–90% of all glucose disposal occurs in skeletal muscle, while under fasting conditions plasma glucose levels are determined by hepatic glucose output (HGO).

Blood glucose levels are established by the balance between the utilization and production of glucose by the body. To achieve equilibrium, the uptake of glucose by the organs of the body must be exactly offset by the release of glucose into the bloodstream. Despite major changes in these variables during the course of a day, the plasma glucose concentration is maintained largely constant due to two opposing hormones, insulin and glucagon. In response to an increase in plasma glucose (after consumption of a meal), insulin is released and increases glucose uptake by the body while suppressing HGO, thus reducing plasma glucose levels. In contrast, when plasma glucose falls (during fasting or exercise), glucagon is secreted and promotes the release of stored and newly synthesized glucose into the bloodstream. These two hormones thus act in concert, to ensure that glucose homeostasis is maintained throughout a wide variety of physiological conditions.

Acute Regulation of Plasma Glucose by Insulin and Glucagon

Despite their opposing actions, insulin and glucagon are both produced by specialized clusters of cells in the pancreas called the islets of Langerhans. The islets are comprised of a central group of β cells, which produce insulin, and are surrounded by α cells, which secrete glucagon. Both cell types are extremely sensitive to plasma glucose concentrations, and can regulate hormonal synthesis and release in response to small changes in blood glucose levels.

Increased plasma glucose levels after a meal directly stimulate insulin secretion by the β cells. The three main target organs for insulin are skeletal muscle, adipose tissue and liver. Insulin stimulates the cellular uptake of glucose into skeletal muscle, and promotes its utilization as energy or storage as long polymers called glycogen. In adipocytes, insulin stimulates glucose transport and storage as both glycogen and lipid. Finally, insulin inhibits the production and release of glucose by the liver into the bloodstream. The stimulation of glucose uptake and inhibition of glucose secretion continue until glucose homeostasis is re-established.

During fasting conditions, the liver is the primary regulator of plasma glucose levels. To maintain homeostasis, the rate of glucose utilization by the body must be precisely matched by the production of glucose by the liver. However, under some conditions, glucose utilization can exceed hepatic production, and plasma glucose levels will fall below $5\,\text{mmol}\,\text{L}^{-1}$. In response, the α cells increase glucagon secretion, while insulin release by the β cells is suppressed. Glucagon acts on the liver, where it promotes gluconeogenesis and glycogenolysis, thus increasing glucose release into the bloodstream. As plasma glucose levels rise, glucagon secretion is diminished.

Regulation of Glucose Transport by Insulin

Insulin increases glucose uptake in skeletal muscle and adipocytes, but is without effect in the liver. Although glucose can cross cellular membranes directly, this process is greatly enhanced by the action of transporters. There are at least six different mammalian glucose transporters (termed GluT 1–6), which act as selective pores in the cell membrane, allowing glucose molecules to pass directly from the bloodstream into the cell. In the basal state, glucose enters skeletal muscle cells through GluT-1. However, insulin increases cellular glucose uptake through the regulation of GluT-4 localization. Basally, GluT-4 is sequestered in intracellular vesicles, and thus is unable to transport glucose into the cell. In response to insulin stimulation, the GluT-4-containing vesicles rapidly migrate to the cell surface, where they fuse with the plasma membrane (see **Figure 2**). The resulting increase in GluT-4 molecules at the cell surface causes a 10–40-fold increase in cellular glucose uptake. Upon entering the cell, glucose is rapidly phosphorylated by an enzyme called hexokinase. The resulting glucose 6-phosphate is trapped within the cell, as it can no longer cross the cell membrane, nor be transported by GluT-4 back into the bloodstream. Glucose 6-phosphate is then further metabolized within the cell, allowing the glucose either to be stored as glycogen or to be oxidized to generate energy in the form of adenosine triphosphate (ATP) synthesis.

The mechanism by which insulin specifically increases GluT-4 vesicle exocytosis is unclear (Rea and James, 1997). At the plasma membrane, the N-ethylmaleimide sensitive fusion (NSF) protein and soluble NSF attachment protein (SNAP) complex with the integral membrane protein syntaxin-4, a SNAP receptor (SNARE). NSF and SNAP act as a bridge between SNARE proteins contained in the GluT-4 vesicles and the plasma membrane, allowing for vesicle docking and fusion. Insulin potentially acts by promoting the release of docked GluT-4 vesicles from intracellular sites and/or increasing the rate of vesicle fusion at the plasma membrane, by enhancing the binding of vesicle SNAREs to the NSF complex. Activation of the enzyme phosphatidylinositol 3′-kinase (PI3 kinase) by insulin plays a crucial role, although the exact downstream target(s) of PI3 kinase involved in GluT-4 vesicle exocytosis remain largely unidentified. Following termination of the insulin signal, the GluT-4 molecules are returned to their intracellular storage compartments via endocytosis and vesicle sorting.

Regulation of Insulin Secretion

Insulin is synthesized and stored within secretory vesicles by the β cells of the pancreas. In response to a number of extracellular stimuli, the secretory vesicles fuse with the plasma membrane, releasing insulin directly into the bloodstream. Insulin secretion is constantly adjusted to a variety of factors, resulting in the tight maintenance of glucose homeostasis over a range of physiological conditions.

Glucose-mediated insulin secretion

Plasma glucose concentration is the most important regulator of insulin secretion. Glucose is constituitively taken up into β cells via the GluT-2 transporter, which has a relatively low affinity for glucose. Therefore, the rate of glucose transport is dictated by plasma glucose levels, and changes in parallel to fluctuations in blood glucose concentration. The β cells also contain a specific glucokinase which phosphorylates glucose, trapping it within the cell, and allowing further metabolism. Unlike other hexokinases, glucokinase possesses three unique attributes: a low affinity for glucose, a sigmoidal relationship between physiological glucose concentration and activity, and a lack of inhibition by product formation. These traits enable glucokinase to adjust its activity in parallel to any change in plasma glucose over a wide range of concentrations. The GluT-2–glucokinase system is therefore termed a 'glucose sensor', since it enables the β cell to gauge plasma glucose concentration directly.

Following consumption of a meal, plasma glucose levels rise from 5 up to 10–15 mmol L^{-1}, and intracellular β-cell glucose levels rise in parallel. The glucose is oxidized, resulting in the generation of ATP molecules, which cause the closure of potassium channels. As intracellular potassium accumulates, the cell depolarizes and voltage-sensitive calcium channels open. Calcium rapidly enters the cell and, through a poorly understood process, stimulates the fusion of the insulin-containing secretory vesicles with the plasma membrane, resulting in the pulsatile release of insulin into the bloodstream (**Figure 1**). There is an initial burst of insulin secretion (first phase) which lasts for several minutes before subsiding, followed by a long plateau phase of insulin release (second phase), which may continue for several hours until the plasma glucose concentration has returned to the homeostatic set point of 5 mmol L^{-1}. The reason for the biphasic nature of the response is unclear. Increases in plasma glucose concentration result in an increase in the frequency, rather than the amplitude, of the insulin secretory pulses (Polonsky *et al.*, 1998). This mechanism allows for exquisite calibration, such that any change in plasma glucose results in the release of an appropriate amount of insulin.

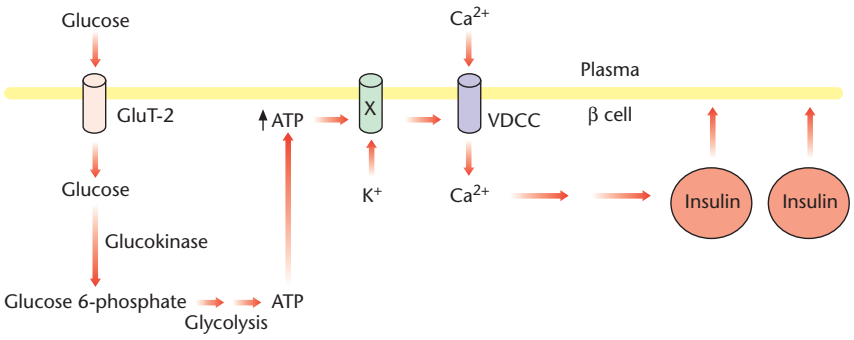

Figure 1 Molecular mechanism of glucose-induced insulin secretion from β cells. ATP, adenosine triphosphate; GluT, glucose transporter; VSCC, voltage-sensitive calcium channel. X indicates that the potassium channel is closed. See text for further details.

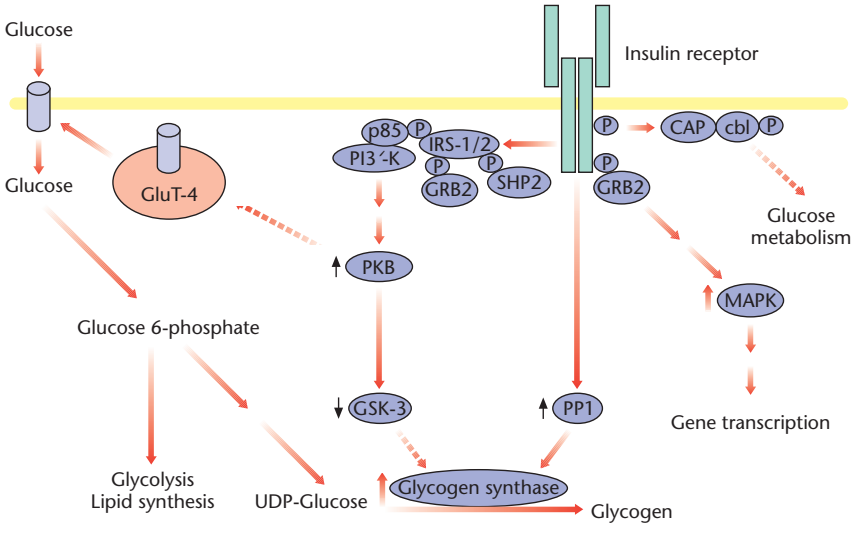

Figure 2 Insulin signal transduction pathways. P, tyrosine phosphorylated residues; GSK-3, glycogen synthase kinase-3; PP1, protein phosphatase type 1; GluT, glucose transporter; PI3′-K, phosphatidylinositol 3′-kinase catalytic subunit; p85, PI3′-K regulatory subunit; PKB, protein kinase B; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase. Defined signalling pathways are shown in solid lines, proposed pathways by dashed lines.

Nonglucose regulators of insulin secretion

The release of insulin from β cells is regulated by a variety of other stimuli. In addition to glucose, digestion of a meal can increase insulin secretion by several mechanisms. An increase in plasma amino acid levels, most notably arginine and leucine, can potently stimulate insulin secretion independently of glucose. Additionally, several peptides released from the gastrointestinal tract during digestion augment insulin release for a given increase in plasma glucose concentration. A variety of hormones can also affect β-cell function. Somatostatin, adrenaline and noradrenaline are all powerful suppressers of insulin release, whereas glucagon, in the presence of raised plasma glucose levels, can stimulate insulin secretion. The β cells are also controlled by the autonomic nervous system, as sympathetic enervation suppresses insulin secretion, while parasympathetic stimulation increases insulin release. Finally, a class of drugs known as sulfonylureas bind to receptors on the β cell, resulting in the closure of a receptor-associated potassium channel, and subsequently insulin release. The natural ligand for these β-cell receptors is not yet known. So, although plasma glucose concentration is the primary signal controlling insulin secretion, a variety of other inputs to the β cell can affect the final amount of insulin released.

The Biochemistry of Insulin Action

Like many other hormones and growth factors, insulin exerts its effects by binding to specific receptors located on the cell surface of target tissues. Occupancy of the insulin receptor stimulates a cascade of intracellular events, which cumulatively mediate insulin signalling. The second-

messenger pathways used by insulin are complex, owing to the multiple effects of the hormone. Although much progress has been made in the past 20 years, the complete mechanisms of insulin action still need to be elucidated. However, insulin signal transduction currently can be broadly divided into four areas: insulin receptor activation, control of glucose transport, stimulation of glucose and lipid storage, and regulation of gene transcription.

Insulin receptor activation

The insulin receptor is comprised of two α and two β subunits, which are linked together by a series of disulfide bridges. The α subunits span the cellular membrane, while the β subunits are located entirely within the cell. The extracellular domain of the α subunits forms the insulin-binding site, whereas the β subunits contain an intrinsic protein kinase activity. Receptor occupancy by insulin-induces a conformational change in the receptor, which is transduced across the membrane, resulting in activation of the intracellular β subunit kinase. The insulin receptor kinase then phosphorylates a number of proteins on tyrosine residues, allowing for their interaction with numerous downstream target enzymes, resulting in the amplification and branching of the initial insulin signal (Saltiel, 1996).

Several signalling adaptor proteins can interact directly with the insulin receptor, and link receptor activation to downstream effectors (**Figure 2**). Several proteins such as growth factor receptor-bound protein 2 (GRB2) have been shown to play crucial roles in the activation of downstream kinase cascades, whereas others such as Casitas β-lineage lymphoma (cbl) protein and cbl-associated protein (CAP) may be involved in metabolic responses. Another key mediator of insulin signalling are a family of insulin receptor substrate (IRS) proteins. IRS-1 and IRS-2 have been the most extensively characterized. Both proteins are phosphorylated on numerous tyrosine residues, and then recruit several key signalling molecules, including enzymes such as PI3 kinase and the tyrosine phosphatase SH_2 domain phosphatase 2 (SHP2), as well as adaptor proteins such as GRB2. PI3 kinase comprises an 85-kDa regulatory subunit which binds to IRS proteins, and a 110-kDa catalytic subunit which is stimulated following p85-IRS binding. Activation of PI3 kinase generates formation of phosphatidylinositol species which play a critical, although ill-defined, role in many of insulin's effect on glucose uptake and storage.

Glucose uptake

The principal physiological effect of insulin is the reduction of plasma glucose levels. As discussed above, insulin stimulates glucose uptake through the translocation of GluT-4 to the cell surface. Recently, links between the activation of PI3 kinase and GluT-4 translocation have been elucidated. The generation of higher-order phosphatidylinositol (PI) products allows the kinase protein kinase B (PKB) to translocate from the cytosol to the plasma membrane where it is phosphorylated and activated. Overexpression of activated PKB in insulin-responsive cells results in increased GluT-4 vesicle translocation, indicating a role for PKB in this process. However, it is still unclear what are the molecular target(s) of PKB, and whether PKB promotes GluT-4 vesicle release from intracellular compartments and/or fusion of the vesicles with the plasma membrane. Additionally, GluT-4 vesicle translocation most likely involves other, unidentified, insulin signalling pathways.

Glucose utilization and storage

Upon entering the cell, glucose is either utilized immediately for the generation of energy or is stored for future use. In addition to increasing glucose uptake, insulin also regulates several enzymatic activities involved in glucose storage and utilization. These metabolic effects of insulin are mediated largely through the activation of protein phosphatases, and the subsequent dephosphorylation of target enzymes.

Within cells, glucose is temporarily stored as glycogen. Insulin stimulates glycogen synthesis by activating glycogen synthase, the rate-controlling enzyme of glycogen formation, and by inactivating glycogen phosphorylase, which catalyses glycogen breakdown. Insulin regulates both enzyme activities by stimulating their dephosphorylation. Activation of protein phosphatase-1 mediates this insulin effect, although inactivation of upstream glycogen synthase-kinases may also be involved. Thus, insulin increases the rate of glycogen synthesis by both increasing substrate (intracellular glucose) and catalytic activity (glycogen synthase).

Insulin also regulates glucose oxidation and lipid synthesis. Through the dephosphorylation and activation of two rate-limiting enzymes, pyruvate kinase and pyruvate dehydroxylase, insulin increases the formation of acetyl coenzyme A (CoA) from glucose. Acetyl–CoA can then enter the citric acid cycle, leading to the generation of energy in the form of ATP. Acetyl–CoA is also a lipid precursor, and insulin increases acetyl–CoA carboxylase (ACC) activity, again through dephosphorylation of the enzyme. ACC is the rate-limiting enzyme in lipid synthesis, and its activation by insulin promotes the storage of glucose as lipid in adipocytes. Finally, insulin inhibits lipid breakdown, through the dephosphorylation and inactivation of hormone-sensitive lipase (HSL). Thus, insulin simultaneously promotes glycogen and lipid synthesis, inhibits lipid breakdown, and stimulates the oxidation of glucose for energy formation.

In contrast, glucagon counterregulates many of these enzymes through increased protein phosphorylation. The glucagon receptor comprises an extracellular ligand-binding site, seven transmembrane domains and an intracellular tail, which binds effector molecules. Via activation of guanine nucleotide-binding protein (G protein), glucagon increases the activity of adenylyl cyclase, which converts ATP into cyclic adenosine monophosphate (cAMP). Increased levels of intracellular cAMP activate cAMP-dependent protein kinase (PKA), which phosphorylates many of the metabolic enzymes dephosphorylated by insulin. PKA mediates the activation of enzymes such as phosphoenolpyruvate carboxykinase (PEPCK), phosphorylase kinase and subsequently glycogen phosphorylase, as well as the inactivation of ACC, HSL and glycogen synthase. The net effect is a reduction in lipid and glycogen synthesis, and an increase in HGO to counteract depressed blood glucose levels.

Gene transcription

In addition to controlling metabolic enzymatic activities by covalent modification, insulin also regulates gene expression of over 100 proteins. PEPCK and ACC are examples of proteins regulated at the level of transcription by insulin. PEPCK is the rate-limiting enzyme in gluconeogenesis, which occurs primarily in the liver and kidneys. Insulin potently inhibits the expression of PEPCK in liver cells, thus decreasing its activity during hyperglycaemic conditions. In contrast, insulin promotes lipid synthesis both by activating ACC directly and by increasing transcription of ACC. Many other key metabolic enzymes are also regulated by insulin in this dual fashion (O'Brien and Granner, 1996). Interestingly, just as glucagon reversed many of the covalent modifications of metabolic enzymes by insulin, glucagon also exerts opposing effects on gene transcription. Thus the increase in hepatic cAMP levels by glucagon increases PEPCK protein expression during hypoglycaemic episodes. Therefore, the activity of many metabolic enzymes reflects the summation of signals from both insulin and glucagon receptors, and is constantly changing in response to alterations in plasma glucose concentration.

Insulin Resistance and Type II Diabetes Mellitus

Type II diabetes mellitus (DM2) is characterized by multiple physiological defects, resulting in the abnormal regulation of glucose homeostasis. DM2 afflicts tens of millions of people worldwide, but is most prevalent among minority groups of industrialized countries. The progression of DM2 from insulin resistance to frank diabetes is characterized by several diagnostic stages (**Figure 3**), which

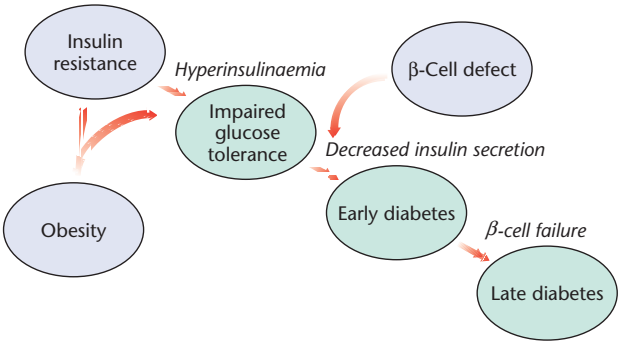

Figure 3 Aetiology of the development of type II diabetes.

occur over the course of years. Although the precise causes of DM2 are poorly understood, both genetic and environmental factors appear to play major roles in the onset and development of the disease.

Insulin resistance

Insulin resistance is defined as a decreased cellular response to a given amount of insulin. In patients, insulin resistance diminishes the ability of insulin to promote glucose utilization and storage, resulting in impaired glucose tolerance. Compared with unaffected individuals, insulin-resistant patients require more time and insulin release to return to basal glucose homeostasis for a given increase in plasma glucose concentration. The β cells become hyperplastic, increasing in size and capacity to secrete insulin, which can result in raised plasma insulin levels (hyperinsulinaemia), despite normal glucose plasma levels. As the insulin resistance worsens over time, basal plasma glucose levels continue to rise, resulting in a combination of hyperglycaemia and hyperinsulinaemia. Eventually, the ability of the β cells to compensate for the insulin resistance is compromised, resulting in β-cell failure and the onset of diabetes.

Insulin resistance results from a decreased signal emanating from the insulin receptor following occupancy. The exact causes of this impairment are unclear. Obesity is the main risk factor for the development of insulin resistance, but additional genetic and environmental factors may also be involved. Recent work has implicated the secretion of factors from adipocytes as potential mediators of insulin resistance in liver and muscle (Peraldi and Spiegelman, 1998).

Type II diabetes mellitus

The reasons for the subsequent failure of the β cell after prolonged insulin resistance are unknown. Potentially, the excessive demand of the body for insulin, coupled with the chronic increase in plasma glucose concentration above the normal set point of $5\,\mathrm{mmol\,L^{-1}}$, could lead to β-cell

desensitization to glucose-stimulated insulin secretion. Despite their insensitivity to plasma glucose levels, initially the β cells respond fully to other secretagogues, such as arginine and sulfonylureas, indicating that the cellular machinery for insulin secretion remains intact. Alternatively, the β cells could possess a second defect unrelated to insulin resistance, which is unmasked by the strain of continually raised insulin secretion. This scenario would explain the varied temporal rates of β-cell failure among patients with similar levels of insulin resistance. Invariably, β-cell function continues to wane, despite the continued increase in plasma glucose levels, resulting in chronic fasting hyperglycaemia. Finally, the β cells fail completely as the islets of Langerhans are destroyed, resulting in the onset of diabetes.

The pathophysiology of DM2 is comprised of a decrease in insulin secretion by the pancreas, a decrease in glucose uptake by skeletal muscle and an increase in glucose production by the liver. All three defects conspire to cause a chronic increase in plasma glucose levels, which in turn leads to a host of secondary complications. The interrelated nature of the defects results in a continued worsening of the disease over time. As insulin levels slowly fall, hyperglycaemia worsens due to a further decrease in glucose uptake by muscle coupled with increased hepatic glucose output. The hyperglycaemia in turn aggravates the insulin resistance, and further diminishes β-cell function. Left untreated, patients suffer from a host of secondary complications, including renal failure, blindness, vasculature obstructions leading to amputation, and ultimately death.

Treatment of DM2

The primary concern in the control of DM2 is the reduction of fasting hyperglycaemia which accounts for most of the secondary complications. Behaviour modification coupled with pharmaceutical intervention can both delay the onset of diabetes, as well as ameliorate the disease state. Obesity is primary risk factor in DM2, and diet restrictions coupled with moderate exercise has been proven significantly to enhance insulin sensitivity in both insulin-resistant and diabetic patients. However, these treatments only slow the progression to frank diabetes, and do not fully restore patients to normal plasma glucose and insulin levels.

Several drugs are also currently used to improve glucose metabolism in DM2 sufferers. During the initial decline of β-cell function, insulin secretagogues, such as sulfonylureas, are used to increase insulin release and reduce plasma glucose levels. However, these drugs are no longer efficacious following β-cell destruction, nor do they address the primary defect of insulin resistance, so the failure rate of sulfonylurea treatment over time is high. Since HGO is the prime determinant of fasting plasma glucose concentration, inhibition of HGO by the drug metformin is used to reduce hyperglycaemic conditions. Finally, a new class of compounds called thiazolidinediones has recently been identified which improve insulin sensitivity. These agents act through increasing gene transcription, and restore insulin signalling in responsive tissues (Saltiel and Olefsky, 1996). In contrast to the downward spiral of DM2, improvement in insulin resistance by thiazolidinedione leads to better glycaemic control, improvement in β-cell function, and a reduction of secondary complications. However, none of these treatments individually cures DM2, although a combination of behaviour and drug therapies has given new hope that the disease can be controlled.

Type I Diabetes Mellitus

A second, less prevalent, form of diabetes is called insulin-dependent diabetes mellitus (DM1). Unlike DM2, which primarily afflicts people over 40 years old, DM1 usually arises in patients before the age of 20 years. The disease results from the destruction of the insulin-secreting β cells by the body's immune system. These patients, therefore, are unable to produce insulin and regulate plasma glucose concentration. The causes of the autoimmune reaction are unclear, although a combination of genetic, environmental and pathogenic factors may be involved (Schranz and Lernmark, 1998). DM1 sufferers usually are not insulin resistant, and are treated by dietary restriction, frequent measurement of plasma glucose levels and insulin injections, in order to maintain proper glucose homeostasis.

Summary

Plasma glucose levels are tightly controlled by the combined action of insulin and glucagon. Despite wide variations in external glucose consumption, and endogenous glucose production and utilization, blood glucose levels are maintained in a narrow physiological range. The secretion of insulin and glucagon is modulated in response to small fluctuations in plasma glucose concentrations, which ensures that glucose homeostasis is preserved. Perturbations in the synthesis and secretion of insulin result in the onset of diabetes, resulting in chronic hyperglycaemia. Current efforts are directed at understanding the causes of diabetes, and discovering pharmaceutical agents that will restore glycaemic control.

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