Pathophysiology of Type 2 Diabetes

8

Jothydev Kesavadev, Fatema Jawad, Asma Deeb, Ankia Coetzee, M. A. Jalil Ansari, Dina Shrestha, Noel Somasundaram, and Sanjay Kalra

Introduction

While in the earlier times type 2 diabetes (T2D) was only considered as a disease related to a disturbance in the functioning of the pancreas, lots of evidences accumulated during the past few decades revealed a plethora of additional factors that contribute to this devastating disease. The understanding of T2D has evolved from recognizing the duo of pancreatic β-cell failure with defective insulin secretion and insulin resistance (IR), to the triumvirate with the addition of hepatic gluconeogenesis. Recently, the ominous octet (addition of deranged adipocyte metabolism, incretin defect, increased glucagon secretion, increased renal glucose reabsorption, and neurotransmitter dysfunction and central appetite dysregulation) and of later the dirty dozen (addition of dopamine, vitamin D, testosterone and renin-angiotensin system) elaborated on the prior simplistic disease models. Furthermore, with the addition of the gut, the unlucky thirteen, suggests that the contributing factors toward T2D pathogenesis are still in the process of being identified [1–9]. In this chapter, we will explore the various factors that have been identified or are being proposed as the underlying contributors to the pathogenesis and pathophysiology of T2D.

J. Kesavadev (✉)
Jothydev's Diabetes Research Center, Trivandrum, India

F. Jawad
Department of Diabetology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan

A. Deeb
Mafraq Hospital, Abu Dhabi, United Arab Emirates
e-mail: adeeb@seha.ae

A. Coetzee
Division of Endocrinology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Cape Town, South Africa

M. A. Jalil Ansari
Department of Endocrinology, Dhaka Medical College & Hospital (DMCH), Dhaka, Bangladesh

D. Shrestha
Department of Endocrinology, Norvic International Hospital, Kathmandu, Nepal

N. Somasundaram
National Hospital of Sri Lanka, Colombo, Sri Lanka

S. Kalra
Department of Endocrinology, Bharati Hospital, Karnal, Haryana, India

Glucose Homeostasis

In healthy individuals, a normal glucose homeostasis in the basal or postabsorptive state is maintained, despite wide fluctuations in supply and demand, by means of a highly regulated and dynamic interaction between tissue sensitivity to insulin and insulin secretion. While maintenance of plasma and tissue glucose levels are required for vital functions of the brain, elevated glucose levels are deleterious or toxic to vascular endothelium and a myriad of other vital tissues. Normally in the postabsorptive state, most of the glucose utilization occurs in the insulin-independent tissues like the brain (50%) and splanchnic areas (25%) while the rest in the insulin-dependent tissues like muscles and adipose tissue. The insulin secretion during the next 2 hours depends on the glucose disposal rate and the degree of suppression of hepatic glucose production (HGP). Additionally, insulin stimulates lipoprotein lipase in the vascular endothelium and promotes lipolysis and removal of chylomicrons and VLDL from the circulation. A derangement in the appropriate β-cell insulin secretion or insulin action at the level of the muscle, liver, and adipose tissue foregoes the hyperglycemic states of prediabetic and diabetic states [10, 11].

Pathophysiology of Type 2 Diabetes

Individuals at risk of T2D are thought to inherit a genetic predisposition to insulin resistance [2, 12]. Chronic fuel excess is the chief pathogenic event that triggers the T2D development in these genetically and/or epigenetically

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susceptible individuals [9]. In states of normal insulin sensitivity, HGP is suppressed by insulin. However, in the event of hepatic IR, gluconeogenesis continues during the basal state even when the fasting insulin level is high and leads to hyperglycemia [13]. During the fed state, suppression of HGP in response to insulin is impaired as well [14]. With peripheral tissue IR, post-meal glucose uptake ensues and postprandial hyperglycemia sets in [14]. The current epidemic of obesity and physical inactivity [15] are IR states [16] that unmask the pancreatic β-cell defect when they fail to augment insulin secretion to offset the effects of IR [2, 3]. As long as the β-cells are able to enhance their insulin secretion to compensate for the impact of IR, glucose tolerance/euglycemia is maintained [17]. However, with time β-cells become unable to compensate for the IR and initially the postprandial plasma glucose (PPG) levels and later the fasting plasma glucose (FPG) levels begin to rise, leading to overt diabetes. Individuals in the upper tertile of impaired glucose tolerance (IGT) are highly insulin-resistant and would have lost 80% of their β-cell function [3, 18].

In 1987 DeFronzo put forward the concept that T2D resulted from deficits in the pancreatic β-cell, the muscle, and the liver, which were collectively referred to as the "triumvirate" (see Fig. 8.1) [1, 18].

In addition to the triumvirate, numerous other factors have been demonstrated to contribute to T2D pathophysiology. In his Banting Lecture, Defronzo revealed some of the other players, viz., adipocytes (accelerated lipolysis), incretin defect, α-cells (hyperglucagonemia), kidney (increased glucose reabsorption), and brain (neurotransmitter dysfunction and central appetite dysregulation), that play important roles in development of glucose intolerance in T2D individuals [3]. Collectively, these eight players were named as the "ominous octet" [18].

The cast list in T2D pathophysiology is still being unraveled. In 2013, Kalra et. al. suggested another four factors responsible for T2D to be added to the list of ominous octet, viz., dopamine, vitamin D, testosterone, and the renin-angiotensin system (RAS), and labeled all the 12 factors as the "dirty dozen" [6]. In 2016, Somasundaram and Wijesinghe proposed a thirteenth mechanism—the role of gut and gut microbiota in T2D [7]. Other factors such as iron overload and gut-derived serotonin have also been proposed to have a role in T2D development [5].

The "Ominous Octet"

β-Cell Dysfunction

β-Cell dysfunction plays a major role in T2D development, across the spectrum of hyperglycemia, from prediabetes to overt diabetes. These cells are in a constant state of dynamic change, with continued regeneration of islets and simultaneous apoptosis. This delicate balance can be disrupted by multiple abnormalities. As the β-cell failure progresses, insulin secretion becomes inadequate to avert the rising blood glucose levels [10]. Although the plasma insulin response to IR is usually increased during the natural history of T2D, this does not imply that the β-cell is functioning normally. In fact, the onset of β-cell failure is found to occur much earlier, and the contribution to hyperglycemia is, in fact, more severe than previously appreciated [3]. For as long as the β-cells are able to augment insulin secretion

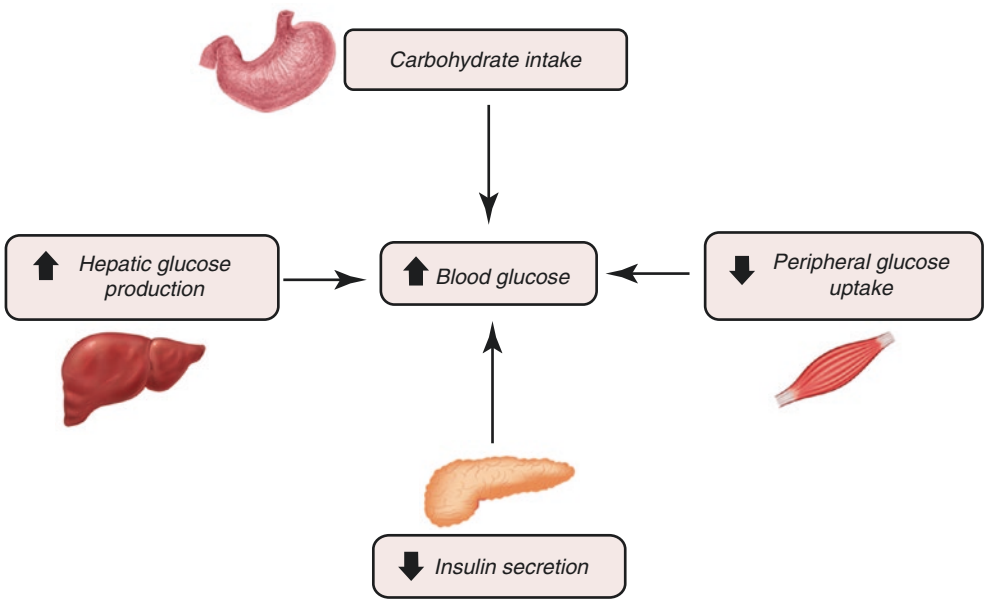

Fig. 8.1 The triumvirate: the core physiological defects that were earlier proposed to be involved in type 2 diabetes pathogenesis [3]. (Adapted from: Chawla [11])

sufficiently to overcome IR, glucose tolerance remains within limits. β-Cell responds to an increment in glucose (ΔG) with an increment in insulin (ΔI), and ΔI/ΔG was initially considered as the measure of β-cell function. The β-cell also takes into account the severity of IR and accordingly adjusts insulin secretion. Thus the gold standard to measure β-cell function is the insulin secretion/IR, i.e., ΔI/ΔG ÷ IR, known as the glucose disposition, index [19]. In individuals susceptible to T2D, there is a limitation to this hypersecretion of insulin. The insulin secretion/IR index seen in normal glucose-tolerant (NGT), IGT, and T2D individuals as a function of the 2-hour PPG during an oral glucose tolerance test (OGTT) is shown in Fig. 8.2. The onset of T2D is not associated with a further deterioration in insulin sensitivity but rather insulin secretion that wanes and fails to compensate for the prevailing IR [20]. Individuals in the upper tertile of "normal" glucose tolerance (2-h PG = 120–139 mg/dL, i.e., 6.7–7.7 mmol/l) would have lost two-thirds of their β-cell function (see first arrow in Fig. 8.2), while subjects in the upper tertile of IGT (2-h PG = 180–199 mg/dL, i.e., 10.0–11.1 mmol/l) would have lost 80–85% of their β-cell function (see second arrow in Fig. 8.2) [3, 21–23]. Concisely, although IR in the liver/muscle is well established early in the natural history of T2D, overt diabetes will not develop in the absence of progressive β-cell failure [18]. Also, with acquisition of recent knowledge, it appears that β-cells may become dedifferentiated in people with T2DM and that these dedifferentiated cells may convert to other cell types such as glucagon-secreting α-cells [24].

Age and genes are two well-known non-modifiable factors which influence the state of β-cell health. A progressive age-related decline occurs in the β-cell function [25], and the incidence of diabetes is found to increase with advancing age. β-Cell failure clusters in families and a number of genes have been associated with T2D in people from multiple ethnic backgrounds. Most common are the transcription factors associated with β-cell dysfunction (e.g., the T-allele of single-nucleotide polymorphism rs7903146 of the TCF7L2 gene) [12, 26, 27]. However, the modifiable contributors to insulin secretion and IR, e.g., lipotoxicity, glucotoxicity, and incretin defects, can improve β-cell function and should be sought [3, 28]. Hypersecretion of islet amyloid polypeptide (co-secreted with insulin) gives way to subsequent amyloid deposition within the pancreas, and it is speculated to be involved with disease progression rather than initiation [29, 30] (see Fig. 8.3).

Insulin Resistance

Insulin resistance is the diminished ability of insulin to exert its biological action across a broad range of concentrations [31]. Relatively high insulin levels observed during fasting and in response to insulin secretagogues are indicative of IR. Insulin resistance is a consistent finding in T2D, and it may appear many years before the disease onset. It is also a well-known associate of obesity, and many obese individuals develop T2D. Interestingly, some patients, despite being obese, never develop T2D highlighting the significant contribution of the β-cell deficit in the individuals who develop T2D. Important to note, physical activity has a significant positive effect on insulin sensitivity, even when correcting for confounding factors such as being overweight. What's more, during the latter half of pregnancy, even in women with normal glucose homeostasis, IR increases due to the production of placentally derived hormones like human placental lactogen. Gestational diabetes mellitus (GDM) ensues if the maternal β-cells are unable to produce sufficient insulin to overcome the IR. Insulin resistance is mediated at three organ levels—the liver, muscle, and adipose tissue. Much more than the mere IR, the triad of factors contributes to the biochemical potpourri of diabetes [5, 10].

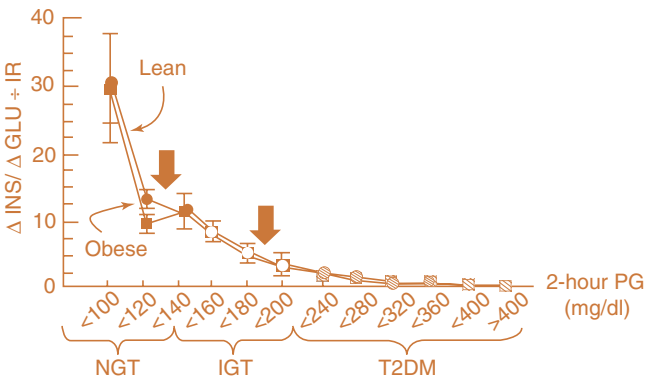

Fig. 8.2 Insulin secretion/insulin resistance (disposition) index in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (T2D) as a function of 2-hour PPG in lean (closed circle) and obese (open circle) individuals [3]. (Adapted from: DeFronzo [3])

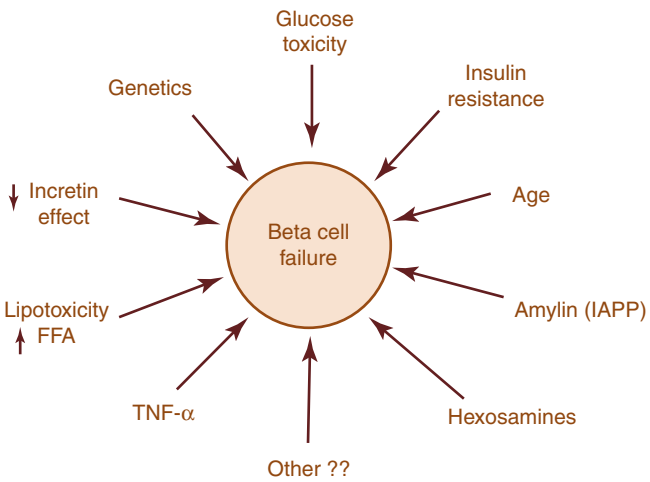

Fig. 8.3 Pathogenic factors implicated in progressive β-cell failure [28]. (Adapted from: DeFronzo et al. [28])

Hepatic Insulin Resistance

Insulin resistance in the liver is manifested by glucose overproduction during the basal state despite fasting hyperinsulinemia [13] and impaired suppression of HGP by insulin [32], following a meal [14]. Due to its obligate need for glucose, brain uses up more than half of the glucose produced. This glucose demand is met primarily by the liver and to a lesser extent by the kidneys [33]. Normally, the liver produces 2 mg/kg per min of glucose, whereas in diabetes-affected individuals, this basal rate of HGP is increased to 2.5 mg/kg per min. This increased HGP occurs even when the fasting plasma insulin levels are increased 2.5 to 3-fold, indicating severe resistance to the suppressive effect of insulin on HGP.

In the early stages of T2D, postprandial hyperglycemia is attributed to reduced glucose uptake by the muscles, and during the postabsorptive period, the fasting levels are maintained by HGP. Both sources of HGP, glycogenolysis and gluconeogenesis are under insulin control. But in IR state, the latter accounts for nearly the entire increase in hepatic glucose output. Around one-fourth of the glucose derived from a meal is extracted by the liver during recycling of portal and systemic blood, and insulin is suggested to facilitate the storage of this glucose as glycogen. Gluconeogenesis accounts for even greater amounts of glycogen. Moreover, during active gluconeogenesis, glycogenolysis is inhibited and results in excess hepatic glycogen [34] in uncontrolled diabetes. The accelerated gluconeogenesis in T2D can be due to elevated circulating levels of precursor molecules like lactate and alanine from the muscles and glycerol from the adipose tissue. Simultaneous upsurge in free fatty acids (FFA) and concomitant hyperglucagonemia facilitate gluconeogenesis. Insulin resistance also stimulates VLDL and apo-B synthesis in the liver, while HDL is lowered due to greater exchange of cholesterol ester transport protein. An increase in small dense low-density lipoprotein particles is also noted during the process [1].

Muscle Insulin Resistance

In the muscle, IR is manifested by impaired glucose uptake after carbohydrate ingestion, resulting in postprandial hyperglycemia [32, 35, 36]. In the insulin-stimulated postprandial state, the skeletal muscle accounts for more than 75% of the excess glucose uptake [37], and in diabetes patients, it accounts for the largest part of impairment of glucose disposal. Adipose tissue mass being smaller in size accounts for the rest, whereas there may not be any change in the case of brain and splanchnic tissues [10]. In T2D the muscle IR accounts for more than 85–90% of the impairment in total body glucose disposal [1, 2, 35, 36].

Adipose Tissue Insulin Resistance

Though deranged adipocyte metabolism was initially not considered to play a significant role in T2D pathogenesis, later on, evidences supported the adipose tissue to be considered as the "fourth musketeer" along with the triumvirate [3, 38, 39]. In healthy individuals, insulin exerts an anti-lipolytic effect on fat cells, whereas in T2D individuals, IR prevents insulin from exerting its anti-lipolytic effect. The result is sustained lipolysis with day-long elevation in plasma free fatty acid (FFA) levels. This, in turn, stimulates gluconeogenesis, induce hepatic and muscle IR, and impair β-cell function (lipotoxicity). FFAs also enhance the activity of glucose-6-phosphatase that ultimately controls the release of glucose by the liver [40]. Dysfunctional adipocytes produce proinflammatory adipocytokines in excess (IL-6, TNF-α, leptin, visfatin, etc.) which induce IR and atherosclerosis. This also induces a feed-forward process in which activation of transcription factors leads to further proinflammatory cytokine production [8, 41]. Deranged adipocytes also tend to secrete subnormal amounts of insulin-sensitizing adipocytokines (e.g., adiponectin). Enlarged fat cells are insulin-resistant and have lesser capacity to store fat. When the storage capacity of adipocyte is exceeded, lipid "overflows" into the muscle, liver, β-cells, and arterial vascular smooth muscle cells leading to muscle and hepatic IR, impaired insulin secretion, and acceleration of atherosclerosis [42].

Meanwhile, the discovery of functional brown adipose tissue raised the possibility for its involvement in human energy homeostasis and in preventing T2D. The detectability of this tissue lessens with age, high BMI and high FPG [9, 43]. A healthy obese state has been entertained, and the theory is based on the existence of differences in IR between adipose deposition sites. In other words, adipose site-specific IR by intra-abdominal adipose tissue seemingly more insulin resistant and harmful than subcutaneous adipose tissue (SAP). The former has particularities related to higher lipolysis, higher release of adipokines, etc. which are longitudinally associated with an increased risk of incident metabolic syndrome (MetS) [44]. In individuals who remain resistant to T2D, excess calories are safely partitioned to SAP rather than to the muscle, liver, heart, and β-cells, thus avoiding damages to the key organs. Major mechanisms for such protective effects include β-cell compensation, maintenance of near-normal blood nutrient levels, development of minimal IR, increased expansion of SAT relative to visceral adipose tissue, and limited increase in liver fat [9].

Alpha Cells (Increased Glucagon Secretion)

In as early as 1970s, it has been established that T2D individuals have elevated plasma glucagon levels [45–47]. While a reduction in β-cell mass is seen in diabetes patients as compared to normal individuals, there occurs no reduction in the α-cell mass. It is also proposed that β-cells dedifferentiate in T2D individuals and get converted to other cell types like glucagon-secreting α-cells [24]. Substantiating these, even when insulin levels progressively decline over the course of T2D, basal glucagon levels tend to remain elevated [48, 49].

The role of hyperglucagonemia in the maintenance of increased rates of HGP in T2D was demonstrated by Baron et al. [50]. In T2D, fasting glucagon levels are elevated, and the postprandial glucagon levels are not suppressed but paradoxically elevated. This raised blood glucagon levels increase the HGP leading to an elevation in FPG and PPG levels resulting in worsening of diabetes [49, 51].

Upon somatostatin infusion, there were declines in plasma glucagon levels by 44% and in basal HGP by 58%. When somatostatin was administered to alloxan-diabetic dogs [52] or to insulin-deprived T2D subjects [53], hyperglucagonemia was suppressed and hyperglycemia was reduced, even though insulin had been reduced or discontinued. Many other studies also support the prime role played by glucagon in T2D pathogenesis [45, 54, 55]. The drugs capable of inhibiting glucagon secretion or blocking the glucagon receptor are now proven effective in treating T2D [56–58].

Incretin Defect

Glucose ingestion can elicit a higher insulin response than an intravenous infusion which is explained by the incretin effect. The incretin hormones, Glucagon-like peptide-1 (GLP-1) secreted by the L-cells of the distal small intestine and Glucose-dependent insulinotropic peptide (GIP) by the K-cells of the more proximal small intestine, collectively act on the pancreatic islet [3, 8, 59, 60]. Of these, the GLP-1 acts on the β-cells to increase insulin and on the α-cells to suppress glucagon secretion [59]. GLP-1 thus imparts an indirect benefit on β-cell workload, since a reduction in glucagon levels leads to a reduced postprandial HGP. Gut hormones, including GLP-1, also have roles in central nervous system (CNS) regulation of energy balance and appetite [9, 61]. GLP-1 delays the rate of gastric emptying, results in a feeling of fullness and satiety, and is therefore associated with control of weight gain [3, 62, 63]. In T2D, the incretin effect is substantially impaired possibly due to impaired GLP-1 production and reduced sensitivity of β-cells to GIP [9, 64, 65]. Dysfunction in the glucagon secretion due to impaired incretin action is also suggested [66]. In subjects with NGT, IGT, or T2D, plasma GLP-1 levels do not seem to differ much [66] which suggests that the β-cell response to GLP-1 following meal ingestion is deficient, as seen during intravenous administration of GLP-1 under controlled conditions [67]. Elevations in GLP-1 levels are reported after bariatric surgery, which might partially explain the multiple beneficial effects of this intervention, especially among T2D individuals [8]. Numerous pharmacologic approaches are available nowadays that effectively harness the potential of incretins to treat diabetes, which include GLP-1 agonists and DPP4 inhibitors [68].

Kidneys (Increased Glucose Reabsorption)

The kidney's adaptive response to conserve glucose, which enables to meet the energy demands of the body, especially the brain and other neural issues, which have an obligate need for glucose, becomes maladaptive in diabetes. Rather than draining out the glucose into urine to correct the hyperglycemia, the kidney retains the glucose. Normally the kidney filters around 162 g of glucose daily, and the high-capacity SGLT2 transporter in the convoluted segment of the proximal tubule reabsorbs almost 90% of the filtered glucose, and the remaining 10% is reabsorbed by the SGLT1 transporter in the straight segment of the descending proximal tubule [69]. In both T1D and T2D, the maximum renal tubular reabsorptive capacity (Tm) for glucose is higher [70–73]. Therefore, in normal individuals, no glucose appears in the urine until the plasma glucose level is >180 mg/dL [74], whereas in T2D this threshold is much higher [72]. Medications to inhibit renal proximal tubular glucose reabsorption were thus thought out to treat T2D [69]. SGLT 2 inhibitors impart multiple benefits like better glycemic control by improving β-cell function and insulin sensitivity, reductions in body weight and blood pressure, etc. [75]. Currently, therapies aimed at inhibiting the SGLT1 receptors in the gut and downstream from SGLT2 receptors in the kidney are also underway [4, 76, 77].

Brain (Neurotransmitter Dysfunction and Central Appetite Dysregulation)

The nervous system also plays a key role in T2D pathogenesis. Sympathetic and parasympathetic nervous systems control glucose metabolism directly through the neuronal input and indirectly via the circulation to regulate insulin and glucagon release and HGP [8, 78]. Severing the vagus nerve impaired the insulin secretion revealing its important role in regulating the islet [79]. Ablation of the hypothalamus leads to β-cell dysregulation and subsequent hyperinsulinemia [80]. Insulin has a powerful appetite suppressing effect [81]. However, in obese individuals with or without diabetes, even though IR results in compensatory hyperinsulinemia, food intake seems to be higher indicating that the appetite centers are also IR. In a functional magnetic resonance imaging study where the cerebral response to ingested glucose was examined [82], consistent inhibition was noted in the lower posterior (which contains the ventromedial nuclei) and upper posterior (which contains the paraventricular nuclei) hypothalamus upon glucose ingestion. Both these areas are key appetite regulation centers, and the extent of inhibitory response upon glucose ingestion was decreased in these areas in obese, insulin-resistant, subjects even when euglycemic. A delay was also observed in the time taken to reach the maximum inhibitory response in these individuals even in the presence of a high plasma insulin response. Further studies have also indicated that cerebral IR leads to increased HGP and reduced muscle glucose uptake [83, 84]. High-fat diet-fed rodents are prone to inflammation-induced neuronal injury, and in humans, structural changes in the hypothalamus

have been observed in keeping with gliosis in obese compared to lean individuals [85]. Reduced dopamine levels in the hypothalamus and increased catecholamine levels in the CNS also contribute to the appetite dysregulation and are suggested to directly cause IR in liver and peripheral tissues [84, 86]. The neuroendocrine hormone amylin is also deficient in T1D and T2D [7], and its effect on appetite dysregulation is suggested to be chiefly mediated via central pathways that include high-affinity binding sites in the area postrema in the hindbrain [87]. It also has direct gut effects through a decrease in the rate of gastric emptying [88]. Clock genes located in the brain which are major determinants of circadian rhythmicity, together with sleep, are now being investigated due to their role in metabolic processes [89, 90].

The "Dirty Dozen," the "Unlucky Thirteen" and Much More

Dopamine

Dopamine the most abundant catecholamine in the brain has been nicknamed as the "forgotten felon" of diabetes [5], and along with the other catecholamines of the autonomic nervous system, this neurotransmitter modulates glycemia. It was Kalra et al. who proposed the specific addition of dopaminergic system as a ninth contributor to the T2D development [91]. Mammalian species have an inherent capacity to alter their metabolism from the insulin-sensitive/glucose-tolerant state to the insulin-resistant/glucose-intolerant state at exactly the right time of the year to meet the varying energy demands. Such seasonal metabolic changes are governed by the changes in monoaminergic concentrations/activity in the suprachiasmatic nuclei (SCN) of the hypothalamus—the mammalian circadian pacemaker—and in the ventromedial hypothalamus (VMH). Development of an IR state during such seasonal changes exactly mimics the T2D state: muscle and hepatic IR, increased HGP/gluconeogenesis, hyperglycemia, adipocyte IR and enhanced lipolysis, increased plasma FFA and triglyceride levels, and obesity. Evidences implicate endogenous dopaminergic and serotonergic rhythms in SCN and VMH in the transition from the insulin-sensitive to insulin-resistant state. In the animals that undergo seasonal changes in metabolism, within the VMH, during the insulin-resistant state, both serotonin and noradrenergic levels and activity are enhanced and decrease to normal levels upon returning to the insulin-sensitive state. On the contrary, dopamine levels are decreased during the IR state and increase to normal following return of the insulin-sensitive state. A selective destruction of dopaminergic neurons in SCN of the hypothalamus resulted in severe IR [86, 92, 93].

Both systemic [94, 95] and intracerebral [96] bromocriptine (a sympatholytic D2-dopamine agonist) administration to insulin-resistant animals decreased the elevated VMH noradrenergic and serotonergic levels with a resultant decline in HGP, reduced adipose tissue lipolysis, and improved insulin sensitivity. In T2D and obese nondiabetic individuals, systemic bromocriptine administration improved glycemic control and dyslipidemia without changes in body weight [97]. It was postulated that in T2D patients hypothalamic dopamine reduces in the early morning leading to elevated HGP and lipolysis resulting in glucose intolerance, IR, and dyslipidemia. Timed bromocriptine (quick release formulation) administration within 2h of awakening augmented low hypothalamic dopamine levels and decreased the sympathetic tone within the CNS, leading to an increase in insulin sensitivity, suppression of HGP, and thereby a reduction in PPG levels [86].

Vitamin D

Vitamin D subserves a range of biological functions like cell differentiation, inhibition of cell growth, and immunomodulation. Both direct and indirect effects of vitamin D on various mechanisms related to the T1D and T2D pathophysiology have been postulated including pancreatic β-cell dysfunction, impaired insulin action, systemic inflammation, and apoptosis. Vitamin D receptors occur in all tissues and organs that are involved in these diseases, and the machinery for producing vitamin D locally is also present in islets, immune cells, and other tissues involved [6, 98–100]. Children receiving recommended dose of vitamin D during the first year of life had an 80% reduced T1D risk [101]. Lower vitamin D levels might result in impaired β-cell function with lowered insulin secretion and sensitivity and with higher IR [102] and might also pose a risk for developing macrovascular and microvascular complications [103]. Among Caucasian children and adolescents, low vitamin D levels were associated with total adiposity, MetS, and hypertension [104]. Vitamin D supplementation to T2D and nondiabetic subjects impart beneficial effects on glucose homeostasis and other markers of MetS like improving β-cell function and insulin secretion and reducing IR [105, 106].

Renin-Angiotensin System

Evidences suggest a role for the RAS in the development of IR and T2D [107, 108]. Detrimental effects that RAS has on insulin secretion is mediated by a decrease in pancreatic blood flow and induction of islet fibrosis, oxidative stress, and inflammation, whereas both impaired skeletal muscle function (disturbances in skeletal muscle blood flow, insulin signaling, and mitochondrial function) and adipose tissue

(AT) dysfunction (adipocyte hypertrophy, inflammation, and impairments in AT blood flow and lipid metabolism) may contribute to RAS-induced IR [108]. Frequent association of T2D with hypertension, retinopathy, nephropathy, and cardiovascular disease (CVD) has also implicated RAS in the initiation and progression of these disorders.

RAS blockade significantly improves insulin sensitivity [109–111] and significantly reduces the incidence of vascular complications in T2D [112–114]. Such improvements are postulated to be due to the improvement of blood flow and microcirculation in skeletal muscles, decrease in adipocyte size, protective actions on pancreatic islets, etc., thereby facilitating insulin signaling at the cellular level and improvement of insulin secretion by pancreatic β-cells [7, 115–118]. Among high-risk populations, RAS blockade using either angiotensin II receptor blockers (ARB) or angiotensin-converting enzyme inhibitors (ACEI) led to a 22% reduction in the incidence of new-onset T2D [119]. The DREAM study in individuals without CVD but with impaired fasting glucose levels or IGT showed that the ACEI ramipril did not significantly reduce the diabetes incidence or death but significantly increased the regression to normoglycemia [120]. The NAVIGATOR study among individuals with IGT and CVD or risk factors showed that ARB (valsartan) use, along with lifestyle modification, caused a relative 14% reduction in the diabetes incidence but did not reduce the rate of CVD events [121]. Despite the lack of consistency in the findings between many of these trials, sub-analyses of some of them have shown that RAS inhibitors improve glucose levels and reduce the risk for diabetes in higher-risk populations [107, 122, 123].

Testosterone

An association between low testosterone and T2DM risk in men is well-proven [124–126]. Morbid obesity imposes negative effects on the hypothalamic-pituitary-gonadal axis in men [127]. A bidirectional relationship between visceral fat and testosterone is suggested, which sets up a self-perpetuating cycle promoting IR and diabetes. High visceral fat increases secretion of proinflammatory cytokines, estradiol, insulin, and leptin, all of which may inhibit the hypothalamo-pituitary gonadal axis activity at multiple levels [128, 129]. Decreased testosterone levels also build up IR via mechanisms involving the muscle [130], liver [131], and bone [132]. Testosterone decreases IR by regulating mature adipocytes and myocytes. Testosterone also increased catecholamine-induced lipolysis in vitro [133] and decreased lipoprotein lipase activity and triglyceride uptake in abdominal adipose tissue in humans [134]. A positive correlation exists between testosterone levels and insulin sensitivity, and the individuals with hypogonadal testosterone levels had higher BMI and higher prevalence of the MetS than their eugonadal counterparts [130]. Other studies, including the landmark studies like Massachusetts Male Aging Study (MMAS) and Multiple Risk Factor Intervention Trial (MRFIT), have all demonstrated an inverse association between low testosterone levels and risk for MetS and diabetes [126, 135–137]. Further, low sex hormone-binding globulin (SHBG) may lead to IR and to lower total testosterone [138]. Among prostate cancer patients subjected to androgen deprivation therapy (ADT), lower testosterone levels were associated with increased IR [139, 140] and an increased diabetes risk [141]. Testosterone substitution in hypogonadal men improved insulin sensitivity and glycemic control [142, 143]. In men with newly diagnosed diabetes, the addition of testosterone to a regimen of diet and exercise significantly improved the outcomes on glycemic control and reversal of the MetS [143, 144].

Interestingly, the effect of testosterone on IR and T2D is opposite between males and females. Its low concentrations in males but high concentrations in females favor IR and T2D [145–147]. In a systematic review and meta-analysis by Ding et.al, endogenous levels of testosterone and SHBG were found to exhibit sex-dependent relations with the risk of T2D. Elevated testosterone levels were linked to greater T2D risk in females but lower risk in males. Meanwhile, SHBG was more protective in females than in males [147]. Polycystic ovary syndrome (PCOS), characterized by chronic anovulation and hyperandrogenism, was also suggested to have partly contributed to this observed positive testosterone association in females. Insulin resistance with compensatory hyperinsulinemia is the key pathogenic factor in PCOS and can lead to the onset of hyperandrogenism by stimulating ovarian androgen production and by decreasing the SHBG levels. Females of reproductive age with PCOS are thus prone to metabolic disorders and T2D [147, 148].

Gut and Gut Microbiota

Gut

Centuries back, the ancient Greek physician Hippocrates had said: “All Disease Begins in the Gut.” Mounting evidence strongly supports the above-quoted hypothesis, and in 2016 Somasundaram et.al. proposed the role played by gut as the thirteenth mechanism in diabetes pathogenesis [7]. Even though the contribution of gastrointestinal (GI) carbohydrate absorption toward T2D pathogenesis had long been known, its contribution was rather underutilized as a target for therapy. Alpha-glucosidase inhibitor (AGI) is the only class of drug that effectively utilizes this mechanism for the treatment

of diabetes and has clear beneficial effects on glycemic control and post-load insulin levels [149]. SGLT1 plays a distinct and complementing role to SGLT2 in glucose homeostasis. Within the GI tract, SGLT1 is responsible for glucose absorption and is also involved in 10% of renal glucose reabsorption. Inhibition of SGLT1 and combined inhibition of SGLT1/SGLT2 is thus a new anti-hyperglycemic concept [4, 76, 77], which further implies the contribution of GI carbohydrate absorption in T2D pathogenesis.

Bile acids also play a significant role in modulating glucose homeostasis, and bile acid homeostasis is altered in T2D. Bile acids act as signaling molecules through receptor-dependent and receptor-independent pathways [150]. They act as endogenous ligands of the farnesoid X receptor (FXR), and their activation of FXR leads to the release of fibroblast growth factor (FGF) [151]. Through FXR, bile acids suppress the in vitro expression of fructose-1, 6-biphosphatase-1, gluconeogenic phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [152]. G-protein-coupled receptors TGR5 (also termed GPR131) located on intestinal L-cells are activated by bile acids, resulting in GLP-1 secretion [153]. Intraduodenal bile acid infusion dose-dependently enhanced the plasma FGF19 concentrations, with smaller effects on GLP-1 and CCK [154, 155]. FGF19 possess insulin-like effects inducing glycogen and protein synthesis while suppressing glucose production [8]. A second-generation bile acid sequestrant colesevelam modestly reduces glucose in T2DM when used as an adjunct to other agents. Suggested mechanisms include its effect on bile acid receptors in the intestine as well as in the liver to reduce endogenous glucose production [7, 156].

Gut Microbiota

Besides the gut, the gut microbiome is also involved in the T2D pathogenesis [7, 8, 157, 158]. Gut dysbiosis, intestinal barrier dysfunction, and subsequent metabolic endotoxemia are all closely related to the inflammation, IR, and finally CVD events in T2D [159, 160]. Individuals with prediabetes or T2D have a moderate degree of gut microbial dysbiosis in terms of a reduction in the abundance of certain universal butyrate-producing bacteria (*Faecalibacterium prausnitzii*, *Roseburia intestinalis*, etc.) and an increase in various opportunistic pathogens (like *Lactobacillus* sp.) [160, 161]. In individuals with MetS, vancomycin treatment decreased the abundance of butyrate-producing gram-positive bacteria, which correlated well with impaired insulin sensitivity [162]. Decreased levels of butyrate-producing gut microbes in T2D individuals were thus suggested to lead to disease pathogenesis. Among the short-chain fatty acids, butyrate acts as a prominent energy source for intestinal epithelial cells and influences a variety of colonic mucosal functions, reinforcing the colonic defense barrier and attenuating oxidative stress [163]. Butyrate also enhances the intestinal barrier by modulating the assembly of tight junctions (TJs) via AMP-activated protein kinase (AMPK) activation [164]. Feces of T2D subjects were relatively enriched with endotoxin-producing gram-negative bacteria (phyla *Bacteroidetes* and *Proteobacteria*) which suggested the role played by these phyla in T2D pathogenesis through an endotoxin-induced inflammatory response pathway [161]. In the liver, cholic acid and chenodeoxycholic acid constitute primary bile acids produced from cholesterol, and the gut microbiota transforms these primary bile acids into secondary bile acids [165]. In line with these facts, 6 weeks after the infusion of intestinal microbiota from lean subjects, an improvement in insulin sensitivity was noted in subjects with MetS [166].

Iron Overload

There is no active mechanism for iron excretion in humans, and the amount of iron absorbed into the body is balanced by the iron lost by means of sloughing of the intestinal mucosa and skin and also lesser amounts that are excreted in the urine and bile [167]. Iron overload is thus a risk factor for T2D [168], whereas its depletion has a protective effect against T2D.

The foremost evidences for this were obtained from studies related to pathologic iron overload disease conditions like hereditary hemochromatosis (HH) [169] and transfusional iron overload [170]. Both insulin deficiency and IR can contribute to the T2D pathophysiology associated with HH [171, 172]. Individuals with HH have an inherent insulin secretory defect, making them highly prone to develop diabetes especially when IR from an independent mechanism such as obesity intervenes [173]. HH individuals have extremely high ferritin levels (1,000–10,000 ng/ml), and around 25–60% of them develop "secondary" T2D [174, 175]. Transfusional iron overload is usually seen in transfusion-dependent chronic hemolytic anemia such as β-thalassemia. Due to the numerous transfusions that are required to maintain adequate erythrocyte levels and the resultant increased iron absorption, these patients become iron overloaded [176]. Individuals with β-thalassemia mostly develop IGT during the second decade of life, and a diabetes prevalence is reported among 6–14% of the patients [177, 178]. T2D is also prevalent among survivors of pediatric bone marrow transplantation [179] and allogeneic hematopoietic cell transplantation [180]. Some rare inherited diseases that cause diabetes such as Friedreich ataxia are associated with iron imbalance and with mutations in the proteins involved in iron metabolism [181].

Positive associations between elevated body iron stores (measured as circulating ferritin), and the risk of T2D and of other IR states such as the MetS, GDM, PCOS, and possibly CVD, have been demonstrated [174, 182, 183]. Moderate increases in iron stores (lower than the levels found in HH subjects) were associated with increases in blood glucose and insulin levels. Furthermore, moderately increased body iron stores at baseline were associated with an elevated risk of developing T2D in the future [175]. In a National Health and Nutrition Education Survey (NHANES), the odds ratios for newly diagnosed diabetes in individuals with higher serum ferritin levels were 4.94 for males and 3.61 for females [184]. A link has also been established between increased dietary iron intake (particularly heme iron) and the risk for T2D and GDM [185–188]. No significant association of dietary intakes of total iron, non-heme, and supplemental iron intake was found with the risk of T2DM, whereas heme iron intake showed a positive association, after adjustment for potential confounders. Individuals who consume meat (a major source of heme iron) are thus reported to be more IR compared to the vegetarians [174, 175].

Iron overload is also implicated in the pathogenesis of many diabetes-associated vascular complications including diabetic nephropathy (DNP) and CVD [185]. In individuals with DNP, an increased proximal tubular lysosomal iron concentration has been observed. In iron-loaded subjects with thalassemia, an early development and accelerated course of DNP is reported. Similarly, mutations for HH appeared to predict the development of DNP [189]. Iron has an adverse effect on endothelium and accelerates the development of atherosclerosis. Elevations have been observed in ferritin gene expression during the course of atherosclerotic plaque formation [189, 190].

Multiple mechanisms have been proposed toward the association between iron and abnormal glucose metabolism, like β-cell dysfunction and IR, possibly mediated through oxidative stress [185, 191]. Being a redox-active transitional metal, excess iron is potentially hazardous. It catalyzes several cellular reactions that lead to the production of reactive oxygen species and thereby to an elevated oxidative stress, which is proposed to contribute to an increased risk of T2D. Pancreatic β-cells, due to their weak antioxidant defense system, are highly susceptible to oxidative damage, and thus iron deposition in these cells can result in impaired insulin secretion. In the muscle, iron overload may diminish glucose utilization, thereby leading to a shift from glucose to fatty acid oxidation, resulting in an increased IR. Increased substrate recycling to the liver may contribute to an elevated HGP. Iron may also impair the action of insulin and interfere with glucose uptake in adipocytes. Elevations in systemic inflammation may also modify iron metabolism. Inflammatory cytokines are found to induce the synthesis of ferritin [174, 175]. As iron influences the action of insulin, insulin also is in turn known to influence iron metabolism. Insulin plays a role in redistributing transferrin receptor (TfR) to the cell surface and thereby increasing the cellular uptake of iron in adipose tissue and the liver. Thus in the IR states, inherent hyperinsulinemia leads to elevations in levels of circulating soluble form of TfR (sTfR), a marker of iron status [174].

The potential benefit of iron depletion on insulin sensitivity and/or T2D has been evaluated by many. Phlebotomy enhanced insulin sensitivity and glycemia, in normal as well as T2D subjects with elevated ferritin levels [174, 192]. In HH patients, phlebotomy and/or iron chelation therapy (to decrease body iron stores) improved their glycemic control, and 30–40% of them achieved either elimination of oral diabetes therapy or a substantial decrease in dosage [193]. Blood donations reduce the circulating ferritin levels and frequent blood donors seem to have a better insulin sensitivity than the non-donors. Increased number of lifetime blood donations was found to be associated with decreased prevalence of T2D [194]. Among T2D individuals who were negative for common HH but had increased serum ferritin concentration, bloodletting improved their insulin sensitivity and reduced their C-peptide levels [189].

Conclusion

With the ever-increasing epidemic of obesity, physical inactivity, and an aging population, the prevalence of T2D is reaching pandemic proportions. This demands rigorous efforts to improve our understanding of this devastating disease. Valiant research endeavors have led to an improved understanding of the causality and pathophysiological aspects of T2D. From humble beginnings, with the recognition of only two factors, defective insulin secretion and IR, we are now able to appreciate the complexity and heterogeneity of the role-players in the pathogenesis of T2D (see Fig. 8.4). It is likely that many more factors are yet to be unveiled. While addressing the modifiable risk factors to prevent T2D, this knowledge empowers us to utilize the existing therapies efficiently and inspires us to explore and develop newer effective therapies. The complexity of T2D demands, a multifaceted therapeutic approach, which combines pharmacological and non-pharmacological interventions in an individualized way.

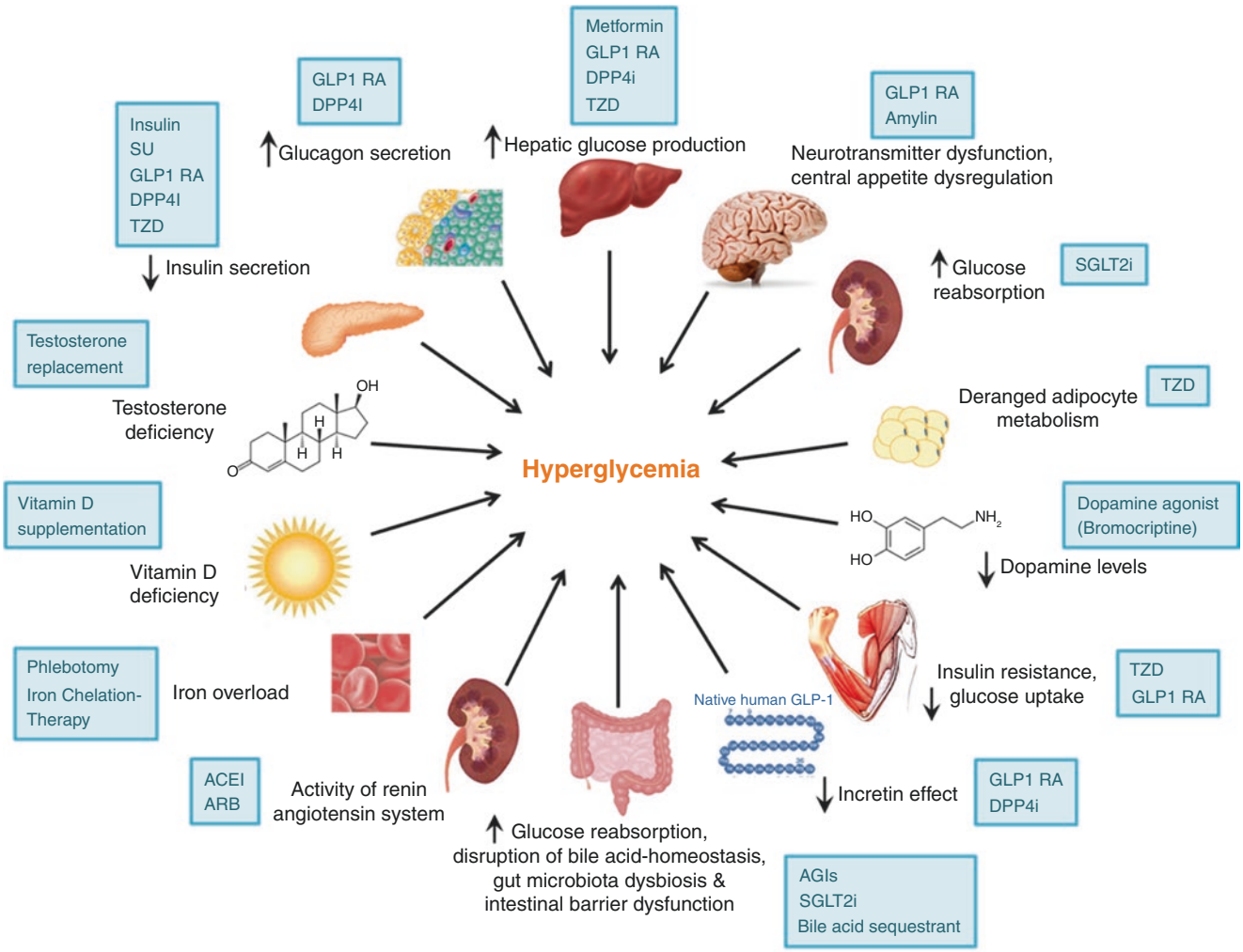

Fig. 8.4 Players in the pathophysiology of T2D

Multiple Choice Questions

1. In the postabsorptive state, most of the glucose utilization occurs:
   (a) In the muscle
   (b) In the brain
   (c) In adipose tissue
   (d) In beta cells
   (e) In red cells
2. Regarding lipoprotein metabolism:
   (a) Insulin has not demonstrated effects.
   (b) Insulin increases circulating VLDL levels.
   (c) Insulin stimulates lipoprotein lipase in adipocytes, promotes lipolysis and removal of chylomicrons.
   (d) Insulin stimulates lipoprotein lipase in vascular endothelium, promotes lipolysis and removal of chylomicrons.
   (e) Insulin increases triglyceride levels.
3. In the event of hepatic insulin resistance:
   (a) Hepatic glucose production is suppressed by low fasting insulin levels.
   (b) Hepatic glucose production initially continues but is suppressed as insulin levels increase.
   (c) Hepatic glucose production is stable.
   (d) Hepatic glucose production continues even when fasting insulin levels are high.
   (e) Hepatic glucose production is suppressed.
4. Postprandial hyperglycemia results:
   (a) From hepatic insulin resistance
   (b) From peripheral tissue insulin resistance
   (c) From beta-cell insulin resistance
   (d) From increased hepatic glucose production
   (e) From decreased transport of glucose in the central nervous system

5. The core physiological defects proposed in the triumvirate concept include the following except:
   (a) The central nervous system
   (b) Pancreatic alpha-cells
   (c) Pancreatic beta-cells
   (d) The liver
   (e) Skeletal muscle
6. Individuals in the upper tertile of "normal" glucose tolerance:
   (a) Maintain 100 percent of beta-cell function
   (b) Have lost 20 percent of beta-cell function
   (c) Have lost 50 percent of beta-cell function
   (d) Have lost 70 percent of beta cell-function
   (e) Have lost 100 percent of beta-cell function
7. Amyloid deposits within the pancreas:
   (a) Have a protective effect on beta-cell function
   (b) Are crucial to initiate type 2 diabetes
   (c) Are involved with disease progression
   (d) Are associated with disease remission
   (e) Indicate glucose toxicity
8. Insulin resistance
   (a) Appears year before the onset of type 2 diabetes
   (b) Is an unusual manifestation of type 2 diabetes
   (c) Is a late manifestation of type 2 diabetes
   (d) Occurs at the same time as beta-cell failure
   (e) Always evolve to type 2 diabetes
9. Hepatic gluconeogenesis is facilitated by:
   (a) The incretin effect
   (b) By overactivity of the beta-cells
   (c) By basal insulin secretion
   (d) By high levels of VLDL lipoproteins
   (e) By high levels of free fatty acids
10. In persons with type 2 diabetes, the largest impairment of glucose disposal occurs:
   (a) In the central nervous system
   (b) In adipose tissue
   (c) In the kidney
   (d) In skeletal muscle
   (e) In erythrocytes

Correct Answers

1. (a) In the muscle
2. (d) Insulin stimulates lipoprotein lipase in vascular endothelium, reduces lipolysis and removal of chylomicrons
3. (d) Hepatic glucose production continues even when fasting insulin levels are high
4. (b) From peripheral tissue insulin resistance
5. (a) The central nervous system
6. (c) Have lost 50 percent of beta-cell function
7. (c) Are involved with disease progression
8. (a) Appears years before the onset of type 2 diabetes
9. (e) By high levels of free fatty acids
10. (d) In skeletal muscle

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