Title: Current findings in first phase insulin secretion and Type 2 Diabetes

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Abstract

Type 2 diabetes (T2D) is a metabolic disorder characterised by the inability of β-cells to secrete enough insulin to maintain glucose homeostasis. Pancreatic β-cells secrete insulin in a biphasic manner, first and second phase insulin secretion, and loss of first phase insulin secretion is an independent predictor of T2D onset. Restoration of first phase insulin secretion has been shown to improve blood glucose in T2D by suppressing hepatic glucose production and priming insulin sensitive tissue to more readily take up glucose and has thus prompted numerous studies into its regulation. First phase insulin secretion is initiated primarily by the classical triggering pathway, a complex system comprised of multiple stimulatory signals. Recent studies have identified a number of novel regulatory factors that are crucial for first phase insulin secretion and glucose homeostasis. These include, among others, hypoxia inducible factor 1α, von Hippel-Lindau, factor inhibiting HIF (FIH), nicotinamide phospho-ribosyl-transferase, and the sirtuin family. Recent publications have described conflicting roles for some of these factors which will be discussed. This review will provide a broad overview of the many facets of first phase insulin secretion and some of the recent discoveries regarding its regulation.

Keywords

Insulin, Insulin secretion, First phase insulin secretion, Aryl hydrocarbon Receptor Nuclear Translocator, Hypoxia Inducible Factor-1α, Glucose homeostasis, Regulatory genes, Type 2 Diabetes

Introduction

Type 2 diabetes (T2D) is characterised by the inability of β-cells to secrete enough insulin to maintain glucose homeostasis, usually accompanied by insulin resistance: impaired action of insulin on target tissues. T2D is a multi-factorial disease, the onset of which is likely to be environmental in a permissive genetic background. Thus, environmental influences such as increased caloric consumption and reduced physical activity are clearly involved [1, 2], and recent evidence from genome wide association studies (GWAS) have identified a number of susceptibility loci and polymorphisms which together increase the risk of developing T2D [3, 4].

Pancreatic β-cells secrete insulin in a biphasic manner, defined as first and second phase (Figure 1). It is thought that the initial insulin spike is crucial for glucose homeostasis. Studies have shown that loss of first phase insulin secretion is an independent predictor of T2D [5-9]. Disease states associated with impairment in first phase insulin secretion such as Huntington's and Alzheimer's diseases are also associated with increased risk of diabetes [10, 11]. Before the development of frank diabetes, people with fasting hyperglycaemia lack first phase insulin secretion [12] and people with impaired glucose tolerance (IGT) have reduced plasma insulin levels after a glucose load [13-15]. An increase in hepatic glucose production (HGP), normally suppressed by first phase insulin secretion, would compound the glucose load on β-cells, possibly leading to β-cell stress and failure observed in T2D. This review will focus on first phase insulin secretion in T2D and recent advances on how it is regulated.

First phase insulin secretion occurs very rapidly, with peak values achieved 1-2 minutes after glucose stimulation [16]. It is generally accepted to be initiated by an increase in the ATP:ADP ratio from glucose metabolism. Quite differently, second phase insulin secretion is more gradual and long lasting, usually reaching a plateau 25-30 minutes after stimulation in people with normal glucose tolerance [16] (Figure 1). While both phases of insulin secretion play an important role in glucose homeostasis, the relative importance of first phase insulin secretion appears to be greater. Studies in humans where first phase but not second phase insulin secretion have been artificially blocked by an infusion of somatostatin had significantly worse glucose tolerance compared with control conditions [17].

In addition to the biphasic manner of insulin secretion, effective control of glucose homeostasis requires secretion of insulin to be oscillatory [18]. It is important to note the difference in first phase insulin secretion and the oscillations in insulin secretion. While first phase insulin secretion is the initial increase in insulin secretion, insulin secretion after this is oscillatory, similar to a "wave". These oscillations occur every 9

– 14 min in humans *in vivo* [19] while others have reported shorter time periods (5 – 8 min) in isolated rat islets [20]. The oscillatory nature of insulin secretion is due to oscillations in glycolysis and its regulatory enzymes [21, 22]. Subjects with T2D have abnormal oscillations in insulin secretion [23] and together with the fact that islets of T2D patients also have reduced glucose oxidation [24] could contribute to inhibited first phase insulin secretion. Some first degree relatives of people with T2D also have abnormal oscillations [25] and thus would suggest some genetic and / or environmental regulation. Insulin infused with an oscillatory pattern has a greater effect than continuous delivery for individuals with diabetes [18] and as such, the less common pulsatile intravenous insulin therapy (PIVIT) or chronic intermittent intravenous insulin infusion therapy (CIIIT) has been used to greater effect [26, 27]. The exact mechanism(s) as to why it is more effective remains to be elucidated but a proposed theory is that PIVIT allows greater expression of insulin receptors than continuously high insulin levels thus increasing sensitivity in peripheral tissues [27].

Measurement of first phase insulin secretion

In order to measure biphasic insulin secretion *in vivo*, the following three methods are commonly employed: a) the intravenous glucose tolerance test (IVGTT), b) the hyperglycaemic clamp [28, 29], and c) mixed meal tolerance tests (MMTT) [30, 31]. The IVGTT is relatively simpler to conduct compared with the hyperglycaemic clamp. It involves a bolus of glucose injected intravenously over a short period (typically 1 minute) and blood samples taken at many, specific time points to measure insulin secretion and/or C-peptide [28]. This method is well suited for testing first phase insulin secretion as after the initial glucose injection, no further glucose is introduced into the system. According to measurements made by Polyzogopoulou and colleages [32], first phase insulin secretion as measured by IVGTT in individuals with normal glucose tolerance was 332.4±39.6 pmol/L compared to 24±22.7 pmol/L in people with T2D. There are issues with variability in readings of the initial insulin spike as glucose decays differently in each subject depending on their glucose tolerance [33]. In normal individuals, approximately 50 % of secreted insulin is cleared by the liver in the first pass of metabolism. So, if C-peptide is measured, deconvolution analysis can be used to estimate actual insulin secretion.

The hyperglycaemic clamp was first described in 1979 by Ralph DeFronzo and colleagues [34] and is a sophisticated technique which requires time and experienced labour. During this procedure, the subject's glucose level is raised to a pre-determined level, for example 125 mg/dl (7 mM/L) above basal levels by a priming dose of intravenous glucose. The hyperglycaemic plateau is then maintained by a constant infusion of

glucose. Insulin concentrations, C-peptide concentrations and the glucose infusion rate (GIR) are used as measures of insulin secretion and action [34]. As the β-cells are being exposed to glucose for a longer period of time, it is a more accurate measurement of both first and second phase insulin secretion and is considered the gold standard for the measurement of insulin secretion *in vivo* [33].

The MMTT, also known as the Boost test, is a relatively simple test and is suitable when performing a hyperglycaemic clamp is not feasible, such as in epidemiologic studies or for following individual changes in insulin secretion [30]. In a MMTT, subjects fast overnight then ingest a liquid meal (e.g. Sustacal^{TM}/Boost^{TM}) with a known proportion of carbohydrates, protein, and fat. Common values are 55 % carbohydrate, 25 % protein, and 20 % fat. Blood samples are taken at specific time points to analyse glucose, insulin, and C-peptide levels [30, 31]. Although not considered the gold standard for the determination of insulin secretion, it represents a more physiological response as the stimulus is delivered orally, and induces the normal incretin response [31]. A recent study showed that MMTT provided comparable results at 15 min after intake to results from a hyperglycaemic clamp in children with normal glucose tolerance [30]. The MMTT is clearly physiologically relevant as the normal means of glucose exposure is oral, not intravenous.

Regulation of first phase insulin secretion

The triggering and amplifying pathways

With the importance of first phase insulin secretion in glucose homeostasis, it is necessary to understand its regulation. The classical triggering pathway of insulin secretion in the β-cell has been extensively studied and is reasonably well understood [35-37]. Circulating blood glucose is taken up into β-cells by a glucose transporter called GLUT2 and is metabolised by the glycolytic pathway in the cytosol (Figure 2). The glucose sensing enzymatic step is catalysed by the hexokinase glucokinase. Together with the tricarboxylic acid (TCA) cycle and the oxidative phosphorylation process located in the mitochondrion, ATP is formed resulting in an increase in the cellular ATP/ADP ratio and closing of the ATP-sensitive potassium channels (K_{ATP} channels) [35, 37, 38]. The cell membrane then becomes depolarised, leading to the opening of the voltage dependent Ca^{2+} channels (VDCC) [39, 40]. An increase in cytoplasmic Ca^{2+} concentration finally leads to the secretion of insulin [41] (Figure 3).

The amplifying pathway was identified in 1992 by two groups working independently of each other [42, 43]. These experiments showed that glucose stimulation of insulin secretion was still possible even when K_{ATP} channels were either closed (with KCl) or kept open (with diazoxide), indicating that another pathway

must exist. The exact mechanisms of the amplifying pathway are still yet to be fully understood but insulin secretion via this pathway seems to involve signalling molecules other than ATP/ADP. Excellent reviews regarding the amplification pathway of insulin secretion have been published [35, 36, 44]. Pyruvate from glucose metabolism can be shuttled off via pyruvate carboxylase to form other intermediates which can trigger insulin secretion without the need for closure of K_{ATP} channels. These include NADPH from the malate-pyruvate shuttle and lipid signalling molecules from the malonyl-CoA/LC-CoA pathway [35].

The triggering and amplifying pathways of insulin secretion are not mutually exclusive but complement each other. However, depending on which phase of insulin is occurring, one of the pathways may play a more significant role. It is generally accepted that the first phase insulin secretion is initiated by the triggering pathway and this has much to do with the timing of the event. As stated earlier, first phase insulin secretion occurs within a very short time *in vivo*. It has been suggested that the signalling involved in the amplification pathway may be too slow for this time-frame and thus the triggering pathway is probably the major initiator of first phase insulin secretion [36, 37]. However, a previous study showed that both the triggering and amplifying pathways are involved [45]. This study examined the different signals in the triggering and amplification pathways of insulin secretion and showed that there are shifts in the cytosolic Ca^{2+}concentration and insulin response curves in first phase insulin secretion, shifts normally associated with the amplification pathway [45].

Insulin granule pools

The insulin that is secreted to maintain glucose homeostasis exists as pre-formed insulin granules within the β-cells. Secretory granules reside in two main pools; the docked and reserve pools [46]. The docked pool can be further divided into granules that are immediately releasable and readily releasable, and whether they are primed or un-primed [37]. Estimates suggest approximately 13000 granules in the docked pool and 50 granules per β-cell in the immediately releasable pool in normal mice [37, 47]. The 50 granules docked and immediately releasable are located in close proximity to the Ca^{2+} channels and are utilised for first phase insulin secretion [47]. The second phase of insulin secretion is thought to be maintained by a subset of approximately 1000 granules which are docked but un-primed [37]. In order for the granules to be primed and readily releasable, they must go through a series of reactions that involve ATP, calcium, and temperature [48, 49].

The fusion of insulin granules to the cell membrane and subsequent exocytosis has been the subject of many excellent reviews [50, 51]. SNARE proteins (soluble-*N*-ethyl-maleimide sensitive factor attachment

protein receptor) form a crucial component of the membrane fusion process and together with other proteins including syntaxin, SNAP-25, and synaptobrevin (or VAMP), allow exocytosis of insulin granules in the immediately releasable pool. In addition to this, SNARE proteins tether the insulin granules within close proximity of the calcium channels to ensure maximal response from minimal Ca^{2+} influx [47].

The role of glycolysis and mitochondrial oxidative phosphorylation

Glycolysis occurs in the cell cytosol and is the process by which glucose is converted to pyruvate, yielding a net of two molecules of ATP per molecule of glucose (Figure 2). The glycolytic pathway is nearly ubiquitous in cellular organisms and is an important process not only to provide a small amount of ATP but to provide precursor components for the TCA cycle and oxidative phosphorylation [52].

Often referred to as the citric acid cycle or the Kreb's cycle, the TCA cycle produces substrates that will be utilised in oxidative phosphorylation to produce ATP under aerobic conditions [52]. Oxidative phosphorylation occurs within the inner mitochondrial membrane and is the process by which ATP is generated by the transfer of electrons from NADH or $FADH_2$ to oxygen by a series of electron transporters. In essence, this is achieved by the generation of an electron-motive force, conversion into a proton-motive force and using this force to drive ATP synthesising assembly [52].

Mitochondrial oxidative phosphorylation plays an important role for insulin secretion, as it provides much of the needed ATP to change the ATP/ADP ratio [53-55]. Additionally, mitochondrial second messengers such as 2-oxoglutarate are known to mediate insulin secretion via the ATP-independent pathway [56] and this has been the subject of many excellent reviews [57, 58]. It has been clearly established that mitochondrial dysfunctions can lead to diabetes [59-61]. Recent experiments have shown that mice deficient in the pancreatic duodenal homeobox 1 (*PDX1*), a β-cell master gene, displayed defective insulin secretion due in part to suppression of the mitochondrial transcription factor A (*TFAM*) [59]. *TFAM* is crucial for the stability and transcriptional activity of mitochondrial DNA [62].

However, the question of the relative contribution of mitochondrial oxidative phosphorylation versus other pathways in physiological insulin secretion remains controversial [63-67]. One such argument is in regards to reactive oxygen intermediates (ROI), which include hydroxyl radicals, hydrogen peroxide, superoxide, and singlet oxygen produced in the mitochondria [68], peroxisomes [69], and plasma membrane-associated NAD(P)H oxidases [70]. Excess ROI levels lead to suppression of insulin secretion [71, 72],

however, absent or very low ROIs also inhibit insulin secretion. Low but not excessive ROIs stimulate insulin secretion [71, 73].

While the fact the mitochondrial derived ROI's having a role in insulin secretion is not disputed, some researchers propose that other sources of ROI's may contribute to a larger extent towards insulin secretion than previously thought. Rotenone, an inhibitor of mitochondrial complex I, stimulates insulin secretion at basal glucose levels [74] and taken together with a previous study showing that rotenone inhibition of mitochondrial activity reduces mitochondrial ROI production in cardiac cells [75] may indicate that extra mitochondrial sources of ROI are responsible for the increased insulin secretion.

Restoration of first phase insulin secretion

First phase insulin secretion is crucial in maintaining glucose homeostasis and a reduction in this early phase may be the first detectable sign of β-cell dysfunction and increased risk of T2D. Therefore, restoration of first phase insulin secretion may have a positive impact on disease development. A study in dogs showed that restoration of first phase insulin secretion (even in the absence of second phase) reduced EGP [76]. Inadequate suppression of EGP can lead to IGT [76-78], so restoration of first phase insulin secretion could be an important first step in the treatment or prevention of type 2 diabetes.

Advances in medical techniques have allowed individuals with morbid obesity and T2D to undergo various forms of bariatric surgery to reduce body weight. In most cases, this either substantially improves or cures T2D [32, 79-81]. These types of surgery may involve a variety of procedures but the general premise is reducing the size of the stomach by using a medical device or removal of a portion of the stomach in morbidly obese patients. This can be combined with a bypass procedure so that the stomach contents do not pass into the proximal small intestine. While weight loss and the control of diabetes after these types of surgeries has been well established [32, 79-81], a surprising finding is that first phase insulin secretion is also restored, often soon after surgery [32, 79]. The exact mechanisms responsible for this are as yet unknown but it was thought that weight loss was not the mechanism due to the relatively short time frame for improvement in first phase insulin secretion (one month after surgery) [79]. "Normal" weight loss obtained through dieting was unable to restore first phase insulin secretion in T2D patients [82, 83]. Various factors including changes in gastrointestinal hormones, adipokines, gluco- and lipotoxicity have been proposed as mechanisms for the restoration of first phase insulin secretion post surgery [79]. However, a new study suggests that pronounced calorie restriction itself, and the associated decreased pancreatic fat content may be the cause [83]. Researchers in this study

showed that patients with T2D who went on a calorie restricted diet (total energy intake of 2.5 MJ (600 kcal)/day)) showed that first phase insulin secretion was restored after 8 weeks on the diet [83]. This may have been due to the decrease in total pancreatic fat observed in the dieting patients and despite the small sample size (11 T2D patients and 9 matched control individuals), it provides fascinating insight into the potential mechanisms for the improvements seen in bariatric surgery patients.

A number of important large scale clinical trials have also been also been performed, including the Diabetes Prevention Program (DPP) [84] and the Troglitazone In the Prevention Of Diabetes (TRIPOD) study [85], and the results of some of these studies are summarised in Table 1.

Regulatory genes

Identifying genes that play a role in insulin secretion is an important research area which has the potential to lead to targets for the treatment of diabetes. A summary of some of these regulatory genes is outlined below.

Genome wide association studies

There is substantial evidence from studies both in families [86] and monozygotic twins [87] for a strong genetic component of type 2 diabetes. Correspondingly, recent studies using large scale, genome wide association studies (GWAS) have identified ~40 susceptibility loci. Polymorphisms within these *loci* increase the risk of T2D and, in the majority of cases; this is through changes in beta-cell function [3, 4, 88, 89]. The identification of such susceptibility *loci* has prompted a number of subsequent studies which have sought to explore the molecular mechanisms through which the implicated genes may impact on β-cell function or survival using rodent models and cellular systems [90-93]. It is unclear which of these genes have an impact on first phase insulin secretion yet but polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) are associated with reduced first phase insulin secretion [94].

Glucose transporter 2

To perform its biological function, glucose needs to be transported across the plasma membrane and this is facilitated by a family of glucose transporters; GLUT1-12. The members of the GLUT family are highly related but they differ in their tissue specificity and function [95]. GLUT2 is highly expressed in the liver and pancreatic β-cells and is an important part of the glucose sensing mechanism necessary for insulin secretion due

to its low affinity and high K_m for glucose [96]. Reduced expression of *GLUT2* has been found in animal models of type 2 diabetes including the neonatal streptozotocin rat [97], the diabetic Zucker rat [98], and the db/db mouse [99]. Mice that have a homozygous deletion of *GLUT2* display characteristics of type 2 diabetes, including a loss of first phase but preserved second phase insulin secretion [100]. The relative importance of GLUT2 in humans is less clear. People with some mutations in *GLUT2* develop Fanconi-Bickel syndrome, which is associated with glucose intolerance and diabetes [101]. A V197I mutation in human *GLUT2* has been shown to abolish glucose transport [102] and thought to be associated with T2D [103]. However, in a small sample of human control and diabetic subjects, no change in *GLUT2* mRNA or protein expression was observed [104, 105] and a recent study has suggested that GLUT1 and GLUT3 are the main glucose transporters in human pancreatic β-cells [106]. Indeed, *GLUT1* expression in human islets was higher than *GLUT2* [105]. Therefore, despite the very clear effects of *GLUT2* disruption in mice with regards to insulin secretion, human GLUT2 may not have the same roles as it does in rodents.

Glucokinase

β-cells secrete insulin in response to increases in blood glucose levels and thus they need to be able to sense changes in glucose concentrations. Glucokinase (GK) is the initial rate determining step of the glycolytic pathway (Figure 2), phosphorylating glucose to glucose 6-phosphate. Glucokinase is thought to be the main glucose sensor for β-cells due to its high K_m of approximately 10 mM and high specificity for glucose [107]. This is supported by the fact that heterozygous mutations in *GK* cause maturity onset diabetes of the young (MODY). In humans, *GK* mutations account for 50 % of cases of MODY [108-110] and cause a right-shift in the GSIS curve.

A number of studies have been performed to identify the role of GK in β-cells [107, 111-115]. Mice with homozygous deletions of *GK* are born diabetic and die shortly after birth. However, mice with one functioning allele of GK survive ($GK^{+/-}$) but are hyperglycaemic and display impaired first and second phase glucose stimulated insulin secretion (GSIS) [107, 112]. This was shown to be caused by the inability of glucose to close the K_{ATP} channels and thus inadequate depolarisation of the β-cell [114]. Mice with targeted deletion of *GK* only in the β-cells display a very similar insulin secretory profile to that of the $GK^{+/-}$ mice [111]. Interestingly, as *GK* is only expressed in the liver and pancreatic β-cells, rescue of $GK^{-/-}$ mice was possible by overexpressing *GK* in the β-cells only, suggesting that β-cell GK was more important than liver GK for glucose homeostasis and survival [107].

K_{ATP} channels

An important step in the triggering pathway of insulin secretion is the closure of the K_{ATP} channels, allowing for subsequent membrane depolarisation and insulin exocytosis. The K_{ATP} channels in general are comprised of a K^+ inward rectifier (Kir6.1 or Kir6.2) and a sulfonylurea receptor (SUR1 or SUR2), with SUR1 and Kir6.2 making up the pancreatic β-cell K_{ATP} channels [116]. It has been shown that disruption of either of these subunits leads to impaired insulin secretion in mice [117, 118] but conversely leads to hypoglycaemia and increased insulin secretion in people [119-121].

Both *SUR1*$^{-/-}$ and *Kir6.2*$^{-/-}$ mice have been generated and display similar phenotypic changes [117, 118, 122, 123]. Glucose stimulated insulin secretion, both first and second phase, was impaired in the *SUR1*$^{-/-}$ and *Kir6.2*$^{-/-}$ mice. Interestingly, euglycaemia was maintained by these animals, as the *Kir6.2*$^{-/-}$ mice had no deviations in their glucose tolerance compared to control littermates [118], and the *SUR1*$^{-/-}$ had normal random fed glucose levels (albeit with slightly impaired glucose tolerance) [117]. This was achieved through different mechanisms. The *Kir6.2*$^{-/-}$ mice was able to maintain normal glucose tolerance in the face of reduced first and second phase GSIS due to an increased insulin sensitivity in peripheral tissue [118]. However, the *SUR1*$^{-/-}$ mice did not display any changes to insulin sensitivity and the authors postulated that euglycaemia was maintained in a fed state by insulin release via K_{ATP} channel independent pathways [117].

Fructose-1,6-bisphosphatatase

Fructose-1,6-bisphosphatase (FBPase) is a gluconeogenic enzyme that catalyses the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. The expression of *FBPase* has been shown to be increased in a number of diabetic mouse models including the New Zealand Obese (NZO) and the BTBR mice [124, 125] and this increase in expression may be induced by an increase in fatty acids [126, 127]. Insulin secretion was reduced in mice and the MIN6 cell model with an overexpression of *FBPase* and it is physiologically relevant to human T2D as human T2D islets have an increase in *FBPase* expression [127]. *FBPase* overexpressing MIN6 cells had reduced glucose utilisation and metabolism, resulting in a reduction in ATP generation, all of which contribute to the observed reductions in insulin secretion [127].

Hypoxia inducible factor-1

We have identified a pair of transcription factors; hypoxia inducible factor-1α (HIF-1α) and the aryl hydrocarbon receptor nuclear translocator (ARNT), which play a role in first phase insulin secretion. ARNT and HIF-1α forms the HIF-1 transcription factor, first identified by Semenza and Wang in 1992 [128]. It was first brought to attention as a regulator of insulin secretion by Gunton and colleagues [105] when it was described that *ARNT* was markedly reduced in islets of T2D patients. Mice with β-cell specific knockout of *ARNT* had reduced glucose tolerance and absent first and impaired second phase GSIS both *in vivo* and in isolated islets. This was supported by evidence in siRNA knock down of *ARNT* in the MIN6 cell culture line, which showed similar results. The reduced insulin secretion was accompanied with decreased expression of *glucose-6-phosphoisomerase (G6PI)* and *aldolase (ALDO)*, two key glycolytic genes [105]. *ARNT* was previously thought to be ubiquitously and constitutively expressed, but it has since been shown that the carbohydrate-responsive element-binding protein (ChREBP) is a negative regulator [129].

Subsequent studies have shown that ARNT's partner HIF-1α also plays an important role in insulin secretion. HIF-1α protein is regulated post translationally in an oxygen dependent manner. Under hypoxic conditions, HIF-1α is stable and can bind to ARNT to commence transcription of target genes. However, at normoxia, HIF-1α is targeted for degradation via an oxygen sensitive ubiquitin-proteasome pathway and has a very short half-life [130]. Studies in our lab have shown that mice with β-cell specific knockout of *HIF-1α* and also siRNA knockdown of *HIF-1α* in MIN6 cells displayed many of the same phenotypic changes seen in the *ARNT* knockout/knockdown models described above. Similar to the *ARNT* knockout mice, mice with β-cell specific knockout of *HIF-1α* also has decreased expressions of the key glycolytic genes *G6PI* and *ALDO*, but also of *GK* and *phosphofructokinase* [131]. Both *ARNT* and *HIF-1α* seem to be regulators of glycolysis, and taken together with that fact they have reduced GSIS, would further provide backing for the importance of glycolysis in insulin secretion.

Interestingly, when HIF-1α protein is stabilised with an iron chelator, insulin secretion was improved and *ARNT* gene expression was restored in type 2 diabetic human islets [131]. As such, HIF-1 seems to be an important regulator of insulin secretion and a possible target for therapy.

Von Hippel-Lindau and Factor Inhibiting HIF

Von Hippel-Lindau protein (VHL) is a tumour suppressor and forms a part of the pVHL-elonginB-elonginC (VBC) complex. Under normoxic conditions, the VBC complex binds to HIF-1α protein and targets it for proteosomal degradation [132]. Deletion of *VHL* stabilises HIF-1α protein under normoxic conditions. A

number of studies have found that mice with homozygous pancreatic or β-cell specific knockout of *VHL* have impaired insulin secretion and significantly impaired glucose tolerance [133-136]. However, mice with β-cell specific knockout of both *VHL* and *HIF-1α* reversed the *VHL* knockout phenotype and had normal insulin secretion, showing that the massive increase in HIF-1α protein was responsible for the deleterious effects [133].

This raises an interesting scenario in which both deletion of *HIF-1α* and increasing HIF-1α protein (by deleting *VHL*) in mice both impaired insulin secretion. We believe this follows a "Goldilocks" paradigm analogous to the effect of reactive oxygen species (ROS), where too much and too little are both deleterious for β-cell function. Interestingly, HIF-1α is both regulated by and regulates ROS [137-139]. Not enough HIF-1α is detrimental [133-136]. However, increasing HIF-1α protein by using an iron chelator to the point where it is "just right" [131], and not to the levels observed by deleting *VHL*, is beneficial for insulin secretion.

This is supported by a study by Zhang et al investigating the effects of knocking out factor inhibiting HIF-1α (FIH) [140]. Under normoxic conditions, HIF-1α protein is highly regulated at the protein level by hydroxlases, with FIH being one of them. Mice with *FIH* knocked out displayed none of the characteristics of a *VHL* knockout, instead these mice had improved insulin sensitivity and decreased weight, even under a high fat diet challenge [140]. These results are similar to those reported by our group using iron chelation to stabilise HIF-1α protein activity [131]. Additional circumstantial support is provided by the fact that people with VHL syndrome in whom there are heterozygous mutations in VHL appear to be protected from diabetes development, especially after pancreatic resections [141-144]. To our knowledge, glucose tolerance in mice with heterozygous *VHL* defects in the pancreas has not been reported, and would be of particular interest.

Insulin receptor

The insulin receptor (IR) is a ubiquitously expressed cell surface protein capable of binding insulin with high affinity, setting off a cascade of insulin signalling reactions in peripheral tissues necessary for glucose homeostasis [145]. In humans, heterozygous mutations in *IR* resulting in loss of function any of the heteroterameric receptors including a mutant allele results in leprechaunism, with traits including growth retardation and diabetes [146]. The phenotype is very severe in mice with homozygous *IR* deletion in that they die shortly after birth (48 – 72 hrs) due to diabetic ketoacidosis [147]. Tissue specific *IR* knockout mice have been made. Muscle specific *IR* knockout mice display some of the characteristics of T2D, including increased triglyceride and serum free fatty acids but glucose tolerance remains normal without additional insults [148]. More interestingly, mice with β-cell specific knockout of *IR* display many of the characteristics of T2D,

including a loss of first phase but retained second phase insulin secretion [149]. This is of particular importance as it provides a model for T2D development, whereby insulin resistance at the β-cell level (replicated in mice with a β-cell specific knockout of *IR*) leads to reduced first phase insulin secretion, a major characteristic of disease development [149].

Nicotinamide nucleotide transhydrogenase

Nicotinamide nucleotide transhydrogenase (NNT) is a nuclear encoded mitochondrial protein responsible for the reduction of $NADP^+$ by NADH and conversion of NADH to NAD^+. This makes it particularly important in terms of insulin secretion because it generates NADPH and thus affecting mitochondrial metabolism [150]. First identified to have a role in insulin secretion by Toye et al [151], *NNT* expression was shown to be significantly lower in C57BL/6J mice, a strain of mice exhibiting impaired glucose homeostasis independent of obesity (including reduced first phase insulin secretion after feeding). In addition, C57BL/6J mice display a 5-exon deletion in NNT, which is not present in other strains of mice including the closely related C57BL/6N [151, 152]. This was subsequently validated by a study showing that mice with mutant forms of *NNT* have reduced glucose tolerance and first phase insulin secretion, with an accompanied increase in glucose utilisation and decreased ATP production [150]. This phenotype was attributed to ROS mediated activation of UCP2 and thus uncoupling of mitochondrial oxidative phosphorylation [150]. In addition to this, upregulation of *NNT* was shown to be a cause for insulin hypersecretion in the DBA/2 mice, a diabetes susceptibility mouse model [153].

Nicotinamide phospho-riboysl-transferase and the Sirtuin family

Recent findings have implicated the enzyme nicotinamide phospho-ribosyl-transferase (NAMPT) and nicotinamide adenine dinucleotide (NAD) biosynthesis in insulin secretion and metabolism [154, 155]. The enzyme NAMPT is the rate limiting step in NAD biosynthesis. Mice with a heterozygous deletion of *NAMPT* have been shown to have impaired glucose tolerance. Isolated islets from $NAMPT^{+/-}$ mice have reduced glucose stimulated insulin secretion at 15 min after stimulation, a time point representing first phase insulin secretion in *in vitro* studies [155]. The authors found that the deficiency in insulin secretion was due to the defects in nicotinamide mononucleotide (NMN) and nicotinamide adenine dinucleotide (NAD) biosynthesis, possibly altering glycolysis or lipid oxidation as a downstream consequence [155]. The deficiency in NAD would have downstream effects on NAD-dependent enzymes, in particular, the sirtuin family.

The sirtuins are a family of deactetylases and mono-ADP-ribosyltransferases, of which there are seven in mammals, that use NAD as a substrate [156]. It has been previously reported that Sirt1 regulates insulin secretion by repressing *UCP2* in β-cells [157] and over expressing *Sirt1* in β-cells improves first phase insulin secretion [158]. More recently, Sirt4 has also been implicated as having a role in metabolism. Sirt4 is a mitochondrial protein that has been shown to regulate insulin secretion by repressing glutamate dehydrogenase activity, thereby reducing the ability of mitochondria to generate ATP from glutamate and glutamine [159, 160]. The generation of ATP is vital for the triggering pathway of insulin secretion and thus Sirt4 could play an important role in first phase insulin secretion. These are the only members of the sirtuin family which have been directly associated with insulin secretion although emerging evidence may shed some light on the role of Sirt3 in this respect. Reduced expression of Sirt3 in streptozotocin induced diabetic mice has been reported [161].

Uncoupling proteins

The uncoupling proteins (UCP) function by dissipating the energy from glucose/lipid oxidation as heat rather than flowing through the electron transport chain to produce ATP [162]. The uncoupling protein UCP1 is the classic and most well known UCP out of five (UCP1 – UCP5) but is predominantly expressed in brown adipose tissue [163, 164]. Of the other UCP's, UCP2 has a definite role in insulin secretion, however research has shown conflicting results. On one side of the argument, *UCP2* expression has been proposed to be a major factor in obesity, β-cell dysfunction, and T2D, negatively regulating insulin secretion [165]. In clear contrast to this, a more recent study reported that over-expressing *UCP2* in mice had no effect on insulin secretion [166]. Another study has shown that mice with homozygous knockout of *UCP2* have impaired β-cell function, possibly due to increased oxidative stress [167]. Therefore, even though UCP2 may have a role in insulin secretion, there are both positive and negative impacts in the current literature.

Interactions between regulatory genes

The regulatory genes described above often have roles within the same metabolic pathway or interact with each other to regulate insulin secretion. GK, HIF-1, NNT, NAMPT, UCPs, and some members of the sirtuin family have either direct or indirect roles in either glycolysis or mitochondrial oxidative phosphorylation, and thus affecting the ability of β-cell to generate ATP. In addition to this, work from our lab has shown that HIF-1 regulates a number of glycolytic and glucose transporter genes, including GK and GLUT2 [105, 131]. In turn,

VHL regulates the proteasomal degradation of HIF-1α [132]. ARNT regulates genes in the insulin signaling pathway, including IR and insulin receptor substrate 2. Mice with deletion of IR in β-cells lose first phase insulin secretion. There are many genes that regulate insulin secretion, shown in (Figure 3).

Summary

It is clear that first phase insulin secretion has an important role in maintaining glucose homeostasis. The initial insulin response after glucose stimulus primes the system, suppressing HGP in the liver and readies insulin sensitive tissue to take up the incoming glucose. Previous research has shown that loss of first phase insulin secretion is one of the earliest detectable symptoms for T2D onset and emerging research has identified restoration of this phase of insulin secretion may benefit disease sufferers, the most pronounced being post-bariatric surgery. Research has identified many factors that play a role in first phase insulin secretion, including ATP/ADP ratios, ROS, HIF-1, VHL, and members of the sirtuin family (Table 2). Therapies which increase first phase insulin secretion include weight loss and GLP-1 receptor agonists. More research will uncover even more regulatory genes and therapies and increase our ability to treat type 2 diabetes.

Acknowledgements

The authors would like to thank Dr. Andrew Dwyer for review and helpful comments. Kim Cheng and Jenny E. Gunton were supported by funding from the National Health and Medical Research Council (NHMRC).

Conflict of interest

The authors have no relevant conflicts of interest.

Abbreviations

ADP	Adenosine diphosphate
ALDO	Aldolase
ARNT	Aryl hydrocarbon receptor nuclear translocator
ATP	Adenosine triphosphate
ChREBP	Carbohydrate-responsive element-binding protein
CIIIT	Chronic intermittent intravenous insulin therapy
FBPase	Fructose-1,6-bisphosphatase
FIH	Factor inhibiting HIF
G6PI	Glucose-6-phosphoisomerase
GIR	Glucose infusion rate
GK	Glucokinase
GLP-1	Glucogon like peptide-1
GLUT	Glucose transporter
GSIS	Glucose stimulated insulin secretion
GWAS	Genome wide association studies
HGP	Hepatic glucose production
HIF-1α	Hypoxia inducible factor-1α
IGT	Impaired glucose tolerance
IR	Insulin receptor
IVGTT	Intravenous glucose tolerance test
K_{ATP}	ATP-sensitive potassium channels
K_m	Michaelis constant
MMTT	Mixed meal tolerance test
NAD	Nicotinamide adenine dinucleotide
NNT	Nicotinamide nucleotide transhydrogenase
NAMPT	Nicotinamide phospho-ribosyl-transferase
PIVIT	Pulsatile intravenous insulin therapy
ROI	Reactive oxygen intermediates
ROS	Reactive oxygen species
siRNA	Short interfering RNA
SNARE	Soluble-*N*-ethyl-maleimide sensitive factor attachment protein receptor
T2D	Type 2 diabetes
TCF7L2	Transcription factor 7-like 2
TCA	Tricarboxylic acid
UCP	Uncoupling protein
VBC	pVHL-elonginB-elonginC
VHL	von Hippel-Lindau
VDCC	Voltage dependent calcium channel

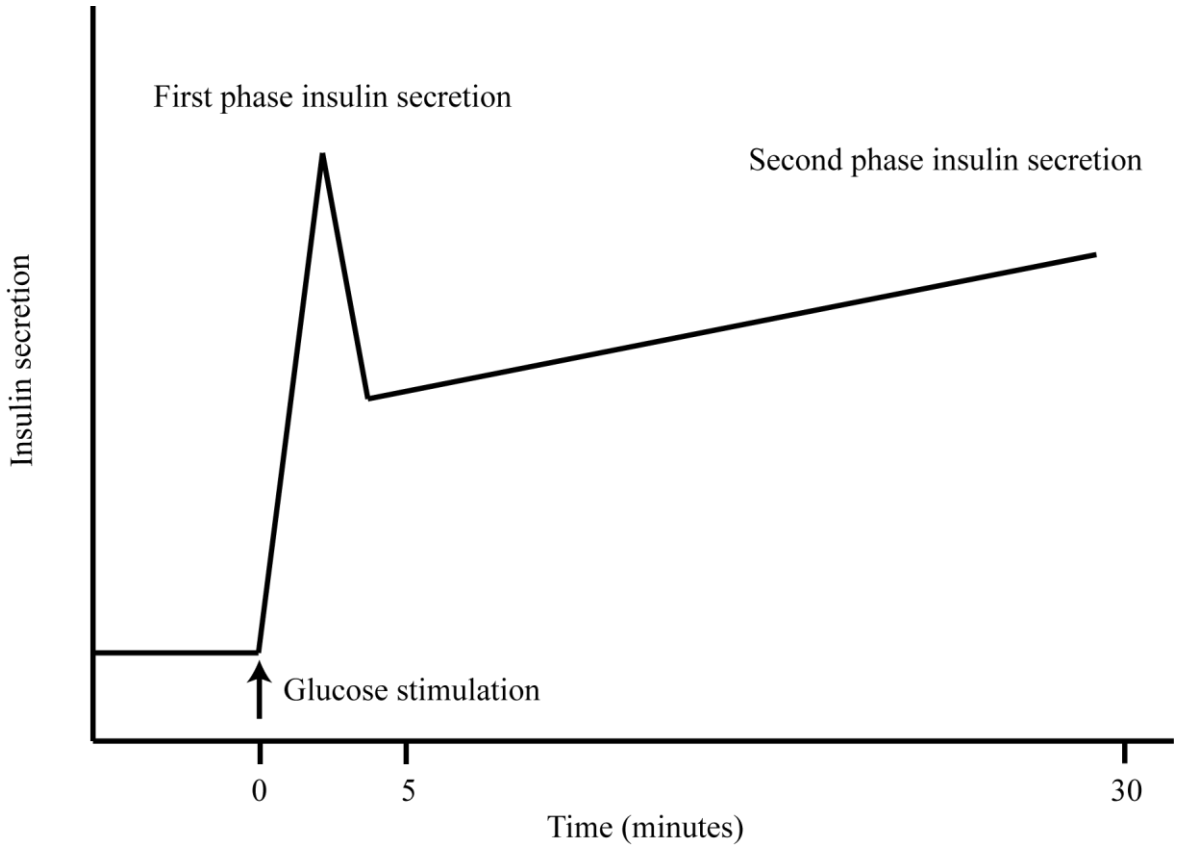

Figure 1 First and second phase insulin secretion in the β-cell. First phase insulin secretion occurs very rapidly, usually within 10 minutes of glucose stimulation. Second phase insulin secretion is more gradual and long lasting, occurring 25 – 30 minutes after glucose stimulation.

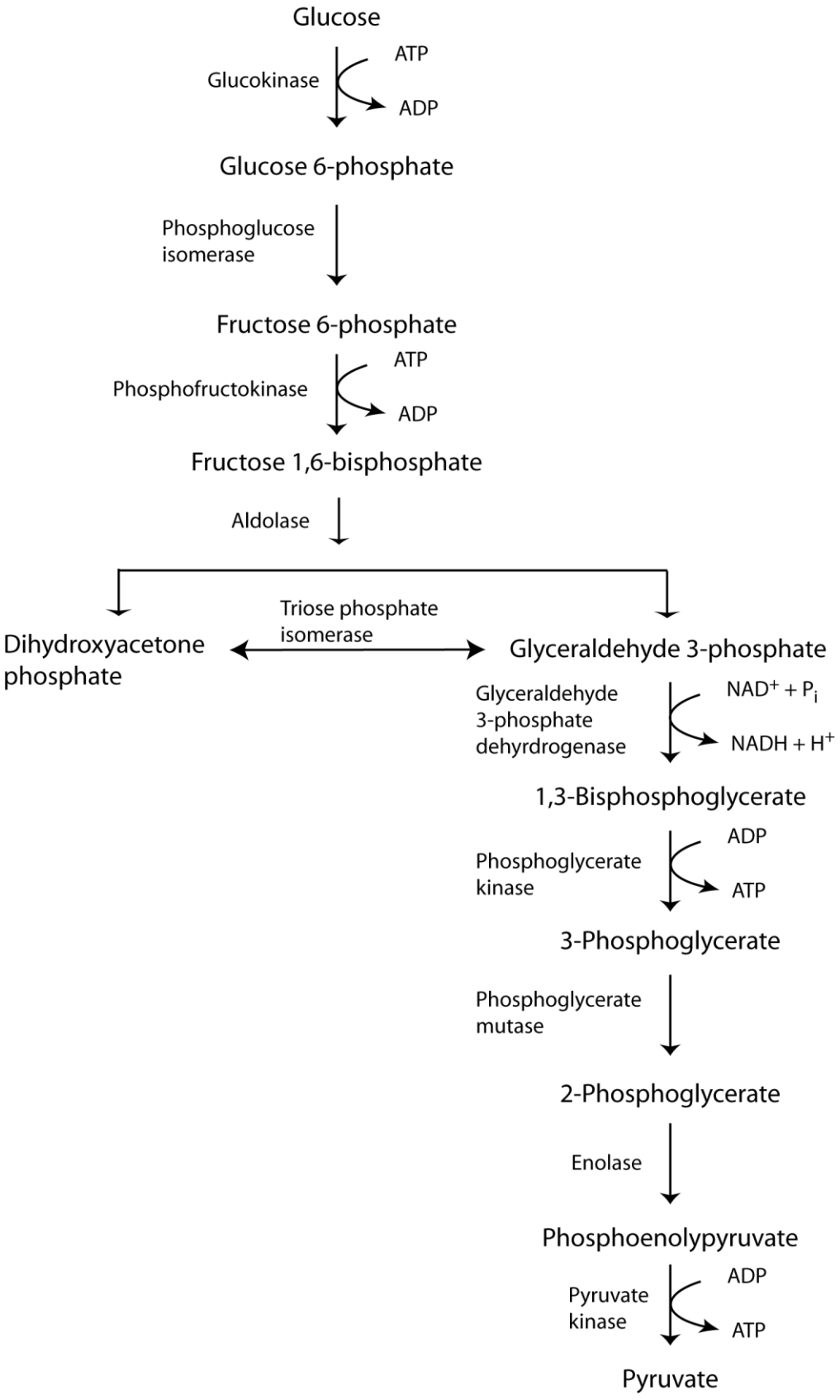

Figure 2 The glycolysis pathway. The glycolytic pathway consists of ten enzymatic reactions to metabolise glucose to pyruvate. For every one molecule of glucose metabolised, one molecule of NADH and two molecules of ATP are produced.

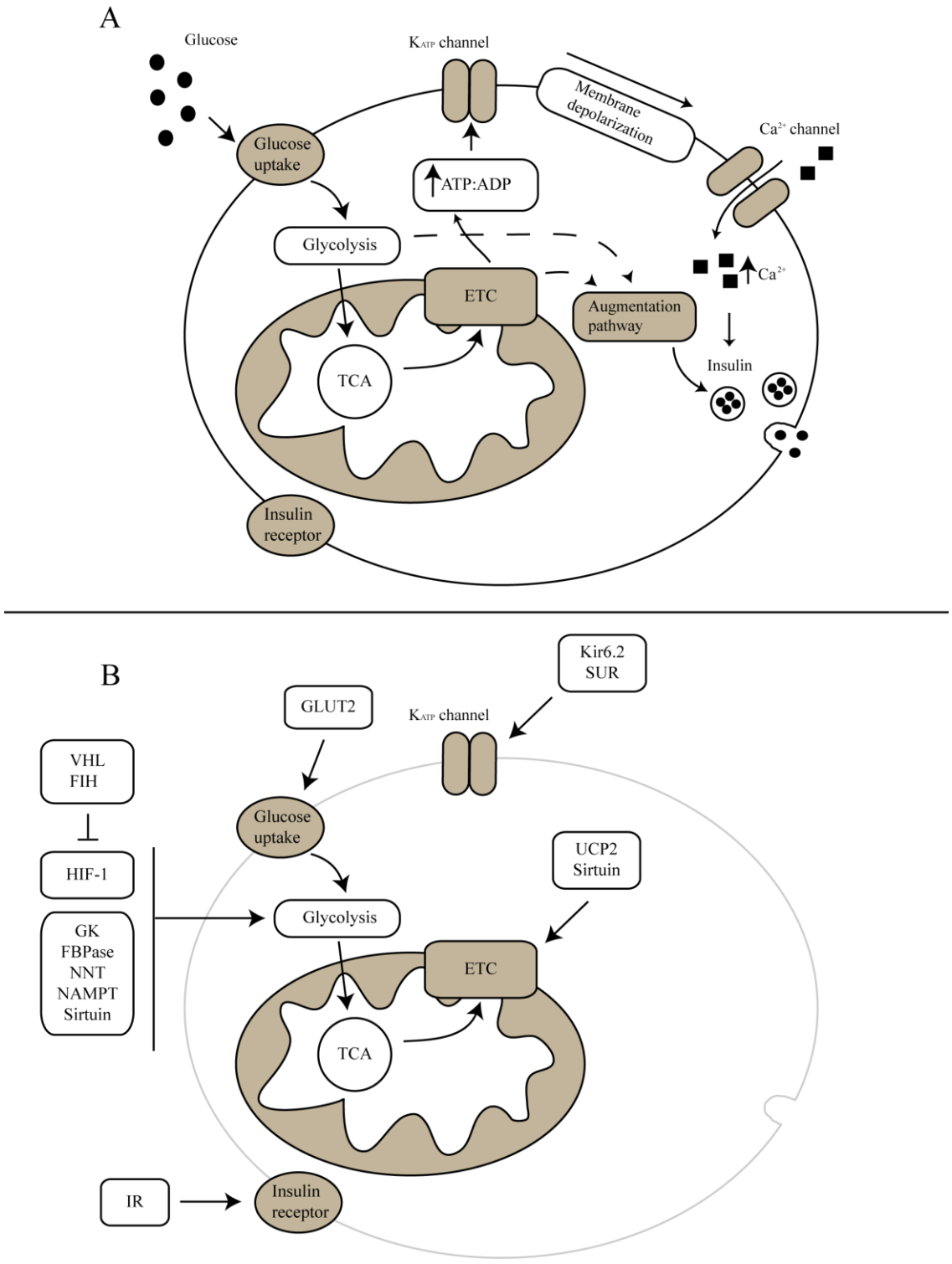

Figure 3 Model of insulin secretion in the β-cell. A) Glucose is taken up and broken down by glycolysis. This provides substrates for the tricarboxylic acid (TCA) cycle, which in turn provides substrates for the electron transport chain (ETC). ATP generation for the ETC increases the ATP/ADP ratio leading to closure of the K_{ATP} channels. Calcium channels open after membrane depolarisation leading to an increase in intracellular calcium and subsequent insulin exocytosis. The amplification pathway of insulin secretion is less well understood but operates independent of changes in ATP/ADP and the K_{ATP} channels. B) The regulatory genes as described in the text and points of interaction with the insulin secretory pathway.

Study	Methods	Overall results	Effects on insulin secretion
Acarbose for the Study To Prevent Non Insulin Dependent Diabetes Mellitus (STOP-NIDDM) [168]	Patients with impaired glucose tolerance were placed on placebo or acarbose treatment	Progression to diabetes was reduced by 25% in the acarbose treatment group. This was accompanied with improved glucose tolerance	Unknown
Diabetes Prevention Program (DPP) [84]	3234 non-diabetic subjects with increased fasting and post-load plasma glucose were placed on a placebo, metformin, or lifestyle modification program	Incidence of diabetes was 11, 7.8, and 4.8 per 100 persons in the placebo, metformin, and lifestyle groups respectively	Unknown
Exenatide [169-171]	13 T2D patients were placed on saline or exenatide treatment	Exenatide restored both first and second phase insulin secretion in T2D patients	Both first and second phase insulin secretion was restored in T2D patients
Glucagon-like Peptide 1 (GLP-1) [172]	20 T2D patients were placed on saline or GLP-1 treatment for six weeks	T2D on GLP-1 treatment had decreased plasma glucose, HbA_{1C}, free fatty acids, and improved insulin sensitivity	First and second phase insulin secretion, as measured by C-peptide, was improved with GLP-1 treatment in T2D patients
Liraglutide [173, 174]	746 individuals with early T2D were placed on liraglutide or glimepiride treatment for 52 weeks	Liraglutide was shown to be a better treatment than glimepiride, with improved HbA_{1C} and blood pressure	Insulin secretion, as measured by C-peptide deconvolution analysis was increased in T2D patients with liraglutide treatment
Troglitazone In the Prevention Of Diabetes (TRIPOD) [85, 175]	Latina women that fit the selection criteria, including prior gestational diabetes, were placed on troglitazone or placebo treatment	Troglitazone reduced incidence of diabetes in test subjects by >50% while preserving β-cell function	First phase insulin secretion was reduced in the placebo group but remained the same in the troglitazone group

Table 1. A summary of some clinical studies regarding T2D prevention and treatment.

Genotype	Effect on first phase insulin secretion	Effect on second phase insulin secretion
β-cell specific knockout of *ARNT* [105]	Absent	Absent
***FBPase* transgenic [127]**	Decreased	Decreased
***GLUT2*$^{-/-}$ [100]**	Absent	Unchanged
***GK*$^{+/-}$ [107]**	Decreased	Decreased
β-cell specific knockout of *HIF-1α* [131]	Decreased	Unchanged
β-cell specific knockout of *IR* [149]	Absent	Unchanged
***Kir6.2*$^{-/-}$ [118]**	Decreased	Absent
***NAMPT*$^{+/-}$ [155]**	Decreased	Decreased
Mutant *NNT* [150]	Decreased	Decreased
***Sirt1*$^{-/-}$ [157]**	Absent	Absent
***Sirt1* transgenic [158]**	Increased	Unchanged
***SUR1*$^{-/-}$[117]**	Decreased	Absent
Polymorphisms in human *TCF7L2* [94]	Decreased	Unknown
***UCP2*$^{-/-}$[165, 167]**	Both increased and decreased	Both increased and decreased
β-cell and pancreas specific knockout of *VHL* [133-136]	Decreased	Decreased

Table 2. A summary of the genes described in the text and its effects on insulin secretion.

References

[1] Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Porte D, Jr., Schwartz MW. Reduced beta-cell function contributes to impaired glucose tolerance in dogs made obese by high-fat feeding. Am J Physiol 1999; 277(4 Pt 1): E659-67.
[2] Kasuga M. Insulin resistance and pancreatic beta cell failure. J Clin Invest 2006; 116(7): 1756-60.
[3] Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010; 42(7): 579-89.
[4] Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008; 40(5): 638-45.
[5] Bunt JC, Krakoff J, Ortega E, Knowler WC, Bogardus C. Acute insulin response is an independent predictor of type 2 diabetes mellitus in individuals with both normal fasting and 2-h plasma glucose concentrations. Diabetes Metab Res Rev 2007; 23(4): 304-10.
[6] Vranic M, Fono P, Kovacevic N, Lin BJ. Glucose kinetics and fatty acids in dogs on matched insulin infusion after glucose load. Metabolism 1971; 20(10): 954-67.
[7] Del Prato S, Tiengo A. The importance of first-phase insulin secretion: implications for the therapy of type 2 diabetes mellitus. Diabetes Metab Res Rev 2001; 17(3): 164-74.
[8] Pratley RE, Weyer C. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. Diabetologia 2001; 44(8): 929-45.
[9] Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 1999; 104(6): 787-94.
[10] Lalic NM, Maric J, Svetel M, Jotic A, Stefanova E, Lalic K, et al. Glucose homeostasis in Huntington disease: abnormalities in insulin sensitivity and early-phase insulin secretion. Arch Neurol 2008; 65(4): 476-80.
[11] Ronnemaa E, Zethelius B, Sundelof J, Sundstrom J, Degerman-Gunnarsson M, Berne C, et al. Impaired insulin secretion increases the risk of Alzheimer disease. Neurology 2008; 71(14): 1065-71.
[12] Kahn CB, Soeldner JS, Gleason RE, Rojas L, Camerini-Davalos RA, Marble A. Clinical and chemical diabetes in offspring of diabetic couples. N Engl J Med 1969; 281(7): 343-7.
[13] Gerich JE. Metabolic abnormalities in impaired glucose tolerance. Metabolism 1997; 46(12 Suppl 1): 40-3.
[14] Pimenta W, Mitrakou A, Jensen T, Yki-Jarvinen H, Daily G, Gerich J. Insulin secretion and insulin sensitivity in people with impaired glucose tolerance. Diabet Med 1996; 13(9 Suppl 6): S33-6.
[15] Yoneda H, Ikegami H, Yamamoto Y, Yamato E, Cha T, Kawaguchi Y, et al. Analysis of early-phase insulin responses in nonobese subjects with mild glucose intolerance. Diabetes Care 1992; 15(11): 1517-21.
[16] Curry DL, Bennett LL, Grodsky GM. Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology 1968; 83(3): 572-84.
[17] Calles-Escandon J, Robbins DC. Loss of early phase of insulin release in humans impairs glucose tolerance and blunts thermic effect of glucose. Diabetes 1987; 36(10): 1167-72.
[18] Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC. Pulsatile insulin has greater hypoglycemic effect than continuous delivery. Diabetes 1983; 32(7): 617-21.
[19] Hansen BC, Jen KC, Belbez Pek S, Wolfe RA. Rapid oscillations in plasma insulin, glucagon, and glucose in obese and normal weight humans. J Clin Endocrinol Metab 1982; 54(4): 785-92.
[20] Cunningham BA, Deeney JT, Bliss CR, Corkey BE, Tornheim K. Glucose-induced oscillatory insulin secretion in perifused rat pancreatic islets and clonal beta-cells (HIT). Am J Physiol 1996; 271(4 Pt 1): E702-10.
[21] Hellman B, Gylfe E, Bergsten P, Grapengiesser E, Lund PE, Berts A, et al. The role of Ca2+ in the release of pancreatic islet hormones. Diabete Metab 1994; 20(2): 123-31.
[22] Jung SK, Kauri LM, Qian WJ, Kennedy RT. Correlated oscillations in glucose consumption, oxygen consumption, and intracellular free Ca(2+) in single islets of Langerhans. J Biol Chem 2000; 275(9): 6642-50.
[23] Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, et al. Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. N Engl J Med 1988; 318(19): 1231-9.
[24] Del Guerra S, Lupi R, Marselli L, Masini M, Bugliani M, Sbrana S, et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes 2005; 54(3): 727-35.
[25] O'Rahilly S, Turner RC, Matthews DR. Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. N Engl J Med 1988; 318(19): 1225-30.
[26] Aoki TT, Grecu EO, Arcangeli MA, Benbarka MM, Prescott P, Ahn JH. Chronic intermittent intravenous insulin therapy: a new frontier in diabetes therapy. Diabetes Technol Ther 2001; 3(1): 111-23.
[27] Mirbolooki MR, Taylor GE, Knutzen VK, Scharp DW, Willcourt R, Lakey JR. Pulsatile intravenous insulin therapy: the best practice to reverse diabetes complications? Med Hypotheses 2009; 73(3): 363-9.
[28] Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 1981; 68(6): 1456-67.

[29] Elahi D. In praise of the hyperglycemic clamp. A method for assessment of beta-cell sensitivity and insulin resistance. Diabetes Care 1996; 19(3): 278-86.
[30] Bacha F, Gungor N, Arslanian SA. Measures of beta-cell function during the oral glucose tolerance test, liquid mixed-meal test, and hyperglycemic clamp test. J Pediatr 2008; 152(5): 618-21.
[31] Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J, et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. Diabetes Care 2008; 31(10): 1966-71.
[32] Polyzogopoulou EV, Kalfarentzos F, Vagenakis AG, Alexandrides TK. Restoration of euglycemia and normal acute insulin response to glucose in obese subjects with type 2 diabetes following bariatric surgery. Diabetes 2003; 52(5): 1098-103.
[33] Caumo A, Luzi L. First-phase insulin secretion: does it exist in real life? Considerations on shape and function. Am J Physiol Endocrinol Metab 2004; 287(3): E371-85.
[34] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979; 237(3): E214-23.
[35] Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 2000; 49(11): 1751-60.
[36] Henquin JC, Ravier MA, Nenquin M, Jonas JC, Gilon P. Hierarchy of the beta-cell signals controlling insulin secretion. Eur J Clin Invest 2003; 33(9): 742-50.
[37] Straub SG, Sharp GW. Glucose-stimulated signaling pathways in biphasic insulin secretion. Diabetes Metab Res Rev 2002; 18(6): 451-63.
[38] Newgard CB, McGarry JD. Metabolic coupling factors in pancreatic beta-cell signal transduction. Annu Rev Biochem 1995; 64: 689-719.
[39] Ashcroft FM, Harrison DE, Ashcroft SJ. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 1984; 312(5993): 446-8.
[40] Cook DL, Hales CN. Intracellular ATP directly blocks K+ channels in pancreatic B-cells. Nature 1984; 311(5983): 271-3.
[41] Kelly RP, Sutton R, Ashcroft FM. Voltage-activated calcium and potassium currents in human pancreatic beta-cells. J Physiol 1991; 443: 175-92.
[42] Gembal M, Gilon P, Henquin JC. Evidence that glucose can control insulin release independently from its action on ATP-sensitive K+ channels in mouse B cells. J Clin Invest 1992; 89(4): 1288-95.
[43] Sato Y, Aizawa T, Komatsu M, Okada N, Yamada T. Dual functional role of membrane depolarization/Ca2+ influx in rat pancreatic B-cell. Diabetes 1992; 41(4): 438-43.
[44] Nolan CJ, Madiraju MS, Delghingaro-Augusto V, Peyot ML, Prentki M. Fatty acid signaling in the beta-cell and insulin secretion. Diabetes 2006; 55 Suppl 2: S16-23.
[45] Henquin JC, Nenquin M, Stiernet P, Ahren B. In vivo and in vitro glucose-induced biphasic insulin secretion in the mouse: pattern and role of cytoplasmic Ca2+ and amplification signals in beta-cells. Diabetes 2006; 55(2): 441-51.
[46] Proks P, Eliasson L, Ammala C, Rorsman P, Ashcroft FM. Ca(2+)- and GTP-dependent exocytosis in mouse pancreatic beta-cells involves both common and distinct steps. J Physiol 1996; 496 (Pt 1): 255-64.
[47] Barg S, Eliasson L, Renstrom E, Rorsman P. A subset of 50 secretory granules in close contact with L-type Ca2+ channels accounts for first-phase insulin secretion in mouse beta-cells. Diabetes 2002; 51 Suppl 1: S74-82.
[48] Parsons TD, Coorssen JR, Horstmann H, Almers W. Docked granules, the exocytic burst, and the need for ATP hydrolysis in endocrine cells. Neuron 1995; 15(5): 1085-96.
[49] Eliasson L, Renstrom E, Ding WG, Proks P, Rorsman P. Rapid ATP-dependent priming of secretory granules precedes Ca(2+)-induced exocytosis in mouse pancreatic B-cells. J Physiol 1997; 503 (Pt 2): 399-412.
[50] Bruns D, Jahn R. Molecular determinants of exocytosis. Pflugers Arch 2002; 443(3): 333-8.
[51] Rorsman P, Renstrom E. Insulin granule dynamics in pancreatic beta cells. Diabetologia 2003; 46(8): 1029-45.
[52] Stryer L. Biochemistry. 4th ed. New York: W. H. Freeman and Company; 1995.
[53] Liang Y, Bai G, Doliba N, Buettger C, Wang L, Berner DK, et al. Glucose metabolism and insulin release in mouse beta HC9 cells, as model for wild-type pancreatic beta-cells. Am J Physiol 1996; 270(5 Pt 1): E846-57.
[54] Maechler P, Kennedy ED, Pozzan T, Wollheim CB. Mitochondrial activation directly triggers the exocytosis of insulin in permeabilized pancreatic beta-cells. Embo J 1997; 16(13): 3833-41.
[55] Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, et al. Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic beta-cells. Potential role in nutrient sensing. J Biol Chem 1994; 269(7): 4895-902.

[56] Odegaard ML, Joseph JW, Jensen MV, Lu D, Ilkayeva O, Ronnebaum SM, et al. The mitochondrial 2-oxoglutarate carrier is part of a metabolic pathway that mediates glucose- and glutamine-stimulated insulin secretion. J Biol Chem 2010; 285(22): 16530-7.
[57] Jitrapakdee S, Wutthisathapornchai A, Wallace JC, MacDonald MJ. Regulation of insulin secretion: role of mitochondrial signalling. Diabetologia 2010; 53(6): 1019-32.
[58] Cline GW. Fuel-Stimulated Insulin Secretion Depends upon Mitochondria Activation and the Integration of Mitochondrial and Cytosolic Substrate Cycles. Diabetes Metab J 2011; 35(5): 458-65.
[59] Gauthier BR, Wiederkehr A, Baquie M, Dai C, Powers AC, Kerr-Conte J, et al. PDX1 deficiency causes mitochondrial dysfunction and defective insulin secretion through TFAM suppression. Cell Metab 2009; 10(2): 110-8.
[60] Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science 2005; 307(5708): 384-7.
[61] Silva JP, Kohler M, Graff C, Oldfors A, Magnuson MA, Berggren PO, et al. Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes. Nat Genet 2000; 26(3): 336-40.
[62] Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol Rev 2008; 88(2): 611-38.
[63] Matschinsky FM, Ellerman J. Dissociation of the insulin releasing and the metabolic functions of hexoses in islets of Langerhans. Biochem Biophys Res Commun 1973; 50(2): 193-9.
[64] Gray JP, Heart E. Usurping the mitochondrial supremacy: extramitochondrial sources of reactive oxygen intermediates and their role in beta cell metabolism and insulin secretion. Toxicol Mech Methods 2010; 20(4): 167-74.
[65] Mertz RJ, Worley JF, Spencer B, Johnson JH, Dukes ID. Activation of stimulus-secretion coupling in pancreatic beta-cells by specific products of glucose metabolism. Evidence for privileged signaling by glycolysis. J Biol Chem 1996; 271(9): 4838-45.
[66] Sener A, Kawazu S, Hutton JC, Boschero AC, Devis G, Somers G, et al. The stimulus-secretion coupling of glucose-induced insulin release. Effect of exogenous pyruvate on islet function. Biochem J 1978; 176(1): 217-32.
[67] Sener A, Malaisse WJ. Stimulation by D-glucose of mitochondrial oxidative events in islet cells. Biochem J 1987; 246(1): 89-95.
[68] Lambert AJ, Brand MD. Superoxide production by NADH:ubiquinone oxidoreductase (complex I) depends on the pH gradient across the mitochondrial inner membrane. Biochem J 2004; 382(Pt 2): 511-7.
[69] Quinn MT, Gauss KA. Structure and regulation of the neutrophil respiratory burst oxidase: comparison with nonphagocyte oxidases. J Leukoc Biol 2004; 76(4): 760-81.
[70] Crane FL, Low H. Reactive oxygen species generation at the plasma membrane for antibody control. Autoimmun Rev 2008; 7(7): 518-22.
[71] Pi J, Bai Y, Zhang Q, Wong V, Floering LM, Daniel K, et al. Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes 2007; 56(7): 1783-91.
[72] Sakai K, Matsumoto K, Nishikawa T, Suefuji M, Nakamaru K, Hirashima Y, et al. Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic beta-cells. Biochem Biophys Res Commun 2003; 300(1): 216-22.
[73] Ebelt H, Peschke D, Bromme HJ, Morke W, Blume R, Peschke E. Influence of melatonin on free radical-induced changes in rat pancreatic beta-cells in vitro. J Pineal Res 2000; 28(2): 65-72.
[74] Leloup C, Tourrel-Cuzin C, Magnan C, Karaca M, Castel J, Carneiro L, et al. Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. Diabetes 2009; 58(3): 673-81.
[75] Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria: central role of complex III. J Biol Chem 2003; 278(38): 36027-31.
[76] Steiner KE, Mouton SM, Bowles CR, Williams PE, Cherrington AD. The relative importance of first- and second-phase insulin secretion in countering the action of glucagon on glucose turnover in the conscious dog. Diabetes 1982; 31(11): 964-72.
[77] Luzi L, DeFronzo RA. Effect of loss of first-phase insulin secretion on hepatic glucose production and tissue glucose disposal in humans. Am J Physiol 1989; 257(2 Pt 1): E241-6.
[78] Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, et al. Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. N Engl J Med 1992; 326(1): 22-9.
[79] Briatore L, Salani B, Andraghetti G, Danovaro C, Sferrazzo E, Scopinaro N, et al. Restoration of acute insulin response in T2DM subjects 1 month after biliopancreatic diversion. Obesity (Silver Spring) 2008; 16(1): 77-81.
[80] Long SD, O'Brien K, MacDonald KG, Jr., Leggett-Frazier N, Swanson MS, Pories WJ, et al. Weight loss in severely obese subjects prevents the progression of impaired glucose tolerance to type II diabetes. A longitudinal interventional study. Diabetes Care 1994; 17(5): 372-5.

[81] MacDonald KG, Jr., Long SD, Swanson MS, Brown BM, Morris P, Dohm GL, et al. The gastric bypass operation reduces the progression and mortality of non-insulin-dependent diabetes mellitus. J Gastrointest Surg 1997; 1(3): 213-20; discussion 20.
[82] Hughes TA, Gwynne JT, Switzer BR, Herbst C, White G. Effects of caloric restriction and weight loss on glycemic control, insulin release and resistance, and atherosclerotic risk in obese patients with type II diabetes mellitus. Am J Med 1984; 77(1): 7-17.
[83] Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. Diabetologia 2011.
[84] Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002; 346(6): 393-403.
[85] Azen SP, Peters RK, Berkowitz K, Kjos S, Xiang A, Buchanan TA. TRIPOD (TRoglitazone In the Prevention Of Diabetes): a randomized, placebo-controlled trial of troglitazone in women with prior gestational diabetes mellitus. Control Clin Trials 1998; 19(2): 217-31.
[86] Pierce M, Keen H, Bradley C. Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. Diabet Med 1995; 12(1): 6-13.
[87] Newman B, Selby JV, King MC, Slemenda C, Fabsitz R, Friedman GD. Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. Diabetologia 1987; 30(10): 763-8.
[88] Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007; 316(5829): 1341-5.
[89] Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007; 445(7130): 881-5.
[90] da Silva Xavier G, Loder MK, McDonald A, Tarasov AI, Carzaniga R, Kronenberger K, et al. TCF7L2 regulates late events in insulin secretion from pancreatic islet beta-cells. Diabetes 2009; 58(4): 894-905.
[91] Lemaire K, Ravier MA, Schraenen A, Creemers JW, Van de Plas R, Granvik M, et al. Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. Proc Natl Acad Sci U S A 2009; 106(35): 14872-7.
[92] Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK, Baldwin JM, Gyulkhandanyan AV, et al. Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes 2009; 58(9): 2070-83.
[93] Zatyka M, Ricketts C, da Silva Xavier G, Minton J, Fenton S, Hofmann-Thiel S, et al. Sodium-potassium ATPase 1 subunit is a molecular partner of Wolframin, an endoplasmic reticulum protein involved in ER stress. Hum Mol Genet 2008; 17(2): 190-200.
[94] Palmer ND, Lehtinen AB, Langefeld CD, Campbell JK, Haffner SM, Norris JM, et al. Association of TCF7L2 gene polymorphisms with reduced acute insulin response in Hispanic Americans. J Clin Endocrinol Metab 2008; 93(1): 304-9.
[95] Gould GW, Holman GD. The glucose transporter family: structure, function and tissue-specific expression. Biochem J 1993; 295 (Pt 2): 329-41.
[96] Efrat S, Tal M, Lodish HF. The pancreatic beta-cell glucose sensor. Trends Biochem Sci 1994; 19(12): 535-8.
[97] Thorens B, Weir GC, Leahy JL, Lodish HF, Bonner-Weir S. Reduced expression of the liver/beta-cell glucose transporter isoform in glucose-insensitive pancreatic beta cells of diabetic rats. Proc Natl Acad Sci U S A 1990; 87(17): 6492-6.
[98] Johnson JH, Ogawa A, Chen L, Orci L, Newgard CB, Alam T, et al. Underexpression of beta cell high Km glucose transporters in noninsulin-dependent diabetes. Science 1990; 250(4980): 546-9.
[99] Thorens B, Wu YJ, Leahy JL, Weir GC. The loss of GLUT2 expression by glucose-unresponsive beta cells of db/db mice is reversible and is induced by the diabetic environment. J Clin Invest 1992; 90(1): 77-85.
[100] Guillam MT, Hummler E, Schaerer E, Yeh JI, Birnbaum MJ, Beermann F, et al. Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. Nat Genet 1997; 17(3): 327-30.
[101] Santer R, Schneppenheim R, Dombrowski A, Gotze H, Steinmann B, Schaub J. Mutations in GLUT2, the gene for the liver-type glucose transporter, in patients with Fanconi-Bickel syndrome. Nat Genet 1997; 17(3): 324-6.
[102] Mueckler M, Kruse M, Strube M, Riggs AC, Chiu KC, Permutt MA. A mutation in the Glut2 glucose transporter gene of a diabetic patient abolishes transport activity. J Biol Chem 1994; 269(27): 17765-7.
[103] Unger RH. Diabetic hyperglycemia: link to impaired glucose transport in pancreatic beta cells. Science 1991; 251(4998): 1200-5.
[104] Ferrer J, Benito C, Gomis R. Pancreatic islet GLUT2 glucose transporter mRNA and protein expression in humans with and without NIDDM. Diabetes 1995; 44(12): 1369-74.

[105] Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, et al. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 2005; 122(3): 337-49.
[106] McCulloch LJ, van de Bunt M, Braun M, Frayn KN, Clark A, Gloyn AL. GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: Implications for understanding genetic association signals at this locus. Mol Genet Metab 2011.
[107] Grupe A, Hultgren B, Ryan A, Ma YH, Bauer M, Stewart TA. Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis. Cell 1995; 83(1): 69-78.
[108] Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, et al. Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. Nature 1992; 356(6365): 162-4.
[109] Velho G, Blanche H, Vaxillaire M, Bellanne-Chantelot C, Pardini VC, Timsit J, et al. Identification of 14 new glucokinase mutations and description of the clinical profile of 42 MODY-2 families. Diabetologia 1997; 40(2): 217-24.
[110] Hattersley AT, Turner RC, Permutt MA, Patel P, Tanizawa Y, Chiu KC, et al. Linkage of type 2 diabetes to the glucokinase gene. Lancet 1992; 339(8805): 1307-10.
[111] Aizawa T, Asanuma N, Terauchi Y, Suzuki N, Komatsu M, Itoh N, et al. Analysis of the pancreatic beta cell in the mouse with targeted disruption of the pancreatic beta cell-specific glucokinase gene. Biochem Biophys Res Commun 1996; 229(2): 460-5.
[112] Bali D, Svetlanov A, Lee HW, Fusco-DeMane D, Leiser M, Li B, et al. Animal model for maturity-onset diabetes of the young generated by disruption of the mouse glucokinase gene. J Biol Chem 1995; 270(37): 21464-7.
[113] Ishihara H, Tashiro F, Ikuta K, Asano T, Katagiri H, Inukai K, et al. Inhibition of pancreatic beta-cell glucokinase by antisense RNA expression in transgenic mice: mouse strain-dependent alteration of glucose tolerance. FEBS Lett 1995; 371(3): 329-32.
[114] Sakura H, Ashcroft SJ, Terauchi Y, Kadowaki T, Ashcroft FM. Glucose modulation of ATP-sensitive K-currents in wild-type, homozygous and heterozygous glucokinase knock-out mice. Diabetologia 1998; 41(6): 654-9.
[115] Gorman T, Hope DC, Brownlie R, Yu A, Gill D, Lofvenmark J, et al. Effect of high-fat diet on glucose homeostasis and gene expression in glucokinase knockout mice. Diabetes Obes Metab 2008; 10(10): 885-97.
[116] Aguilar-Bryan L, Bryan J. Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocr Rev 1999; 20(2): 101-35.
[117] Seghers V, Nakazaki M, DeMayo F, Aguilar-Bryan L, Bryan J. Sur1 knockout mice. A model for K(ATP) channel-independent regulation of insulin secretion. J Biol Chem 2000; 275(13): 9270-7.
[118] Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, et al. Defective insulin secretion and enhanced insulin action in KATP channel-deficient mice. Proc Natl Acad Sci U S A 1998; 95(18): 10402-6.
[119] Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. Science 1995; 268(5209): 426-9.
[120] Dunne MJ, Kane C, Shepherd RM, Sanchez JA, James RF, Johnson PR, et al. Familial persistent hyperinsulinemic hypoglycemia of infancy and mutations in the sulfonylurea receptor. N Engl J Med 1997; 336(10): 703-6.
[121] Nestorowicz A, Inagaki N, Gonoi T, Schoor KP, Wilson BA, Glaser B, et al. A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. Diabetes 1997; 46(11): 1743-8.
[122] Norman M, Moldovan S, Seghers V, Wang XP, DeMayo FJ, Brunicardi FC. Sulfonylurea receptor knockout causes glucose intolerance in mice that is not alleviated by concomitant somatostatin subtype receptor 5 knockout. Ann Surg 2002; 235(6): 767-74.
[123] Ravier MA, Nenquin M, Miki T, Seino S, Henquin JC. Glucose controls cytosolic Ca2+ and insulin secretion in mouse islets lacking adenosine triphosphate-sensitive K+ channels owing to a knockout of the pore-forming subunit Kir6.2. Endocrinology 2009; 150(1): 33-45.
[124] Andrikopoulos S, Rosella G, Gaskin E, Thorburn A, Kaczmarczyk S, Zajac JD, et al. Impaired regulation of hepatic fructose-1,6-bisphosphatase in the New Zealand obese mouse model of NIDDM. Diabetes 1993; 42(12): 1731-6.
[125] Lan H, Rabaglia ME, Stoehr JP, Nadler ST, Schueler KL, Zou F, et al. Gene expression profiles of nondiabetic and diabetic obese mice suggest a role of hepatic lipogenic capacity in diabetes susceptibility. Diabetes 2003; 52(3): 688-700.
[126] Andrikopoulos S, Proietto J. The biochemical basis of increased hepatic glucose production in a mouse model of type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1995; 38(12): 1389-96.
[127] Kebede M, Favaloro J, Gunton JE, Laybutt DR, Shaw M, Wong N, et al. Fructose-1,6-bisphosphatase overexpression in pancreatic beta-cells results in reduced insulin secretion: a new mechanism for fat-induced impairment of beta-cell function. Diabetes 2008; 57(7): 1887-95.

[128] Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992; 12(12): 5447-54.
[129] Noordeen NA, Khera TK, Sun G, Longbottom ER, Pullen TJ, da Silva Xavier G, et al. Carbohydrate-responsive element-binding protein (ChREBP) is a negative regulator of ARNT/HIF-1beta gene expression in pancreatic islet beta-cells. Diabetes 2010; 59(1): 153-60.
[130] Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 2004; 5(5): 343-54.
[131] Cheng K, Ho K, Stokes R, Scott C, Lau SM, Hawthorne WJ, et al. Hypoxia-inducible factor-1alpha regulates beta cell function in mouse and human islets. J Clin Invest 2010; 120(6): 2171-83.
[132] Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 2001; 292(5516): 464-8.
[133] Cantley J, Selman C, Shukla D, Abramov AY, Forstreuter F, Esteban MA, et al. Deletion of the von Hippel-Lindau gene in pancreatic beta cells impairs glucose homeostasis in mice. J Clin Invest 2009; 119(1): 125-35.
[134] Choi D, Cai EP, Schroer SA, Wang L, Woo M. Vhl is required for normal pancreatic beta cell function and the maintenance of beta cell mass with age in mice. Lab Invest 2011; 91(4): 527-38.
[135] Puri S, Cano DA, Hebrok M. A role for von Hippel-Lindau protein in pancreatic beta-cell function. Diabetes 2009; 58(2): 433-41.
[136] Zehetner J, Danzer C, Collins S, Eckhardt K, Gerber PA, Ballschmieter P, et al. pVHL is a regulator of glucose metabolism and insulin secretion in pancreatic {beta} cells. Genes Dev 2008; 22(22): 3135-46.
[137] Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, et al. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. J Biol Chem 2000; 275(33): 25130-8.
[138] Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, et al. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab 2005; 1(6): 401-8.
[139] Goyal P, Weissmann N, Grimminger F, Hegel C, Bader L, Rose F, et al. Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. Free Radic Biol Med 2004; 36(10): 1279-88.
[140] Zhang N, Fu Z, Linke S, Chicher J, Gorman JJ, Visk D, et al. The asparaginyl hydroxylase factor inhibiting HIF-1alpha is an essential regulator of metabolism. Cell Metab 2010; 11(5): 364-78.
[141] Woodward ER, Maher ER. Von Hippel-Lindau disease and endocrine tumour susceptibility. Endocr Relat Cancer 2006; 13(2): 415-25.
[142] Chetty R, Kennedy M, Ezzat S, Asa SL. Pancreatic endocrine pathology in von Hippel-Lindau disease: an expanding spectrum of lesions. Endocr Pathol 2004; 15(2): 141-8.
[143] Perigny M, Hammel P, Corcos O, Larochelle O, Giraud S, Richard S, et al. Pancreatic endocrine microadenomatosis in patients with von Hippel-Lindau disease: characterization by VHL/HIF pathway proteins expression. Am J Surg Pathol 2009; 33(5): 739-48.
[144] Hammel PR, Vilgrain V, Terris B, Penfornis A, Sauvanet A, Correas JM, et al. Pancreatic involvement in von Hippel-Lindau disease. The Groupe Francophone d'Etude de la Maladie de von Hippel-Lindau. Gastroenterology 2000; 119(4): 1087-95.
[145] Rosen OM. After insulin binds. Science 1987; 237(4821): 1452-8.
[146] Wertheimer E, Lu SP, Backeljauw PF, Davenport ML, Taylor SI. Homozygous deletion of the human insulin receptor gene results in leprechaunism. Nat Genet 1993; 5(1): 71-3.
[147] Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, et al. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 1996; 12(1): 106-9.
[148] Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, et al. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell 1998; 2(5): 559-69.
[149] Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 1999; 96(3): 329-39.
[150] Freeman H, Shimomura K, Horner E, Cox RD, Ashcroft FM. Nicotinamide nucleotide transhydrogenase: a key role in insulin secretion. Cell Metab 2006; 3(1): 35-45.
[151] Toye AA, Lippiat JD, Proks P, Shimomura K, Bentley L, Hugill A, et al. A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. Diabetologia 2005; 48(4): 675-86.
[152] Wong N, Blair AR, Morahan G, Andrikopoulos S. The deletion variant of nicotinamide nucleotide transhydrogenase (Nnt) does not affect insulin secretion or glucose tolerance. Endocrinology 2010; 151(1): 96-102.

[153] Aston-Mourney K, Wong N, Kebede M, Zraika S, Balmer L, McMahon JM, et al. Increased nicotinamide nucleotide transhydrogenase levels predispose to insulin hypersecretion in a mouse strain susceptible to diabetes. Diabetologia 2007; 50(12): 2476-85.
[154] Garten A, Petzold S, Korner A, Imai S, Kiess W. Nampt: linking NAD biology, metabolism and cancer. Trends Endocrinol Metab 2009; 20(3): 130-8.
[155] Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A, et al. Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab 2007; 6(5): 363-75.
[156] Guarente L, Picard F. Calorie restriction--the SIR2 connection. Cell 2005; 120(4): 473-82.
[157] Bordone L, Motta MC, Picard F, Robinson A, Jhala US, Apfeld J, et al. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. PLoS Biol 2006; 4(2): e31.
[158] Moynihan KA, Grimm AA, Plueger MM, Bernal-Mizrachi E, Ford E, Cras-Meneur C, et al. Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. Cell Metab 2005; 2(2): 105-17.
[159] Ahuja N, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, et al. Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. J Biol Chem 2007; 282(46): 33583-92.
[160] Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, et al. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell 2006; 126(5): 941-54.
[161] Yechoor VK, Patti ME, Ueki K, Laustsen PG, Saccone R, Rauniyar R, et al. Distinct pathways of insulin-regulated versus diabetes-regulated gene expression: an in vivo analysis in MIRKO mice. Proc Natl Acad Sci U S A 2004; 101(47): 16525-30.
[162] Andrews ZB, Diano S, Horvath TL. Mitochondrial uncoupling proteins in the CNS: in support of function and survival. Nat Rev Neurosci 2005; 6(11): 829-40.
[163] Klingenberg M, Echtay KS. Uncoupling proteins: the issues from a biochemist point of view. Biochim Biophys Acta 2001; 1504(1): 128-43.
[164] Rousset S, Alves-Guerra MC, Mozo J, Miroux B, Cassard-Doulcier AM, Bouillaud F, et al. The biology of mitochondrial uncoupling proteins. Diabetes 2004; 53 Suppl 1: S130-5.
[165] Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, et al. Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. Cell 2001; 105(6): 745-55.
[166] Produit-Zengaffinen N, Davis-Lameloise N, Perreten H, Becard D, Gjinovci A, Keller PA, et al. Increasing uncoupling protein-2 in pancreatic beta cells does not alter glucose-induced insulin secretion but decreases production of reactive oxygen species. Diabetologia 2007; 50(1): 84-93.
[167] Pi J, Bai Y, Daniel KW, Liu D, Lyght O, Edelstein D, et al. Persistent oxidative stress due to absence of uncoupling protein 2 associated with impaired pancreatic beta-cell function. Endocrinology 2009; 150(7): 3040-8.
[168] Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet 2002; 359(9323): 2072-7.
[169] Fehse F, Trautmann M, Holst JJ, Halseth AE, Nanayakkara N, Nielsen LL, et al. Exenatide augments first- and second-phase insulin secretion in response to intravenous glucose in subjects with type 2 diabetes. J Clin Endocrinol Metab 2005; 90(11): 5991-7.
[170] DeFronzo RA, Okerson T, Viswanathan P, Guan X, Holcombe JH, MacConell L. Effects of exenatide versus sitagliptin on postprandial glucose, insulin and glucagon secretion, gastric emptying, and caloric intake: a randomized, cross-over study. Curr Med Res Opin 2008; 24(10): 2943-52.
[171] Kim D, MacConell L, Zhuang D, Kothare PA, Trautmann M, Fineman M, et al. Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. Diabetes Care 2007; 30(6): 1487-93.
[172] Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet 2002; 359(9309): 824-30.
[173] Garber A, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez-Pattzi H, Olvera-Alvarez I, et al. Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. Lancet 2009; 373(9662): 473-81.
[174] Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, et al. Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. Diabetes 2002; 51(2): 424-9.
[175] Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, et al. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. Diabetes 2002; 51(9): 2796-803.