Metabolic signaling of insulin secretion by pancreatic β-cell and its derangement in type 2 diabetes

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Abstract. Pancreatic β-cell is responsible for insulin secretion in response to the availability of nutrients. Type 2 diabetes mellitus (T2D) is the result of pancreatic β-cell failure to supply sufficient amount of insulin accompanied with decreased sensitivity of the body tissues to respond to insulin. The insulin secretion apparatus of β-cell is uniquely equipped with multiple metabolic and signaling steps that are under rigorous control. The metabolic machinery of β-cell is designed to sense the fluctuations in blood glucose level and supply insulin accordingly to the needs of body. Besides glucose, amino acids including glutamine and leucine and also fatty acids are known to either stimulate the β-cells directly or potentiate the glucose stimulated insulin secretion (GSIS) response. Glucose metabolism dependent GSIS is linked with the production of ATP that is needed for K\textsuperscript{+} ATP channel inhibition and influx of calcium, necessary for insulin granule exocytosis. Besides glucose metabolism, amino acid metabolism and lipid metabolism derived metabolites mediate the optimal glucose response of β-cells to secrete insulin. Metabolites derived from nutrient secretagogues that directly or indirectly participate in the enhancement of GSIS are considered as metabolic coupling factors. In this review, we will discuss the regulation of insulin secretion by β-cell keeping the recent developments in metabolic signaling in focus. The relevant metabolic pathways in pancreatic β-cell and their role in the control of fuel-stimulated insulin secretion will be reviewed to arrive at a consensus picture with respect to the metabolic signaling of insulin secretion.

Key Words: Pancreatic β-cell, Insulin secretion, Type 2 diabetes mellitus.

Introduction

Type 2 diabetes mellitus (T2D) is the manifestation of pancreatic islet β-cell failure to supply sufficient amount of insulin and decreased sensitivity of the body tissues to insulin. It results from genetic susceptibility and epigenetic changes in the context of toxic environmental factors such as malnutrition and reduced physical activity\textsuperscript{1-3}. The islet of Langerhans is a key fuel sensing micro-organ that constantly adjusts the release of insulin in response to the levels and stimulus strength of nutrients and hormonal factors. The insulin secretion apparatus of β-cells is uniquely equipped with multiple metabolic and signaling steps that are under rigorous control. The glucose response of β-cell to secrete insulin is considered to be highest in comparison to other calorigenic nutrient secretagogues\textsuperscript{4,5}. Thus, the metabolic machinery of β-cell is designed to sense the fluctuations in blood glucose level and supplies insulin accordingly to the needs of the body\textsuperscript{5,6}. Besides glucose, some amino acids including glutamine and leucine and also fatty acids are known to either stimulate the β-cells directly or potentiate the glucose stimulated insulin secretion (GSIS) response\textsuperscript{5,7}. The early pre-absorptive phase of insulin release, seen within few minutes after food ingestion, is due to the parasympathetic nerves supplying the islets\textsuperscript{8}.

Unlike in most cell types where activation of an energy consuming biological process (e.g., contraction) lowers the ATP/ADP ratio, which in turn promotes cellular metabolism to produce ATP, in β-cell metabolic activation is primarily driven by substrate (fuel) availability\textsuperscript{9,10}, rather than as a secondary effect to enhanced insulin release\textsuperscript{11}. Glucose metabolism in β-cell is linked to the production of ATP and a rise in the cytoplasmic ATP/ADP ratio that is needed for K\textsuperscript{+} ATP channel inhibition and depolarization of the plasma membrane. The im-
portance of $K^+_{ATP}$ channel closure for opening up the voltage dependent L-type calcium channels in the plasma membrane with the resultant $Ca^{2+}$ influx, as one of the primary events for insulin granule exocytosis, is well established\cite{2,12,13} (Figure 1). Glucose metabolism driven $K^+_{ATP}$ channel inhibition has also been implicated in the regulation of $\beta$-cell mass\cite{14,15}. The ability of the $\beta$-cell to respond to the alterations in the blood glucose levels in the (patho)physiological range (3 to 16 mM) is accomplished because of the affinities of two key regulatory proteins for glucose. These are Glut-1 and Glut-2 glucose transporters that have high Km (17 mM), in human and in rodent $\beta$-cells, respectively, which rapidly equilibrate external and internal glucose and glucokinase (hexokinase IV), the rate limiting enzyme that catalyzes the first step of glycolysis, which has a Km of ~8 mM for glucose\cite{16}. The Glut-1/2-Glucokinase tandem ensures a steady increase in glycolysis and ATP production in the $\beta$-cell with increasing blood glucose levels and, thus, the glucose concentration dependent insulin secretion response\cite{17}. GSIS in $\beta$-cells is achieved by a tight link between glycolysis and mitochondrial metabolism for the quantitative direction of glucose carbons into mitochondria, due to the very low expression of lactate dehydrogenase\cite{18,19,20}.

The exocytotic process in the $\beta$-cell is orchestrated by several components, in particular $Ca^{2+}$ ions and exocytotic effector proteins located at the surfaces of secretory granules and plasma membranes\cite{20}, which facilitate the fusion of the insulin containing large dense-core vesicles with the plasma membrane. Besides, synaptic-like micro vesicles (SLMV) are also present in $\beta$-cells and these vesicles contain small molecules like $\gamma$-aminobutyric acid (GABA) and may release their contents – in a manner similar to that of synaptic vesicles of neurons\cite{21,22}. However, not much is known about how fuel stimuli influence the release of SLMV.

In this review, we will specifically discuss the regulation of insulin secretion by fuel stimuli in the $\beta$-cell keeping the recent developments in metabolic signaling in focus. The relevant metabolic pathways and their role in the control of nutrient stimulated insulin secretion will be reviewed to arrive at a consensus picture with respect to the biochemical basis of insulin secretion promoted by glucose, amino acids and fatty acids.

**Biphasic modes of insulin secretion**

A step rise in glucose concentration induces the release of insulin in a biphasic pattern, both in vitro and in vivo\cite{23,24}, consisting of a rapid 3-10 min peak initially, followed by a slowly developing second phase. The first phase secretion is reduced in prediabetes, whereas it is almost completely abolished in T2D along with significantly reduced second-phase secretion\cite{23}. However, the biphasic pattern of insulin secretion probably does not exist in vivo under physiological conditions, where glucose concentration does not rise in a step-wise manner. However, this feature observed in vitro, helps in understanding the biochemical basis of fuel-induced insulin secretion and in identifying prediabetes condition. The first phase corresponds to the exocytosis of a small number of the secretory granules already docked on the plasma membrane that release insulin, whereas the second phase involves mobilization of granules from the storage pool. There are 10,000-12,000 insulin granules in a $\beta$-cell, with a small pool of them (~500) docked to the plasma membrane, of which 50-100 granules, considered as the ready-releasable pool, are tethered to the membrane in close association with $Ca^{2+}$ channels and contribute to the rapid first phase secretion\cite{23}. The ready-releasable pool is replenished with fresh granules from the storage pool, which eventually contribute to the second sustained phase of secretion.

The molecular basis of how granules pools are linked to phasic secretion is currently being worked out. Recent studies showed that in human islets, nascent insulin granules contribute to first phase and the mature granules to the second phase insulin secretion\cite{23}. Different pools of insulin granules that are functionally distinct have been described in $\beta$-cells. Regions of $\beta$-cells with preassembled soluble NSF-attachment protein receptor (SNARE) proteins showed fast exocytosis in response to rise in $Ca^{2+}$, but in regions without preassembled SNAREs, the exocytosis is slower.\cite{23} Whether these granule pools related to biphasic secretion is uncertain. Recently, the model of phasic secretion with respect to granule pools has been questioned\cite{23}. The current model proposes that first phase insulin secretion results from a readily-releasable pool composed of granules docked to the plasma membrane, whereas the second phase results from a reserve pool of granules located farther away that are recruited upon stimulation, docked, and followed by fusion with the plasma membrane. In a new currently accepted model, insulin granules are recruited upon $\beta$-cell stimulation and immediately fused to the plasma membrane, in both the phases. This model promotes the idea that the second phase secretion actually consists of iteration of the first phase.
Figure 1. Fuel metabolism and production of metabolic coupling factors in the β-Cell. Glucose, fatty acids and glutamine are metabolized through the Krebs cycle, pyruvate cycle and triglyceride/fatty acid (TG/FA) cycle in the β-cell. Glutamine is converted by glutaminase in cytosol to glutamate, which is oxidized by glutamate dehydrogenase (GDH) in the mitochondria to produce α-ketoglutarate that enters Krebs cycle. Further oxidation of α-ketoglutarate in Krebs cycle leads to production of GTP, which inhibits GDH. Generation of α-ketoglutarate by GDH functions as anaplerosis as it provides substrate for Krebs cycle. Reducing equivalents (NADH) produced by Krebs cycle are oxidized by electron transport chain (ETC) to generate ATP and as a side product, reactive oxygen species (ROS). ROS can directly stimulate insulin exocytosis. ATP exits mitochondria and in cytosol, it inhibits K+ ATP channel and triggers Ca2+ influx and insulin granule exocytosis. ATP is also used by adenylate cyclase to produce cyclic AMP (cAMP), when activated in response to stimuli like binding of GLP1 to its Gs-coupled receptor. cAMP activates exchange protein directly activated by cyclic AMP (EPAC2) and also protein kinase-A (PKA), both of these promote exocytosis. Fatty acids are activated to fatty acyl-CoA by acyl-CoA synthase, long chain enzymes present on the surface of different subcellular membranes. Under conditions of low glucose, fatty acyl-CoAs are oxidized to generate acetyl-CoA, which enters Krebs cycle at citrate synthase step. Fatty acyl-CoAs are channeled to the TG/FA cycle under conditions of increased glucose availability, when β-oxidation is reduced. Glucose enters β-cell via Glut1 or Glut 2 transporters (in human or rodent, respectively) and enters glycolysis after its conversion to glucose-6-phosphate by glucokinase (GK); glycolysis gives rise to pyruvate and acetyl-CoA, which enters Krebs cycle. Approximately 50% of pyruvate is also converted to oxaloacetate via the anaplerotic enzyme pyruvate carboxylase inside mitochondria. Pyruvate participates in pyruvate cycles exchanging substrates, viz., citrate, isocitrate and malate, with Krebs cycle. Transport of these metabolites from mitochondria to the cytoplasm (anaplerosis) facilitates pyruvate cycles. NADPH produced by pyruvate cycles either directly or in combination with glutaredoxin, or via ROS formation through the action of NADPH oxidase, promotes insulin granule exocytosis. Dihydroxyacetone phosphate (DHAP), produced during glycolysis, is reduced to glycerol 3-phosphate using NADH, by glyceraldehyde-3-phosphate dehydrogenase. Glycerol-3-phosphate and fatty acyl-CoA react together to form lysophosphatidic acid and to enter TG/FA cycle. Malonyl-CoA, which is formed from acetyl-CoA, as part of pyruvate/citrate cycle, inhibits fatty acid oxidation and positively influences TG/FA cycle by diverting fatty acyl-CoA into TG/FA cycle, with participating enzymes distributed in endoplasmic reticulum (ER), mitochondria, cytosol and plasma membrane. TG/FA cycle produces several lipid signaling molecules including monoacylglycerol (MAG), which stimulate insulin secretion. MAG activates Munc13-1, an exocytosis facilitating protein. MAG can also be produced by the hydrolysis of diacylglycerol (DAG), produced at plasma membrane from phosphoinositides (PIP2) during the activation of Gq-coupled receptors like GPR40, the fatty acid receptor. Nutrient metabolism in β-cell generates metabolic coupling factors (MCF) that positively influence insulin granule exocytosis at different steps.
**Metabolic coupling factors**

Insulin secretion is a multi-component process and different steps are influenced by metabolites produced during glucose, amino acid and lipid metabolism. It has recently been suggested that the metabolic signals can be ‘early or late effectors’, on the basis of whether the corresponding affected step is an early event or late step in the process of insulin exocytosis. A metabolic coupling factor (MCF) can be a signal that contributes to the regulation of nutrient-stimulated insulin secretion process, either by modulating the nutrient metabolism (early regulators) or by directly influencing the component(s) of exocytotic machinery (late regulators). Table I gives examples of various MCFs and their proposed targets and roles in insulin secretion. Considering the importance of insulin secretion, β-cell harbors metabolic pathways that generate these multiple MCFs to ensure proper insulin secretion.

As mentioned above, the relatively low affinities of Glut1 and 2 and glucokinase for glucose control the flux of glucose metabolism in the β-cell, which in turn dictates the rate and magnitude of insulin secretion in response to blood glucose level. Glucose metabolism produces ATP, which closes the K<sub>ATP</sub> channel with the resultant Ca<sup>2+</sup> influx that promotes insulin granule exocytosis. Production of ATP in mitochondria is facilitated by the efficient transfer of glucose-derived NADH from cytosol to mitochondria by the glycerol-3-phosphate and malate/aspartate shuttles. Activated mitochondrial metabolism, consisting of anaplerosis and cataplerosis, is central for enhanced Krebs cycle activity and ATP production and also for the generation of additional MCF that take part in the amplification of GSIS (Figure 1).

**Signals from anaplerosis and cataplerosis**

Anaplerosis is the process that contributes to the replenishment of Krebs cycle intermediates. Once elevated, certain Krebs cycle intermediates, like citrate, not only enhance the cycle activity catalytically, but also participate in additional metabolic pathways that lead to the production of different MCFs (e.g., malonyl-CoA, Glutamate, NADPH) in the cytoplasm. This process is complemented by cataplerosis, which refers to the exit of Krebs cycle intermediates from the mitochondrial matrix to cytoplasm. Various transporters located on the mitochondrial inner membrane facilitate the transmembrane movement of the Krebs cycle intermediates, such as the di- and tri-carboxylate carriers. In the cytoplasm, some of the Krebs cycle intermediates (citrate, isocitrate, α-ketoglutarate and malate) participate in pyruvate cycling processes that generate cytoplasmic NADPH (Figure 1), an important MCF.

Pyruvate metabolism via pyruvate carboxylase (PC), which is highly expressed in β-cells, is central to anaplerosis. Many studies have shown that as compared to the rates of decarboxylation and oxidation of pyruvate, the rate of pyruvate carboxylation correlates well with the glucose dose dependence of GSIS. Studies using PC inhibitor phenylacetic acid, RNAi knockdown and overproduction of PC in INS-1 cells and islets clearly demonstrated the significance of PC in GSIS. Besides formation of oxaloacetate by PC, the oxidative deamination of glutamate to α-ketoglutarate by mitochondrial glutamate dehydrogenase (GDH) is also a significant contributor to anaplerosis by amino acids as discussed below. Mitochondrial GDH is important for

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**Table I.** Metabolic coupling factors implicated in insulin secretion.

<table>
<thead>
<tr>
<th>MCF</th>
<th>Target of action</th>
<th>Mode and site of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>AMPK</td>
<td>Negative regulation of GSIS</td>
</tr>
<tr>
<td>ATP</td>
<td>K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signaling</td>
</tr>
<tr>
<td>cAMP</td>
<td>PKA, Epac2</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;, channels, exocytosis proteins</td>
</tr>
<tr>
<td>Citrate</td>
<td>ACL, ACC</td>
<td>Pyruvate cycling</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>PKC, Munc13-1</td>
<td>Channels, exocytosis</td>
</tr>
<tr>
<td>Fatty acyl-CoA</td>
<td>Lipogenic enzymes</td>
<td>TG/FA cycling</td>
</tr>
<tr>
<td>Glutamate</td>
<td>GDH</td>
<td>Anaplerosis</td>
</tr>
<tr>
<td>GTP</td>
<td>GDH, GTP-SCS</td>
<td>Anaplerosis, Ca&lt;sup&gt;2+&lt;/sup&gt; signaling</td>
</tr>
<tr>
<td>Inositol poly-PO₄</td>
<td>IP3 receptor, L-type Ca&lt;sup&gt;2+&lt;/sup&gt; channel.</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; influx, exocytosis</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>α-KG dehydrogenase, HIF1α-hydroxylase</td>
<td>Exocytosis</td>
</tr>
<tr>
<td>Malonyl-CoA</td>
<td>CPT-1, FAS</td>
<td>Fatty acyl group partitioning</td>
</tr>
<tr>
<td>MgADP decrease</td>
<td>SURI1</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; signaling</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>Munc13-1</td>
<td>Vesicle fusion, Exocytosis</td>
</tr>
<tr>
<td>NADPH</td>
<td>Glutaredoxin, Kv</td>
<td>Exocytosis redox control, Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
</tr>
<tr>
<td>ROS</td>
<td>Exocytosis proteins</td>
<td>Exocytosis complex redox control</td>
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amino acid (glutamine plus leucine) induced insulin secretion and gain of function mutation of GDH is associated with a hyperinsulinemic hyperglycemic syndrome.

**Pyruvate cycles and cytosolic NADPH**

There are four pyruvate cycling processes, viz., pyruvate/citrate, pyruvate/malate, pyruvate/isocitrate and pyruvate/phosphoenol-pyruvate cycles, which are critical for anaplerosis/catabolism-derived signaling and MCF production and for producing NADPH in the cytosol using mitochondrial NADH. Inasmuch as PC converts approximately 50% of the pyruvate to OAA in β-cell mitochondria and citrate levels both in cytosol and mitochondria are elevated in proportion to glucose concentration, and because pharmacological intervening at different steps of the pyruvate/citrate cycle causes reduced GSIS in β-cells, it has been suggested earlier that pyruvate/citrate cycling is quantitatively important in the production of MCF and in GSIS. This cycle may also be linked to metabolic oscillations and, thus, contribute to the pulsatile insulin release from β-cells that parallels oscillations in [Ca^{2+}], ATP, NAD(P)H and citrate levels. In the pyruvate/isocitrate cycle, cytosolic isocitrate dehydrogenase (cICDH) converts isocitrate to α-ketoglutarate and NADP to NADPH. The precise role of this cycle in GSIS is uncertain as cICDH RNAi-knockdown studies were shown to both decrease GSIS, as well as increase GSIS. It has been shown that knockdown of cytosolic malic enzyme, which takes part in the pyruvate/malate cycle, reduces GSIS.

The fourth pyruvate cycle, the “pyruvate/phosphoenolpyruvate” cycle, is linked to ATP formation in the cytoplasm and GTP hydrolysis in the mitochondria. The relative importance of these cycles is currently controversial.

Glucose stimulation of β-cell causes a rapid rise in cytosolic NADPH that occurs even prior to β-cell depolarization, increased intracellular Ca^{2+} and insulin granule fusion to plasma membrane. The pentose phosphate shunt pathway, which can also generate NADPH is unlikely to be important for GSIS, as flux through this pathway in β-cell is much lower, thus suggesting pyruvate cycling pathways to be the major source of cytosolic NADPH in these cells. NADPH is also formed in mitochondrial matrix by the reduction of NADP by nicotinamide nucleotide transhydrogenase (NNT) and by mitochondrial NADP-dependent isocitrate dehydrogenase. NNT mutant mice show glucose intolerance and reduced insulinemia due to a glucose tolerance test, suggesting a role for NNT and NADPH in GSIS. NNT-generated NADPH can be used by mitochondrial NADP-dependent isocitrate dehydrogenase, which using α-ketoglutarate, can produce isocitrate, followed by the export of isocitrate (or citrate) to the cytoplasm, for regenerating NADPH via pyruvate cycling processes (Figure 1). There is strong evidence implicating a role for cytosolic NADPH in GSIS. Thus, NADPH was shown to directly promote exocytosis in patch-clamped β-cells (Ivarsson et al, 2005) and RNAi silencing of cytosolic NADPH generating malic enzyme and isocitrate dehydrogenase enzymes reduces GSIS. NADPH likely targets glutaredoxin and voltage-dependent K_v (Kv) channels. Glutaredoxin is important for the post-translational modifications of exocytotic proteins and the Kv channel β-subunit, which can bind NADPH (Figure 1), is thought to be the sensor of intracellular redox potential that in turn regulates the channel and, thus, controls glucose-stimulated action potentials in β-cells.

**Pyruvate cycling and malonyl-CoA**

Acetyl-CoA formed by ATP-citrate lyase (ACL) is carboxylated by acetyl-CoA carboxylase (ACC) to malonyl-CoA, which has signaling role in the control of GSIS by inhibiting carnitine palmitoyltransferase-1 (CPT-1). Inhibition of CPT-1 diverts fatty acids from β-oxidation to lipid synthesis, and some of these lipids play important role in the amplification of GSIS. Build up of fatty acyl-CoA due to CPT-1 inhibition, can lead to the activation of protein kinase-C enzymes and K_v channel. The view that malonyl-CoA/CPT-1 interaction is needed for optimal GSIS was supported by the studies showing impaired GSIS in INS cells overexpressing a mutant CPT-1 that is insensitive to malonyl-CoA. However, overexpression of cytosol-directed malonyl-CoA decarboxylase appears to lower GSIS only in the presence of fatty acids. Fatty acids also directly bind to cell surface GPCRs, GPR40 and GPR120 and stimulate GSIS.

**Glutamate, GDH and GTP**

Inasmuch as glucose stimulation of islets is accompanied by augmented glutamate levels supports the view that glutamate is an MCF. In addition, reduction of β-cell glutamate levels by glutamate decarboxylase overexpression reduces insulin secretion. Islets from β-cell-specific GDH KO mice display reduced GSIS, indicating glutamate metabolism via GDH reaction is necessary.
for the stimulation of GSIS\textsuperscript{68}. In mitochondria, allosteric inhibition of glutamate dehydrogenase by GTP inhibits oxidative deamination of glutamate, thereby negatively affecting insulin secretion (Figure 1; Table I). Gain of function mutations of GDH, which render GDH to be less susceptible to GTP inhibition are associated with hyperinsulinemia\textsuperscript{36,69}. Both cytosolic and mitochondrial GTP has an effect on insulin secretion. Mitochondrial GTP is predominantly produced by the GTP-specific succinyl-CoA synthase (GTP-SCS), whereas in cytosol nucleoside diphosphate kinase is responsible for GTP formation. On the other hand, mitochondrial GTP has also been shown to modulate mitochondrial metabolism and Ca\textsuperscript{2+} and to positively influence GSIS in β-cells\textsuperscript{70}. Thus, RNAi-knockdown of GTP-SCS lowers β-cell ATP levels and reduces GSIS\textsuperscript{70}. GTPase enzymes associated with insulin exocytosis utilize cytosolic GTP to promote secretion and it has been shown that GTP levels in cytosol rise at high glucose concentration. Besides, recent evidence strongly implicated a role for cGMP in stimulating insulin secretion. Thus, activation of AMPA receptors by glutamate can lead to elevated cGMP, which inhibits K\textsuperscript+ATP channel and stimulate secretion\textsuperscript{41} and the presence of guanylate cyclases, which produce cGMP from GTP in β-cells has been confirmed\textsuperscript{72}.

**ATP, ADP, AMP and AMPK**

Inasmuch as K\textsuperscript+ATP channel inhibition by ATP is a necessary step for insulin secretion, alterations in ATP/ADP ratio in the vicinity of K\textsuperscript+ATP channel are relevant and are regulated by adenylate kinase-1, which likely associates with Kir6.2 subunit of K\textsuperscript+ATP channel\textsuperscript{73}. Adenine nucleotides, whose cellular levels are dependent on the metabolic state of the β-cell, directly influence the insulin exocytotic machinery at the plasma membrane. Overall regulation of K\textsuperscript+ channels by the adenine nucleotides depends on the net inhibitory effect of ATP and the net activating effect of MgADP on SUR1 component of the channel\textsuperscript{74}.

Besides ATP/ADP ratio, ATP/AMP ratio is also important in the overall regulation of insulin secretion in β-cells as this influences the activity of AMP activated protein kinase (AMPK), which is a master controller of cellular energy metabolism\textsuperscript{75}. There have been several excellent reviews on AMPK\textsuperscript{76} and we focus here exclusively on the recent developments. Cellular AMP levels are regulated via its utilization by adenylate kinase and through its formation during fatty acid and amino acid activation. Activation of AMPK triggers enhanced FFA β-oxidation and reduces lipolysis, thereby reducing the production of lipid signals for the amplification of GSIS\textsuperscript{77}. Recent work indicated that AMPK activation causes metabolic deceleration in the β-cell by slowing down the glucose metabolism and, thus, insulin secretion at glucose concentrations < 10 mM, whereas at higher glucose levels above 16 mM, this metabolic deceleration effect is absent\textsuperscript{78}, suggesting that AMPK activation offers protection to β-cells from the toxicity of fuel surplus and exhaustive overstimulation\textsuperscript{76}. Recent studies suggested that AMPK also likely controls the activity of SIRT1, which also is known to regulate insulin secretion in β-cells\textsuperscript{76,78}. LKB1/AMPK enzymes have been implicated as negative regulators of insulin secretion\textsuperscript{79}.

**Reactive Oxygen Species (ROS)**

ROS include superoxide and hydroxyl radicals and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and these are produced physiologically in many cells, during nutrient oxidation. In β-cells, it has been proposed that ROS acts as MCF for promoting GSIS\textsuperscript{79,80}. However, chronic production of elevated levels of ROS can be detrimental for β-cell function. Mitochondrial electron transport chain components Complex-I and Complex-III are the major site for ROS formation\textsuperscript{36,81}. Besides mitochondria, ROS can also be produced by plasma membrane electron transporting NADPH oxidase complex\textsuperscript{79} and in peroxisomes. It has been suggested that in β-cells peroxisomal fatty acid oxidation is the major source for H\textsubscript{2}O\textsubscript{2}, which leads to β-cell dysfunction and death whereas mitochondrial β-oxidation does not contribute significantly to ROS\textsuperscript{82}. In mitochondria, nicotinamide nucleotide transhydrogenase, which produces NADPH, contributes to free radical detoxification\textsuperscript{83} and altered activity of this enzyme is associated with proportional changes in insulin secretion\textsuperscript{77,83}.

The rise in influx of Ca\textsuperscript{2+}, while necessary for insulin granule exocytosis, can also cause NADPH oxidase activation resulting in increased production of H\textsubscript{2}O\textsubscript{2}\textsuperscript{79}. Attenuation of ROS signal is mainly accomplished by superoxide dismutase, glutathione peroxidase, thioredoxin and peroxiredoxins in human β-cell whereas these enzymes are expressed at low levels in rodent islets\textsuperscript{79}. The positive effects of ROS, in particular H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2–} on GSIS include enhancement of Ca\textsuperscript{2+} influx\textsuperscript{80} and activation of volume regulated anion channels\textsuperscript{84} even though the precise targets are not identified. The detrimental effects of ROS are activation of mitochondrial UCP2, oxidative modi-
fication and inhibition of aconitase, adenine nucleotide translocase and glyceraldehyde-3-phosphate dehydrogenase, and oxidation of mitochondrial cardiolipin resulting in reduced ATP levels, decreased insulin secretion and apoptosis. Production of ROS is likely to be elevated in the pancreatic islets from T2D patients than from nondiabetic subjects. Thus, ROS can have dual function in the regulation of GSIS (Figure 1) – both in stimulating secretion as well as in causing β-cell dysfunction, when chronically produced in high amounts.

Glucose stimulation of β-cell has been shown to cause a rapid turnover of inositol containing lipids, particularly the plasma membrane associated phosphatidylinositol-4,5-bisphosphate (PIP2) and -3,4,5-trisphosphate (PIP3) and has it been suggested that these polyphosphoinositides likely play a facilitating role in GSIS, probably by controlling intracellular Ca2+ levels and also DAG levels. Recent studies implicated inositol-triphosphate and other inositol polyposphates in the regulation of GSIS, probably by their direct action on L-type Ca2+ channels.

Triglyceride/fatty acid cycling and lipid MCF signals

The triglyceride/fatty acid (TG/FA) cycle consists of two segments – lipid synthesis and lipid breakdown and produces many lipid intermediates (Figure 2). Lipogenic arm of TG/FA cycle is initiated by the fatty acid esterification of glycerol-3-phosphate, arising from the action of cytosolic glycerol-3-phosphate dehydrogenase on glycolytic intermediate, dihydroxyacetone phosphate (Figures 1 and 2). This first step of esterification, catalyzed by glycerol-3-phosphate acyltransferase iso-enzymes forms lysophosphatidic acid (LPA), that is further converted to phosphatidic acid by LPA acyltransferase, followed by removal of phosphate by lipin to sn1,2-diacylglycerol (DAG) and then conversion to triglyceride (TG) by DAG acyltransferase. TG, then, enters lipolytic arm, by sequential hydrolysis to produce either sn2,3-DAG or sn1,3-DAG (by adipose triglyceride lipase), followed by the formation of either 2-monoacylglycerol (MAG) or 1-MAG (by hormone sensitive lipase) and finally glycerol and FA by the recently discovered α/β-hydrolyase containing-6 (Figure 2). TG in β-cells is stored as micro lipid droplets, distributed beneath the cell membrane (Pinnick et al, 2010). Many of the intermediates of TG/FA cycle show signaling functions and some are known to participate in insulin secretion in β-cells. Thus, agents that block TG/FA cycle at different steps are known to reduce GSIS. It is important to note that lipid intermediates of lipogenic arm have various signaling functions and disruption of the any of the involved enzymes has varying effects on GSIS (Figure 2). Lysophosphatidic acid is known to affect Ca2+ influx while phosphatidic acid was thought to directly influence exocytosis. However, agents that block lipolysis arm of the TG/FA cycle have been shown to reduce GSIS.

Recent studies demonstrated that ATGL produces 1,3- and 2,3-DAG species, which have no known signaling function, rather than the signaling competent sn1,2-DAG that activates C-kinase enzymes. In addition, intracellularly, HSL primarily hydrolyzes DAG at position 1 or 3, thus producing 1- or 2-MAG (Figure 2). It has been proposed that sn1,2-DAG is the lipid signal for insulin secretion as this lipid can activate certain protein kinase C iso-enzymes and also activate the exocytotic protein Munc13-1 in the β-cells. However, considering that conditions that lead to accumulation of DAG generally cause a decrease in GSIS rather than increase suggests that MAG is the likely lipid signal derived from lipolysis. Recent studies confirmed this as suppression of the MAG hydrolase ABHD6 in β-cells led to enhanced GSIS, both in vitro and in vivo and also added MAG stimulated GSIS in islets by activating Munc13-1.

Glucose stimulation of β-cells increases lipolysis and the release of FFA, which can activate GPR40, the Gq-coupled FFA receptor leading to sn1,2-DAG production and subsequent activation of protein kinase C enzymes or protein kinase D, that have been implicated in GSIS (Figure 2). The role of released FFA acting as autocrine/paracrine signals in GSIS is yet to be established.

Cyclic AMP and hormonal modulators: Several hormonal and neurotransmitter stimuli to β-cells lead to elevated cyclic AMP levels via Gα-coupled GPCR activation, without influencing intracellular Ca2+. Incretins like glucagon-like peptide-1 (GLP-1), which are released from intestinal L-cells in response to high blood glucose potentiate insulin secretion by β-cell by stimulating production of cAMP, which activates protein kinase A (PKA)-dependent and -independent mechanisms of exocytosis, and KATP-channel closure, cAMP also acts via PKA-independent mechanism mediated by the cAMP-sensing protein Epac2 (Figure 1), which has been shown to be a target of sulphonureas.
Insulin secretory defects in diabetes: Under conditions of excess fuel supply and insulin resistance due to obesity, pancreatic β-cell from normal non-diabetic individuals responds by compensatory hypersecretion of insulin in order to maintain normoglycemia. Loss of this ability of β-cells for compensatory elevated insulin secretion ultimately culminates in T2D. Thus, it has been shown that in subjects who potentially develop T2D, there is an increase in blood insulin levels during the prediabetic stage, where normoglycemia is maintained and this is followed with time by a steady decline in circulating insulin levels due to β-cell failure, associated with elevated

Figure 2. Lipid signals and insulin secretion. Continuous synthesis and breakdown of triglycerides and other glycerolipids is termed as triglyceride/fatty acid (TG/FA) cycle. This metabolic pathway starts with the condensation of glucose derived glycerol-3-phosphate with fatty acyl-CoA (FA-CoA), produced from fatty acid by acyl-CoA synthase, long chain (ACSL). This first step of TG/FA cycle is catalyzed by glycerol-3-phosphate acyltransferase isoenzymes located on mitochondria and endoplasmic reticulum, to form lysophosphatidic acid. Lysophosphatidic acid is further acylated to phosphatidic acid on ER by lysophosphatidic acid acyltransferase (LPAAT) isoenzymes. Formation of 1,2-diacylglycerol (1,2-DAG) from phosphatidic acid is catalyzed by lipin enzymes, which are distributed in lipid droplets, ER, nucleus and cytosol. Reversal of this reaction, i.e., formation of phosphatidic acid from 1,2-DAG is conducted by several DAG kinase (DGK) isoenzymes, distributed throughout the cell. TG is formed by the acylation of 1,2-DAG, the final step of lipogenic segment, by diacylglycerol acyltransferases-1 & 2 (DGAT) present on ER and lipid droplets. Lipolysis of TG is initiated by adipose triglyceride lipase (ATGL), which hydrolyzes TG to DAG. Depending on its activation by Comparative Gene Identification 58 protein (CGI-58) on the surface of lipid droplets, ATGL generates either 2,3-DAG (with CGI58) or 1,3-DAG (in the absence of CGI58) from TG. DGAT can use 1,3-DAG also to form TG. Hormone sensitive lipase (HSL) hydrolyzes 1,3-DAG and 2,3-DAG to 1-monoacylglycerol (MAG) and 2-MAG, respectively. MAG can be converted to lysophosphatidic acid by acylglycerol kinase (AGK). Plasma membrane associated α/β-domain containing hydrolase-6 (ABHD6) catalyzes the hydrolysis of MAG to glycerol and FFA in β-cells. Glycerol is not further metabolized in the β-cells, due to the lack of glycerol kinase, and leaves the cell via aquaglyceroporins. TG/FA cycle generates several lipid signals that promote insulin secretion. These include (1), fatty acyl-CoA, which targets KATP channels; (2), lysophosphatidic acid, which influences Ca2+ levels; (3), phosphatidic acid, which affects exocytosis; (4), 1,2-DAG, which likely binds and activates exocytosis promoting protein Munc13-1, protein kinase-C (PKC) and protein kinase-D (PKD); (5), 1-MAG, which activates Munc13-1 and (6), FA, which activates GPR40.
fasting glycemia above 5.5 mM. The mechanisms involved in \( \beta \)-cell compensation are not clear but animal studies implicated both \( \beta \)-cell mass expansion as well as enhanced \( \beta \)-cell function\(^7\). The elevated compensatory insulin secretion by pancreatic islets can be due to increased fuel (glucose and fatty acids) supply, increased growth factor and incretin signaling. Several monogenic forms of obesity and diabetes, including maturity-onset diabetes of the young (MODY) have been described\(^7\). However, T2D is a polygenic disease and shows more complex genetics, in which variations within multiple genes, each independently contributing some risk for disease development\(^8\).

The insulin secretory defect in T2D is multifactorial and likely involves reduced \( \beta \)-cell mass, impaired \( \beta \)-cell glucose sensing, and defective \( \beta \)-cell secretory machinery and MCF production. These defects are not readily recognized in \textit{in vivo} studies, making it difficult to understand the specific disease mechanisms coupled to T2D risk loci. A recent study in T2D patients revealed that such T2D risk imposing genetic variants can affect either glucose sensing, exocytosis or structural elements of secretory machinery\(^9\).

**Conclusions**

Despite much knowledge and technological advancement the precise mechanisms involved in GSIS are not entirely clear. This lack of understanding stems from the complexity and degeneracy of multiple metabolic pathways, that are linked to signaling processes to control GSIS. Such high level of organization consisting of structural proteins, metabolic enzymes, metabolites, ion channels and metal ions, ensures the delivery of the needed amounts of insulin into circulation, as excess or low insulin can be detrimental. Considering the multitude of signaling processes involved in GSIS, defects at one or more steps of these pathways can contribute to the development of T2D. Failure of \( \beta \)-cell can occur when any of these metabolic signaling pathways are compromised either because of genetic, environmental or epigenetic factors. Further research should focus on \( \beta \)-cell metabolic signaling mechanisms that are altered in T2D and to prevent or reverse such pathological alterations in metabolism. Identification of these pathways will help in understanding the molecular basis of \( \beta \)-cell failure in diabetes and to discover new targets to develop antidiabetic drugs.

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**Conflict of interest**

The Authors declare that they have no conflict of interests.

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