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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 [illegible]el and supply insulin accordingly to the n[illegible] body. Besides glucose, amino acids inc[illegible]ng glutamine and leucine and also fatty acid[illegible]e known to either stimulate the β-cells dire[illegible] or potentiate the glucose stimulated insu[illegible] secretion (GSIS) response. C[illegible] metab[illegible]lism dependent GSIS is lin[illegible]he pro-duction of ATP that is ne[illegible]d for [illegible] chan-nel inhibition and influx [illegible]alcium [illegible]essary for insulin granule exo[illegible]is. [illegible]cose metabolism, a[illegible] a[illegible]bolism and lipid metabolism [illegible]ed me[illegible]es mediate the optimal gluc[illegible]esponse o[illegible]lls to se-crete insulin. [illegible]es derived [illegible]n nutri-ent secretag[illegible]es [illegible]directly or indirectly participate in the enh[illegible]ment of GSIS are consider[illegible]s metabolic[illegible]ling factors. In this re[illegible], we will discuss [illegible]e regulation of insul[illegible]ecreti[illegible]y β-cell keeping the recent dev[illegible]ment[illegible] metabolic signaling in focus. The [illegible]etaboli[illegible]athways in pancreat-ic β-ce[illegible] their [illegible]n the control of fuel-[illegible]ulate[illegible]uli[illegible]cretion will be reviewed to[illegible]ve at [illegible]nsus picture with respect to [illegible] metabo[illegible] signaling of insulin secre-tio[illegible]**

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

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Type 2 [illegible]abetes [illegible]itus (T2D) is the manifestation of pancreatic [illegible]-cell failure to supply suf[illegible]mount of insu[illegible]d decreased sensitivity of the body tissues to insulin. It results from [illegible]etic susceptib[illegible]ty and epigenetic changes in [illegible]ontext of to[illegible] environmental factors such as [illegible]trition and [illegible]uced physical activity[1-3]. The islet [illegible] is a key fuel sensing micro-organ that [illegible]tly adjusts the release of insulin in [illegible]sponse to the levels and stimulus strength of nu-[illegible]d hormonal factors. The insulin secretion [illegible]s 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 secretogogues[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[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[5,7]. The early preabsorptive phase of insulin release, seen within few minutes after food ingestion, is due to the parasympathetic nerves supplying the islets[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[9,10], rather than as a secondary effect to enhanced insulin release[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^+_{ATP} channel inhibition and depolarization of the plasma membrane. The im-

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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[2,12,13] (Figure 1). Glucose metabolism driven K^+_{ATP} channel inhibition has also been implicated in the regulation of β-cell mass[14,15]. The ability of the β-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 β-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[16]. The Glut-1/2-Glucokinase tandem ensures a steady increase in glycolysis and ATP production in the β-cell with increasing blood glucose levels and, thus, the glucose concentration dependent insulin secretion response[17]. GSIS in β-cells is achieved by a tight link between glycolysis and mitochondrial metabolism for the quantitative dir n of glucose carbons into mitochondria, du very low expression of lactate dehydrogenase

The exocytotic process in the β-cell is orche ted by several components, in particular Ca^{2+} i and exocytotic effector proteins lo t the surf ces of secretory granules and r branes[20], which facilitate the fusion o insulin taining large dense-core vesicles he plas ne. Besides, synaptic-li e m s (S are also present in β- and t sicles contain small molecules li aminobuty d (GABA) and may release ents – in a m similar to that of syna vesic neurons[21,22]. However, not much i own about fuel stimuli influence the re of SLMV.

In t eview ve will specifically discuss the reg n of i n secretion by fuel stimuli in the p k g the r t developments in metabolic ng in f . The relevant metabolic ays e in the control of nutrient sti ted in ecretion will be reviewed to arri at a cons sus picture with respect to the b is of insulin secretion promoted glucose, amino acids and fatty acids.

Basic 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*[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 r cond-phase secretion[1]. However, the asic pa tern of insulin secretion probably d ot exist *in vivo* under physiological conditio re glucose concentration does not rise in a st e manner. However, this feature o ved *in* v lps in understanding the bio ical basis of duced insulin secretio in id ying pre betes condition. The hase espond to the exocytosis of a sm nu e secr gra- nules already d ed on a sma mbrane that release i whereas t phase involves mo f granules the storage pool. The re 10, 2,000 insulin granules in a β-cell, with a small p f them (~500) docked to t a membrane, hich 50-100 granules considered as the ready-releasable pool, are ered to the m brane in close association with channels an ntribute to the rapid first phase retion[25]. T eady-releasable pool is replenis granules from the storage pool, which e y contribute to the second sustained phase of secretion.

olecular basis of how granules pools are lik o 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[26]. Different pools of insulin granules that are functionally distinct have been described in β-cells. Regions of β-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[21]. Whether these granule pools related to biphasic secretion is uncertain. Recently, the model of phasic secretion with respect to granule pools has been questioned[24]. 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 β-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.

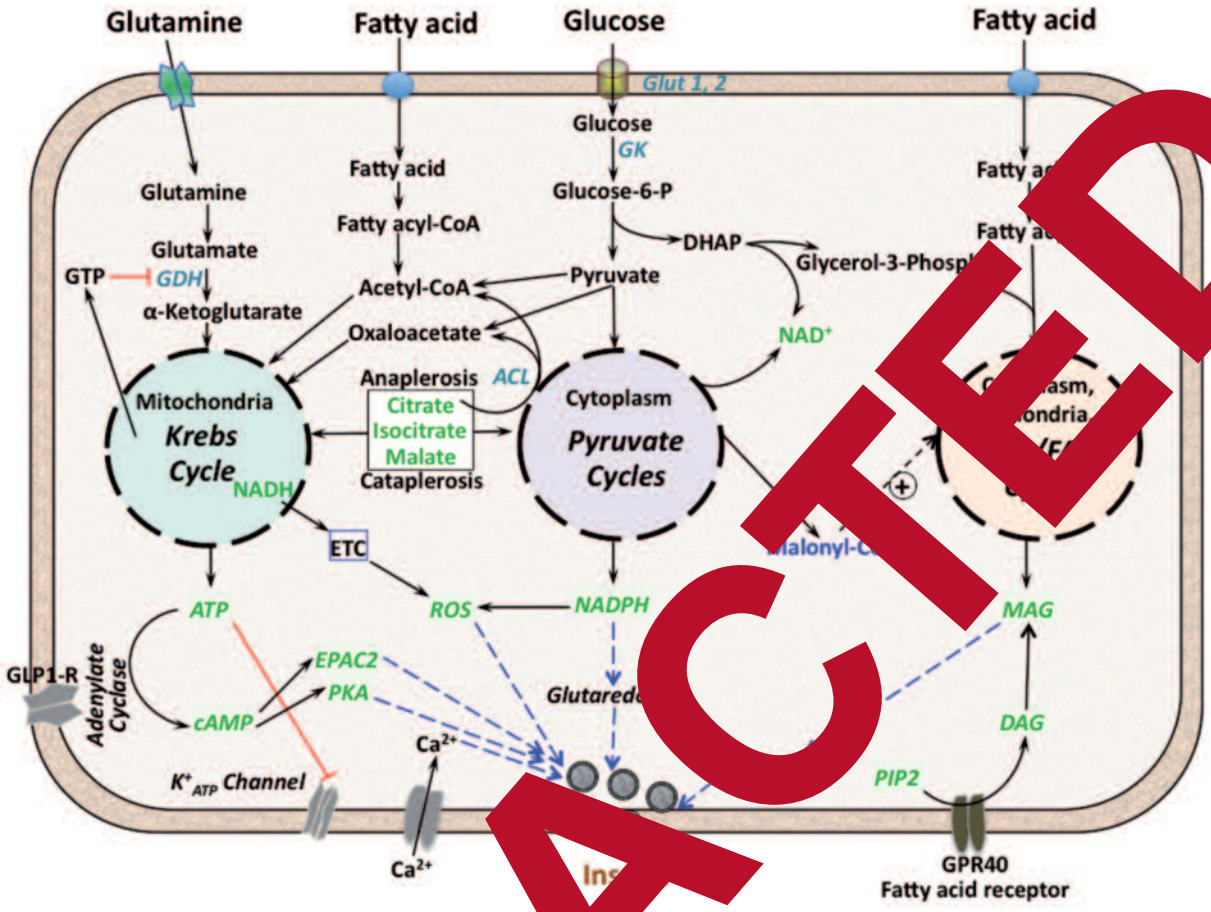

Figure 1. Fuel metabolism and produc[tion] of metabolic c[oupl]ing factors in the β-Cell. Glucose, fatty acids and glutamine are metabolized through the Krebs cycle [and] cle and tri[glyc]ide/ fatty acid (TG/FA) cycle in the β-cell. Glutamine is converted by glutaminase in cytosol t[o] tamate [w]hich is oxid[ized] by glutamate dehydrogenase (GDH) in the mitochondria to produce α-ketoglutarate that en[ters] Krebs cy[cle]. Further oxid[at]ion of α-ketoglutarate in Krebs cycle leads to production of GTP, which inhibits GDH. Ge[ner]n of α[-K]GDH functions as anaplerosis as it provides substrate for Krebs cycle. Reducing equivalent[s] NA[DH] [and] by K[rebs] cycle are oxidized by electron transport chain (ETC) to generate ATP and as a side product, re[active] oxyg[en] s (ROS). ROS can directly stimulate insulin exocytosis. ATP exits mitochondria and in cytosol, it inhib[its] K^+_{ATP} channe[l] [t]riggers Ca^{2+} influx and insulin granule exocytosis. ATP is also used by adenylate cyclase to produce c[AM]P (cAMP), w[hich] [is] vated in response to stimuli like binding of GLP1 to its Gs-coupled receptor. cAMP activates e[ffe]g[ec]in directly act[iva]ed by cyclic AMP (EPAC2) and also protein kinase-A (PKA), both of these promote exocyto[sis]. Fatty a[cid] activated to fatty acyl-CoA by acyl-CoA synthase, long chain enzymes present on the surface of diffe[rent] subcellular m[em]es. Under conditions of low glucose, fatty acyl-CoAs are oxidized to generate acetyl-CoA, whi[ch] ers Krebs cycle at [citrate] synthase step. Fatty acyl-CoAs are channeled to the TG/FA cycle under conditions of increas[ed] cose av[ail]ability, when [fatty acid] xidation is reduced. Glucose enters β-cell via Glut1 or Glut 2 transporters (in human or roden[ts] pectivel[y]) enters glycolysis after its conversion to glucose-6-phosphate by glucokinase (GK); glycolysis gives rise t[o] vate a[nd] tyl-CoA, which enters Krebs cycle. Approximately 50% of pyruvate is also converted to oxaloacetate via the an[aplerotic] me pyr[uvate] carboxylase inside mitochondria. Pyruvate participates in pyruvate cycles exchanging substrates, viz., [citrate] socitrate, malate, with Krebs cycle. Transport of these metabolites from mitochondria to the cytoplam (cataplerosis) [is] s [pyruvate] te cycles. NADPH produced by pyruvate cycles either directly or in combination with glutare[doxin] r via R[edox] ion through the action of NADPH oxidase, promotes insulin granule exocytosis. Dihydroxyacetone phos[phate] e (DHAP) oduced during glycolysis, is reduced to glycerol 3-phosphate using NADH, byglycerol-3-phosphate deh[ydro]ase. Glycerol-3-phosphate and fatty acyl-CoA react together to form lysophosphatidic acid and to enter TG/FA cy[cle]. [Citrate], which is formed from acetyl-CoA, as part of pyruvate/citrate cycle, inhibits fatty acid oxidation and positi[vely] 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 involved 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[27]. 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 MCF 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_{ATP} channel with the resultant Ca^{2+} 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 glycerol-phosphate and malate/aspartate shuttles activated mitochondrial metabolism, existing anaplerosis and cataplerosis, is central for enhancing Krebs cycle activity and ATP production, also the generation of additional MCF plays a key role 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 enzyme activity catalytically, but also participate in additional metabolic pathways that lead to the production of different MCF (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 the cytoplasm. Various transporters located on the mitochondrial inner membrane facilitate the transmitochondrial movement of the Krebs cycle intermediates, such as the di- and tri-carboxylates. In the cytoplasm, some of the Krebs cycle intermediates (citrate, isocitrate, α-ketoglutarate and malate) participate in pyruvate recycling processes that generate cytoplasmic NADPH (Figure 1), an important MCF[27].

Pyruvate metabolism via pyruvate carboxylase (PC), which is highly expressed in β-cells[28,29], is coupled to anaplerosis. Many studies have shown that GSIS correlates to the rates of decarboxylation and oxidation of pyruvate[30], the rate of pyruvate carboxylation correlates well with the glucose dependence of GSIS[31,32]. Studies using PC inhibitor phenylacetic acid[32], RNAi knockdown and overproduction of PC in INS-1 cells and islets clearly demonstrated the significance of PC in GSIS[33-35]. 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[36]. Mitochondrial GDH is important for

Table I. Metabolic coupling factors implicated in insulin secretion.

MCF	Target of action	Mode and site of action
AMP	AMPK	Negative regulation of GSIS
ATP	K_{ATP}	Ca^{2+} signaling
cAMP	PKA, Epac2	Ca^{2+}, channels, exocytosis proteins
Citrate	ACL, ACC	Pyruvate cycling
Diacylglycerol	PKC, Munc13-1	Channels, exocytosis
Long-chain acyl-CoA	Lipogenic enzymes	TG/FA cycling
Glutamate	GDH	Anaplerosis
GTP	GDH, GTP-SCS	Anaplerosis, Ca^{2+} signaling
IP_3	IP3 receptor, L-type Ca^{2+} channel.	Ca^{2+}influx, exocytosis
α-Ketoglutarate	α-KG dehydrogenase, HIF1α-hydroxylase	Exocytosis
Malonyl-CoA	CPT-1, FAS	Fatty acyl group partitioning
MgADP decrease	SUR1	Ca^{2+} signaling
Monoacylglycerol	Munc13-1	Vesicle fusion, Exocytosis
NADPH	Glutaredoxin, Kv	Exocytosis redox control, Ca^{2+}
ROS	Exocytosis proteins	Exocytosis complex redox control

amino acid (glutamine plus leucine) induced insulin secretion and gain of function mutation of GDH is associated with a hyperinsulinemic hypoglycemic syndrome[37].

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/cataplerosis-derived signaling and MCF production[36,38-40] 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[41] 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[32,38,42]. This cycle may also be linked to metabolic oscillations and, thus, contribute to the pulsatile insulin release from β-cells that parallels oscillations in $[Ca^{2+}]_i$, ATP, NAD(P)H a... trate levels[43-45]. In the pyruvate/isocitrate cy... tosolic isocitrate dehydrogenase (cICDH) co... ts isocitrate to α-ketoglutarate and NAD... NADPH. The precise role of this cycle in GSI... uncertain as cICDH RNAi-kn... n studi... were shown to both decrease G... ell as in... crease GSIS[47]. It has been s... n that ...ckdown of cytosolic malic enzym... ich tak... the pyruvate/malate cycl... re... s fourth pyruvate cycl... e "py... hosphoenol-pyruvate" cycle[51] i... ked to ATP... tion in the cytoplasm and... rolysis in th... tochondria. The rel... im... ce of these cycles is currently c... roversial[38,...

Gluc... imulation of β-c... uses a rapid rise in cyt... c NAD...H[55] that occu...s even prior to β-cell... olariz...n, increased intracellular Ca^{2+} and ... ule fusi... to plasma membrane[56]. The pe... hospha... unt pathway, which can ...ener... AD... is unlikely to be important fo... S, as ... ough this pathway in β-cell is mu... lower, th... suggesting pyruvate cycling p... e the major source of cytosolic ...DPH in these cells. NADPH is also formed in ...tochondrial matrix by the reduction of $NADP^+$ ...cotinamide nucleotide transhydrogenase (N...) and by mitochondrial NADP-dependent isocitrate dehydrogenase. NNT mutant mice show glucose intolerance and reduced insulinemia during a glucose tolerance test[57], suggesting a role for NNT and NADPH in GSIS. NNT-generated NADPH can be used by mitochondrial NADP-dependent isocitrate dehydrogenase, whic... ketoglutarate, can produce isocitrate... owed ... the export of isocitrate (or citrate)... e cytoplasm, for regenerating NADPH via p... te cycling processes (Figure 1). There is strong ...ce implicating a role for cytoso... NADPH ...SIS. Thus, NAPDH was sho... to directly p... exocytosis in patch-cl... ed β-c... (Ivarsso... al, 2005) and RNAi s... g of ...osolic NADPH generating malic ...yme, ...citrate ...ydrogenase enzyme... uces G... [50]. NA... H likely targets glutam... in[58] and vo... endent K^+ (Kv) chan... redoxin is ...ortant for the post-trans... onal ... cations of exocytotic proteins and the Kv cha... β-subunit, which can bin... H (Figure 1), ...ought to be the sens... intracellular redox potential that in turn re... tes the chan... and, thus, controls glucose-sti... ted action p... tials in β-cells[59].

Pyr... g and malonyl-CoA

Acet... formed by ATP-citrate lyase (ACL) ...carboxylated by acetyl-CoA carboxylase (ACC) ...yl-CoA, which has signaling role in the ...n of GSIS[10] 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[10]. Build up of fatty acyl-CoA due to CPT-1 inhibition, can lead to the activation of protein kinase-C enzymes[60] and K^+_{ATP} channel[61] and also stimulate GSIS[62]. 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[63]. However, overexpression of cytosol-directed malonyl-CoA decarboxylase appears to lower GSIS only in the presence of fatty acids[64,65]. Fatty acids also directly bind to cell surface GPCRs, GPR40 and GPR120 and stimulate GSIS[66].

Glutamate, GDH and GTP

Inasmuch as glucose stimulation of islets is accompanied by augmented glutamate levels supports the view that glutamate is an MCF[67,68]. 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[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[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^{2+} and to positively influence GSIS in β-cells[70]. Thus, RNAi-knockdown of GTP-SCS lowers β-cell ATP levels and reduces GSIS[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^+_{ATP} channel and stimulate secretion[71] and the p… of guanylate cyclases, which produce cGM… m GTP in β-cells has been confirmed[72].

ATP, ADP, AMP and AMPK

Inasmuch as K^+_{ATP} channe… by ATP is a necessary step for ins… secret… altera-tions in ATP/ADP ratio… vici… K^+ channel are relevant a… l a… l… by … late kinase-1, which… y ass… with Kir6.2 subunit of K^+_{ATP} …nel[73]. Aden… cleotides, whose cellular … dependent … e metabolic state of … β-ce… ctly influence the insulin exoc… tic machine… the plasma membrane. C… ll regulation o… hannels by the adeni… cleoti… s depends on the net inhibitory effe… f ATP… the net activating effect of Mg… n… 1 com… ent of the channel[74].

Besi… P/ADP… , ATP/AMP ratio is also …tant … regulation of insulin secretio… β-cel… s influences the activity of AM… ctivated p…tein kinase (AMPK), which is a m… r of cellular energy metabolism[75]. …re have been several excellent reviews on …MPK[76] and we focus here exclusively on the re… …velopments. Cellular AMP levels are regulate… a 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[77]. Recent work indicated that AMPK activation causes met… celeration in the β-cell by slowing d… the gl… cose metabolism and, thus, insul… ecretion at glucose concentrations < 10 mM… eas at higher glucose levels above 16 mM, t… tabolic deceleration effect is abs… ; sugge… that AMPK activation offers … ction to β-cel… the toxicity of fuel sur… and ex… tive ove… mulation[78]. Recent s… sug… d that AMPK also likely contro… he … of SIRT… which also is known t… gulate … se… on in β-cells[76,78]. LKB… MPK enzym… een implicated as ne… lators of insu… secretion[76].

Reactive Oxygen S… es (ROS)

R… tude superoxi… d hydroxyl radicals a… ydrogen peroxide (H_2O_2) and these are pro-…ed physiolog…lly in many cells, during nu-…t oxidation. …β-cells, it has been proposed t… OS acts a… CF for promoting GSIS[79,80]. Ho… h… production of elevated levels of ROS ca… rimental for β-cell function. Mitochondrial electron transport chain components …x-I and Complex-III are the major site for O… mation[36,81]. Besides mitochondria, ROS can also be produced by plasma membrane electron transporting NADPH oxidase complex[79] and in peroxisomes. It has been suggested that in β-cells peroxisomal fatty acid oxidation is the major source for H_2O_2, which leads to β-cell dysfunction and death whereas mitochondrial β-oxidation does not contribute significantly to ROS[82]. In mitochondria, nicotinamide nucleotide transhydrogenase, which produces NADPH, contributes to free radical detoxification[57] and altered activity of this enzyme is associated with proportional changes in insulin secretion[57,83].

The rise in influx of Ca^{2+}, while necessary for insulin granule exocytosis, can also cause NADPH oxidase activation resulting in increased production of H_2O_2[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[79]. The positive effects of ROS, in particular H_2O_2 and $O_2^{\bullet-}$ on GSIS include enhancement of Ca^{2+} influx[80] and activation of volume regulated anion channels[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 [36, 79, 81]. Production of ROS is likely to be elevated in the pancreatic islets from T2D patients than from nondiabetic subjects[85]. 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 it has been suggested that these polyphosphoinositides likely play a facilitating role in GSIS, probably by controlling intracellular Ca^{2+} levels and also DAG levels[86]. Recent studies implicated inositol-triphosphate and other inositol polyphosphates in the regulation of GSIS[87], probably by their direct action on L-type Ca^{2+} 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 the cycle is initiated by the fatty acid esterification of glycerol-3-phosphate, arising from the acylation of cytosolic glycerol-3-phosphate by glycerol kinase or glycolytic intermediate dihydroxyacetone phosphate (Figures 1 and 2). This first step of esterification, catalyzed by glycerol-3-phosphate acyltransferase isozymes, forms lysophosphatidic acid (LPA) that is further converted to phosphatidic acid by LPA acyltransferase, followed by removal of phosphate by lipin to *sn*1,2-diacylglycerol (DAG) and then conversion to triglyceride (TG) by DGAT acyltransferase. TG, then, enters lipolytic cycle by sequential hydrolysis to produce *sn*2,3-DAG and *sn*1,3-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-hydrolase domain containing-6 (Figure 2)[88]. TG in β-cells is stored in 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[77]. Thus, agents that block TG/FA cycle at different steps are known to reduce GSIS[78,89-91]. It is important to note that lipid intermediates of lipogenic arm have signaling functions and disruption of any of the involved enzymes has varying effects on GSIS (Figure 2). Lysophosphatidic acid is known to affect Ca^{2+} influx while phosphatidic acid was thought to directly influence exocytosis. However, agents that block lipogenic arm of the cycle have been shown to reduce GSIS[27].

Recent studies demonstrated that ATGL produces 1,3- and 2,3-DAG which have no known signaling function, rather than signaling competent sn-1,2-DAG that activate C-kinase enzymes. Likewise, intracellular HSL primarily hydrolyzes DAG at position 1 or 3, thus producing 1- or 2-MAG (Figure 2). It has been proposed that sn-1,2-DAG is the lipid signal for insulin secretion as this lipid can activate certain protein kinase-C isoenzymes and also activate the exocytotic protein Munc13-1 in the β-cells. However, considering that conditions that lead to accumulation of DAG would cause a decrease in GSIS rather than increase suggests that MAG is the likely lipid signal derived from lipolysis. Recent studies confirm 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[88].

Glucose stimulation of β-cells increases lipolysis[92] and the release of FFA, which can activate GPR40, the Gq-coupled FFA receptor leading to *sn*1,2-DAG production and subsequent activation of protein kinase C enzymes or protein kinase D[93], that have been implicated in GSIS (Figure 2)[94]. 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 G_s-coupled GPCR activation, without influencing intracellular Ca^{2+}. 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 K_{ATP}-channel closure[95]. 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 sulphonylureas[96].

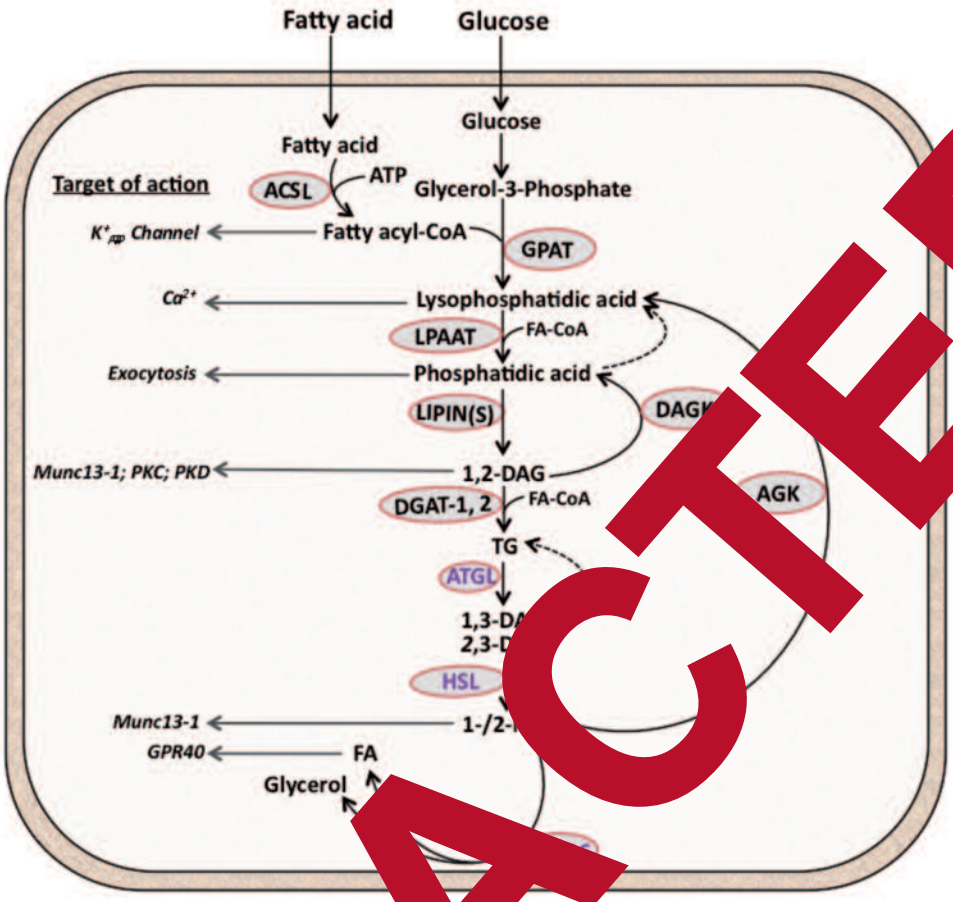

Figure 2. Lipid signals and insulin secretion. Continuou[s] ...esis and breakdown of triglycerides and other glycerolipids is termed as triglyceride/fatty acid (TG/F... This metab... athway starts with the condensation of glucose derived glycerol-3-phosphate with fatty acyl-CoA ... Co... oduced fr... ty acid by acyl-CoA synthase, long chain (ACSL). This first step of TG/FA cycle is catalyzed ... glycerol-... osphate ac... ansferase isoenzymes located on mitochondria and endoplasmic reticulum, to form lysop... atidic ac... hosphatidic acid is further acylated to phosphatidic acid on ER by lysophosphatidic acid acyltransfe... PAA... ormation of 1,2-diacylglycerol (1,2-DAG) from phosphatidic acid is catalyzed by lipin enzy... whi... ibuted in lipid droplets, ER, nucleus and cytosol. Reversal of this reaction, i.e., formation of phosphati... id from ... G is conducted by several DAG kinase (DAGK) isoenzymes, distributed throughout the cell. TG is ... d by the acyla... 1,2-DAG, the final step of lipogenic segment, by diacylglycerol acyltransferases-1 & 2 (DGAT)... ER and lipid d... s. Lipolysis of TG is initiated by adipose triglyceride lipase (ATGL), which hydrolyzes TG t... G. D... g on its activation by Comparative Gene Identification 58 protein (CGI-58) on the surface of lipid droplets. ATGL generate... 2,3-DAG (with CGI58) or 1,3-DAG (in the absence of CGI58) from TG. DGAT can use 1,3 DAG al... form TG. Horm... sitive lipase (HSL) hydrolyzes 1,3-DAG and 2,3-DAG to 1-monoacylglycerol (MAG) and 2-M... espectively. MAG ca... converted to lysophosphatidic acid by acylglycerol kinase (AGK). Plasma membrane associ... /β-dom... containing hydrolase-6 (ABHD6) catalyzes the hydrolysis of MAG to glycerol and FFA in β-cells. Glycerol ... ot furth... tabolized in the β-cells, due to the lack of glycerol kinase, and leaves the cell via aquaglyceroporins. TG/... e s... tes seve... lipid signals that promote insulin secretion. These include (1), fatty acyl-CoA, which targets K^+_{ATP} c...), lysop... atidic acid, which influences Ca^{2+} levels; (3), phosphatidic acid, which affects exocytosis; (4), ...DAG, ... likely ... and activates exocytosis promoting protein Munc13-1, protein kinase-C (PKC) and protein kinase D (PKD); (... which activates Munc13-1 and (6), FA, which activates GPR40.

...tory defects in diabetes: Under ...ditions of excess fuel supply and insulin resi... nce due to obesity, pancreatic β-cell from nor... on-diabetic individuals responds by compen... tory 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

fasting glycemia above 5.5 mM. The mechanisms involved in β-cell compensation are not clear but animal studies implicated both β-cell mass expansion as well as enhanced β-cell function[17]. 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[97]. 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[98]. The insulin secretory defect in T2D is multifactorial and likely involves reduced β-cell mass, impaired β-cell glucose sensing, and defective β-cell secretory machinery and MCF production. These defects are not readily recognized in *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[99].

Conclusions

Despite much knowledge a[illegible]nologic[illegible] advancement the precise mec[illegible]olved in GSIS are not entirely clea[illegible]is lac[illegible] understanding stems from the [illegible]lexity [illegible] racy of multiple met[illegible]bo[illegible]s; th[illegible]e linked to signaling [illegible]cesse[illegible]ntrol GSIS. Such high level [illegible]rganizatio[illegible]sisting of structural prote[illegible]bolic enzym[illegible]etabolites, ion chan[illegible]and [illegible]ions, ensures the delivery of the [illegible]eded amou[illegible]insulin into circulation, a[illegible]cess or low insu[illegible]n be detrimental. C[illegible]dering [illegible]e multitude of signaling processes involve[illegible]GSIS, defects at one or more step[illegible]thway[illegible]n contribute to the development T2D. [illegible]ure of β-cell can occur any [illegible]se[illegible]abolic signaling pathways are [illegible]nprom[illegible]ner because of genetic, environ[illegible]tal or epigenetic factors. Further research s[illegible]n β-cell metabolic signaling mechanisms that are altered in T2D and to prevent reverse such pathological alterations in meta[illegible]. Identification of these pathways will help in [illegible]derstanding the molecular basis of β-cell failure in diabetes and to discover new targets to develop antidiabetic drugs.

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Conflict of interest

The Authors declare that th[illegible]ve no c[illegible] of interest[illegible]

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