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# Insulin Secretion and Actions

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## 1. Introduction

### 1.1 Insulin biosynthesis

The islets of langerhans are the clusters of the endocrine tissue that scatter among the exocrine cells in the pancreas. The islets occupy approximately 1-2% of the total pancreatic tissue. Approximately, 1 million islets are scattered in the 25 cm long human pancreas. The insulin-producing cells make up 80% of each islet, while the remaining includes glucagon-producing  $\alpha$ -cells, somatostatin-producing  $\delta$ -cells and the pancreatic polypeptide (PP) F-cells (Unger et al., 1978). Insulin is first synthesized as a 110-amino acid polypeptide chain known as pre-proinsulin. This precursor form contains a hydrophobic 24-amino acid at its N-terminus known as the signal peptide. This signal peptide is removed during translocation from the cytoplasm to the endoplasmic reticulum, producing the proinsulin which comprises of chains A, B and C with three disulfide bonds. Further proteolysis of proinsulin in the secretory vesicles by the prohormone convertases (PC1 and PC2) and the carboxypeptidase E, removes the C-peptide from the rest of the molecule while still retains three disulfide bonds. This remaining part or mature insulin contains 21 amino acids on chain A and 30 amino acids on chain B (Steiner, 1969).

### 1.2 Biphasic insulin secretion and insulin exocytosis

The mature insulin is stored in the secretory granules which can be divided into two distinct pools, i.e. the reserve pool (RP) and the readily releasable pool (RRP) (Barg et al., 2002; Bratanova-Tochkova et al., 2002). The RRP is located close to the plasma membrane and is a rather small pool of insulin, comprising only 1-10% of total insulin in the cell. In contrast, the RP is located intracellularly and is a largest insulin pool. Once insulin granules in the RRP are released, the RP moves close to the plasma membrane to replenish the RRP (Barg et al., 2002; Bratanova-Tochkova et al., 2002).

Unlike other endocrine cells in the pancreas,  $\beta$ -cells secrete insulin. This occurs not only under low glucose conditions (non-stimulatory conditions) (3-5 mM glucose) but also when the glucose concentration in plasma is high during the postprandial period (10-25 mM glucose) when  $\beta$ -cells secrete much larger amounts of insulin into the circulation. Although several nutrients including glucose, some amino acids and non-esterified fatty acids can stimulate insulin secretion, glucose appears to be the most potent insulin

secretagogue. The mechanism of glucose-induced insulin secretion (GSIS) is the most extensively studied. Secretion of insulin in response to the elevated levels of glucose in plasma is rapid and occurs in a two-step process known as *biphasic insulin secretion* (Straub and Sharp, 2002; Straub and Sharp, 2004). The first phase occurs very rapidly within a first few minutes upon stimulation. At this stage, the insulin granules in the RRP are fused very rapidly with the plasma membrane, resulting in a sharp release of insulin in the blood circulation. The first phase lasts only for a few minutes before the second phase begins and is sustained to the peak at 30-40 min or longer, depending on whether the concentration of plasma glucose is still high. The amount of insulin released during the second phase is much higher than the first phase. It is estimated that 99% of total insulin is secreted in this second phase, with an approximate release rate of 5-40 granules/cell/minute (Barg et al., 2002; Straub and Sharp, 2004). Therefore the second phase of insulin secretion is more physiologically important. Not unexpectedly, this biphasic insulin secretion appears to be impaired in the patients with type 2 diabetes. The translocation of the insulin granules in the RP to become the RRP, as well as the docking of secretory vesicles to the plasma membrane are dynamic processes, requiring the rearrangement of cytoskeleton proteins inside the  $\beta$ -cell (Wang and Thurmond, 2009). During basal conditions, the F-actin filaments are polymerized as a dense network below the plasma membrane. This web structure of filamentous F-actin not only blocks the access of insulin granules in the reserved pool to the plasma membrane but also prevents the interaction of the v-SNARE protein, VAMP2, in the insulin granule vesicles with the t-SNARE proteins (syntaxin 1 and 4) on the plasma membrane. This process is a prerequisite for granule exocytosis. Under glucose stimulation conditions, F-actin filaments are depolymerized and there is an increased microtubule polymerization rate, allowing the RP of insulin granules to translocate to the plasma membrane where the interaction of vSNAREs and tSNAREs are maximized (Farshori and Goode, 1994; Howell and Tyhurst, 1979; Thurmond et al., 2003).

## 2. Biochemical basis of glucose-induced insulin secretion

### 2.1 $K_{ATP}$ -dependent GSIS: Roles of glycolysis, mitochondrial metabolism and ATP-sensitive potassium channels

Unlike other ligands, glucose does not require a cellular receptor to mediate signal transduction to stimulate insulin secretion in  $\beta$ -cells. This signal transduction is initiated by the rapid uptake of glucose through the glucose transporter 2 (GLUT2) in rodents (Chen et al., 1990) or glucose transporter 1 (GLUT1) in humans (De Vos et al., 1995) located on the plasma membrane of  $\beta$ -cells. GLUT2 transporters allow the high-capacity and low affinity transport needed to equilibrate glucose concentrations across the plasma membrane and to support the  $\beta$ -cell's very high metabolic rate. Glucose then undergoes phosphorylation by a glucokinase which possesses a high  $K_m$  for glucose, allowing the elevated levels of plasma glucose present during the postprandial period to enter  $\beta$ -cells for glycolysis (Matschinsky, 1990). As glucokinase has low binding affinity for glucose, this means that the glycolytic rate is never saturated during the postprandial period. Because the  $\beta$ -cell contains very low activity of lactate dehydrogenase, most glycolysis-derived pyruvate enters the mitochondria and is oxidized to acetyl-CoA by the pyruvate dehydrogenase complex. Acetyl-CoA is then oxidized in the TCA cycle, concomitant with the production of the reducing equivalents, NADH. In contrast to other cell types,

$\beta$ -cells possess very high mitochondrial glycerol-3-phosphate dehydrogenase activity, which is a key enzyme in the mitochondrial-3-phosphate dehydrogenase shuttle (MacDonald, 1981). This allows NADH formed during glycolysis to be transported to the mitochondria for oxidative phosphorylation. The reducing equivalents obtained from glycolysis and TCA cycle are subsequently oxidized through the electron transport chain to produce cellular ATP. The key component that links the metabolic signal and the insulin granule exocytosis is the ATP-sensitive potassium channel ( $K_{ATP}$ ). This channel is an octamer comprising four pore forming subunits of Kir6.2 and four subunits of the sulfonylurea receptor (SUR1) (Aguilar-Bryan et al., 1995). Under unstimulated conditions,  $K_{ATP}$  channels are opened, allowing the diffusion of  $K^+$  across plasma membrane of  $\beta$ -cells near equilibrium. However, when the ratio of ATP:ADP ratio is high due to a high rate of glucose oxidation, ATP binds to the Kir6.2 component of the  $K_{ATP}$  channel, causing the channel to close. The depolarization of the membrane caused by the closure of the  $K_{ATP}$  channel opens the voltage-gated  $Ca^{2+}$  channel, causing  $Ca^{2+}$  influx into the cells (Ashcroft et al., 1984). This electrophysiological cascade results in the exocytosis of the insulin granules in the RRP. The increase of intracellular  $Ca^{2+}$  also stimulates the calmodulin-dependent protein kinase II which can phosphorylate several targets including the myosin light chain kinase that controls the cytoskeletal or secretory vesicle proteins (Easom, 1999). The insulin release triggered by the  $K_{ATP}$ -dependent mechanism corresponds to the first phase of the biphasic insulin secretion (Straub and Sharp, 2004).

## 2.2 $K_{ATP}$ -independent GSIS: Anaplerosis and coupling factors

Although the  $K_{ATP}$  channel appears to control the GSIS, several lines of evidence suggest that GSIS can be operated independently of  $K_{ATP}$  channel. Treating  $\beta$ -cells with a  $K_{ATP}$  channel opener, diazoxide, does not completely eliminate GSIS (Gembal et al., 1992). Furthermore, mice lacking Kir6.2 or the SUR1 component of  $K_{ATP}$  are still capable of secreting insulin in response to glucose albeit not as robust as the wild type mice (Seghers et al., 2000; Shiota et al., 2002). It has now become clear that GSIS requires “coupling factors” or “metabolic factors” that act as the amplifying signal of insulin secretion. Those coupling factors include NADPH, GTP, long chain acyl-CoA and glutamate. The biochemical pathways or cycles that lead to production of the coupling factors are described below.

**NADPH:** The pentose phosphate pathway is the major pathway that produces NADPH, however the  $\beta$ -cell does not possess glucose-6-phosphate dehydrogenase to produce this reducing equivalent. Instead,  $\beta$ -cells possess a very high activity of pyruvate carboxylase and pyruvate dehydrogenase (MacDonald, 1993). Although equal proportions of the glycolysis-derived pyruvate enters mitochondria is carboxylated by PC and decarboxylated by pyruvate dehydrogenase, only the flux via the carboxylation reaction is correlated with GSIS (Lu et al., 2002). In  $\beta$ -cells, there is a high rate of the export of TCA cycle intermediates, i.e. citrate and malate from mitochondria to cytoplasm which is known as cataplerosis (MacDonald, 2003). The exported citrate and malate are then recycled back to the mitochondria as pyruvate known as pyruvate cycling as shown in Figure 1. This pyruvate cycling can be shuttled to the pyruvate via pyruvate/malate, pyruvate/isocitrate or pyruvate citrate cycles (MacDonald et al., 2005). As noted in Figure 1, NADPH is a common reducing equivalent produced by malate dehydrogenase, malic

enzyme and isocitrate dehydrogenase in the above three cycles, respectively. Pyruvate then re-enters the mitochondria and is carboxylated by pyruvate carboxylase, which is as highly abundant as in the gluconeogenic tissue. Deficiencies in pyruvate carboxylase, cytosolic malic enzyme and cytosolic isocitrate dehydrogenase result in impaired GSIS, indicating the importance of pyruvate cycling in  $\beta$ -cells (Jitrapakdee et al., 2010). Glucose sharply increases the NADPH:NADP ratio proportion to the level of insulin secretion. The mechanism by which NADPH acts on insulin secretion is thought to be mediated through the glutaredoxin and thioredoxin redox pairs. The maintenance of the extra-mitochondria redox state via glutaredoxin and thioredoxin is required to support insulin granule exocytosis. Furthermore, NADPH is also associated with the voltage-dependent potassium channel ( $K_v$ ) which works in an opposite way to the  $K_{ATP}$  channel. This channel functions as  $K^+$  efflux, causing the repolarization of the  $\beta$ -cell plasma membrane for the next cycle of GSIS. Binding of NADPH to the  $K_v$  causes the conformational change of its regulatory subunit, reducing the efficacy of this channel for repolarization of the  $\beta$ -cell plasma membrane and enhancing the action of  $K_{ATP}$  channel [reviewed by Jitrapakdee et al., 2010].

**Long chain acyl-CoA:** Long chain acyl-CoA is another coupling factor thought to be required for GSIS (Brun et al., 1996; Corkey et al., 1989; Prentki et al., 1992). Evidence for this is derived from the following observations. Acute exposure of  $\beta$ -cells to glucose sharply increases intracellular levels of malonyl-CoA and long chain acyl-CoA. In supporting this observation, exposure of permeabilized  $\beta$ -cells to long chain acyl-CoA or non-esterified fatty acids also stimulates  $Ca^{2+}$ -evoked insulin exocytosis. This is accompanied by elevated levels of acetyl-CoA carboxylase 1 (ACC1), a rate-limiting enzyme of *de novo* fatty acid synthesis. ACC condenses two molecules of acetyl-CoA to malonyl CoA and this enzyme is rapidly induced by high concentrations of glucose in  $\beta$ -cells. Because malonyl-CoA is a potent inhibitor of the carnitine palmitoyl transferase I (CPT-1), the rapid increase of malonyl-CoA level by ACC1 would inhibit  $\beta$ -oxidation of fatty acids, resulting in the elevated levels of long chain acyl-CoA in  $\beta$ -cells (Brun et al., 1996; Corkey et al., 1989; Prentki et al., 1992). This long chain acyl-CoA can be used as the precursor for synthesizing diacyl glycerol and phospholipids. Consistent with this idea, acute exposure of  $\beta$ -cells to glucose also modifies the concentrations of phospholipids and cholesteryl esters. These modifications could affect membrane fluidity and exocytosis of the secretory vesicles. Furthermore diacyl glycerol can also activate protein kinase C which in turn phosphorylates its downstream targets including ion channels.

Although inhibition of ACC1 and fatty acid synthetase activities result in a marked reduction of GSIS, suppression of ATP-citrate lyase expression does not appear to affect GSIS, suggesting the presence of another pathway that can supply acetyl-groups for *de novo* fatty acid synthesis in  $\beta$ -cells [reviewed by Jitrapakdee et al., 2010]]. An alternate pathway that provides acetyl-groups for long chain acyl-CoA synthesis lies within the acetoacetate production catalyzed by acetoacetyl-CoA synthetase. This was demonstrated by the knockdown experiment in which suppression of this enzyme expression impairs GSIS in  $\beta$ -cells (MacDonald et al., 2005). Acute exposure of  $\beta$ -cells to glucose not only stimulates rapid lipogenesis but also alters phospholipid and cholesteryl ester contents in the plasma membrane which in turn affects insulin granule exocytosis and  $\beta$ -cell plasma membrane fluidity (MacDonald et al., 2008).



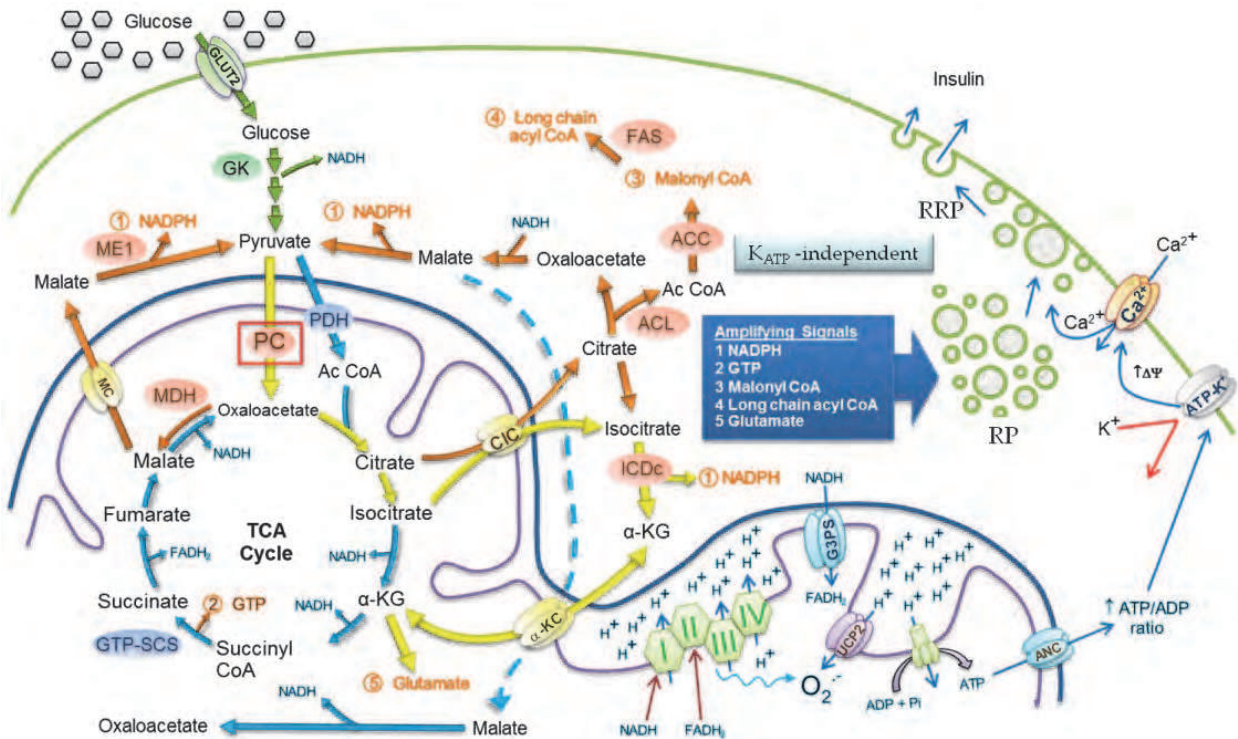


Fig. 1. Biochemical basis of glucose-induced insulin secretion (GSIS). Glucose enters  $\beta$ -cells through GLUT2 transporter and is metabolized to pyruvate by glycolysis. Pyruvate enters the mitochondria where it is oxidized in the TCA cycle. The NADH produced by both glycolysis and TCA cycle are oxidized to produce the cellular ATP. The increased level of ATP:ADP triggers the closure of ATP-sensitive potassium channels resulting in membrane depolarization. This in turn opens the voltage gate-dependent  $\text{Ca}^{2+}$  channels, causing the influx of  $\text{Ca}^{2+}$  which triggers the immediate exocytosis of insulin granules in the readily releasable pool, corresponding to the 1<sup>st</sup> phase of biphasic insulin secretion. Some components of the TCA cycle, i.e. malate, citrate and isocitrate are also exported from the mitochondria to cytoplasm (cataplerosis) where these exported products are converted back to pyruvate (pyruvate cycling) concomitantly with the production of NADPH via pyruvate-malate, pyruvate-citrate and pyruvate-isocitrate shuttles, respectively. PC replenishes OAA in the TCA cycle when malate, citrate and isocitrate are removed for the pyruvate cycling. The exported citrate is converted to oxaloacetate and acetyl-CoA. ACC1 converts acetyl-CoA to malonyl-CoA which is subsequently converted to long chain acyl-CoA by FAS. The NADPH malonyl-CoA, long chain acyl-CoA together with the mitochondrial GTP produced by succinyl-CoA synthetase and glutamate produced by glutamate dehydrogenase serve as “amplifying signals” that correspond to the 2<sup>nd</sup> phase of biphasic insulin secretion. ACC, acetyl-CoA carboxylase; ACL, ATP-citrate lyase; cICD, cytosolic isocitrate dehydrogenase; CIC, citrate/isocitrate carrier; GTP-SC, GTP-succinate dehydrogenase; FAS, fatty acid synthase, MDH, malate dehydrogenase; ME, malic enzyme, PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase complex; RP, reserve pool; RRP, readily releasable pool.

**Other coupling factors:** Acute exposure of  $\beta$ -cells to glucose causes a sharp increase in the level of glutamate, suggesting that glutamate might be a second messenger that promotes insulin secretion (Maechler and Wollheim, 1999). This intracellular source of glutamate is derived from the conversion of  $\alpha$ -ketoglutarate by the glutamate dehydrogenase. Although there is a strong correlation between the rapid increase of an intracellular level of glutamate upon an acute stimulation by glucose, there is no direct evidence indicating that the rise of glutamate level results in the insulin secretion (MacDonald and Fahien, 2000). Incubation of  $\beta$ -cells with glutamine, a precursor of glutamate production does not increase GSIS. Furthermore the GDH ablated mice showed only 30-40% loss of GSIS indicating that glutamate may not be the second messenger for insulin secretion (Carobbio et al., 2009).

The level of mitochondrial GTP may be one of the coupling factors that regulate GSIS. The succinyl-CoA synthetase catalyzes the conversion of succinyl-CoA to succinate, concomitant with the production of GTP. Suppression of the succinyl-CoA synthetase expression results in impaired GSIS in  $\beta$ -cells, indicating the importance role of mitochondrial GTP in GSIS (Kibbey et al., 2007).

Incubation of  $\beta$ -cells with high concentrations of glucose not only stimulates ATP production via electron transport system but also triggers the production of the reactive oxygen species in the mitochondria. There is evidence that the reactive oxygen species may be an obligatory signal for insulin secretion (Leloup et al., 2009; Pi et al., 2007). Incubation of  $\beta$ -cells with certain reactive oxygen species stimulates insulin secretion. Because these reactive oxygen species are toxic to the cells and they are removed very quickly by the antioxidant enzymes in the  $\beta$ -cells the question remains whether this transient increase of reactive oxygen species is a bona fide coupling factor for GSIS.

### 3. Other insulin secretagogues

Although glucose is the most potent insulin secretagogue, certain amino acids including leucine and glutamine can also stimulate insulin secretion (Fahien et al., 1988; Malaisse-Lagae et al., 1982). Leucine stimulates insulin secretion because it acts as an allosteric activator of the glutamate dehydrogenase, an anaplerotic enzyme that converts glutamate to  $\alpha$ -ketoglutarate in the TCA cycle. Glutamine by itself cannot stimulate insulin secretion, however combination of glutamine and leucine stimulates insulin secretion as robustly as glucose because glutamine can be converted to glutamate, and leucine acts as allosteric activator of glutamate dehydrogenase in the presence of excess glutamate substrate. Unlike leucine, arginine can stimulate only the 1<sup>st</sup> phase but not the amplifying phase of insulin secretion. The reason for this is because arginine is not metabolized in the mitochondria in the glycolysis or TCA cycle.

Free fatty acids by themselves cannot stimulate insulin secretion but low concentrations of them augment glucose-induced insulin secretion (Deeney et al., 2000; Poitout, 2003). Free fatty acids can be metabolized to long chain fatty acyl-CoA which is one of the coupling factors as described earlier. However, chronic exposure of  $\beta$ -cells to high concentration of fatty acids promotes  $\beta$ -cell apoptosis via the formation of ceramides or other reactive lipids (Giacca et al., 2011; Poitout and Robertson, 2002).

Apart from the nutrient secretagogues, some hormones can stimulate insulin secretion. The well known insulinotropic peptide hormones include the glucagon-like-peptide-1 (GLP-1) and gastric-inhibitory peptide (GIP) (Holst, 2007). GLP-1 is secreted from the enteroglucagon-

producing cells (L-cells) in the lower intestine, while GIP is secreted from K-cells in the upper gastrointestinal tract. These two peptides are secreted in response to the ingestion of glucose. GLP-1 acts to increase insulin secretion via the circulation acting directly on pancreatic  $\beta$  cells and also via the sensory afferent neurons acting on the central nervous system (Holst et al. 2007). In the brain GLP-1 acts a neuropeptide to promote neuroendocrine actions on the autonomic nervous system including regulation of food intake, satiety and pancreatic secretions. GLP-1 is controlled by the dipeptidyl protease 4 (DPP4). DPP4 cleavage renders GLP-1 unable to bind to its target receptor, the glucagon-like peptide-1 receptor (GLP-1R) and thus tightly controls the levels of GLP-1 in the intestine and the circulation. GLP-1 also promotes metabolic control by inhibiting glucagon secretion.

#### 4. Insulin oscillation

In humans, mouse and rat the majority of insulin (>70%) is released in a pulsatile manner with a periodicity of 3-5 min (Matveyenko et al., 2008; Porksen et al., 1997; Song et al., 2000). This pattern is observed both before and after meals, however the amplitude of oscillation is higher during the postprandial period. The oscillation of insulin secretion is believed to be a mechanism to prevent down-regulation of insulin receptors in the target tissues. The pulsatile insulin secretion is most obviously detected in the portal vein and can also be detected in the isolated islets. Remarkably, the oscillations of insulin secretion are also synchronized among one million islets. These tightly synchronized oscillations require the complex factors including the soluble factor, gap junction and intra-pancreatic nerves. However, this pulsatile pattern becomes less obvious in the peripheral blood (Tengholm and Gylfe, 2009). These oscillations are intrinsic to the islets, and are regulated by the concentrations in individual  $\beta$ -cells of cytoplasmic  $\text{Ca}^{2+}$ , intracellular cAMP and plasma membrane phosphoinositide lipids, as well as the activity of phospholipase C (Tengholm and Gylfe, 2009). The oscillations of insulin secretion from pancreatic islets correlate very well with the oscillation of intracellular  $\text{Ca}^{2+}$  concentration (Bergsten et al., 1994; Bergsten and Hellman, 1993). Although it is widely accepted that the insulin oscillation is the result of intracellular  $\text{Ca}^{2+}$  oscillation, it is unclear whether the oscillation of  $\text{Ca}^{2+}$  levels results from the oscillations of glycolytic and/or mitochondrial intermediates. The oscillation of phosphofructokinase (PFK) activity is well known to produce the oscillation of its product, fructose-1,6-bisphosphate which may in turn regulate oscillation of intracellular  $\text{Ca}^{2+}$  concentrations (Tornheim, 1997). However, suppression of PFK activity in  $\beta$ -cells did not affect the oscillation of intracellular  $\text{Ca}^{2+}$  concentration, suggesting that oscillation of PFK activity may not control the pulsatile manner of insulin secretion. However, recent reports have shown that there are oscillations of key metabolic products in the mitochondria including citrate, ATP, NAD(P)H, and  $\text{O}_2$  consumption (Bertram et al., 2007; Tengholm and Gylfe, 2009). Notably, the oscillations of these metabolic products are also in the same range as that of intracellular  $\text{Ca}^{2+}$  oscillation. Although the oscillations of key products of mitochondrial metabolism are likely to regulate the oscillations of intracellular  $\text{Ca}^{2+}$ ,  $\text{Ca}^{2+}$  by itself may feedback inhibit or stimulate the mitochondrial metabolism, resulting in the decrease or increase insulin secretion. The intracellular  $\text{Ca}^{2+}$  raised upon glucose-induced insulin secretion can enter mitochondria via the uniporter and depolarize the electrochemical potential in the inner membrane of the mitochondria thereby reducing mitochondrial ATP production (Bertram et al., 2007; Tengholm and Gylfe, 2009). However,



the intra-mitochondrial  $\text{Ca}^{2+}$  can also stimulate the activities of several mitochondrial enzymes including the pyruvate dehydrogenase complex, isocitrate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase (Bertram et al., 2007; Tengholm and Gylfe, 2009). These synchronous oscillations of products of mitochondrial metabolism are believed to orchestrate the oscillation of insulin secretion.

Normoglycaemia is more efficiently maintained when insulin is delivered in a pulsatile fashion, most probably because of enhanced expression on the target tissues of insulin receptors that have a similar recycling periodicity. Significantly, this pulsatile delivery of insulin is lost or severely diminished in type 2 diabetes. This contributes to insulin resistance and the requirement for compensatory hypersecretion by the islets, potentially leading to their exhaustion (Bertram et al., 2007; Tengholm and Gylfe, 2009).

## 5. Insulin signaling

Insulin signaling controls metabolism as well as growth and survival in many mammalian tissues. It also plays a vital role in controlling lifespan (Longo et al., 2008). In humans, perturbation of insulin signaling results in diabetes but is also implicated in neoplasia (Pollak, 2008). Signaling via the insulin receptor (IR) results in activation of two main signalling pathways: the phosphoinositide 3-kinase/Akt (PI3K/Akt) and the mitogen activated protein kinase (MAPK) pathways. Both mitogenic and metabolic signalling outcomes are activated via the IR and the response arising depends on expression levels of the receptor and downstream signaling molecules by the cells within the target tissues.

The IR exists in two isoforms arising from alternative splicing (Belfiore et al., 2009; Denley et al., 2003). The exon 11+ (IR-B) isoform is expressed in insulin sensitive tissues and primarily in the liver. This receptor is responsible for the metabolic control processes classically associated with insulin's action. The exon 11- (IR-A) isoform, which lacks the 12 amino acids normally encoded by exon 11, is expressed mainly in fetal tissues including liver, kidney and muscle. Interestingly, both insulin and insulin-like growth factor-II (a structurally related mitogenic growth factor) can bind to the IR-A with high affinity to promote cell proliferation and survival. The foetal co-expression of IGF-II and IR-A suggests both may act together to play an important role in foetal growth. Interestingly, expression of both IGF-II and the IR-A is often upregulated in cancer and this represents an additional mechanism by which cancer cells grow and survive (Avnet et al., 2009; Denley et al., 2003).

### 5.1 Insulin receptor structure

The IR is a transmembrane glycoprotein with tyrosine kinase activity. It is a homodimer with each subunit consisting of an extracellular  $\alpha$  subunit and a transmembrane spanning  $\beta$  subunit (see Figure 2) (De Meyts and Whittaker, 2002; Ward and Lawrence, 2009). The receptor is produced from a single proreceptor protein that is glycosylated, dimerised and proteolytically processed into separate  $\alpha$  (~135kDa) and  $\beta$  chains (95kDa, mature receptor ~460kDa). The ligand binding region is located in the extracellular  $\alpha$  subunits and the tyrosine kinase domain is located in the cytoplasmic region of the  $\beta$  subunits. The stoichiometry of ligand binding is 1:1. A recent crystal structure of the extracellular portion of the IR revealed a folded over conformation with two potential ligand binding pockets (McKern et al., 2006; Smith et al., 2010). The residues important for ligand binding have been identified by a series of detailed site-directed mutagenesis studies, with the use of IR:IGF-1R

chimeras and using antibody competition for ligand binding (De Meyts and Whittaker, 2002). Within a single binding pocket ligand contacts the receptor at two sites. Site 1 is made up of residues within the L1 domain (large domain 1 leucine rich region) and ID (insert domain), with each derived from opposite receptor monomers. Site 2 is located within the Fn-III-1 and Fn-III-2 domains (derived from the same monomer as the ID of site 1).

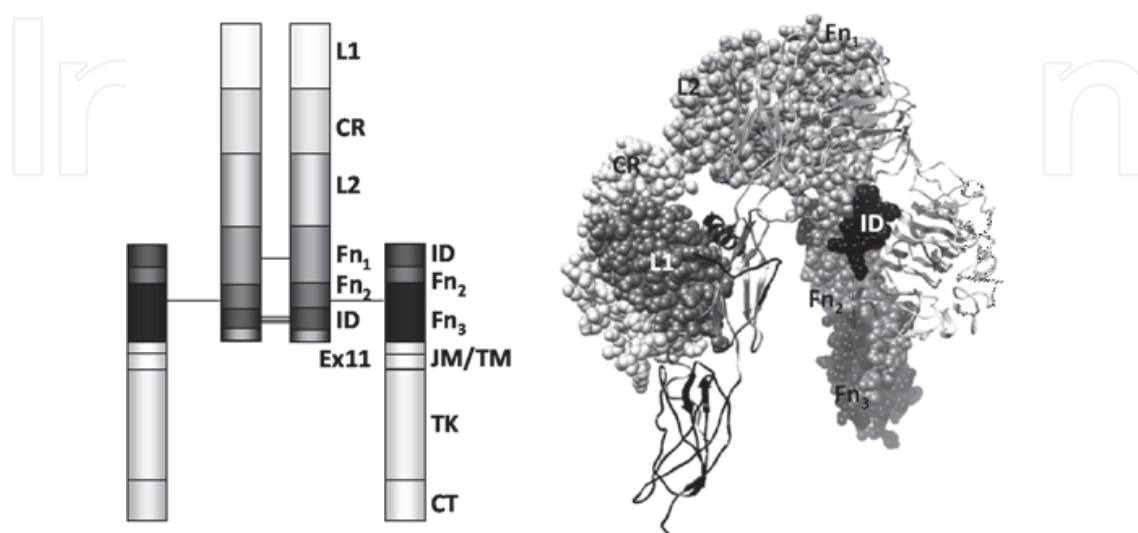


Fig. 2. The IR structure. The IR consists of 2  $\alpha$  and intracellular  $\beta$  subunits made up of the following domains (labelled on one receptor monomer, Left): L1 and L2, large domains 1 and 2 (leucine-rich repeats); CR, Cys-rich domain; Fn1, Fn2, Fn3, fibronectin type III domains 1-3 (also referred in the text as FnIII-1, FnIII-2, FnIII-3); ID, insert domain; Ex11, 12 residues encoded by exon11 (IR-B only); TM, transmembrane; JM, juxtamembrane; TK, tyrosine kinase; CT, C-terminal domains (adapted from Denley et al., 2003). The ligand binding regions are found in the L1, Fn1 and Fn2 (also referred in the text as FnIII-1 and -2) and the ID. (Right) The folded over conformation of the receptor is revealed in the IR ectodomain crystal structure (McKern et al., 2006), pdb 3LOH). The two binding pockets evident in the folded over structure include residues from each receptor monomer. One monomer is depicted in ribbon mode and the other is in surface filled mode.

While there is currently no structure of insulin bound to the intact IR, chemical cross-linking data and the structure of the IR ectodomain have allowed the development of a structural model of the interaction (Lou et al., 2006). Ligand binding cross-links the two receptor monomers leading to a structural change in the ectodomain and precluding binding of a second ligand molecule in the unoccupied binding pocket (as described in the mathematical model by Kiselyov et al., (Kiselyov et al., 2009).

The receptor structural change is transduced across the transmembrane region to the intracellular domain leading to activation of the intracellular tyrosine kinase domain. Crystal structures of the inactive and activated forms of the IR tyrosine kinase domain reveal that the first step in the activation process is the movement of an inhibitory arm reaching from the juxta membrane region that maintains the tyrosine kinase domain in a basal, low activity state. Removal of juxtamembrane domain Tyr984 from its contacts with the amino terminal kinase lobe allows coordination of ATP and subsequent trans autophosphorylation of Tyr1146, Tyr1150 and Tyr1151 within the activation loop of the tyrosine kinase domain (Hubbard, 2004).

## 5.2 Insulin signaling components

Following tyrosine kinase domain activation several other residues are phosphorylated and these act as docking sites for downstream signaling molecules (Siddle, 2011; Taniguchi et al., 2006). In fact at least 7 tyrosine residues, 12 serine residues and a single threonine have been shown to be phosphorylated in response to insulin (Kohanski, 1993; Lewis et al., 1990; Tavaré and Denton, 1988; Tornqvist et al., 1987). Initial autophosphorylation of Tyr960 within a NPXY motif of the transmembrane domain provides an important docking site for insulin receptor substrate 1 and 2 (IRS-1 and IRS-2) and Shc (both substrates for the IR tyrosine kinase). The Grb2-associated binder 1 (Gab 1) and Cas-Br-M (murine) ecotropic retroviral transforming sequence homologue (Cbl) proteins also are substrates of the IR tyrosine kinase and play a role in glucose uptake (see section 6.1). IRS-1 and IRS-2 contain a phosphotyrosine-binding domain and a plekstrin-homology domain which facilitate the interaction with activated IR via phosphoTyr960. IRS and Shc proteins are phosphorylated on multiple sites by the IR and these phosphotyrosines then act as docking sites for different SH2 containing signaling molecules including PI3K and growth receptor binding protein-2 (Grb2). These proteins are the first molecules of the two main insulin stimulated signaling cascades: the PI3K-AKT/protein kinase B (PKB) pathway, which leads to protein translation, metabolic control, cell cycling and cell survival, and the Ras-mitogen-activated protein kinase (MAPK) pathway, which controls cell growth and differentiation.

There are six IRS proteins with IRS-1 and IRS-2 being the most widely expressed (Taniguchi et al., 2006). Knockdown studies *in vivo* and *in vitro* indicate that IRS-1 and IRS-2 play different roles of in insulin signaling. For example, *in vivo* knockdown of hepatic IRS-1 expression is associated with increased gluconeogenesis whereas down-regulation of hepatic IRS-2 is associated with expression of genes involved in lipogenesis. IRS-1 knockout mice are small and insulin resistant with normal glucose homeostasis due to compensatory insulin secretion. IRS-2 knockout mice are normal in size but develop diabetes and are insulin resistant due to reduced  $\beta$ -cell mass (reviewed in (Taniguchi and White, 2008)). Although they do recruit many of the same binding partners the signalling differences of the two IRS isoforms may be explained by their differing abilities to bind certain downstream signalling molecules. Subcellular localisation or activation kinetics may also play a role. IRS signalling is controlled by feedback mechanisms predominantly involving serine phosphorylation of the IRS proteins by downstream kinases including Akt, S6K1 and GSK3, and leading to IRS inactivation (Taniguchi et al., 2006).

Several proteins regulate signaling via the IR (Taniguchi et al., 2006). SOCS-3 (suppressor of cytokine signalling-3), induced by cytokine signaling, regulates IR signaling by competing for binding with IRS proteins to phosphorylated Tyr960, thereby down-regulating insulin's action. SOCS proteins have attracted significant interest as they are up-regulated in cases of insulin resistance. Growth factor receptor bound proteins (Grb10/Grb14) act as pseudo substrates for the tyrosine kinase domain of activated IR and thereby inhibit further phosphorylation of downstream signaling molecules including IRS-1. They also protect the phosphotyrosines in the tyrosine kinase domain from dephosphorylation by phosphatases, thus potentially prolonging receptor activation (Holt and Siddle, 2005). One such phosphatase is PTP1B, which directly interacts with the IR tyrosine kinase domain thereby reducing IR signalling activity (Yip et al., 2010). Grb10 also promotes receptor downregulation via its interaction with the ubiquitin ligase NEDD4 (Ramos et al., 2006; Vecchione et al., 2003). Simultaneous knockout of Grb10 and Grb14 improved glucose homeostasis due to enhanced IR signalling (Holt et al., 2009; Holt and Siddle, 2005).

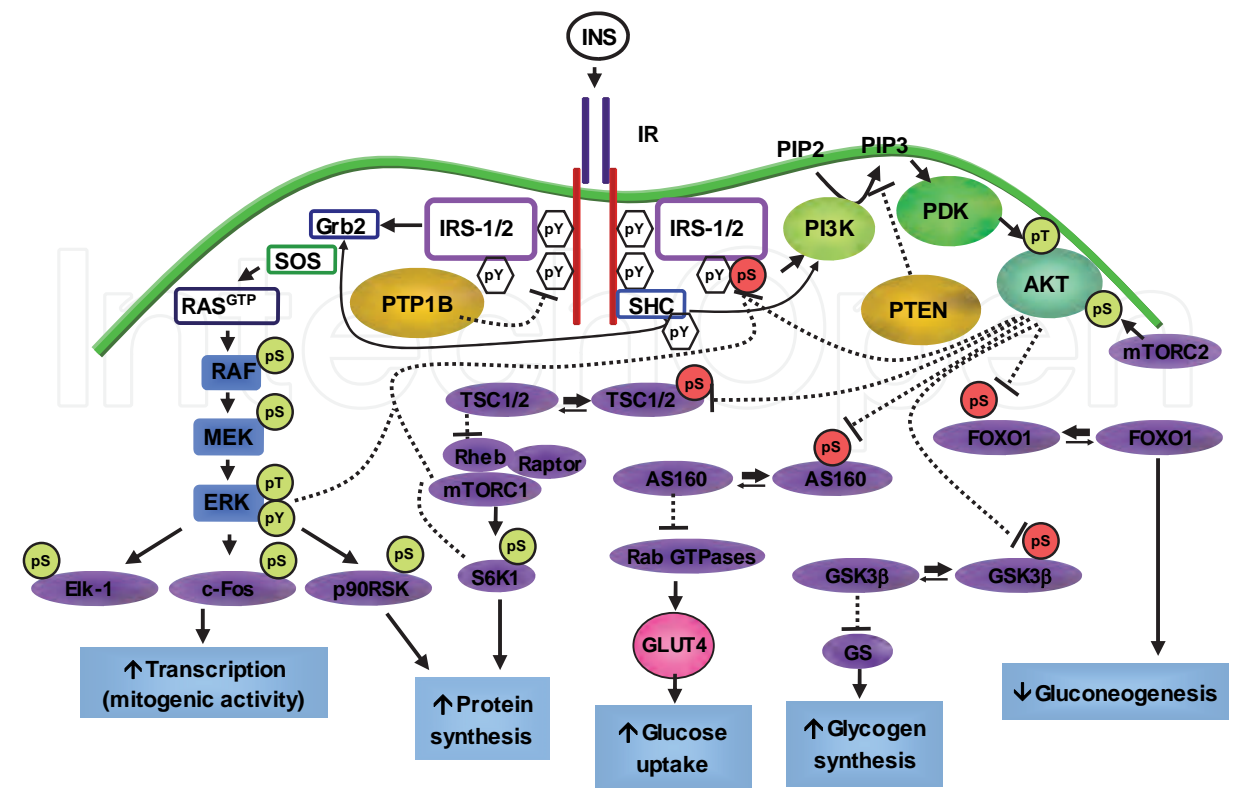


Fig. 3. The canonical insulin receptor signalling pathways (PI3K/ AKT and MAPK pathways). Binding of insulin (INS) to the insulin receptor (IR) leads to activation of the intracellular receptor tyrosine kinase. Subsequent autophosphorylation leads to recruitment of IRS-1/2 and Shc. Activated PI3K then converts phosphatidyl inositol (4,5) biphosphate (PIP2) to phosphatidyl inositol (3,4,5) trisphosphate which then recruits PDK and AKT to the membrane. AKT is phosphorylated on Thr308 by PDK and Ser473 by mTORC2. There are many target substrates of the serine kinase AKT including TSC1/2, which when phosphorylated by AKT becomes inactive and thereby promotes activation of the mTORC1 complex and subsequent protein synthesis. Inactivation of the GTPase activating protein AS160 by AKT relieves the inhibition of RAB GTPase to promote GLUT4 translocation and glucose uptake. Glycogen synthase promotes glycogen synthesis when AKT inactivates GSK3β and phosphorylation of FOXO1 prevents its translocation to the nucleus and thus inhibits gluconeogenesis. AKT signalling is switched off by the phosphatases PTEN (converts PIP3 to PIP2) and PTP1B (direct action on the IR). Mitogenic signalling involves recruitment of Grb2 by activated IRS-1/2 and Shc. SOS bound to Grb2 acts as a guanine exchange factor promoting the formation of active RAS GTP. Activation of RAF and the downstream MAPK signalling cascade follows leading to activation of p90RSK and protein synthesis as well as the transcription factors Elk-1 and c-FOS. IRS proteins are negatively regulated upon serine phosphorylation by AKT, mTORC1, pS6K and activated ERK1/2. Activation is indicated by a solid line and inhibition by a dashed line. IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; PDK, protein dependent kinase; mTORC, mammalian target of rapamycin complex; PTEN, phosphatase and tensin homologue; FOXO1, forkhead box O1; GSK3β, glycogen synthase kinase 3β; GS glycogen synthase; AS160, AKT substrate of 160kDa; TSC1/2, tuberous sclerosis complex-1 and -2; Rheb, Ras homologue enriched in brain; Raptor, regulatory associated protein of mTOR; PTP1B, protein tyrosine phosphatase 1B; Grb2, growth receptor binding protein 2; SOS, son-of-sevenless; MEK, MAPK kinase; ERK, extracellular signal-regulated kinase 1 and 2; p90RSK, p90 ribosomal protein S6 kinase; pY, phosphotyrosine; pS, phosphoserine; pT, phosphothreonine.



Activation of the IR by insulin also leads to internalization of the ligand:receptor complex and results in endosomal breakdown of insulin. Internalised receptor is either degraded or recycled back to the membrane for further signaling events (Foti et al., 2004). Interestingly, rapid receptor recycling is linked to sustained Akt signalling (Romanelli et al., 2007), whereas there is evidence that receptor internalization plays a role in prolonged signalling associated with the MAPK pathway and mitogenic activity (Jensen et al., 2007).

## **6. Insulin actions**

### **6.1 Mitogenic effects**

Insulin receptor signaling via the MAPK pathway leads predominantly to mitogenic biological effects such as cell growth, survival and differentiation (Belfiore et al., 2009; Siddle, 2011). Binding and activation of IRS proteins leads to recruitment of Grb2 and the guanyl nucleotide exchange factor SOS (son-of-sevenless). SOS then activates the small GTPase Ras which in turn activates Raf and the MAPK, MEK and Erk1/2 signaling cascade. Activated Erk1/2 phosphorylates a series of targets including p90 ribosomal protein S6 kinase (p90RSK), which promotes protein synthesis, and the transcription factors Elk-1 and c-Fos (Figure 3) (Shaul and Seger, 2007). Erk1 and Erk2 have both overlapping and unique functions. Erk1 knockout mice develop normally and are born a normal size probably due to compensation by Erk2. However, Erk1 knockouts do have deficient thymocyte maturation and some neurological defects. In contrast knockout of Erk2 is embryonic lethal. In relation to metabolism, Erk1 appears to play specific roles in adipogenesis (Taniguchi et al., 2006).

While insulin stimulates mitogenic effects such as promoting pancreatic  $\beta$  cell health through signalling via the IR (Belfiore et al., 2009), it is also able to activate mitogenic pathways upon binding to the highly similar type 1 IGF receptor (IGF-1R) (Pollak and Russell-Jones, 2010). IGFs promote cell proliferation, survival and migration upon activation of the IGF-1R. IGFs are essential for normal growth and development and also promote cancer cell proliferation and survival. Elevated circulating IGF-I levels have been associated with an increased risk of cancer and up-regulation of IGF-I, IGF-II and the IGF-1R is commonly seen in many types of cancer (Pollak, 2008). The affinity of insulin for the IGF-1R is at least 100-fold lower than the affinity of IGF-I for its receptor. Therefore activation of the IGF-1R by insulin only occurs in situations of high insulin concentrations. For this reason there is growing concern that hyperinsulinemia associated with Type 2 diabetes leads to an elevated risk of cancer, highlighting the need for tight glucose control in these patients. Furthermore the potential increased cancer risk is being assessed for patients currently treated with long acting insulin mimetics such as glargine which have increased IGF-1R binding affinities (Pollak and Russell-Jones, 2010).

### **6.2 Metabolic effects**

Insulin exerts its metabolic effects in three major tissues including liver, skeletal muscle and adipose tissues. Those effects include the stimulation of glucose transport, glycolysis, lipogenesis and protein synthesis while inhibiting gluconeogenesis, glycogenolysis, lipolysis and protein breakdown (see Figure 4).

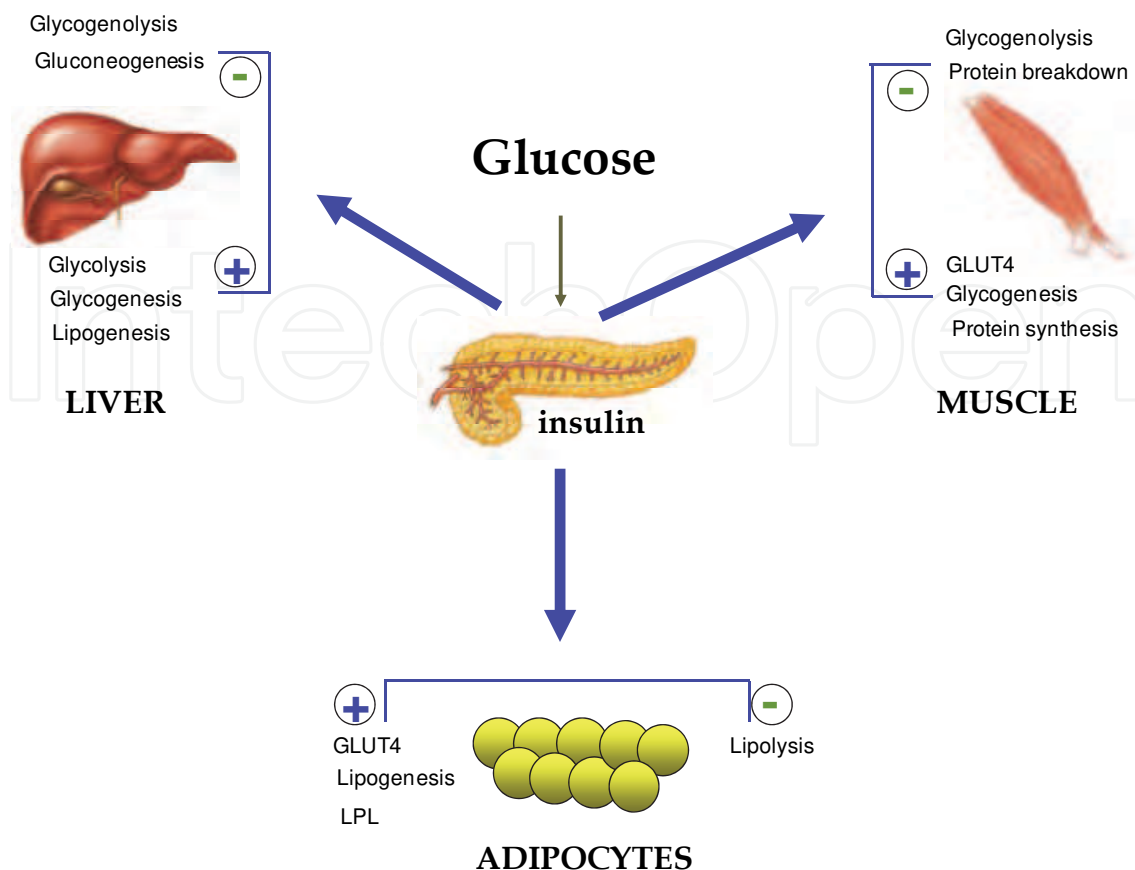


Fig. 4. Metabolic effects of insulin in liver, adipose tissue and skeletal muscle. In liver, insulin stimulates glycogenesis, glycolysis and lipogenesis (*de novo* fatty acid synthesis) but inhibits glycogenolysis and gluconeogenesis. In muscle, insulin stimulates glucose uptake via GLUT4 transporter, glycogenesis and protein synthesis but inhibits protein breakdown and glycogenolysis. In adipose tissue, insulin stimulates glucose uptake via GLUT4, lipogenesis (*de novo* fatty acid synthesis), and triglyceride synthesis by stimulating LPL activity. -, inhibition; +, stimulation.

### 6.2.1 Glucose transport

Glucose transporter 4 (GLUT4) is the most abundant transporter isoform in adipocytes and myocytes and is the only isoform that is regulated by insulin (Bryant et al., 2002). GLUT4 acts as the gate that allows extracellular glucose to enter the cells. During nutrient restriction or unstimulated conditions, 5-10% of GLUT4 is located on the plasma membrane while 90-95% is sequestered in an intracellular vesicle-bound form. However, when the concentration of extracellular glucose becomes high and the insulin is released, GLUT4 is translocated from intracellular sites to the plasma membrane (Holman and Cushman, 1994). The molecular mechanism by which insulin promotes the translocation of GLUT4 depends upon phosphorylation of downstream kinases including PI3K and Akt. The production of phosphoinositol(3,4,5) triphosphate by PI3K facilitates the release of the vesicle-bound GLUT4 and allows trafficking to the cell surface. The increased phosphoinositol (3,4,5)-triphosphate also promotes actin polymerization, resulting in the mobilization of the vesicle-bound GLUT4 near the plasma membrane. The other signal that promotes translocation of GLUT4 involves phosphorylation of Cbl that is associated

with an adaptor protein, CAP. The CAP-phosphorylated Cbl complex is then translocated to the lipid raft on the plasma membrane where this complex further interacts with three more adaptor proteins, namely Crk, C3G and TC10. Here, TC10 functions as the second signal independent of PI3K activation, facilitating the trafficking of GLUT4 to the plasma membrane. Furthermore, fusion of the vesicle bound GLUT4 to the plasma membrane also requires the interaction between SNARE on the GLUT4 vesicles and the plasma membrane (Huang and Czech, 2007; Ishiki and Klip, 2005; Shisheva, 2008). The translocation of GLUT4 to the plasma membrane occurs within a few minutes after insulin stimulation and this allows rapid uptake of extracellular glucose to the muscle cells. In fact 75% of insulin-dependent glucose disposal occurs through GLUT4-mediated transport into muscle cells.

### 6.2.2 Glycogen synthesis

Glycogenesis is an important means to store excess glucose in liver and skeletal muscle (Pagliassotti and Cherrington, 1992). Insulin stimulates glycogen synthesis in liver and skeletal muscle through the glycogen synthase kinase 3 (GSK3). There are two isoforms of GSK3, i.e. GSK3 $\alpha$  (51 kDa) and GSK3 $\beta$  (47 kDa), both of which share over 98% sequence identity in their kinase domains but different in their N-termini (Forde and Dale, 2007). Both isoforms are capable of phosphorylating glycogen synthase. During starvation when glucose is low, glycogen synthase is phosphorylated by casein kinase II at Ser657. Phosphorylation at this residue of glycogen synthase primes GSK3 to phosphorylate three more serine residues namely, Ser641, Ser645 and Ser649, making glycogen synthase becomes catalytically inactive (Forde and Dale, 2007). However, when insulin is released in response to hyperglycemic conditions, this leads to the activation of the Akt/PKB signaling cascade as mentioned earlier. Akt/PKB in turn phosphorylates Ser21 residue of GSK $\alpha$  or Ser9 residue of GSK3 $\beta$ , causing them become catalytically inactive, and no longer able to phosphorylate glycogen synthase (Sutherland et al., 1993). The non-phosphorylated glycogen synthase can now convert the UDP-glucose into glycogen in muscle and hepatocytes. Insulin also stimulates the activity of protein phosphatase 1 (PP1) specifically localized near the glycogen granules (Brady and Saltiel, 2001; Ragolia and Begum, 1998). This enzyme removes a phosphate group from glycogen synthase, rendering it catalytically active.

Insulin not only stimulates glycogen synthesis but also inhibits the process of glycogen breakdown known as glycogenolysis which is important for the supply of glucose during short term starvation. Glycogen phosphorylase releases one unit of glucose in the form of glucose-1-phosphate from the glycogen chain. During starvation when the level of glucagon is high, binding of glucagon to its G-protein couple receptor activates adenylyl cyclase activity to convert ATP to cAMP. cAMP in turn stimulates PKA activity to phosphorylate an inactive form of glycogen phosphorylase (known as the phosphorylase b form), transforming it to become an active form (glycogen phosphorylase a form) (Johnson, 1992). However, during postprandial period when the level of insulin is high, insulin activates phosphodiesterase which subsequently converts cAMP to AMP. As the level of cAMP is low, PKA is no longer activated, the glycogen phosphorylase remains in an inactive form. Furthermore, insulin activates protein phosphatase-1 to remove phosphate from phosphorylase a, transforming it to an inactive form and results in the inactivation of glycogenolysis (Brady and Saltiel, 2001; Ragolia and Begum, 1998).

### 6.2.3 Gluconeogenesis

Gluconeogenesis is the pathway that converts non-carbohydrate precursors including glycerol (product from triglyceride hydrolysis), lactate (end product from anaerobic glycolysis in skeletal muscle) and alanine (from protein breakdown in muscle) to glucose through the combined reverse reactions of glycolysis and the four additional reactions catalyzed by pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase, respectively (Pilkis and Granner, 1992). Gluconeogenesis is absolutely essential for survival during prolonged fasting because red blood cells and the brain primarily rely on glucose as fuel. The genes encoding these four enzymes are regulated by two transcription factors, i.e. the forkhead transcription factor box O (FoxO) and the cAMP-responsive element binding protein (CREB). During prolonged starvation, glucagon triggers the production of cAMP which in turn stimulates PKA to phosphorylate Ser133 of CREB (Altarejos and Montminy, 2011; Mayr and Montminy, 2001; Montminy, 1997). This phosphorylated CREB together with its coactivator, the transcriptional coactivator of regulated CREB activity 2 (TORC2) (Koo et al., 2005) then binds to the cAMP-responsive element (CRE) in the promoters of PC, PEPCK and G6Pase genes and stimulate their transcription.

Under fasting conditions, FoxO also binds to its responsive element known as the insulin-responsive element (IRE) [(T/C)(G/A)AAACAA] in the promoters of PEPCK and G6Pase genes and stimulate their expression (Barthel et al., 2005; Nakae et al., 2008). Combined actions of CREB and FoxO result in the robust stimulation of gluconeogenic pathway. However, when the level of extracellular glucose is high and the level of insulin is high, the gluconeogenic rate is inhibited. As the result of insulin signaling, Akt phosphorylates FoxO protein at Thr24, Ser256 and Ser319 residues (Barthel et al., 2005; Nakae et al., 2008), preventing its entry to the nucleus thus inhibiting transcription of PEPCK and G6Pase genes (Zhang et al., 2006). Furthermore Akt also phosphorylates TORC2 via another kinase, SIK2, at Ser 171 residue, inhibiting its entry to the nucleus (Dentin et al., 2007; Koo et al., 2005). This in turn prevents transcriptional activation of PC, PEPCK and G6Pase genes. In summary insulin inhibits hepatic gluconeogenesis by inhibiting the entry of FoxO and TORC2 to the nucleus, thereby preventing transcriptional activation of gluconeogenic enzyme genes.

### 6.2.4 Coordinate control of glycolysis and fatty acid synthesis

Excess glucose is not only stored as glycogen in the liver but is also stored as fat through the *de novo* fatty acid synthesis. Insulin stimulates this effect by stimulating the glycolytic pathway through the increased expression of some glycolytic enzymes including glucokinase and L (liver)-type pyruvate kinase. This allows the production of acetyl-CoA which subsequently enters TCA cycle. A high rate of acetyl-CoA oxidation in the mitochondria accelerates the export of citrate from mitochondria to cytoplasm where it is oxidatively decarboxylated back to oxaloacetate and acetyl-CoA by an ATP-citrate lyase. Acetyl-CoA is converted to malonyl-CoA by the first rate-limiting step enzyme of lipogenesis, namely the acetyl-CoA carboxylase (ACC). Malonyl-CoA formed by the activity of ACC are condensed together to form fatty acyl-CoA in the cytoplasm. Insulin is able to increase both glycolysis and *de novo* fatty acid synthesis by stimulating the expression of two transcription factors, namely the sterol regulatory element binding protein 1c (SREBP1c) and the carbohydrate responsive element binding protein (ChREBP) (Dentin et al., 2005;



Desvergne et al., 2006). SREBP1c binds to the promoters of glucokinase, acetyl-CoA carboxylase and fatty acid synthase genes while ChREBP binds to the promoters of L-type pyruvate kinase, ATP-citrate lyase, acetyl-CoA carboxylase and fatty acid synthase genes (Postic et al., 2007; Towle et al., 1997). The coordinate regulation of glycolysis, citrate export and *de novo* fatty acid synthesis by insulin through the use of ChREBP and SREBP1c transcription factors allows fuel partitioning toward fat storage in hepatocytes.

Insulin can also stimulate glycolysis through the stimulation of one of glycolytic enzymes, i.e. phosphofructokinase 1 (PFK1) activity. PFK1 phosphorylates glucose-6-phosphate to fructose-1,6-bisphosphate thus increasing the level of this intermediate for the glycolytic pathway. Fructose-2,6-bisphosphate is an allosteric activator of PFK1 while it is an inhibitor of FBPase1 (Okar et al., 2001; Pilkis et al., 1995). This fructose-2,6-bisphosphate intermediate is produced by the bifunctional enzyme, phosphofructokinase 2/fructose bisphosphatase 2 (PFK2/FBPase2). PFK2 converts fructose-6-phosphate to fructose-2,6-bisphosphate while FBPase2 converts fructose-2,6-bisphosphate back to fructose-6-phosphate. Glucagon acting via cAMP-dependent protein kinase phosphorylates PFK2, converting it to an inactive form, allowing FBPase2 to convert fructose-2,6-bisphosphate to fructose-6-phosphate. The lower level of fructose-2,6-bisphosphate de-represses the FBPase1 activity, promoting gluconeogenesis. In contrast, insulin stimulates PFK2 activity of this bifunctional enzyme through phosphoprotein phosphatase, dephosphorylating PFK2, converting it into a catalytically active form. This results in the rise of fructose-2,6-bisphosphate level which stimulates PFK1, promoting glycolysis (Okar et al., 2001; Pilkis et al., 1995).

### 6.2.5 Triglyceride synthesis

Similar to liver, insulin also stimulates expression of some key enzymes in glycolytic pathway and *de novo* fatty acid synthesis, resulting in the increase in the synthesis of fatty acids in adipose tissue.

The dietary fat transported with chylomicrons as well as the *de novo* fat that is synthesized from liver and transported with very low density lipoprotein are taken up in adipose tissue. Because triglycerides cannot be readily transported to adipocytes, they must be first hydrolyzed to free fatty acids and glycerol by the lipoprotein lipase (LPL). The LPL is synthesized by adipocytes and is secreted in the circulation where it is associated with the extracellular matrix on the endothelial cells. After the hydrolysis of triglycerides by LPL, free fatty acids can now be transported across the plasma membrane of endothelial cells to adipocytes. Here, the monoacylglycerol acyltransferase and diacylglycerol acyltransferase re-esterify free fatty acids with glycerol back to triglycerides (Jin et al., 2002). Insulin promotes fat storage by stimulating adipocyte LPL activity, resulting in the uptake of dietary fat and *de novo* fat from liver to adipose tissue. The molecular mechanism by which insulin regulates LPL activity in adipocyte is not well defined, although there is clear evidence that insulin does not stimulate transcription of LPL gene (Raynolds et al., 1990; Semenkovich et al., 1989) but rather enhances the secretion of LPL from adipocytes to the extracellular matrix where the enzyme becomes active (Camps et al., 1990; Chan et al., 1988; Knutson, 2000; Nielsen et al., 1997). It is noted that insulin does not stimulate LPL activity in other tissues such as muscle and myocardium which possesses different LPL. This explains why the fat deposition rate is high in adipose tissue during fed period. In addition to stimulating the LPL activity, insulin inhibits the activity of another lipase called the hormone sensitive

lipase (Watt and Steinberg, 2008). Hormone sensitive lipase is inhibited when the level of insulin is high. However, when the level of insulin is low, which is counterbalanced by the high level of glucagon, hormone sensitive lipase becomes active and it hydrolyzes triglycerides to free fatty acids which are released to the blood circulation during prolonged starvation.

### 6.2.6 Protein synthesis

Another metabolic effect of insulin is protein synthesis in muscle cells. Insulin promotes this anabolic process through the activity of mammalian target of rapamycin (mTOR) which is activated by Akt. Activation of mTOR in turn regulates different steps in the protein synthesis including translation initiation, elongation and ribosome biogenesis (Proud, 2004; Proud, 2006). The initiation factor 4 (eIF4E) functions as the protein that recognizes the CAP structure at the 5'-end of eukaryotic mRNA thus allowing translation to occur in a CAP-dependent manner. During non-stimulated conditions, eIF4E is bound to its inhibitor protein, PHAS1 or 4E-binding protein, resulting in the suppression of translation initiation. However when the level of insulin is high, PHAS1 is phosphorylated by mTOR, causing the dissociation of eIF4E from PHAS1. This results in the initiation of protein synthesis in a CAP-dependent manner (Lin et al., 1994; Wang et al., 2005). Insulin also regulates the recognition of the methionyl tRNA (tRNA<sup>Met</sup>) to the initiation codon through the initiation factor, eIF2. In general, the GTP-bound eIF2 carries the tRNA<sup>Met</sup> to the initiation codon where the engagement between first codon and tRNA<sup>Met</sup> occurs, concomitant with the release of the GDP-eIF2. The GDP-eIF2 is then converted back to GTP-eIF2 by another initiation factor, eIF2B. During unstimulated conditions, GSK3 phosphorylates eIF2B, inhibiting its activity to convert GDP-eIF2 to GTP-eIF2, resulting in translational inhibition. However, when the level of insulin is high, GSK3 is phosphorylated by Akt/PKB resulting in the loss of its activity. This causes the de-repression of eIF2B activity, enabling it to activate eIF2B (Cohen and Frame, 2001). Insulin can also regulate the elongation step of protein synthesis by modulating the activity of elongation factor, eEF2 (Proud, 2006; Redpath et al., 1996). In general eEF2 facilitates the translocation of ribosome along the mRNA so that the next codon can be engaged by the corresponding aminoacyl tRNA. This translocation process is regulated by the eEF2 kinase which phosphorylates Thr56 residue of eEF2, inhibiting its activity to translocate the ribosome to the next site. When the level of insulin is high, mTOR phosphorylates eEF2 kinase, allowing eEF2 to regain its activity. Insulin also promotes the dephosphorylation of eEF2, leading to the stimulation of polypeptide chain elongation.

In addition to promoting initiation and elongation steps in the protein synthesis, insulin again stimulates ribosome biosynthesis through mTOR. mTOR phosphorylates p70 ribosomal S6 kinase (p70) and PHAS1 as mentioned earlier. p70 subsequently phosphorylates S6 ribosomal protein in the 40s subunit of the ribosome, resulting in the biosynthesis of active ribosomes (Proud, 2004; Proud, 2006).

Insulin has long been known to inhibit cellular protein breakdown. This is important when considering muscle loss in association with the increased proteolytic activity has been seen in the type 2 diabetic patients. Administration of insulin to the patients can reverse the muscle loss. Insulin inhibits protein breakdown through the inhibition of the non-ubiquitin and ubiquitin-mediated proteolytic activity in the proteasome (Bennett et al., 2000; Duckworth et al., 1994; Hamel et al., 1997). Although an insulin-degrading enzyme has been proposed to be

involved in this action, the exact mechanism by which this enzyme mediates the anti proteolytic activity of insulin remains unclear. Signaling through the Akt phosphorylation is also a critical step to control the ubiquitin-mediated proteolytic activity (Faridi et al., 2003). In muscle, two isoforms of ubiquitin ligase (E3) namely the atrogin-1 (also known as MAFbx), MuRF-1 are transcriptionally regulated by insulin via FoxO (Sandri et al., 2004). As mentioned earlier, insulin stimulates phosphorylation of FoxO, inhibiting its entry to the nucleus and results in the transcriptional repression of atrogin-1 and MuRF-1 genes. As such, the abundance of these two proteins is low, limiting the availability of ubiquitin-mediated proteolytic machinery. Furthermore insulin also inhibits the lysosomal-mediated proteolytic activity (autophagy) through the activation of mTOR protein (Meijer, 2008).

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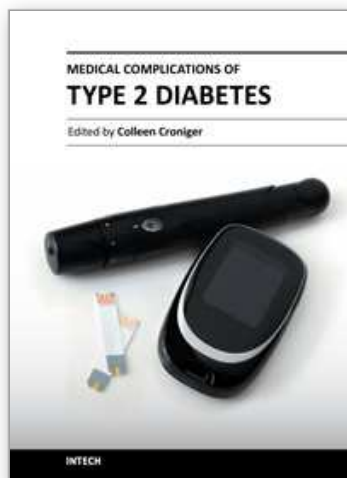
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## **Medical Complications of Type 2 Diabetes**

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Obesity and type 2 diabetes are increasing worldwide problems. In this book we reviewed insulin secretion in both healthy individuals and in patients with type 2 diabetes. Because of the risk associated with progression from insulin resistance to diabetes and cardiovascular complications increases along a continuum, we included several chapters on the damage of endothelial cells in type 2 diabetes and genetic influences on endothelial cell dysfunction. Cardiovascular complications occur at a much lower glucose levels, thus a review on the oral glucose tolerance test compared to other methods was included. The medical conditions associated with type 2 diabetes such as pancreatic cancer, sarcopenia and sleep disordered breathing with diabetes were also discussed. The book concludes with several chapters on the treatments for this disease offering us hope in prevention and successful alleviation of the co-morbidities associated with obesity and type 2 diabetes.

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