INSULIN RESISTANCE RELATED SIGNALING PATHWAYS IN THE LIVER

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Abstract

Over the past 20 years, the worldwide toll of diabetes has tripled to more than 400 million, which makes it one of the fastest-growing health challenges of the 21st century. There are three main categories of diabetes: type 1, type 2 and gestational diabetes mellitus. Among them, Type 2 diabetes(T2D) makes up to 90% of diabetes worldwide. Hyperglycemia can be effectively controlled by giving insulin injection for type 1 and gestational diabetes mellitus. However, because insulin resistance is one of the causes of T2D, those with T2D do not respond as well to insulin as those with T1D or gestational diabetes. Furthermore, our lack of knowledge about the underlying physiology of T2D makes it difficult to find reliable treatments.

While high blood glucose concentration is one of the major symptoms of T2D, changes in lipid metabolism are characteristic of insulin resistance(IR). In the human body, the liver plays a major role in glucose homeostasis and lipid metabolism. Hence, this essay provides an overview of signaling pathways in the liver and presents their interrelationship to better understand the underlying IR mechanism.

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Dedication

This thesis is dedicated to my parents, Xinsheng Wang and Weixia Chen,

and my love, Zirui Li,

for their eternal love, trust and support.

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Chapter 1

Introduction

The incidence of Type 2 Diabetes has been rising rapidly worldwide. According to the International Diabetes Federation(IDF), the number of people estimated to have diabetes in the 20–79 age group was 151 million in 2000 and tripling to an estimated 463 million people by 2019. Estimates for the future are that this number will increase to 700 million by 2045. This is 50 million more than the projected just two years ago [1]. Moreover, diabetes now is seen frequently in children and adolescents under the age of 20.

Diabetes mellitus, often called diabetes in short, is a chronic disease that occurs when the human body, particularly muscle and adipose tissue, stops responding to the insulin produced by the pancreas. Specifically, despite binding of insulin to the insulin receptors on cells, the cells stop deploying GLUT4 receptors to the cell surface and hence the blood glucose level remains high after meals. Hyperglycemia, which is the hallmark of diabetes, can lead to serious damage to many of the body's systems including the peripheral nervous system and blood vessels which can lead to severe complications like cardiovascular disease, loss of sensation in the limbs and blindness [2][3]. According to the World Health Organization, an estimated 1.6 million deaths were caused by diabetes in 2016 [4].

There are three main categories of diabetes: type 1, type 2, and gestational diabetes mellitus. Among them, type 2 diabetes makes up the majority of diabetes, about 90% [5]. It is widely believed that excess body weight and physical inactivity is the consequence of T2D. However, the underlying mechanism remains unclear though various experiments

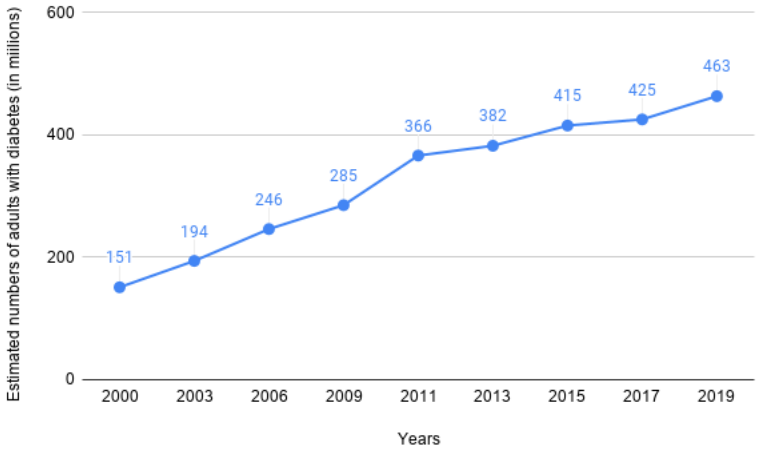

Figure 1-1. Estimated numbers of adults with diabetes (in millions)

have been done to figure out how T2D is developed and how the body's systems work under T2D.

The liver, one of the largest organs in the body, plays a major role in metabolism of many different species including that of carbohydrates and lipids. For example, the liver synthesizes glycogen from glucose when there is hyperglycemia and then breaks it down for release into the blood when there is hypoglycemia. Similarly, the liver packages lipids and cholesterol into lipoprotein micelles for distribution to other parts of the body. In addition to insulin resistance, people with T2D often also have dyslipidemia and alterations in lipid metabolism. Hence, the liver plays a critical part in the mechanism of IR [6].

Here, this article summarizes insulin, glucagon, glucose, and lipoprotein signaling pathways in the liver to help provide a better understanding of the insulin resistance physiology.

Chapter 2

Liver insulin signaling pathway

The insulin signaling pathway is one of the major foci of research in liver metabolism. Insulin, a peptide hormone secreted by beta cells in the pancreatic islets of Langerhans, controls a variety of biological processes, like coordinating the metabolic response to feeding in the liver, by acting through insulin receptors on the hepatocytes' plasma membrane. This binding complex of insulin and insulin receptor then activates the recruitment of insulin receptor substrates(IRS) [7]. And the tyrosine-phosphorylated IRs later activates phosphoinositide 3-kinases(PI3K), on which phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) can form phosphatidylinositol (3,4,5)-triphosphate(PIP3) rapidly [8]. PIP3 recruits the inactive protein kinase B (Akt) to the plasma membrane, where it is activated by phosphorylation and induces downstream signaling [9]. Here, major researches on Akt downstream signaling pathway include the activation of the forkhead box O 1(FOXO1) [10], which controls the gene expression of lipid synthesis and gluconeogenesis, the phosphorylation of the tuberous sclerosis complex protein (TSC) [11], which regulates the protein synthesis, and the activation of glycogen synthase kinase-3 (GSK3) [12], which mediates the glycogen synthesis.

Insulin receptors

Insulin receptors are part of the family of tyrosine kinase receptors. The receptor has two

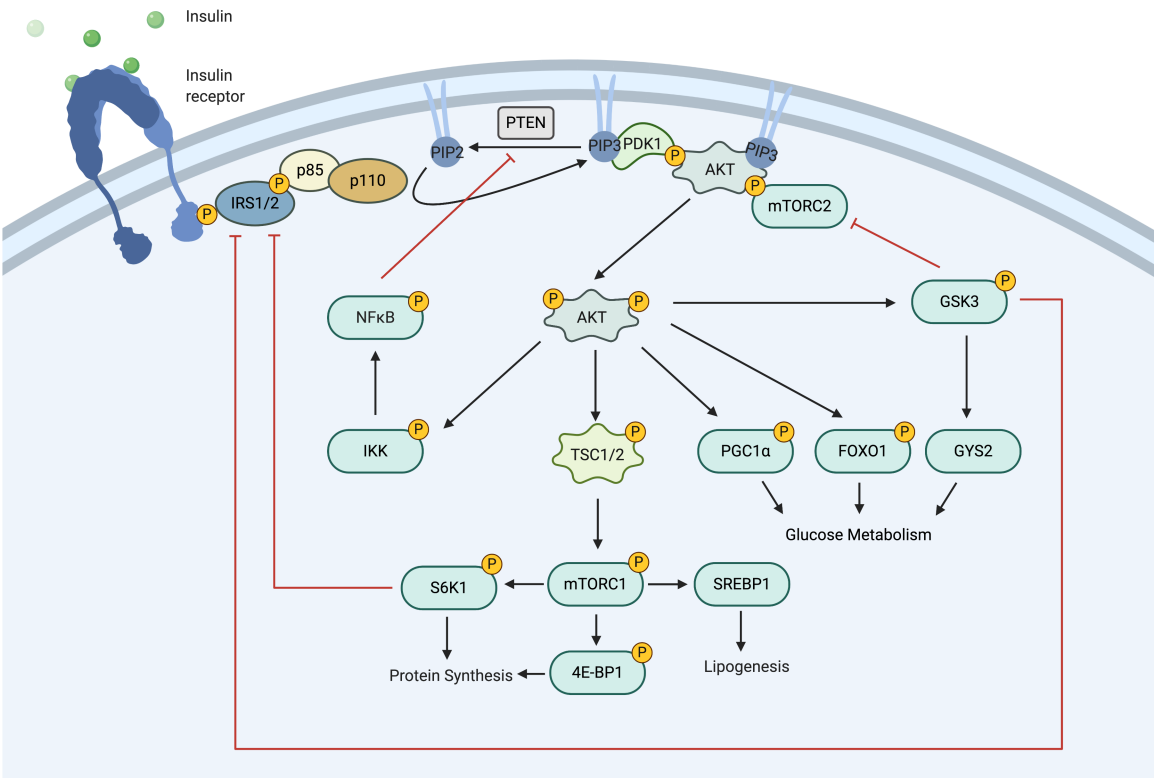

Figure 2-1. Liver insulin signaling

extracellular alpha-subunits linked to a beta-subunit individually by disulfide bonds. There are two isoforms of these transmembrane receptors encoded by a single gene named INSR, IR-A or IR-B, which is generated through differential slicing [13]. Almost every cell in the human body has IR-A, IR-B, and hybrid receptors of the two, but the number and composition of insulin receptors differ. For example, fat, muscle, and liver cells have mainly IR-B embedded in the plasma membrane [14]. However, cancer cells have more IR-A than IR-B [15]. This phenomenon leads to different functions of insulin receptors. The binding of insulin to IR-A mostly triggers insulin production, while insulin binding to IR-B functions largely as the onset of the intracellular glucose sensor [16]. Moreover, it's possible that the increased ratio of IR-A/IR-B in insulin target tissues involves the reduction of glucose uptake and insulin signaling [17]. In addition, IR-A and IR-B also bind to Insulin-like growth factor 1(IGF-1) and Insulin-like growth factor 2(IGF-2) [18]. Although

both promote growth in children and anabolic effects in adults, IGF-1 is secreted by the liver and regulated by the growth hormones whereas IGF-2 is not regulated by the growth hormones [19].

When insulin binds to its receptor, insulin receptors are activated by ligand binding [20]. Although the specific mechanism of this binding process remains unclear due to the unavailability of the structure of completed unliganded and ligand receptors, it is known that this insulin receptor tyrosine kinase phosphorylation creates kinase domains including src-homology 2(SH2) domains or phosphotyrosine- binding(PTB) domains, which are binding sites for intracellular signaling molecules.

Insulin receptor substrates

The insulin receptor is a large protein constituted of two protein chains, which remain in a constrained position when insulin is not bound to it. When insulin binds to the insulin receptor, which is embedded on the cell membrane, at the site of α-subunit, the constraints are released as β-subunit is activated through conformational change and then phosphorylates receptor substrates inside the cell by binding [21]. And the activated receptor substrates then triggers the signaling network inside the cell subsequently. The insulin receptor substrate(IRS) proteins are a family of cytoplasmic adaptor proteins, the most prevalent are IRS1 to IRS6 and they function as protein scaffolds to regulate signaling complexes. In specific, the recruitment of the insulin receptor substrates then activates the insulin receptor through pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains in their N-termini [22]. Afterward, they are phosphorylated by activated receptor kinases on tyrosine residues in their C-termini to create binding sites for the recruitment of downstream signaling molecules such as PI3K [23].

IRS1 and IRS2 are the most prevalent substrates of the six, quite similar in the structure and function but different in different tissue cells [24]. They both have close involvement

in the insulin-dependent inhibition of gluconeogenesis, and the activation of lipogenesis in the liver cells. IRS1 knockout mice develop impaired insulin action, and IRS2 knockout mice show defective insulin signaling in the liver [25].

The first identified member of the insulin receptor substrates was IRS1. It has been characterized as a 131 kDa phosphoprotein including the amino acid sequence of 1242 residues. Tyrosine phosphorylation sites on IRS1 function as docking sites for various SH2 domain proteins such as PI3K, Growth factor receptor-bound protein 2(Grb2), and SH2-containing phosphatase 2(SHP2) [26]. Then the binding of IRS1 to PI3K or Grb2 triggers PI3K/Akt and mitogen-activated protein kinase(MAPK) pathway, respectively, while the binding of IRS1 with SHP2 negatively affect the binding of IRS1 to other SH2 domain proteins [27]. Besides, in the N-termini of IRS1, it shares high homology with other IRS family members as well as among different species [28]. It is shown that IRS1, which has no intrinsic kinase activity, plays an important role in the signaling of the late feeding period after first feeding in the liver [29]. IRS2 was discovered as an alternative route from signaling pathway mediated by the insulin receptor in IRS1 knockout mice [30]. It has been identified as a 180 kDa adapter protein. The kinase regulatory loop binding domain(KRLB), which activates to recruit cellular receptors, makes IRS2 different from the other IRS family members, which is characterized by a PH domain and a PTB domain. Especially, unlike IRS1, IRS2 plays an important part in the period shortly after food intake and fast feeding [31]. It is recognized that after insulin binds to its receptor, IRS2 combines with p85, which is an 85-kD subunit making up PI3K along with a 110-kD catalytic subunit, generally called p110, then activate the PI3K/Akt and MAPK pathway [32].

Interestingly, it has been demonstrated that hepatic IRS2-knockout mice show a selective insulin resistance in which insulin doesn't suppress the generation of glucose from certain non-carbohydrate carbon substrates but still activates the synthesis of hepatic lipid [33]. Whereas mice lacking in IRS1, or both IRS1 and IRS2, show total insulin resistance [34]. In addition, studies have found that in diabetic mice, the expression of IRS2 was

decreased under hyperinsulinemia in the periportal and perivenous zones, while the expression of IRS1 mostly in the perivenous zone was not affected [34][35]. This suggests that the phenomenon of selective insulin resistance might result from the different expression of IRS1 and IRS2 within different parts of the liver lobule, compared with normal insulin sensitivity.

Phosphoinositide 3-kinases

Phosphoinositide 3-kinases(PI3K), discovered by Lewis Cantley and colleagues [36], are a family of intracellular enzymes that are able to phosphorylate the 3 position hydroxyl group of phosphatidylinositol. There currently are four different classes of PI3K, Class I to Class IV that have different structures and functions [37]. However, only Class I PI3Ks, which activates the lipid phosphorylation, catalyze the conversion of PIP2 into PIP3 in hepatocytes [38]. Besides, the mammalian target of rapamycin (mTOR), which is a member of Class IV PI3Ks, is required for the full activation of Akt phosphorylation at Ser-473 [39]. Class II and III PI3Ks are not significantly involved in the insulin signaling pathway of hepatocytes.

PI3K

Class I PI3Ks, ususally referred to as just PI3K, is a heterodimer consisting of two subunits, a p85 regulatory subunit, and a p110 catalytic subunit. Each of the subunits has several variants. In detail, p85 has five isoforms named p85α, p55α, p50α, p85β, and p55γ, while p110 has three isoforms named p110α, p110β, and p110δ. The most prevalent expressed types of p85 and p110 in the liver are p85α, p110α, and p110β. When PI3K has not been activated, its regulatory subunit develops the stability of its catalytic subunit for the PI3K to stay inactive. After tyrosine on the IRS1/2 is phosphorylated, the p85 subunit, which contains two SH2 domains, binds to the phosphorylated tyrosine. Re-

sults suggest that IRS1 is down-regulated by monomeric p85 through the formation of a sequestration complex between p85 and IRS1 [40]. The phosphorylated p85 subunit then reduces the inhibition on the p110 catalytic subunit. The p110 subunit can also be activated by the Ras-GTP when being recruited independently of the p85 subunit [41].

mTOR

Mammalian target of rapamycin complex(mTORC) plays a key role in the regulation of liver metabolic homeostasis. It serves as a node to connect the insulin signaling pathway and energy homeostasis related pathways like the AMPK pathway [42]. It consists of mammalian target of rapamycin(mTOR), which is a highly conserved serine and threonine protein kinase, and the catalytic subunit. Based on different catalytic subunits, mTOR can be divided into two kinds of complexes: mTORC1 and mTORC2 [43]. mTORC1, involved in the acute metabolic regulation, is a rapamycin-sensitive protein complex containing RAPTOR, which is a regulatory associated adapter protein. It is suppressed by the phosphorylation of TSC1 and TSC2 via Akt [44]. Also, Akt can induce the suppression of mTORC1 through the phosphorylation of the proline-rich Akt substrate of 40 kDa(PRAS40) [45]. mTORC2, involved in the long-term regulation of metabolism, is a rapamycin-insensitive protein complex containing RICTOR, which is not directly inhibited by rapamycin [46]. It is shown there's a mSin1 subunit of mTORC2, which is inhibited in the absence of insulin, will be phosphorylated by active Akt to trigger downstream signalings like the regulation of FOXO1 [47].

PIP2 and PIP3

Phosphatidylinositol 4,5-bisphosphate(PIP2) and phosphatidylinositol (3,4,5)-triphosphate (PIP3), which are bound to the inner side of lipid bilayer, are minor components of phospholipids in the cell membrane (less than 1%), but they play a crucial role not only in the

PI3K-Akt pathway but also in the regulation of various processes at the plasma membrane [48]. In specific, PIP2 serves as the precursor of diacylglycerol and inositol [49]. It can be converted into the second messenger PIP3 under the catalyzation of the active enzyme PI3K, which is an energy-consuming process. PIP3 serves as the effector of the PI3K pathway, docking phospholipids that recruit proteins such as the protein 3-phosphoinositide-dependent protein kinase-1(PDK1) and Akt to the plasma membrane and then activate downstream signalings [50]. In the PI3K/Akt pathway, both PDK1 and Akt protein can bind to PIP3 on the 3 position phosphate group, which then triggers the activation of Akt [51].

As PIP2 can be phosphorylated by the enzyme PI3K to PIP3, this process is reversible through the dephosphorylation with the main enzyme phosphatase and tensin homolog (PTEN) [52]. Observations have shown that decreased expression of PTEN that could be rescued by specific inhibition of the Nuclear factor-B(NFκB) pathway, which suggests that the inhibition of PTEN expression requires the activation of NFκB [53]. The mechanism of this positive feedback of PI3K-Akt pathway can be roughly concluded that NFκB is phosphorylated by the Akt phosphorylated IKKα [54], which is one of the two kinase subunits of the IκB kinase(IKK) complex. However, the detailed signaling pathway remains uncertain. For instance, it has been shown that IKKα is the requirement for phosphorylation of the p65 induced by Akt in response to Akt-mediated NFκB [55]. In contrast, LPS-induced p65 phosphorylation on serine 536 was not reduced by LY294002 in wild-type as reported [56].

PDK1

3-phosphoinositide-dependent protein kinase-1(PDK1), a master kinase, plays a crucial role in catalyzing various signaling pathways including insulin signaling. Studies have shown that PDK1 gene knockout mice can not survive during embryonic development,

while mice possessing PDK1 hypomorphic alleles survive but have slight glucose intolerance [57]. There is a PH domain on the PDK1, which is the binding site to PIP3. Subsequently, PDK1 bound to the PIP3 phosphorylates the residue T308, which activates Akt partially [58]. To develop the full activation of Akt, mTORC2 is required to phosphorylate Akt at the residue S473 [59]. Besides, the active PDK1 can activate atypical protein kinase C(PKC), the atypical PKCs in specific, by the phosphorylation at a critical Thr residue in the activation loop, which is one of the three conserved positions that have to be phosphorylated before PKC is catalytically competent [60].

Protein kinase B and its downstream signalings

Protein kinase B(PKB or Akt in short), is a serine/threonine-specific protein kinase that activates or suppresses other signalings involved in multiple cellular processes like cell growth and death through phosphorylation [61][62]. There are mainly three highly related isoforms of the Akt family members: Akt1, Akt2 and Akt3. Akt1 plays a role in the inhibition of apoptotic processes and activation of protein synthesis [63]. Akt2 is a crucial signaling molecule that induces glucose transport in the regulation of glucose homeostasis the insulin signaling pathway [64]. Akt2 deficient mice show a diabetic phenotype of hyperglycemia and loss of pancreatic beta cells and loss of adipocytes compared with controls and Akt1 deficient mice [65]. The role that Akt3 plays remains unclear. It has been shown that it plays an important role in the development of the brain since Akt3 deficient mice were observed to have significantly small brains [66][67]. After Akt is fully phosphorylated on the plasma membrane by mTORC2 and PDK1 phosphorylated on PIP3, Akt has the ability to translocate from the cell membrane to the cytoplasm and nucleus where it controls downstream signalings including negative and positive feedback of the PI3K-Akt pathway [68].

GSK3 and its downstream signalings

Glycogen synthase kinase-3(GSK3) is a serine/threonine protein kinase, whose phosphorylation mediates various aspects of cellular metabolism. GSK3 regulation by AKT was first demonstrated in L6 myotubes stimulated with insulin [69]. In mammals, GSK3 has two isoforms, GSK3α and GSK3β. Encoded by different genes, they both have a highly conserved catalytic domain but different termini [70]. Data have suggested that they are abundant in most human cells [71]. There are approximately 50,000 or 15,000 molecules of GSK3β and around 5,000 molecules of GSK3α per human bone osteosarcoma epithelial cell [72][73]. It has been demonstrated that the phosphorylation of GSK3 is activated via PI3K at its N-terminal serine residues(Ser9/Ser21), which is autoinhibitory, in GSK3 isoforms β and α respectively [74]. However, some data have shown that the inhibitory phosphorylation of GSK3 via Akt is partial since a greater decrease in the phosphorylation activity of GSK3 substrates comes after a great decrease in GSK3 activity under growth factor stimulation [74][75].

The active GSK3 can directly phosphorylate broad kinds of proteins of the insulin signaling pathway including the Akt upstream complex mTORC2, the Akt downstream signaling protein TSC, and even Akt itself [76]. Besides, GSK3 can also phosphorylate IRS1 and IRS2 to reduce the insulin-stimulated activation of PI3K and AKT, which then might increase insulin resistance [77]. The phosphorylated GSK3 can phosphorylate glycogen synthase 2(GYS2) [78], a key enzyme in hepatic glycogen synthesis. However, the regulation of GYS2 is a quite complex interplay between glucose-6-phosphate(G6P), protein phosphatase 1(PP1), and many other signalings proteins like PKC that can phosphorylate GSK3. It has been identified that G6P-mediated activation of GYS2 plays a key role in up-regulating glycogenosis through the fact that glucose intolerance(metabolic conditions which result in high blood glucose levels) increases and glycogen accumulation decreases with feeding or glucose in G6P-mediated pathway impaired mice [79], but the detailed GSK3 related signalings require further research.

FOXO1

Forkhead box protein O1(FOXO1), also referred to forkhead in rhabdomyosarcoma(FKHR), is one of 4 mammalian FOXO protein family members, FOXO1, FOXO3, FOXO4, FOXO6. FOXO1 plays a key role in down-regulating liver insulin signaling, specifically in the up-regulation and downregulation of gluconeogenesis and glycogenolysis [80]. All FOXO proteins can be phosphorylated by active Akt on different phosphorylation sites like S249, S322/325, and S329 in FOXO1 [81]. And these site of action also present the binding sites for 14-3-3 proteins [81]. When FOXO1 in the nucleus is phosphorylated, it can not be unphosphorylated and subsequently excluded outside the nucleus accompanied by the blockage of its transcriptional activity [82]. Then the transcription of glucose-6 phosphatase(G6Pase) decreases, which subsequently negatively affects glycogenesis and glycogenolysis in the liver. This has been demonstrated in glucose tolerance and gene regulation experiments that liver-specific insulin receptor knockout (LIRKO) mice have higher expression of G6Pase compared with IR floxed and IR/FoxO1 double knock-out (LIRFKO) mice [83]. Also, LIRKO mice have severe insulin resistance along with hepatic glucose production and glucose utilization while those defects are restored in LIRFKO mice [83][84], which suggests the importance of FOXO1 in hepatic gluconeogenesis and glycogenolysis.

TSC 1/ 2 and its downstream signalings

Tuberous sclerosis complex protein 1 and 2, TSC1 and TSC2 in short respectively, form a heterodimeric complex that acts as an integral node from Akt to downstream protein mTORC1. Unlike TSC2 which has a GTPase Activating Protein (GAP) domain and shows active state, TSC1 does not have a GAP domain and stabilizes TSC2 from degradation by excluding the HERC1 ubiquitin ligase [85], which is a 532-kDa protein with an E3 ubiquitin ligase homology to E6AP carboxyl terminus domain, from the TSC2 complex to inhibit the

interaction between TSC2 and the HERC1 [86]. Like FOXO proteins, TSC2 is also shown to be regulated by 14-3-3 protein, which results from the overlap between the recognition motifs of AKT and 14-3-3.

In PI3K-Akt signaling, the phosphorylated Akt activates the phosphorylation of TSC2 on its 2-5 sites of action, which consequently inhibit the mTORC1 signaling by converting Rheb into its inactive GDP-bound state [87]. Besides, prolinerich Akt substrate 40(PRAS40) phosphorylated by Akt also functions as a direct inhibitor of substrate binding to regulate mTORC1 [88]. Experiments have shown that insulin promotes a marked decrease in PRAS40 association with mTORC1, while rapamycin also reduces the association of PRAS40 and mTORC1 [89]. The activation of the mTORC1 complex then phosphorylates the eukaryotic initiation factor 4E(eIF4E)-binding protein 1(4E-BP1) and the p70 ribosomal S6 kinase 1(S6K1) to promote protein synthesis [90]. The amount of eIF4E bound to 4E-BP1 decreases and then promote cap-dependent translation since the phosphorylated 4E-BP1 can not bind to eIF4E. The activation of S6K1 leads to an increase in mRNA biogenesis and cap-dependent translation. Also, it has been proved that mTORC1 positively regulates the activity of sterol regulatory element-binding protein 1(SREBP1) [91], which later induces lipogenesis in the liver.

Otherwise, S6K1 downregulates IRS1 through phosphorylation of IRS1 at multiple residues with a preference for RXRXXS/T over S/T,P sites [92]. This serine phosphorylation promotes IRS1 degradation and thus inactivates IRS1 activity with PI3K, which is a negative feedback mechanism of the PI3K-Akt pathway.

Other substrates of Akt downstream signalings

Apart from those substrate proteins above, there are various kinds of proteins that involve in the insulin signaling pathway, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha(PGC1α) [93]. Unlike FOXO1 which induces significant insulin resistance both in the liver, PGC1α does not significantly affect insulin sensitivity as well

as GSK3β as data shows [94]. On the other hand, PGC1α triggers stronger expression of gluconeogenic enzymes G6Pase than active FOXO1 [95].

The Akt substrate of 160 kDa (AS160), also called TBC1D4, is phosphorylated by Akt at the TBC1D4 residue Thr-642 and Ser-588. It subsequently regulates insulin, glucose transport in the uptake of glucose. Among other members of the TBC1 domain family, TBC1D1, sharing homology with TBC1D4, is the one also phosphorylated by Akt at Thr-596 corresponding to Thr-642 in TBC1D4 to involve in glucose transport [96]. Several experiments have shown that the detailed mechanism of active TBC1D1/4 in vesicle transport seems to depend on small GTPase phosphorylation by increasing the GTP load on GLUT4 vesicle [97]. However, those two Akt substrate proteins mediate in the insulin signaling of muscle cells [98]. In the insulin signaling of the liver, TBC1D4 and TBC1D1 are not observed [98].

Chapter 3

Liver glucagon signaling pathway

Glucagon, a hormone released by pancreas β cells, is the main activator of the regulation of liver glucose metabolism. In contrast to the role that the insulin signaling pathway plays in liver glucose regulation, which promotes glycogen synthesis, the glucagon signaling pathway is the one that inhibits catabolism. When glucagon binds to its receptor on hepatocytes membrane, which is a G protein-coupled receptor, it triggers the conformational change in the receptor and thus leads to releasing its α subunit from the β and γ subunits by the binding of α subunit to guanosine-5'-triphosphate(GTP) as a replacement of guanosine diphosphate(GDP) molecule originally bound in GTP molecule [99]. The α subunit is the activator for the next enzyme. Depending on the G protein type, it has been demonstrated that there are two different downstream signaling pathways. If the G_s protein-coupled receptor is bound, then $G_s\alpha$ stimulates the cAMP-dependent pathway by activating adenylyl cyclase [100]. If the G_q protein-coupled receptor is bound to glucogen, $G_q\alpha$ subsequently stimulates the phospholipase C-inositol triphosphate channel (PLC-INSP3) pathway, also known as the cAMP-independent pathway, which then causes intracellular calcium signaling [101]. They both play a role in liver glucagon signaling. Only cAMP-dependent pathway will be described here since it is more well-established and seemingly more dominant than the other one.

In the cAMP-dependent pathway, the active adenylyl cyclase(AC) then activates the conversion of ATP to cyclic adenosine monophosphate(cAMP), which then leads to the acti-

vation of protein kinase A(PKA), also known as cAMP-dependent protein kinases [102]. The active PKA subsequently activates the phosphorylase kinase(PPK) [103], which is an energy-consuming process. Then glycogen phosphorylase b(PYGb) is phosphorylated to its active form glycogen phosphorylase a(PYGa) by the phosphorylated PPK [104]. RYGa is the enzyme that acts as an enzyme converting α-1,4 glucosyl unit which is formed from glycogen to G1P. Besides, as described in the chapter 4, PKA can also affect the glycogen synthase to influence glycogen synthesis.

In individuals with T2D, they have shown an increased glucagon level compared with normal ones, which raises interest in the relationship between glucagon and T2D [105]. And it has been suggested that hyperglucagonemia contributes to the hyperglycemic state of patients with T2D. Therefore, glucagon antagonism is indicated as a potential therapeutic target in type 2 diabetes. Furthermore, in transgenic glucagon receptor and leptin receptor deficient mice, T2D is developed. However, in transgenic leptin receptor deficient mice, T2D is developed [106]. It suggests that glucagon and its receptor play a role in the development of T2D, yet the mechanism behind hyperglucagonemia in T2D remains unclear.

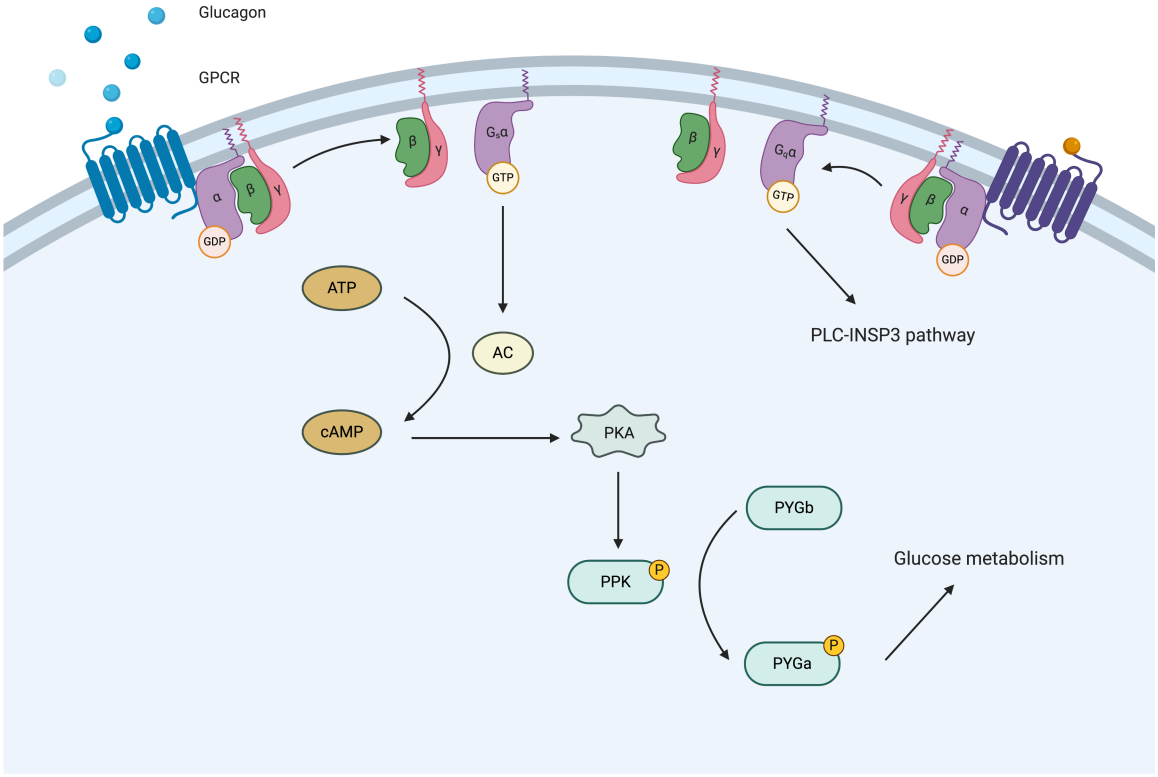

Figure 3-1. Liver glucagon signaling

Chapter 4

Liver glucose signaling pathway

Glucose, a small simple sugar, is the key energy source for almost every cell of the human body to sustain life, especially brain cells and muscle cells. About 60% of the energy needed by the human body is provided by sugar [107]. It is mainly absorbed from carbohydrates and sugar, which come from foods such as vegetables and fruits, through absorptive cells in the small intestine. This process of glucose transport belongs to facilitated diffusion, which is the consequence of the sodium ion relative concentration changes between absorptive cells and intestinal lumen as well as cells and blood. Besides, glucose acts as a substrate to produce structural molecules such as glycoproteins, which have carbohydrate groups attached to the polypeptide chain, and glycolipids, which are the carbohydrate-attached lipids. Those structural molecules play an important role in the stability of the human body by involving various biological processes. For example, glycoproteins on the surface of skin cells stabilize the attachment of our skin cells to forming a tough barrier, while glycolipids initiate the immune response through the binding of selectins to its carbohydrates site. The glucose level is under close control of the body's glucose homeostasis since hyperglycemia might lead to many complications like T2D and eye diseases, also hypoglycemia causes various problems like weakness and headaches [108].

In the body's glucose homeostasis, the liver is a crucial part of the homeostasis system functioning as the glucose reservoir to supply glucose to the whole body. It is the

main place that regulates the blood glucose level signaled by the hormones insulin and glucagon. The liver directly stores glucose in glycogen via glycogenesis and releases glucose to the bloodstream via glycogenolysis. When blood glucose concentration decreases, α cells of the pancreas secrete glucagon, which binds to the G protein-coupled receptors(GPCR) on the hepatocytes and thus trigger glycogenolysis. When blood glucose concentration increases, the hormone insulin that is secreted by pancreas β cells activates insulin signaling pathways in the liver as described in Chapter 1, and also glycogen in the liver is converted into glucose. Besides, the liver can facilitate amino acids, fat byproducts, and even waste products to produce glucose through the process of gluconeogenesis.

Glycogenesis

Glucose in the blood, which is absorbed from the intestine, is transported into hepatocytes via the glucose transporter isoform 2(GLUT2), which is widely expressed both in the liver and intestine as well as in the central nervous system like neurons [109]. In specific, GLUT2, a high-capacity but low-affinity isoform of the big family of glucose transporter, facilitates glucose diffusion into the hepatocytes due to the glucose concentration gradient from the outside cell and the inside cell. Once inside the hepatocytes, glucose is immediately phosphorylated by glucokinase(GCK) into G6P. G6P catalyzed by phosphoglucomutase(PGM) is then converted into Glucose 1-phosphate(G1P). G1P subsequently is synthesized with uridine-5'-triphosphate(UTP) by the enzyme UTP—glucose-1-phosphate uridylyltransferase(UGPase) into uridine diphosphate glucose(UDP-glucose) and the byproduct pyrophosphate(PPi). Together with the enzyme glycogenin and glycogen synthase, UDP-glucose synthesizes (1,4-α-D-glucosyl)$_{n+1}$, which is α-1,4 glucosyl unit on glycogenin. It is then catalyzed by glycogen branching enzyme(GBE) transferase, which adds branches to the growing glycogen molecule. Thus glycogen, a storage form

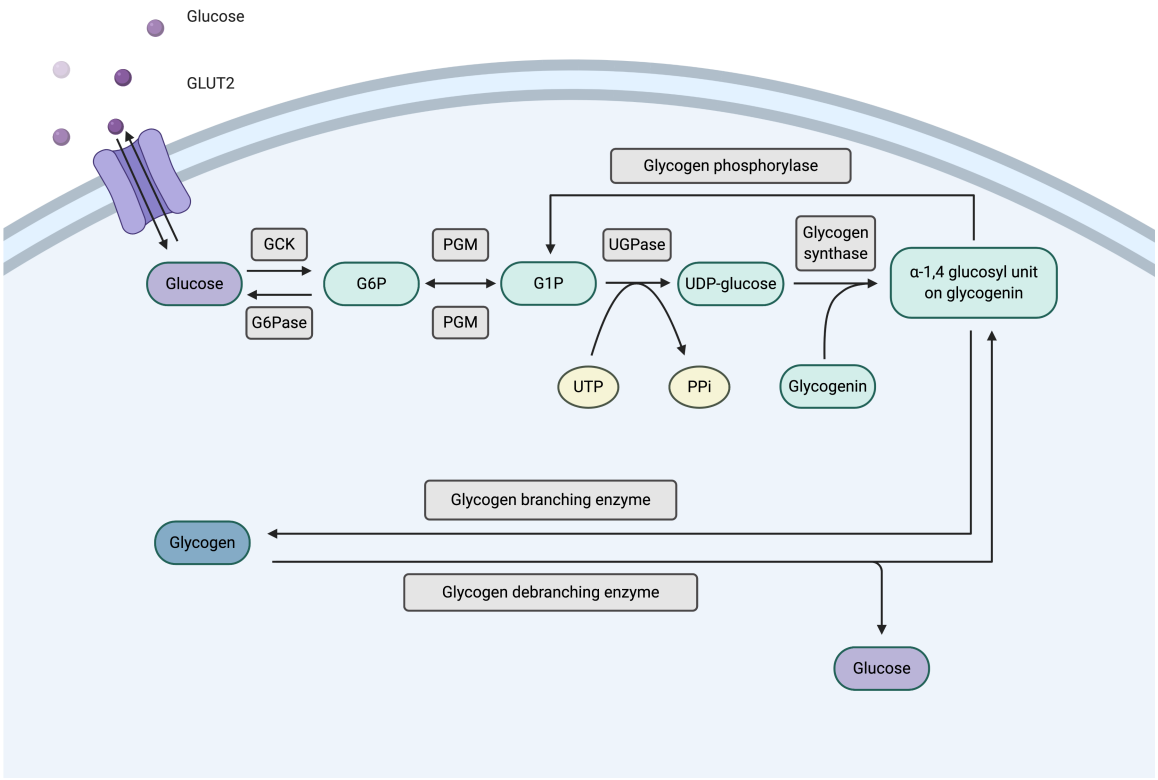

Figure 4-1. Liver glycongenesis and glycogenolysis

of glucose in the synthesis of glycogen, is produced, whose storage is closely linked with insulin action and blood glucose homeostasis.

Glucose transporter 2

Glucose transporter isoform 2(GLUT2), also known as solute carrier family 2 (facilitated glucose transporter), member 2(SLC2A2), exists mainly in the liver and pancreatic β cells. The glucose-sensing system in hepatocytes and pancreatic β cells is constituted of GLUT2 and glucokinase to instantly alter the rate of glucose into the cell. The system helps hepatocytes absorb glucose via facilitated diffusion from the high concentration side, outside the cell, to the low concentration, inside the cell, or from the low one to the high one via adenosine triphosphate(ATP) consumption. And the process does not rely on insulin. GLUT2 has a high K_m (about 15 to 20 mM) [110], which is an indicator of

the affinity as higher K_m indicates lower affinity. However, both GLUT1 that embedded in the blood-brain barrier and GLUT3 that is specifically expressed in neurons have a low K_m (around 6.9 mM and 1.8 mM respectively) [111]. It is shown that GLUT2 has a high transport capacity which exceeds the trapping response of glucokinase, resulting in the fact that the rate-limiting step for glucose uptake in hepatocytes and beta cells is the phosphorylation of glucose, not the glucose during GLUT2 transport [112]. Interestingly, it has been identified in a tamoxifen-dependent recombination system(LG2KO mice) that the inactivation of GLUT2 in hepatocytes didn't have an influence on the glucose homeostasis though with suppression of hepatic glucose uptake [113]. Genome-wide association studies(GWAS) have shown that the risks of various diseases, such as T2D, hypercholesterolemia and cardiovascular diseases, increase if GLUT2 transport mutates [114]. And persons with GLUT2 variants have a preference for foods containing sugar [115].

Glucokinase

Glucokinase(GCK), a hexokinase isozyme, plays a crucial role in storing glucose as glycogen, particularly in the postprandial state. It acts as a metabolite sensors o catalyze the phosphorylation of glucose to glucose-6-phosphate(G6P), in specific the transfer of phosphate from ATP to glucose. The expression of GCK has been well justified to be controlled by a single gene with two organ-specific promoters, an active neuroendocrine promoter(NE-GCK-promoter) that controls the expression in the pancreas and brains, and an insulin-dependent promoter controls expression in liver(HEP-GCK-promoter) [116]. While hexokinase is prevalent in almost all kinds of tissue cells in the human body, especially brain cells and red blood cells, the expression of GCK is highest in the liver as well as in the pancreatic β cells [117]. Unlike hexokinase which has a high affinity to allow glucose into the cells, GCK has a low affinity which results in the minimum glucose uptake by the liver during fasting and thus avoiding the excess synthesis of glycogen, which might lead to hypoglycemia. Importantly, this reaction has been identified as the first rate-

limiting step in glucose metabolism [118].

Experiments have been conducted on GCK, which might be a new target for the treatment of T2D [119]. As early as in the 1990s, researches and discoveries discovered glucokinase activators (GKAs) as a new class of potentially antidiabetic drugs. GKAs, nonessential allosteric activators, can increase glucose affinity as well as the maximal velocity of GCK, which is the velocity when the enzyme's active sites are all saturated with substrates, which then promote liver glucose metabolism. Although the Phase I trial of GKAs seemed to work well in T2D, patients with T2D were diagnosed with other diseases like hyperlipidemia and vascular hypertension and the drug didn't work after months in the new Phase II trial [120]. Even so, it is acceptable to keep an eye on researches of GKAs which might still lead to novel antidiabetic medicine.

Glucose-6-phosphate

Glucose-6-phosphate(G6P), also called the Robison ester, is a glucose sugar formed by phosphorylation of glucose via GCK on the sixth carbon, which happens immediately once glucose enters the cell. The main reason for this process is to prevent inside glucose from diffusing outside. This reaction between glucose and G6P is reversible and energy-consuming since G6P can act as a substrate for glucose-6-phosphatase(G6Pase) in the liver to be converted back into glucose, which is an important part of the glycogenolysis to release free glucose into the bloodstream.

G6P is also an important substrate for phosphoglucomutase(PGM), which is a family of enzymes that transfers a phosphate group from C-3 to C-2, to produce glucose 1-phosphate(G1P) and thus lead to interchange between not only glycogen but also galactose and uronic acid metabolism. There are four distinct isoforms of phosphoglucomutase that have a difference in proportions in different tissues. It is suggested that the enzyme PGM1 is the key regulator of cellular glucose homeostasis in human [121]. Besides, G6P can be oxidized by glucose 6-phosphate dehydrogenase(G6PD), thus beginning the pen-

tose phosphate pathway. Studies on the relationship between diabetes and G6PD have shown that patients aging from 45 to 65 years old with G6PD deficiency are more likely to have diabetes compared with the total population [122]. Besides, G6PD deficiency and T2D showed a significant relationship ($p<0.05$) in male patients while there was no significant ($p>0.05$) interaction between G6PD deficiency and T2D to influence oxidative stress in patients [123].

Glucose-1-phosphate

Glucose 1-phosphate(G1P), also known as cori ester, is a glucose molecule that formed from G6P serves as a substrate for the enzyme UTP—glucose-1-phosphate uridylyltransferase(UGPase) to produce uridine diphosphate glucose(UDP-glucose). In detail, one molecule of G1P reacts with one molecule of UTP on UDP-glucose pyrophosphorylase, and then the phosphate group on G1P exchanges the phosphoanhydride bond on UTP, thus producing one molecule of UDP-glucose and one molecule of pyrophosphate(PPi), which comes from the outer two phosphoryl residues of UTP. This reaction is readily reversible and the Gibbs Free Energy is close to zero. However, the conversion from UDP-glucose to G1P is not reversible in the human body [104], let alone in the liver. The consequence is that PPi is rapidly converted into pyrophosphatase(Pi), which is inorganic, via hydrolyzation. One molecule of PPi reacts with one molecule of water producing two molecules of Pi. Since the hydrolysis is irreversible, the general synthesis of UDP-glucose is irreversible in the hepatocytes. Also, G1P can be converted back into G6P via the enzyme PGM, which is the same enzyme catalyzing G6P to G1P. This reversible reaction plays a key role in the later included glycogenolysis. Studies on biochemistry indicate that PGM in various organisms works more efficiently on this process compared with the corresponding PGM [124].

UGPase

UTP—glucose-1-phosphate uridylyltransferase(UGPase), also known as glucose-1-phosphate uridylyltransferase, is a nucleotidyltransferase that plays a pivotal role in all organisms. It serves as an enzyme in the conversion of G1P to UDP-glucose. This enzyme is mainly controlled by the genes, which mainly include two kinds: UGP1 and UGP2. They are widely expressed in various organs but with differential expression. Although it has been demonstrated that between the two UGP1 is the predominant one in Arabidopsis [125], it is questionable whether in the liver UGP1 is still dominant. However, studies on the developmental and/or epileptic encephalopathies(DEEs) have demonstrated that the normal UGP2 is an essential gene for keeping the full expression of UGPase protein since the mutations on UGP2 can cause genetic diseases like DEEs [126].

UDP-glucose

Uridine diphosphate glucose(UDP-glucose), also known as uracil-diphosphate glucose, is a nucleotide sugar, serves as the glycosyl donor for glycogen that is formed from G1P and can then be converted into (1,4-α-D-glucosyl)$_{n+1}$ with glycogenin and glycogen synthase, which produce the byproduct of uridine diphosphate(UDP) via the enzyme glycogen synthase. In detail, glucose moiety of glycogen molecule, which is added to tyrosine residues of glycogenin, can form a 1-4 glycosidic linkage with UDP-glucose bound to glycogen synthase at the nonreducing end of core glycogen, and then free UDP is released, which leads to the generation and elongation of glycogen when each reaction adds one glucose moiety to glycogen. Therefore, UDP-glucose is the scaffolding protein in glycogen synthesis.

In experiments, UDP-glucose flux is widely measured as an indicator of glycogenesis [127][128]. It has been shown that UDP-glucose flux is approximately 35% lower in patients with T2D compared with nondiabetic ones [128], which leads to the conclusion that

hepatic glucose metabolism is impaired in people with T2D.

Glycogenin

Glycogenin, a protein and an enzyme, acts as a primer molecule in self-glucosylation to catalyze the position change of glucose residues from UDP-glucose to it. Besides, the self-glucosylation includes the modification of one subunit by the other, by which the enzyme glycogenin can elongate various enzymes like glycogen synthase as a substrate [129]. There are two types of glycogenin in human, type 1, and type 2. While type 2 is mainly expressed in the liver as well as the pancreas, type 1 is commonly expressed [130]. It has been demonstrated that overexpression of type2 causes the increase of glycogen in mice cells [130]. Besides, UDP-glucose also serves as a key intermediate in many other metabolisms like the biosynthesis of starch [131].

Glycogen synthase

Glycogen synthase(GYS), also called UDP-glucose-glycogen glucosyltransferase, serves as the rate-limiting enzyme in glycogenesis, which catalyzes UDP-glucose into α-1,4 glucosyl unit on glycogenin with the byproduct of UDP. The regulation of the function of glycogen synthase is via phosphorylation and dephosphorylation.

When the dephosphorylation of glycogen synthase occurs, the activity of glycogen synthase is enhanced, and thus glycogen synthesis increases. The phosphatases protein phosphatase-1(PP1) catalyzes the dephosphorylation of glycogen synthase. This process is insulin-dependent, yet the detailed signalings between PP1 and insulin remain unclear. Besides, the dephosphorylation of glycogen phosphorylase, which results in its inactivity, is also catalyzed by PP1. The specific glycogen targeting subunits of PP1 varies when it functions, like PP1R3A for glycogen in mice skeletal muscle. It has been shown that the mutations of this gene, which results in mutations of the relevant regulatory subunit of PP1, account for obese mice and its glucose intolerance as well as insulin

resistance [132]. Although it is concluded in some studies, it can be concluded that PP1 is a possible target for T2D [133].

On the other hand, the phosphorylation of glycogen synthase inhibits glycogen synthase activity, and thus glycogenesis decreases. GSK3 is one of the enzymes that phosphorylate glycogen synthase and PP1 as well, leading to an increase in glycogen. As described in Chapter 1, GSK3 regulated via inhibitory phosphorylation by active Akt can phosphorylate glycogen synthase 2(GYS2) in specific. Besides, GSK3 can be regulated through the other two pathways. First, PKA, which is known to be involved in hepatic lipid metabolism and formed from adenylate cyclase regulated by adrenaline and forskolin, can suppressively phosphorylate GSK3 [134]. Second, PKC formed from phospholipase C(PLC) regulated by lysophosphatidic acid(LPA) can suppressively phosphorylate GSK3 [135].

Apart from the phosphorylation pathways above, glycogen synthase can also be regulated by G6P via allosteric control. Interestingly, mutations in glycogen synthase can lead to hypoglycemia in infancy [136]. And it has been indicated that GSK3 is a possible target for the novel treatment of T2D according to the fact that inhibition of GSK3 improves insulin and glucose metabolism in human skeletal muscle [137].

Glycogen synthase is the rate-limiting enzyme for glycogen synthesis [138]. GYS2 is a key glycogen synthase enzyme in liver glycogenesis. There are probably more than nine sites of phosphorylation on glycogen synthase. It has been identified that Arg 582 is a key residue on GYS2 to be phosphorylated or dephosphorylated [79]. Further experiments on GYS2 Arg582Ala knockin (+/R582A) mice model have shown the impaired activation of GYS2, modest glucose intolerance, and significantly reduced glycogen formation with feeding or glucose load [79].

Glycogen branching enzyme

Glycogen branching enzyme(GBE), also known as 1,4-alpha-glucan-branching enzyme, serves as a catalyst in the final step of glycogen formation. In detail, it works by chang-

ing the short glycosyl chain from α-1,6 glycosidic bonds to peripheral chains of nascent glycogen. This process of branching can also increase the solubility and decrease the osmotic strength of glycogen, which results in a stable way of storing glucose that can be easily transported outside hepatocytes. GBE is encoded and controlled by the gene GBE1. Mutations in GBE1 gene could lead to GBE deficiency, which is referred to as glycogen storage disease type IV or Andersen's disease. It is characterized by liver cirrhosis occurring in childhood.

Glycogenolysis

When the concentration of bloodstream decreases, there is an increase in glucagon secreted by the pancreas α cells, which thus decreases the insulin secretion by the pancreas β cells due to the regulation mechanism among pancreas cells. This decrease in insulin secretion later induces a decrease in insulin signaling while the increase in glucagon enhances glucagon signaling which thus leads to the regulation of glycogen by affecting the activity of glycogen related enzymes. Under reduced insulin signaling and enhanced glucagon signaling, the glycogen breakdown pathways are enhanced, which thus plays a more major part than insulin-induced glycogen synthesis pathways in hepatocytes.

In glycogenolysis, glycogen which has branches of the glucose chain is catalyzed into α-1,4 glucosyl unit via the glycogen debranching enzyme. And then the glucosyl unit with n numbers of glucose residues is phosphorylated to remove glucose via glycogen phosphorylase, which subsequently forms glycogen with n-1 numbers of glucose residue left and G1P. While the glycogen with n-1 numbers of glucose residue can continue to be catalyzed via glycogen phosphorylase until no glucose residue left, G1P is converted back into G6P via the reaction with the enzyme PGM since this reaction is reversible as described in glycogenesis. Subsequently, G6P is dephosphorylated into glucose via G6Pase. Like glycogenesis, GLUT2 serves as a facilitated transporter to secrete glucose

into the blood.

Glycogen debranching enzyme

Glycogen debranching enzyme is a molecule that plays a key role in glycogenolysis together with glycogen phosphorylase. It cleaves α-1,6 linkages on α-1,4 glucosyl unit on glycogenin before the activity of glycogen phosphorylase, thus converting the glycogen structure from a branched one into a linear one. The process paves the way for the later catalyzation of α-1,4 glucosyl unit via glycogen phosphorylase, which can only cleave α-1,4- glycosidic bond between glucose molecule.

The process of glycogen debranching enzyme also produces a small amount of free glucose when hydrolyzing the α-1,6 linkage. It has been demonstrated that glycogen storage disease type III is the consequence of glycogen debranching enzyme deficiency, which mainly results from its gene mutations with a characteristic of low blood glucose [139].

Glycogen phosphorylase

Glycogen phosphorylase, an enzyme existing in at least one active phosphorylated form with the rest in dephosphorylated form, acts as a cleavage tool of α-1,4- glycosidic bond, which connects adjacent glucose molecules forming linkages within branched chains. This process, which leads to the conversion of α-1,4 glucosyl unit to G1P via phosphorolytic cleavage of α-1,4-glycosidic linkages, happens after the glycogen debranching enzyme takes function.

Apart from phosphorylation of glycogen phosphorylase, there are some other ways in the liver that mediate the activity of glycogen phosphorylase through allosteric ligands like adenosine monophosphate(AMP)-activated protein kinase and G6P. In detail, the activation of glycogen phosphorylase is through phosphorylation via phosphorylase kinase, which is activated via phosphorylation by cyclic adenosine monophosphate(cAMP)-dependent protein kinases(PKAs), also known as PKA signaling pathways. Unlike adrenaline

as the main activator of cAMP signaling pathways in muscle cells, glucagon is the foremost one in the liver. However, there is almost no difference in the activation of PKA which then leads to glycogen phosphorylase activation [140].

LIke PI3K, PKA is also constituted of two kinds of subunits, the catalytic subunit and the regulatory subunit. The former one acts as the site of phosphate-adding reaction while the later subunit serves as a sensor of the cAMP level inside the cell which in turn switches the catalytic subunits on or off to mediate the activity of PKA [141]. In specific, when cAMP level increases, it binds to the regulatory subunits of the inactive PKA, and thus leads to releasing the activated catalytic subunit through a conformational change of the regulatory subunits. And when cAMP level is below the normal level, two copies of the catalytic subunits are bound to a dimer of the regulatory subunits, and thus results in storing the inactive PKA. After PKA is activated, it then phosphorylates glycogen phosphorylase b (PYGb) and converts it into phosphorylase a(PYGa), which is an active form of PYGb, thus leading to the enhancement of glucose formation.

Also, there are three isoforms of the catalytic subunit and four types of the regulatory subunit: α, β and γ; type I α, type I β, type II α, type II β. And it has been identified that the null-mutation of the catalytic subunit β gene leads to a decreased accumulation of visceral fat in mice fed a high caloric diet [142].

Experiments have shown that glycogen phosphorylase deficiency in the liver accounts for glycogen storage disease type VI while the deficiency in the muscle is the cause of glycogen storage disease type V, which shows intolerance to exercise [143].

G6Pase

Glucose-6-phosphatase(G6Pase), also known as d-Glucose-6-phosphate phosphohydrolase, is an enzyme mainly found in the liver and kidneys, which serves as a key enzyme to dephosphorylate G6Pase to glucose. Liver G6Pase is an integral membrane protein with its active site exist inside the lumen of the endoplasmic reticulum. It contains multi-

components, which is constituted of two units: one catalytic unit and a G6P-specific bidirectional translocase(G6PT). The latter one serves as a regulatory part in the activation of G6Pase to mediate the binding of G6P to the catalytic unit of G6Pase. Like previous descriptions of deficiency of the glucose metabolism enzyme, the G6Pase deficiency, also known as glycogen storage disease type I or Von Gierke disease, can also result in the reduced formation of glucose into the bloodstream, and thus leads to hypoglycemia. Recent researches have identified that G6Pase deficiency could affect the brain and thus lead to carbohydrate and fatty acid metabolism disorders [144]. It has been suggested that the increased content of G6Pase inside cells might explain the increased production of endogenous glucose. [145] It is consistent with the experiment on the relationship between rates of endogenous glucose and the net flux of G6Pase in T2D, which have concluded that the increased G6Pase flux can increase the rates of endogenous glucose production [146].

Gluconeogenesis

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors such as lactate, pyruvate, amino acids as well as glycerol. It plays a key role in maintaining blood glucose levels under fasting and especially under starvation when dietary intake is insufficient since normal concentrations of blood glucose are important to support the brain and erythrocytes. Otherwise, higher or lower levels can lead to brain dysfunction. Recent studies have suggested that the lack of glucose might have a relationship with cognitive impairment diseases like Alzheimer's and dementia [147]. Although gluconeogenesis mainly occurs in the liver, it also occurs in the kidney and some small intestines, which acts as a crucial gluconeogenesis organ in liver failure.

Among those main gluconeogenic precursors, glycerol is the one that will be described in detail below. Glycerol acts as a key polyol compound, which is responsible for fatty acid

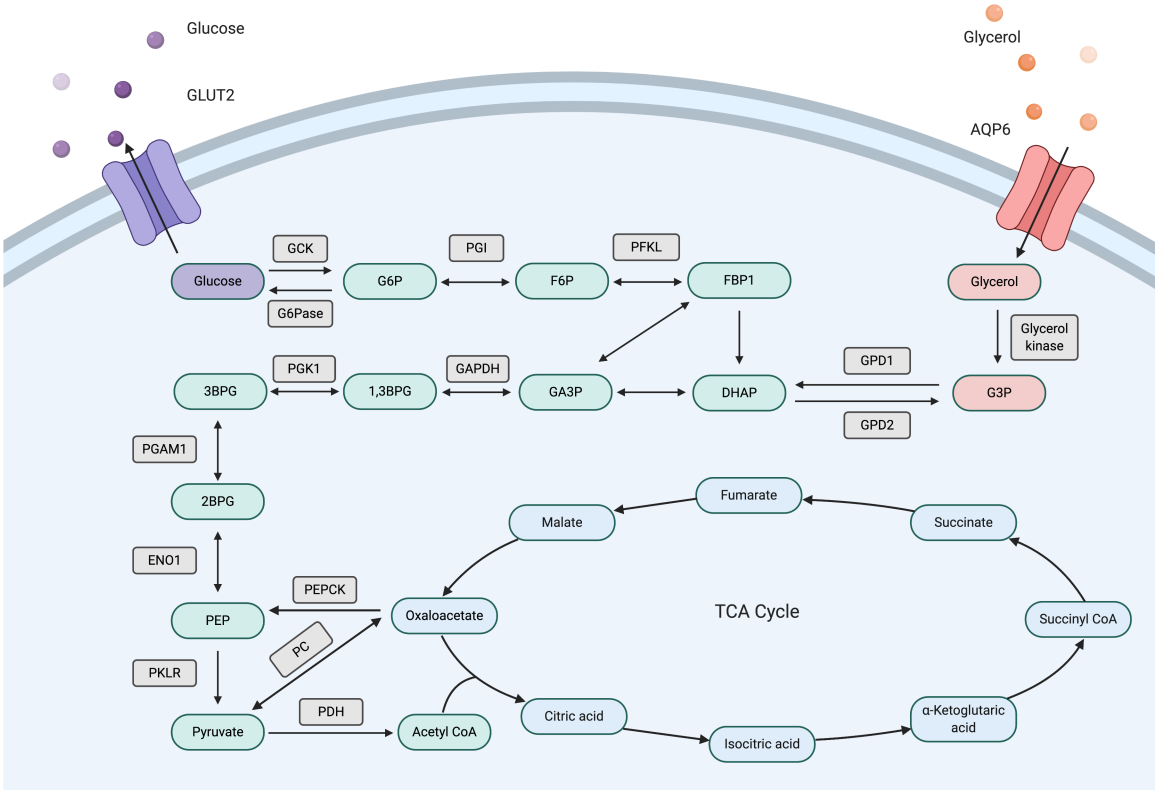

Figure 4-2. Liver gluconeogenesis

metabolism in the formation of lipoprotein such as very low density lipoprotein(VLDL), one of the three main types of lipoproteins.

Gluconeogenesis signaling pathway

The release of glucose in gluconeogenesis is directly through the conversion of G6P to glucose via the enzyme G6Pase. As the reaction in the glycogen synthesis and breakdown, this process is reversible since glucose is convertible into G6P via GCK, and then it can be secreted into the bloodstream from the hepatocytes via GLUT2 or be facilitated in other intracellular metabolic processes. While G6P is formed from fructose-6-phosphate(F6P) via glucose-6-phosphate isomerase(PGI), PGI also acts as the enzyme to catalyze G6P to F6P unlike glycogenesis, in which PGM which catalyzes G6P to G1P. This is also a reversible process.

F6P is formed from the dephosphorylation of fructose-1,6-bisphosphate(FBP1) via 6-phosphofructokinase liver type(PFKL), and also F6P can be phosphorylated into FBP1 by PFKL. It is reported that F1,6BPase has two isoforms: F1,6BPase1 and F1,6BPase2 which is formed inside the muscle [148][149]. And including PFKL, the enzyme phosphofructokinase also has two isoforms: phosphofructokinase, platelet(PFKP) in fibroblasts and 6-phosphofructokinase, muscle type(PFKM) in muscle.

Subsequently, FBP1 can be converted into dihydroxyacetone phosphate(DHAP) or glyceraldehyde 3-phosphate(GA3P) via aldolase reaction. Also, GA3P can be converted back into FBP1 via aldolase. Here, aldolase is a cytoplasmic protein and also belongs to class I proteins. There are three forms that have been found in human: aldolase A that is preferentially expressed in muscle and brain [150], aldolase B which is expressed in liver, kidney, and enterocytes, and aldolase C expressed only in the brain. There is also a reversible reaction between GA3P and DHAP through the use of triose isomerase(TMI). Especially, DHAP can be formed from glycerol, which is the process to get glycerol involved in gluconeogenesis thus leading to the function of glycerol in liver glucose metabolism.

Via the enzyme glyceraldehyde 3-phosphate dehydrogenase(GAPDH), GA3P is convertible from and to 1,3-bisphosphoglycerate(1,3BPG). This is a two-step conversion and both of the two are reversible via GAPDH [151]. The first step is the oxidation of GA3P at its position-1, in which an aldehyde is converted into a carboxylic acid, generating D-glycerate 1,3-bisphosphate. The second step is the exergonic oxidation to form 1,3BPG from D-glycerate 1,3-bisphosphate, which requires a supply of nicotinamide adenine dinucleotide(NAD) plus hydrogen(H), which is referred to as NADH in short.

Then, 1,3BPG is formed from the 3-phosphoglyceric acid(3PG) via the catalyst phosphoglycerate kinase 1(PGK1). In turn, 1,3BPG can be converted back into 3PG via the same enzyme PGK1. This reversible reaction requires energy from ATP. In human, there are two types of PGK which have been identified: PGK1 and PGK2. The former is widely expressed in all human cells [152].

Also, 3PG can be produced from 2-Phosphoglyceric acid(2PG) via the catalyzation of phosphoglycerate mutase (PGAM) to transfer the phosphate group from C2 to C3, which thus results in the conversion of 2PG into 3PG. This process is also reversible via PGAM. In human, there are two isoforms of PGAM: PGAM1 which is expressed in the liver, and PGAM2 in the muscle [153].

Then enolase(ENO) functions as a catalyst to convert phosphoenolpyruvic acid(PEP) into 2PG, which is reversible via the same enzyme. In human, there are three isoforms of enolase: ENO1, ENO2, and ENO3 [154]. ENO1, also called alpha-enolase, is widely expressed in cells. And ENO2, also known as γ-enolase, is found in mature neurons and neuronal origin cells. ENO3, also named β-enolase, is found in skeletal muscle cells of adults.

PEP then can be converted into pyruvate via pyruvate kinase and this reaction is not reversible. In human, pyruvate kinase can be divided into two types: pyruvate kinase isozymes R/L(PKLR) pyruvate kinase isozymes M1/M2(PKM2). The former is active in the live and red blood cells while the latter is expressed in differentiated tissues like the pancreas.

With the use of pyruvate carboxylase (PC), pyruvate subsequently is converted into oxaloacetate, which can be catalyzed into PEP via phosphoenolpyruvate carboxykinase(PEPCK) with the carbon dioxide byproduct.

Glycerol in gluconeogenesis

Glycerol is passively transported into hepatocytes by aquaporin-9(AQP9) embedded in the plasma membrane of hepatocytes. Once inside hepatocytes, glycerol is converted into glycerol-3-phosphate(G3P) via glycerol kinases, which is an energy-consuming process. Following the formation of G3P, G3P then is oxidized to dihydroxyacetone phosphate(DHAP) by glycerol-3-phosphate dehydrogenase 1(GPD1) that exists in the mitochondrial inner membrane space or cytosol, which requires a energy supply of NADH. Be-

side GPD1, there is also another glycerol-3-phosphate dehydrogenase, named mitochondrial glycerol-3-phosphate dehydrogenase(GPD2), embedded in the outer surface of the inner mitochondrial membrane. It serves as an enzyme to catalyze the conversion of G3P to DHAP with the oxidation of flavin adenine dinucleotide(FAD) to $FADH_2$(hydroquinone form of FAD). The GPD1 and GPD2 reactions with glycerol are referred to as the glycerol phosphate shuttle.

Other glucose signalings

Apart from glycogenesis, glycogenolysis, and gluconeogenesis, there are other important liver glucose signalings, which make the actual liver glucose metabolism more complex [155]. The general liver glucose also includes the involvement of the TCA cycle(tricarboxylic acid cycle) or CAC(citric acid cycle) or the Krebs cycle, which is a series of chemical reactions used to release energy through the oxidation of acetyl-CoA.

As described in the section of gluconeogenesis, pyruvate is converted into oxaloacetate. Beside, pyruvate can also be catalyzed to acetyl coenzyme A(acetyl-CoA) by pyruvate dehydrogenase(PDH), which requires a supply of NADH. Acetyl-CoA then reacts with oxaloacetate and water through citrate synthase to produce citrate and coenzyme A(CoASH). The product citrate is subsequently dehydrated into cis-aconitate and water through aconitase. Aconitase also catalyzes the hydration of cis-aconitate to D-isocitrate. Both of the two reactions are reversible isomerization. D-isocitrate afterward is oxidized to oxalosuccinate by isocitrate dehydrogenase, which is a process generating NADH as a storage of energy. Then the decarboxylation of oxalosuccinate via isocitrate dehydrogenase produces α-ketoglutarate and carbon dioxide. α-ketoglutarate later can be converted into succinyl-coenzyme A(succinyl-CoA) via α-ketoglutarate dehydrogenase with the generation of NADH. Succinyl-CoA synthetase catalyzes succinyl-CoA into succinate with the generation of ATP or GTP. Through succinic dehydrogenase, succinate is oxidized into fu-

marate, which then can be hydrated into malate via fumarase. The hydration of fumarate is to hydrate the double carbon bond. Afterward, malate is catalyzed into oxaloacetate through the oxidation via malate dehydrogenase. This is a reversible conversion, which generates NADH. Oxaloacetate then can be converted into acetyl-CoA, which restarts the cycle.

Amino acids as one of the major precursors of gluconeogenesis involve liver glucose metabolism through the metabolism of amino acids. Specifically, amino acids supply pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate. Besides, fatty acids can also get involved in glucose signaling through fatty acid degradation through oxidation including β oxidation and peroxisomal β oxidation, as another precursor of gluconeogenesis [156]. In detail, the degradation of fatty acids forms acetyl-CoA while citrate is facilitated to synthesize fatty acid, which will be described in the chapter of the fatty acid signaling pathway.

Chapter 5

Liver lipoprotein signaling pathway

Fatty acid metabolism, also known as lipid metabolism, is a series of catabolic and anabolic processes involving fatty acids (FAs). Fatty acids have a long aliphatic chain with a carboxylic acid group at one end. Fatty acids usually are present in humans as esters in mono-, di-, tri-glycerides(MAG, DAG, TAG), phospholipids, and cholesterol esters(CE). Fatty acids play important roles in providing energy through fatty acid oxidation and cellular membranes [157]. Unsaturated fatty acids, particularly Omega-3 and Omega-6 fatty acids, help to ensure fluidity, flexibility, and permeability, of cell membranes. This affects molecule transport into and out of cells especially the passive transport through the membrane. Fatty acid metabolism includes both fatty acid synthesis and fatty acid beta oxidation.

Another important pathway involving fatty acids in the liver is the formation of very-low-density lipoprotein micelles (VLDLs), which transport hepatic lipids to peripheral tissues. This process is related to diacylglycerol (or diglyceride) (DAG), which is important in the connection between lipoprotein signaling and insulin signaling.

In patients with T2D, it has been suggested that there is a disturbance in fatty acid metabolism accompanied by lower glucose intolerance [158]. In addition, the increase in fatty acid concentrations may be responsible for impaired insulin-mediated glucose metabolism, increased rate of hepatic glucose production, inhibition of insulin secretion, hyperlipidemia, and hypertension [159]. Furthermore, fatty acid may account for the apop-

tosis in the pancreas β cells [160]. Studies provide evidence for the possible explanation of lipotoxicity to T2D [161].

Fatty acid synthesis

There are various types of fatty acids involved in daily life. For instance, animal fat and coconut oil mainly contain unsaturated fatty acids. The monounsaturated fatty acid makes up about 92% and 75% in algal oil and olive oil, respectively [162][163]. There are also polyunsaturated fatty acids, such as omega-3 fatty acids from fish oils and grass-fed beef, omega-6 fatty acids from fast foods. Besides, trans fatty acids are the ones that the human body has difficulty metabolizing. They come from the polyunsaturated oils that have been industrially hydrogenated. However, unlike industrially synthesized fatty acids [164], in the human body fatty acid synthesis, which is also known as lipogenesis, is an anabolic pathway whereby fatty acids are formed from acetyl-CoA and NADPH.

Almost all fatty acids can be synthesized inside the human body and absorbed through diet. However, essential fatty acids, which can not be formed by humans, have to be absorbed from the diet such as linoleic acid and alpha-linolenic acid [165]. Besides, there are two other types of division of fatty acids in human: saturated fatty acids and unsaturated, straight-chain fatty acids and branched-chain fatty acids. The unsaturated fatty acids are as common in the bilayer interior of membranes as saturated fatty acids. The branched-chain fatty acids are usually saturated and most often found in bacteria and in human infants [166]. Therefore, saturated straight-chain fatty acids and unsaturated straight-chain fatty acids signalings are concluded here.

Since saturated fatty acid is formed before the formation of unsaturated fatty acids could happen, saturated fatty acid synthesis is described first, in specific saturated straight-chain fatty acid synthesis [167]. It occurs both in the cytoplasm and endoplasmic reticulum of the cell. In detail, acetyl-CoA is catalyzed by the enzyme acetyl-CoA carboxylase

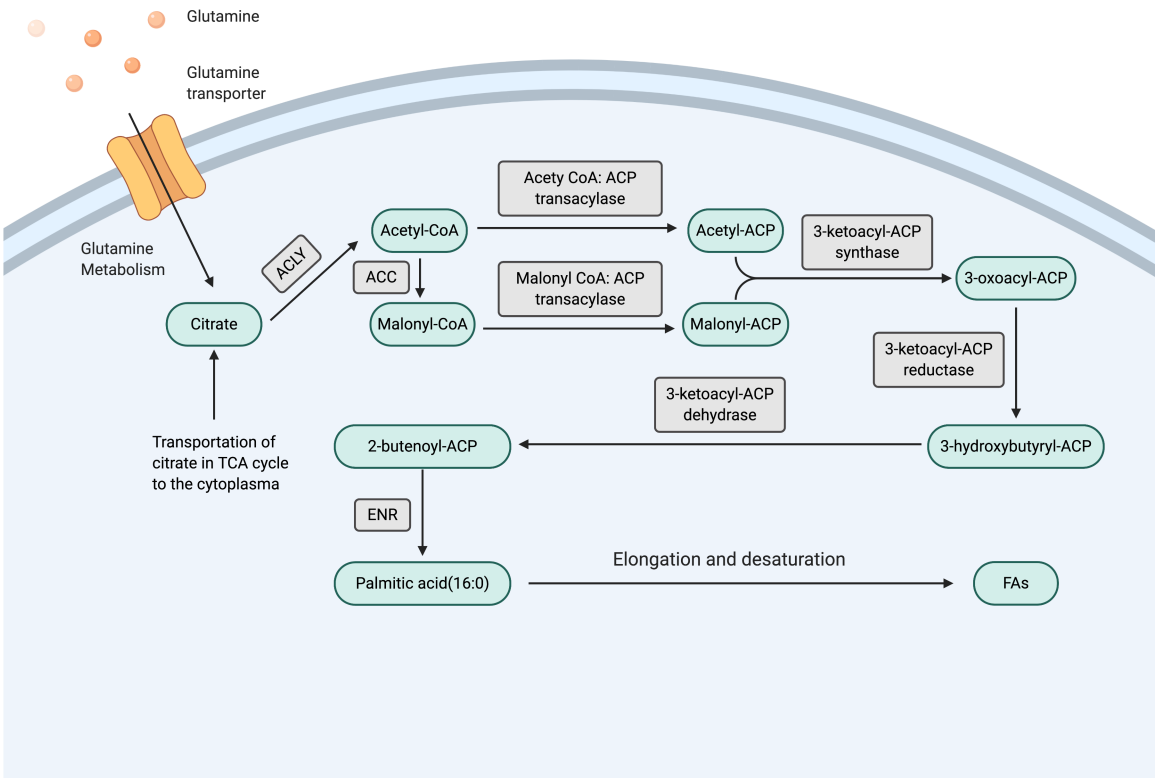

Figure 5-1. Liver fatty acid synthesis

(ACC) to form malonyl-CoA. Then both acetyl-CoA and malonyl-CoA can be converted into acetyl-ACP and malonyl-ACP, respectively. Those two molecules are then catalyzed together into a fatty acyl-ACP, acetyl-ACP here. Acetyl-ACP is subsequently converted into a 16:0 carbon fatty acid, which then undergoes various modifications including desaturation and elongation to form longer or shorter, saturated, or unsaturated fatty acids.

Saturated straight-chain fatty acid synthesis

The first step for hepatic saturated straight-chain fatty acid synthesis is the conversion of cytoplasmic citrate to cytoplasmic acetyl-CoA via the enzyme ATP-citrate lyase(ACLY). Importantly, the cytosolic citrate, which is an intermediate substance in TCA cycle, can be transported from the mitochondria to the cytoplasm. The other source of cytoplasmic citrate is through glutamine metabolism, in which glutamine is catalyzed into citrate via a

series of biological processes [168]. Furthermore, this step requires a supply of energy from ATP, and also forms oxaloacetate that can not exchange with the one in TCA cycle. The acetyl-CoA, on the one hand, is activated to acetyl-ACP by the enzyme Acetyl CoA:ACP transacylase with CoA portions replaced by acyl-carrier protein(ACP). On the other hand, acetyl-CoA is carboxylated to malonyl-CoA by the enzyme acetyl-CoA carboxylase(ACC). There are two isoforms of ACC: ACC1(also known as ACC-α, ACACA) and ACC2(also known as ACC-β, ACACB). ACC1 has been identified as the enzyme for fatty acid synthesis while ACC2 might be involved in the regulation of fatty acid oxidation. Also, the resulting malonyl-CoA is activated to malonyl-ACP by the enzyme Malonyl CoA:ACP transacylase like the conversion of acetyl-CoA to acetyl-ACP.

Then acetyl-ACP and malonyl-ACP are joined to react via the enzyme 3-ketoacyl-ACP synthase. The resulting 3-oxoacyl-ACP, with the byproduct carbon dioxide, is subsequently catalyzed to 3-hydroxybutyryl-ACP via the enzyme 3-ketoacyl-ACP reductase, in which the carbon 3 ketone is reduced to a hydroxyl group. And this process requires a supply of NADPH. Afterward 3-hydroxybutyryl-ACP is catalyzed to 2-butenoyl-ACP via 3-Hydroxyacyl ACP dehydrase, and then 2-butenoyl-ACP can be condensed to palmitic acid (16:0)—a basic 16'carbon saturated fatty— via the enzyme enoyl-ACP reductase(ENR) and thioesterase in order. Then through further elongation and desaturation of palmitic acid, it can form more complicated fatty acids like docosahexaenoic acid(DHA).

Interestingly, all the enzymes, which are involved in the catalyzation from acetyl-CoA to the palmitic acid except the one catalyzing the conversion of acetyl-CoA to malonyl-CoA, are in fact different structures of one multi-enzyme protein, fatty acid synthase(FASN) [169]. It has been identified in animals yet in bacteria, these reactions occur on separate enzymes [170].

Studies have indicated that blood cholesterol levels decrease when saturated fatty acids are absorbed less [171]. Furthermore, it has been suggested that a high intake of saturated fatty acids increases the risk of T2D [172]. However, the reverse relationship is

identified in some experiments [173]

Unsaturated straight-chain fatty acid synthesis

Unsaturated fatty acids, which contain at least one double bond within the chain, are a component of phospholipids in cell membranes. The double bond is responsible for the decrease in the stability of unsaturated fatty acid compared with saturated fatty acid. They serve as a contributor to membrane fluidity, Generally, unsaturated fatty acids are predominant ones, especially in animals occupying cold environments [174]. And all of the unsaturated fatty acids, which can be synthesized in the human body, have double bonds within carbon 9 [175]. It suggests the explanation for the synthase deficiency of essential fatty acids, which is that the human body lacks the enzymes required to introduce double bonds beyond carbon 9.

Unsaturated fatty acids are typically synthesized via the enzyme fatty acid desaturase through a dehydrogenation reaction called desaturation. The enzyme exists in the endoplasmic reticulum, which also requires oxygen and either NAD^+ or NADPH. Inside the body, after saturated straight-chain fatty acids are formed through the process of saturated straight-chain fatty acid synthesis as described above, they can then be desaturated into unsaturated straight-chain fatty acids through aerobic desaturation. The process, which involves saturated fatty acid, oxygen, nicotinamide adenine dinucleotide phosphate hydrogen, and cytochrome$_b$, is to oxidize single carbon bonds into double bonds and is catalyzed by enzymes named desaturases.

It has been suggested in some experiments that when saturated fat is replaced with polyunsaturated fatty acids in diet, blood cholesterol decreases [176]. And it has been identified that there are beneficial effects of both marine and vegetable-derived PUFAs on glycemic control in people with T2D [177].

Fatty acid degradation

Fatty acid degradation, also known as lipolysis, is the process that fatty acids are broken down into acetyl-CoA. It includes the activation of fatty acids and transport into peroxisomes or mitochondria as well as β-oxidation. There are two kinds of oxidation: mitochondrial β-oxidation and peroxisomal β-oxidation. They depend on the length of the fatty acid chains [178]. When fatty acid chains are too long to be converted by mitochondria usually with carbon chain length no less than 22, fatty acids will be transported into peroxisomes to perform peroxisomal β-oxidation. While peroxisomal β-oxidation happens inside peroxisomes, mitochondrial β-oxidation occurs via the mitochondrial trifunctional protein, an enzyme complex associated with the inner mitochondrial membrane. Short-chain fatty acids(SCFAs)(carbon chain no larger than 5) and medium-chain fatty acids(MCFAs)(carbon chain between 6 to 12) are supposed to enter mitochondria by diffusion before being activated, while long-chain fatty acids(LCFAs)(carbon chain between 13 to 21) and very-long-chain fatty acids(VLCFAs) have to be activated outside peroxisomes or mitochondria before transported into organelles respectively. Therefore, like in peroxisomes, the differences in the steps for fatty acids with different chains in mitochondria are the order between transport and the activation of fatty acids [179].

Furthermore, there are three major sources for hepatic fatty acids: dietary lipids, fatty acids secreted by adipose cells and fatty acids synthesis inside hepatocytes [180]. Dietary lipids are the major source after a meal, which are hydrolyzed in the lumen and then absorbed by intestines as lipoproteins via re-esterification. Adipose cells and muscles are the main places to uptake lipoproteins under this circumstance while the rest goes to the liver. Also, there is intracellular synthesis of fatty acids and secretion of very low density lipoprotein(VLDL) which is transported into muscle. Under the state of fasting, adipose cells are the major source of providing the liver fatty acids whereas hepatic synthesis also exists and the liver also releases VLDL for muscle.

Mitochondrial β-oxidation

Mitochondrial β-oxidation occurs in the mitochondrial matrix. However, unlike SCFAs and MCFAs, LCFAs can not be transported through organelle membranes, thus LCFAs have to be activated before getting into mitochondria whereas SCFAs and MCFAs are activated after getting into mitochondria by diffusion. Except for the order of activation and transport, Mitochondrial β-oxidation of fatty acids can be generalized as below.

For the activation of fatty acid, it is first converted into acyl-CoA via acyl-CoA synthetases, and then the resulting acyl-CoA will be catalyzed to acylcarnitine by carnitine palmitoyltransferase 1(CPT1). CPT1 is an integral membrane protein, which is embedded in the outer mitochondrial membrane. It serves to translocate acylcarnitine, which is formed by the activation of fatty acids, from the cytosol to the mitochondrial matrix. It has been shown that CPT1 can be inhibited by malonyl CoA while the mechanism of this action remains unknown [181]. However, studies have shown that the expression of CPT1 in liver cells and muscle cells differ using inhibitor constants for malonyl-CoA as an indicator [182].

After acyl-CoA is activated, the mitochondrial carnitine/acylcarnitine carrier (CACT) promotes the transport of carnitine, which is also the other substrate in CPT1 catalyzed reaction, and acylcarnitines, from the outside mitochondria to the inside mitochondria. The process is passive transport as part of the carnitine shuttle system. The deficiency of CACT results in the accumulation of fatty acid in muscles and liver and hypoglycemia [183]. Importantly, for SCFAs and MCFAs, this step occurs before acyl-CoA is activated. Afterward, acyl-CoA in the cytosolic of mitochondria is formed via CPT2 from the acylcarnitine transported inside the mitochondrial, resulting in the conversion of acylcarnitine to acyl-CoA.

Then the β oxidation inside mitochondria starts. First, the cytosolic acyl-CoA can be oxidized into trans-Δ^2-enoyl-CoA(Δ^2 designates the position of the double bond) through the enzyme acyl-CoA dehydrogenases(ACADs). It is a dehydrogenation, accompanied

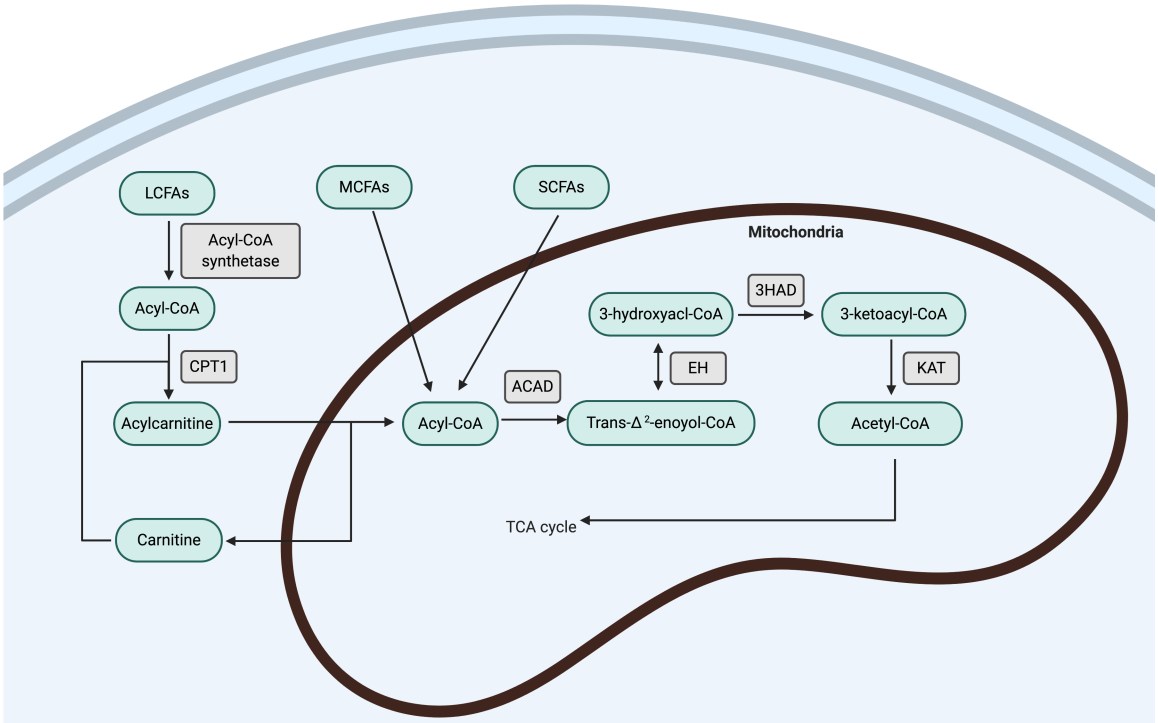

Figure 5-2. Liver fatty acid degradation in mitochondria

by energy generation of FAD to $FADH_2$. In detail, the enzyme catalyzes the formation of a double bond between carbons. Besides, it has been identified that there are 9 major kinds of ACAD enzymes and five of them are involved in β oxidation like very-long-chain acyl-CoA(VLCAD) [184]. And it has been suggested that ACAD9 is highly expressed in the human brain [185].

Then enoyl-CoA hydratases(EHs) catalyze the resulting enoyl-CoA into L-β-hydroxyacyl-CoA(also known as 3-hydroxyacyl-CoA) with the hydration of the double bond, which formed in the previous step. This reaction is reversible. Mitochondria contain different EHs such as the short-chain enoyl-CoA hydratase and long-chain enoyl-CoA hydratase [186], which hydrolyze SCFAs and LCFAs respectively. The former enzyme shows high activity with the C4:1 substrate while the latter enzyme with C16:1.

Subsequently, 3-hydroxyacyl-CoA is then oxidized into 3-ketoacyl-CoA via 3-hydroxyacyl-

CoA dehydrogenases(3HADs), in which the hydroxyl group is converted into the keto group. It is oxidation with energy generation in the form of NADH.

Afterward, 3-hydroxyacyl-CoA is catalyzed by 3-ketothiolase(KAT) into acetyl-CoA and shortened acyl-CoA, in which the thiol group is inserted between C2 and C3. Especially for mitochondrial oxidation, cytosolic acetyl-CoA can also involve in TCA cycle, thus affecting glucose metabolism [187].

Peroxisomal β-oxidation

Generally, reactions of mitochondrial β-oxidation and peroxisomal β-oxidation are the same, except the transporter, some of the involved enzymes and the formation of H_2O_2, which are marked in red in Figure 5-3.

Unlike other fatty acids that are transported by diffusion or via CACT into mitochondria,

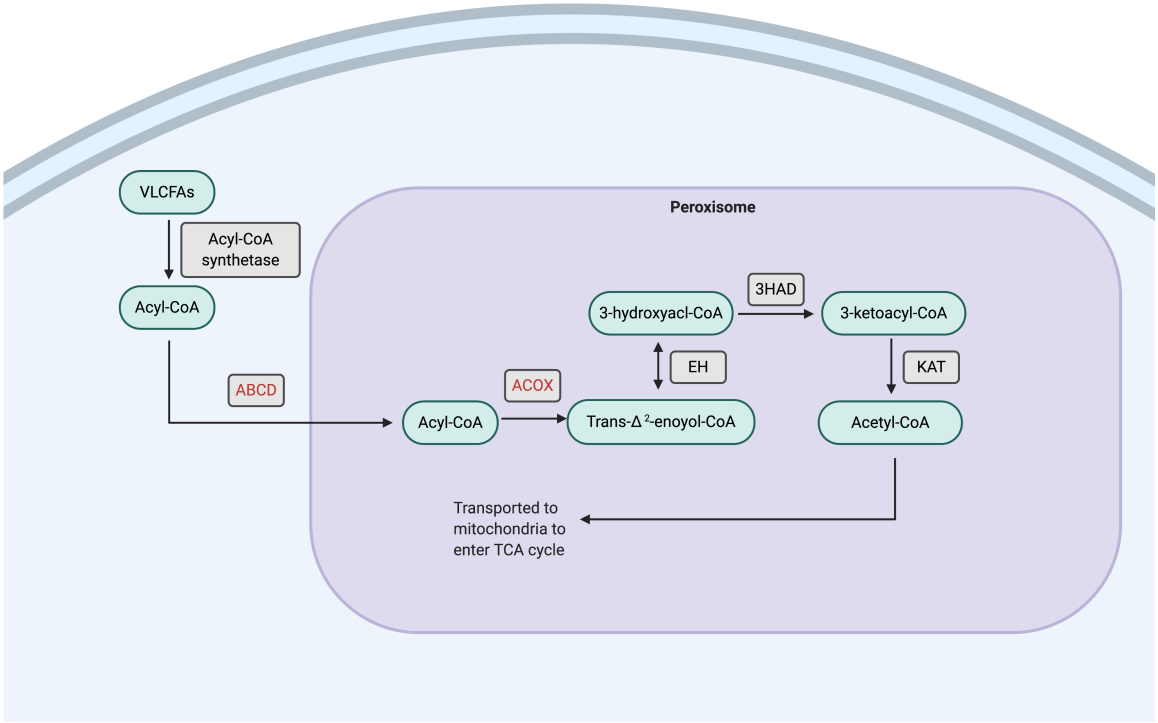

Figure 5-3. Liver fatty acid degradation in peroxisome

VLCFAs are transported into the peroxisome via the transporter ABCD(ATP-binding cassette subfamily D), which is expressed exclusively in the peroxisome. Also, like LCFAs, VLCFAs are activated before being transported.

Furthermore, the enzyme catalyzing the oxidation of the cytosolic acyl-CoA is acyl-CoA oxidase(ACOX) in peroxisome whereas ACAD in mitochondria. The difference is that in ACOX the electrons from FAD are transferred directly to O_2 thus forming H_2O_2, which results from the bonds of FAD to its α-helical domain, while ACAD transfer electrons to respiratory chain through electron transferring flavoprotein [188].

Lipoprotein signalings

Lipoproteins are a biochemical assembly, which enables the transport of triglycerides(TAG, also known as triacylglycerol or TG) and cholesterol from cell to cell through the water-based solution of the bloodstream. There are mainly five types of lipoproteins in the human: very-low-density lipoproteins(VLDs), low-density lipoproteins(LDLs), intermediate-density lipoproteins(IDLs), high-density lipoproteins(HDLs), and chylomicrons. Among the five, VLDLs, which are newly synthesized and secreted by the liver, carry 90% of the serum TGs in the fasting state [189]. And then HDLs, IDLs, and LDLs act as intermediates to promote the maturity of VLDLs in the plasma and interact with peripheral cells directly to transport TAG and cholesterol [190]. It has been suggested that insulin resistance increases hepatic lipase activity such as increased fatty acid flux to cells [191].

VLDL synthesis and secretion

The synthesis of VLDL can be divided into two steps including the formation of pre-VLDL and the assembly of nascent VLDL. The first step occurs in the rough endoplasmic reticulum lumen, in which apolipoprotein B-100(apo B) is translated and then assembled together with TAG by the microsomal triglyceride transfer protein (MTP). Especially the in-

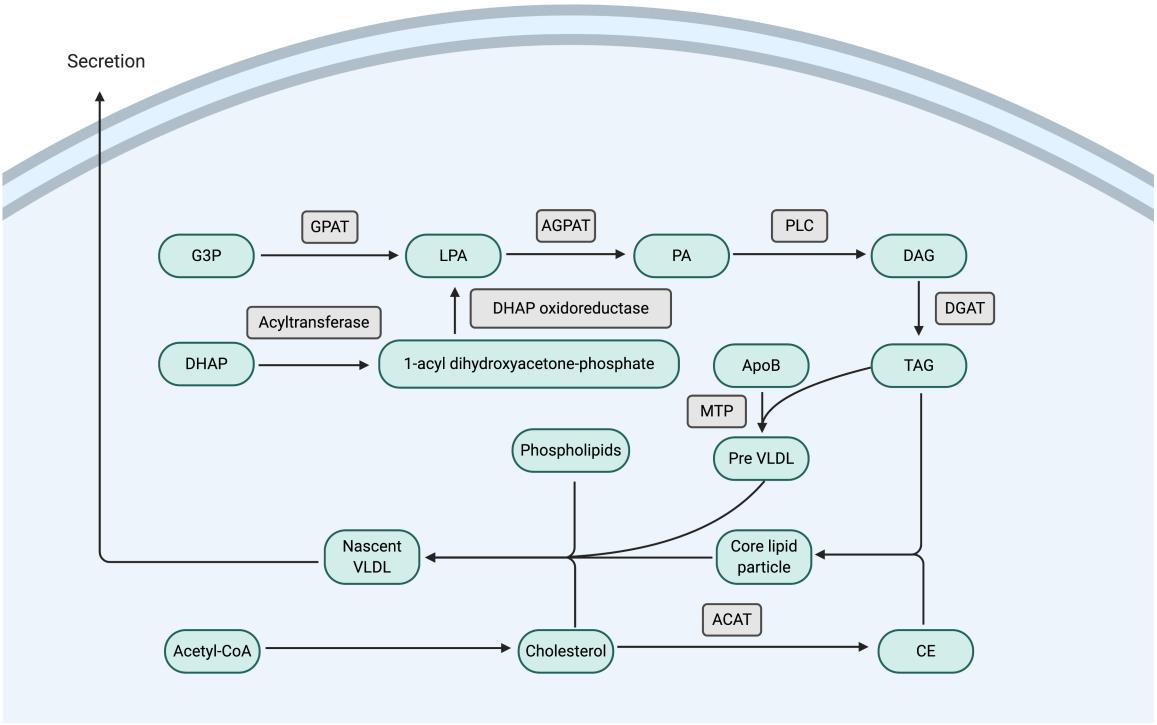

Figure 5-4. Liver lipoprotein signalings

teractions between apoB and MTP rely on their homodimerization surfaces, which result from the fact that they are homologs of lipovitellin, the egg yolk storage protein. Therefore, this step forms pre-VLDL droplets since the complete VLDL particle is composed of various fatty acids, to be specific, triglycerides (50-60%), cholesterol (10-12%), cholesterol esters (4-6%), phospholipids (18-20%), and apolipoprotein B (8-12%) [192].

Then after the pre-VLDL was transferred to the smooth endoplasmic reticulum, the second step occurs. This process requires a core lipid particle that lacks apoB, which consists of an inner core of TAG and cholesterol ester. The pre-VLDL droplet fuse with the core lipid particle accompanied by the assembly of phospholipids and cholesterol [193]. However, the enzymology of VLDL synthesis remains unclear. After the assembly is complete, nascent VLDL goes through the Golgi apparatus to be secreted from the liver into the circulation. In the plasma, VLDL has to acquire more lipoproteins to be a mature one. In

detail, nascent VLDL acquires apolipoprotein C-II(apo C-II) and additional apoE donated from high-density lipoprotein(HDL) [194]. Then the mature VLDL is able to contract with lipoprotein lipase(LPL) in the capillary beds, by which LPL removes triglycerides from VLDL into other cells.

TAG synthesis

There are two main pathways for the synthesis of TAG in the liver: one is known as the Kennedy pathway and the other one is via dihydroxyacetone-phosphate [195].
In the former process, the synthesis starts with glycerol-3-phosphate(G3P), which is catalyzed to lysophosphatidic acid(LPA) via the enzyme acyl-CoA:G3P acyltransferase(GPAT). Then LPA is acylated into phosphatidic acid(PA) by an acylglycerophosphate acyltransferase(AGPAT). In turn, PA is catalyzed by phosphatidic acid phosphohydrolase(PAP) and forms DAG, which then can be acylated into TAG by diacylglycerol acyltransferases (DGAT).
In the latter pathway, the difference is the source of LPA, in which LPA doesn't come from G3P yet dihydroxyacetone-phosphate(DHAP) in peroxisomes or endoplasmic reticulum. DHAP is acylated by a specific acyltransferase to form 1-acyl dihydroxyacetone-phosphate, which then is catalyzed into LPA via DHAP oxidoreductase.

Other related pathways

Besides TAG, cholesterol and cholesteryl esters also play essential roles in VLDL synthesis. It has been shown that about 80% of total daily cholesterol production occurs in the liver and the intestines [196]. The synthesis of cholesterol starts with two molecules of acetyl-CoA. Since the overall cholesterol synthesis in the liver is quite complex and there is no overlap between the substance involved in cholesterol synthesis and previously listed pathways, the detailed synthesis of cholesterol is not described here and it is simplified to be the formation from acetyl-CoA [197].

As for cholesteryl esters, it is also a major part of liver fatty acid synthesis. In hepatocytes, the enzyme acyl-CoA:cholesterol acyltransferase(ACAT) catalyzes cholesterol to cholesteryl esters(CE). There are mainly two isoforms of ACAT: ACAT1 which is widely expressed in many cells, and ACAT2 which is found only in the liver and small intestine [198].

As described in TAG synthesis, DAG acts as a precursor of TAG. DAG is also synthesized from the hydrolysis of the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2), which plays a key role in the PI3K/Akt pathway, through the enzyme phospholipase C(PLC). The hydrolysis occurs at the cell membrane. Also, the resulting DAG can activate protein kinase C(PKC) by binding [199].

Different DAG downstream signaling can be triggered depending on the different types of PKC that DAG binds to. There are two isozymes of PKC that require the activation of DAG: conventional PKC(cPKC), and novel PKC(nPKC). It has been shown that the JNK pathway can be inhibited through PKC [200]. Also, it has been indicated that DAG and PKC-mediated transphosphorylation of PKD act synergistically to promote PKD catalytic activation [201]. Furthermore, PKD can have a direct negative effect on mTORC1/2 and IKK, which negatively affects Akt via the NFκB pathway [202][203]. And both mTORC1/2 and Akt have been reported to positively regulate SREBP1c, which is identified as a protein regulating fatty acid metabolism [204][205].

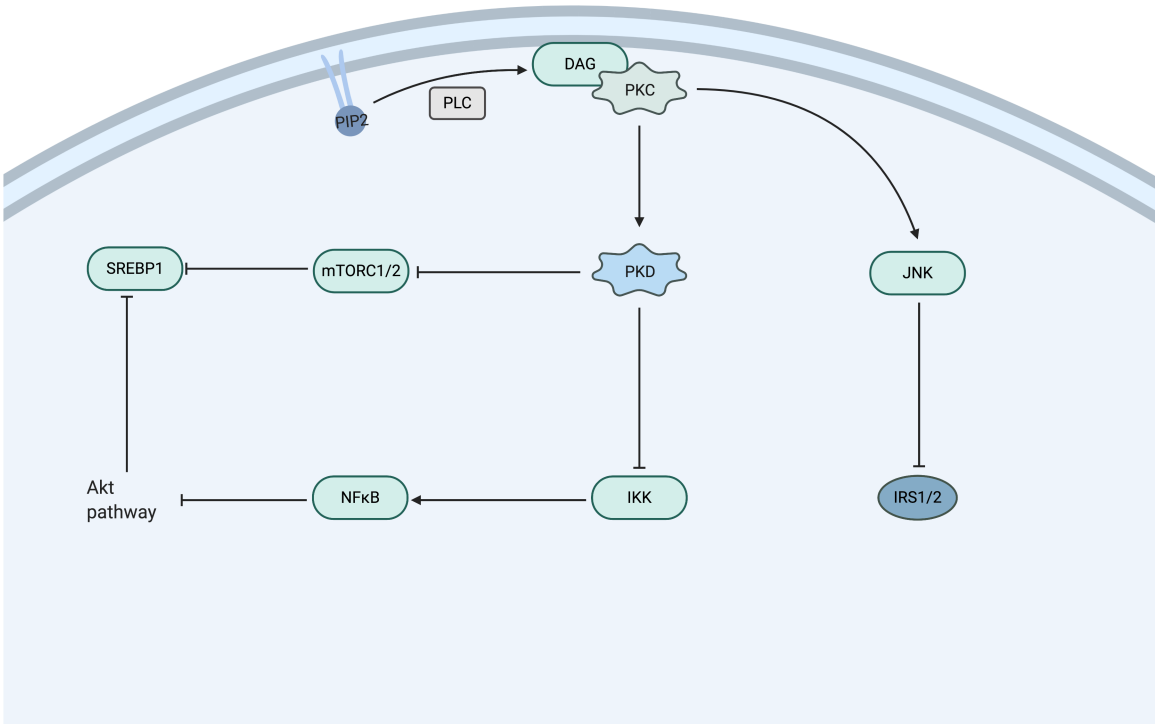

Figure 5-5. Liver DAG on the cell membrane and its downstream signaling

Chapter 6

Conclusion

As insulin, glucagon, glucose, and lipoprotein signaling pathways are summarized, it is clear that those four signaling pathways though quite different are not independent of each other. For example, GYS2 is activated via insulin signaling pathways while it is also the glycogen synthase to form α-1,4 glucosyl unit on glycogenin from UDP-glucose in glycogenesis. This can explain how insulin accelerates the consumption of glucose, thus reducing the plasma concentration of glucose. Also, in the glucagon signaling pathways, RYGb is activated into the active form, RYGa. It is the glycogen phosphorylase which catalyzes α-1,4 glucosyl unit on glycogenin to G1P in the production of glucose. This explains how glucagon increases the plasma concentration of glucose. Cytosolic acetyl-CoA formed in the TCA cycle via gluconeogenesis also can be used to produce cholesterol and cholesteryl esters in the lipoprotein signaling pathways. Here, insulin resistance related signaling pathways can be roughly concluded. However, detailed mechanisms behind insulin resistance remain unclear and require further studies.

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EDUCATION AND DEGREES

2018–Present Graduate student, Department of Chemical and Biomolecular Engineering
Johns Hopkins University, Baltimore MD

2013–2017 Undergraduate student, School of Chemical Engineering and Technology
Tianjin University, Tianjin China

RESEARCH EXPERIENCE

Johns Hopkins University, Whiting School of Engineering
Department of Chemical and Biomolecular Engineering *(Sep 2018-Present)*
Graduate Student under the supervision of Prof. Marc D. Donohue

- Constituted comprehensive type 2 diabetes signaling pathway and inflammatory response pathway to explain how T2D develops and progresses.
- Built PK/PD models in MATLAB/python to predict concentration changes of key molecules in patients.
- Tested and optimized the model using patients' data to ensure simulated results are within mean±SE.
- Identified rate-limiting steps in the signaling pathway as potential drug targets to guide drug development.
- Verified the connection between insulin resistance and T2D through modeling.

Tianjin University, Tianjin China *(Nov 2016-Jun 2017)*
Researcher in the laboratory of Prof. Yue Wang

- Applied the Automatic film element rolling technology on producing and testing the RO membrane
- Identified optimal conditions for filtration efficiency using IMSDesign software.
- Demonstrated that the desalination membrane system could improve the current efficiency by 100%.
- Carried out minimum energy analysis and provided the basis for energy saving methods.

Tianjin University, Tianjin China *(Sep 2016-Jun 2017)*
Project leader of IChemE design under the supervision of Prof. Shaoyi jia

- Composed production plan and quality control setup, designed rectification towers for the production of 2,4-di-tert-butylphenol based on McCabe-Thiele method.
- Designed process flow diagram and plant layout, evaluated the safety of the factory including the main measures to control pollution and toxic substances, achieved 99.5% purity of the product.

TECHNIQUES AND SKILLS

Experiment

Pharmacokinetic and pharmacodynamic analysis, Western Blot Experiment, Chemical instrumentation such as rotameters, Orthogonal experiment

Computing and Other Skills

MATLAB, Python, ASPEN PLUS, Origin, CAD, ChemOffice, LabVIEW